MOLECULAR MECHANISMS IN DNA REPLICATION AND RECOMBINATION

Organizers: Charles Richardson and Robert Lehman

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Molecular Mechanisms in DNA Replication and Recombination

DNA Structure

L 001 UNUSUAL DNA STRUCTURES IN VITRO AND IN VIVO, R.D. Wells, S. Amirhaeri, J.A. Blaho, D.A. Collier, A. Dohrman, J.A. Griffin, J.C. Hanvey, A. Jaworski, J.E. Larson, A. Rahmouni, M. Rajagopalan, M. Shimizu, F. Wohlrab and W. Zacharias, Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294

Unorthodox DNA secondary structures may play important roles in gene regulation and other cellular processes. The types of unusual DNA structures under investigation include left-handed Z-DNA, triplexes and other non-B structures at oligopurine-oligopyrimidine inserts, cruciforms, bent DNA, and anisomorphic DNA. Enzymatic, chemical, and physical investigations are focused on inserts in recombinant plasmids. Left-handed Z-DNA exists in E. coli and has biological consequences. This conclusion is based on four in vivo assays (MEcoRI probe, change in linking number, chemical probes, plasmid insert-specific deletions). Intramolecular DNA triplexes exist at oligopurine-oligopyrimidine inserts in plasmids. Studies will be described with a family of simple repeating sequence inserts, analogs of the murine immunoglobin alpha switch region, and inserts containing point mutations specifically designed to evaluate the presence of triplexes. In summary, unusual DNA structures exist and function in vivo.

Discussion: Structure of Centromeres and Telomeres

L 002 THE TELOMERASE RIBONUCLEOPROTEIN OF TETRAHYMENA

Elizabeth H. Blackburn, Carol W. Greider, Margaret Lee and Guo-Liang Yu, Department of Molecular Biology, University of California Berkeley, CA 94720

We are analyzing the reaction catalyzed by the ribonucleoprotein enzyme telomerase (telomere terminal transferase) isolated from Tetrahymena, and its control in vivo. Tetrahymena telomerase adds TTGGGG repeats, one nucleotide at a time, to synthetic G-rich telomeric DNA oligonucleotide primers in vitro(1,.2). We measured the K_m of telomerase for different telomere sequence primers . Under normal assay conditions, the K_m for $(TTGGGG)_4$ was 1.5 nanomolar, for $(TTGGGG)_2$ was 10 nanomolar, and for $(TTTTGGGG)_4$ was 2 nanomolar. When 100 mM K^+ ion replaced the 100 mM Na^+ in the assay, the kinetics for the (TTGGGG)2 - primed reaction became non-Michaelis-Menten. With a non-telomeric primer that could form a duplex involving G.G base-pairs, telomerase exhibited bimolecular kinetics. An RNA primer, $ribo(UUGGGG)_3$, was no more active as a primer than non-telomeric DNA oligonucleotide primers. The chimaeric DNA-RNA primer 5' $d(TTGGGGTTGGGG)\text{-}rU_{OH}$ 3' was an efficient primer; hence the inactivity of the RNA primer was not due to inability to bind a primer bearing a 2' OH group. These results support the hypothesis that a structural component of telomeric G-rich oligonucleotides is recognized by telomerase.

The cloned telomerase RNA gene(C. W. Greider and E. H. Blackburn, submitted) was introduced back into Tetrahymena on a high copy ribosomal RNA gene vector. We are analyzing phenotypes of such transformants and the expression of this gene.

1. C.W. Greider and E. H. Blackburn, (1985) Cell 43, 405-413.
2. C.W. Greider and E. H. Blackburn, (1987) Cell 51, 887 - 898.

Molecular Mechanisms in DNA Replication and Recombination

DNA Topology

L 003 DNA SUPERCOILING: STRUCTURE IN SOLUTION AND ROLE IN RECOMBINATION, T. Christian Boles[1], Nicholas R. Cozzarelli[1,3], Clem Donahue[1], Peter Droge[1], Roland Kanaar[1], Pieter van de Putte[2], and James H. White[3], [1]Department of Molecular Biology, University of California, Berkeley; [2]Laboratory of Molecular Genetics, State University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands; [3]Department of Mathematics, University of California, Los Angeles, CA 90024

We have investigated the structure of free supercoiled DNA by electron microscopy and topological methods. The DNA is a very long and thin interwound right-handed superhelix with occasional branches. With increasing linking deficit, writhe, twist, and double helical repeat change proportionally. The structure of supercoiled DNA in solution differs fundamentally from that in nucleosomes even when they are topologically identical. Supercoiling is required for site-specific recombination by the resolvase and Gin systems. In the synaptic intermediates, there are a characteristic number and arrangement of trapped supercoils that have the interwound structure of supercoils in solution rather than those in nucleosomes. The critical feature of these complexes is their geometry and not their topology. An attractive model for how the Gin recombinational sites and the Gin enhancer interact in the synaptic intermediate postulates that all three meet at a branch in the supercoiling. We shall discuss the topological and electron microscopic evidence for this intermediate.

L 004 VISUALIZATION OF THE EFFECTS OF SEQUENCE ARRANGEMENTS AND DNA DAMAGE ON DNA TOPOLOGY AND PROTEIN BINDING. Jack D. Griffith, Carl Bortner, Cheng-Hsilin Hsieh, and Randy Thresher. Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina 27514. The binding or kinking of DNA by sequence arrangements, DNA damage and DNA binding drugs can be followed using a number of different assays. In an approach using polyacrylamide gel electrophoresis, short DNA fragments containing damage at a single site are ligated head-to-tail and the migration of the linear multimers are followed by electrophoresis in polyacrylamide gels. The size distribution of circular multimers can be determined by electron microscopy. Using these assays we have examined the effects of mismatches and extra bases in duplex DNA. We find that whereas mismatches have no detectible effect on DNA bending, single extra bases produce sharp kinks of 30 to 40 degrees. Placement of two extra bases 5 bp apart on one strand of a duplex DNA cancel any net effect as seen by gel electrophoresis, while their separation by 10 bp produces a more highly kinked DNA. Using direct EM visualization of natural DNA we have prepared a complete "bending" map of SV40 DNA which provides a mathematical measure of curvature at each point on the 5243 bp genome. The relation of natural sequences which cause DNA to be bent or curved to the binding of topoisomerase II will be discussed as well as the effects of intercalating drugs such as ethidium bromide on the binding of the RecA protein to duplex DNA.

L 005 THE DNA HELICASES OF *E. coli*. S.W. Matson, J. W. George, K.A. Kaiser-Rogers, E.E. Lahue, E.R. Wood and J.E. Yancey, Department of Biology, University of North Carolina, Chapel Hill, NC 27599.

E. coli cells encode at least seven enzymes with duplex DNA unwinding activity; F+ cells encode an eighth helicase. It is likely that each helicase has a specific role in DNA metabolism accounting for the large number of enzymes with the same basic biochemical activity within a single organism. We have concentrated our efforts on four helicases; helicase I, helicase II, Rep protein and helicase IV (previously the 75-kDa helicase). Using an *in vitro* strand displacement assay we are able to distinguish three distinct biochemical mechanisms by which these enzymes unwind duplex DNA. Helicase I (F factor *traI* gene product) is a processive, catalytic helicase. Unwinding of long partial duplex substrates is as efficient as unwinding of short partial duplex molecules. Helicase II (*uvrD* gene product) unwinds duplex DNA by a protein-dependent, distributive mechanism. The amount of duplex DNA unwound by this enzyme is directly proportional to protein concentration. Thus large amounts of helicase II are necessary to unwind significant regions of duplex DNA. Helicase II also unwinds DNA:RNA hybrid molecules in a reaction that is considerably faster than unwinding of the corresponding DNA partial duplex. Preliminary data suggest that this unwinding reaction may be processive. Helicase II is the only helicase of the four able to catalyze this reaction. This previously undescribed activity may suggest an additional role(s) for helicase II in the cell. Rep protein and helicase IV are examples of the third biochemical unwinding mechanism we observe. Both helicases unwind short (71 bp) duplex molecules efficiently but unwinding decreases dramatically when the length of the duplex region is increased. Helicase IV is stimulated about 4-fold by *E. coli* SSB, however Rep protein is not stimulated by SSB under these conditions. A small polypeptide has been purified that markedly stimulates the unwinding reaction catalyzed by Rep protein. This protein interacts with the DNA substrate to stimulate the unwinding reactions catalyzed by Rep protein and helicase II but fails to stimulate either helicase I or helicase IV.

The gene encoding the recently described helicase IV has been cloned, sequenced and mapped on the *E. coli* chromosome. The predicted amino acid sequence is similar to both Rep protein and helicase II but may have an additional amino terminal domain not found on the other proteins. The gene encoding this helicase maps to 22 minutes on the *E. coli* genetic map. The physiological role of helicase IV is currently being investigated.

DNA Replication Proteins

L 006 **EFFECTS OF ACCESSORY PROTEINS ON THE FUNCTIONAL PROPERTIES OF DNA POLYMERASE DELTA**, Kathleen M. Downey, David M. Andrews, Xiangyang Li, Cheng-Keat Tan and Antero G. So, Departments of Medicine and of Biochemistry/Molecular Biology, University of Miami School of Medicine, Miami, FL 33101

Recent evidence suggests that DNA polymerases alpha and delta are structurally, functionally and immunologically distinct enzyme species and that both polymerases are involved in replication of the cellular genome. Based on their functional properties, we have proposed that DNA polymerases alpha and delta act as lagging strand and leading strand replicases, respectively. The association of accessory proteins with DNA polymerase delta markedly affects the functional properties of the core enzyme. The proliferating cell nuclear antigen (PCNA) converts the essentially distributive core polymerase to a highly processive polymerase on both homopolymer and natural DNA templates. The results of studies on the mechanism by which PCNA affects the processivity of DNA polymerase delta suggest that the stability of the enzyme-primer terminus complex is dramatically increased in the presence of PCNA.

A second accessory protein which affects the ability of DNA polymerase delta to carry out strand displacement synthesis has been isolated from fetal calf thymus. The strand displacement factor specifically affects DNA polymerase delta and has no effect on the activity of DNA polymerase alpha. The protein is a DNA helicase and shows specificity for ATP or dATP. Strand displacement synthesis by DNA polymerase delta requires PCNA in addition to the strand displacement factor.

L 007 MUTATIONAL ANALYSIS OF REVERSE TRANSCRIPTASE ENZYMES: STRUCTURE AND FUNCTION OF DNA POLYMERASE AND RNASE H DOMAINS. Naoko Tanese, Monica Roth, Vinayaka Prasad, and Stephen P. Goff, Department of Biochemistry and Molecular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY 10032.
We have expressed the reverse transcriptase (RT) of Moloney murine leukemia virus in bacterial cells, and have shown that the enzyme exhibits both the RNA-dependent DNA polymerase and RNAse H activities associated with the authentic, monomeric, enzyme. Mutagenesis showed that the enzyme could be divided into two functional domains: the DNA polymerase activity was contained in the N-terminal two-thirds of the molecule, while the RNAse H activity was contained in the C-terminal third. The two activities were fully independent as demonstrated by separate expression of appropriate non-overlapping sections of the enzyme. In these studies a large number of mutations have been generated in the expression construct and characterized; many have been transferred back into the viral genome, and the effects on replication have been determined. Mutants lacking RNAse H were blocked at a well-defined step during reverse transcription: strong stop DNA did not translocate but was held in RNA:DNA hybrid form. One mutation near the C-terminus of RT yielded an exquisitely temperature-sensitive phenotype. Examination of the virion proteins in this mutant showed that the proteolytic cleavage that normally separates RT from the adjacent integrase protein (IN) was itself temperature-sensitive and blocked at the nonpermissive temperature.

We have also expressed the RT of HIV in bacterial cells. This enzyme is normally a heterodimer of 66- and 51-kd subunits; our construct generates a trpE-RT fusion protein containing the 66-kd version of the enzyme. Analysis showed that the bacterial protein exhibited both DNA polymerase and RNAse H activities, with template and divalent cation preferences diagnostic of the authentic enzyme. Mutational studies showed further that the HIV RT was arranged similarly to the murine enzyme, with the polymerase function localized to the N-terminus; constructs that expressed the p51 subunit did show normal levels of polymerase although the natural p51 has only shown very little activity. To facilitate the isolation of rare variant enzymes with selected properties, we have developed an *in situ* assay technique that permits the screening of large numbers of bacterial colonies for the DNA polymerase activity of the HIV RT expressed by their variant plasmids. We are currently using the screen for the isolation of revertants of linker-insertion mutants carrying second-site suppressors of the original mutations; and for the isolation of variant enzymes that are resistant to inhibitors such as AZTTP.

L 008 THE TWO ACTIVE SITES OF THE KLENOW FRAGMENT OF DNA POLYMERASE I OF *E. COLI*. Catherine M. Joyce, Andrea H. Polesky, Victoria Derbyshire, Nigel D.F. Grindley, Lorena S. Beese, Jonathan M. Friedman and Thomas A. Steitz. Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.
The Klenow fragment of DNA polymerase I provides a simple and well-characterized model system for exploring the molecular details of template-directed DNA synthesis. The molecule has two enzymatic activities, the polymerase function and an editing (3′-5′) exonuclease, which reside on separate structural domains (1, 2). The polymerase domain is the larger of the two and contains a large cleft, which has been proposed as the binding site for duplex DNA (2). More recently, crystallographic experiments have demonstrated that single-stranded DNA binds to the 3′-5′ exonuclease domain, with the 3′-terminus at the exonuclease active site (3).

The 3′-5′ exonuclease active site was originally identified crystallographically as the binding site for a deoxynucleoside monophosphate inhibitor (2). Site-directed mutagenesis of carboxylate side-chains in this region yielded two mutant derivatives devoid of exonuclease activity but with normal polymerase activity (4). Biochemical and structural studies of the mutant proteins demonstrated that the effect of these mutations is to perturb the binding of the two divalent metal ions at the exonuclease active site. The results suggested a role for one of these metal ions in catalysis, and for the other in DNA binding and possibly also in catalysis. Additional mutations in this active site are currently being studied.

The position of the polymerase active site has been inferred from biochemical experiments since there is currently no direct crystallographic information on the binding of a duplex DNA primer terminus or of deoxynucleoside triphosphates. Site-directed mutagenesis of residues in the putative active site region suggests that the proposed location is probably correct. Mutagenesis of a cluster of residues on the floor of the binding cleft has a profound effect on k_{cat} for the polymerase reaction. Close by, on one side of the cleft, is another cluster of residues which, when mutated, affect the K_m of the deoxynucleoside triphosphate substrate.

1. Joyce, C.M. and Steitz, T.A. (1987) Trends Biochem. Sci. 12:288-292.
2. Ollis, D.L. *et al*. (1985) Nature 313:762-766.
3. Freemont, P.S. *et al*. (1988) Proc. Natl. Acad. Sci., in press.
4. Derbyshire, V. *et al*. (1988) Science 240:199-201.

Molecular Mechanisms in DNA Replication and Recombination

L 009 FUNCTIONS AND REGULATION OF THE DNA POLYMERASE III HOLOENZYME, AN ASYMMETRIC DIMERIC DNA POLYMERASE,

Charles S. McHenry, Henry Tomasiewicz, Ann Flower, Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver CO 80262

In 1983, my laboratory proposed that the DNA polymerase III holoenzyme is an asymmetric dimer with distinguishable leading and lagging strand polymerases. I will review progress in testing this hypothesis. To date, the hypothesis is supported by our demonstration of (i) an asymmetry in function of two populations of holoenzyme in solution in their ability to use the ATP analog, ATPγS, to support initiation complex formation, (ii) the stabilization of a dimeric polymerase structure by the τ subunit, (iii) allosteric communication between polymerase halves and (iv) the coexistence of γ and the τ, subunits which share common sequences, within the same holoenzyme assemblies. This latter observation may provide a structural basis for holoenzyme asymmetry. I will discuss the implications of the asymmetric dimer hypothesis to the solution of problems encountered by polymerases at the replication fork.

Parallel with these studies, we are investigating the genetic regulation of holoenzyme components. I will present evidence that the *dna*E and *dna*Z components are on complex operons with other components required for macromolecular synthesis and cell division.

Protein-DNA Interactions

L 010 ZINC FINGERS, Barbara T. Amann and Jeremy M. Berg, The Johns Hopkins University, Department of Chemistry, 34th and Charles Sts., Baltimore, MD 21218

In recent years, a family of proteins has been discovered that are characterized by the presence of one or more sequences of the form (Tyr,Phe)-X-Cys-X_{2-4}-Cys-X_3-Phe-X_5-Leu-X_2-His-X_{3-4}-His-X_n where X represents a relatively unconserved amino acid and n is often 5. Based on analysis of the zinc content of the archetypal protein of this family Xenopus Transcription Factor IIIA (TFIIIA), it was proposed that each of these sequences forms a structural domain around a zinc ion coordinated to the invariant Cys and His residues. These domains were termed "zinc fingers". A variety of evidence now supports this hypothesis. A three-dimensional structure for the "zinc finger" domains was deduced based on analysis of the structures of crystallographically characterized metalloproteins. The structure consists of a two stranded beta sheet followed by an alpha helix. This stucture has been supported and extended by detailed analysis of the "zinc finger" sequences and by experimental studies of single "zinc finger" peptides. This structure has considerable implications with respect to the interactions between "zinc finger" proteins and nucleic acids. In particular, it appears that "zinc finger" proteins with consensus linker regions wrap around nucleic acids in the major groove.

Molecular Mechanisms in DNA Replication and Recombination

L 011 STRUCTURE AND DYNAMICS OF DNA-PROTEIN INTERACTIONS WITHIN THE T4 DNA REPLICATION COMPLEX. Peter H. von Hippel, Mary Kay Dolejsi, Frederic R. Fairfield, Joel W. Hockensmith, Thale C. Jarvis, William L. Kubasek, Sigrid B. Kuhl, Michael K. Reddy, and Mark C. Young. Institute of Molecular Biology, Department of Chemistry and Department of Biology, University of Oregon, Eugene, OR 97403.

The DNA replication system encoded by bacteriophage T4 consists of seven proteins. As initially defined and reconstituted by the Alberts and Nossal groups, this seven-protein system can carry out leading and lagging strand DNA elongation synthesis at rates, fidelities and processivities that closely approximate in vivo values. We have been attempting to characterize the molecular mechanisms of this system by reducing it to functional subassemblies and studying their behavior. Aspects of these studies that will be described include: (i) analysis of the processivity of both the DNA synthesis and the 5'-3' editing activities of the DNA polymerase (gene 43 protein) at a primer-template junction; (ii) structure and function of the components and specificities of binding of the polymerase accessory proteins complex (gene 44/62 and 45 proteins); (iii) UV laser crosslinking studies to analyze protein-DNA contacts within the polymerase accessory proteins complex at the primer-template junction and the dynamics of the conformational changes within this complex that accompany the addition of ATP; and (iv) the effects of the addition of single-stranded DNA binding protein (gene 32 protein) on these processes.

Enzymes of Recombination

L 012 ON THE MECHANISM OF recA PROTEIN-MEDIATED DNA STRAND EXCHANGE, Michael M. Cox, Department of Biochemistry, University of Wisconsin, Madison, WI 53706

RecA protein is unusual among DNA-binding proteins in that its function is to bind not one but two DNA molecules. Ultimately, DNA strands are exchanged between them in a reaction that involves ATP hydrolysis. The first complex (primary binding, DNA1) is generally formed on a DNA molecule that is at least partially single-stranded. Binding is sequence-independent and stoichiometric, resulting in a recA nucleoprotein filament. Binding to the second DNA (secondary binding, DNA2) is largely dependent on the presence of sequences homologous to some region of DNA1. The two DNAs are bound asymmetrically. Homology between DNA1 and DNA2 is aligned prior to any exchange of strands in a paranemic joint. When DNA1 is single-stranded, paranemic joints can span thousands of homologous base pairs. A decade of research has not yet provided unambiguous answers to several key questions, answers required for an understanding of recA protein-promoted DNA strand exchange. These questions include: (a) Are three- or four-stranded DNA helices intermediates in the reaction (in the paranemic joint)? (b) What is the molecular function of ATP hydrolysis in this system? and (c) How does the recA nucleoprotein filament structure facilitate this reaction? Recent results bearing on these questions will be discussed and used as a framework for a modified model for DNA strand exchange.

L 013 THE ROLE OF GIN AND FIS IN PHAGE MU SITE-SPECIFIC RECOMBINATION, R. Kahmann, A. Klippel, F. Rudt and C. Koch, Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, D-1000 Berlin 33, West-Germany

Host range switching in phage Mu is controlled by site-specific inversion of the G segment. The reaction is catalyzed by the phage encoded DNA invertase Gin. Recombination is stimulated by an enhancer sequence in cis, relatively independent of its distance and orientation with respect to the recombination sites. The enhancer effect requires the E. coli Protein FIS which interacts with the enhancer. A specific threedimensional structure of the enhancer FIS complex which involves a highly bent conformation of the DNA is necessary for enhancer function. Recombination requires a supercoiled DNA substrate and directionality e.g. recombination is efficient only when the sites are arranged as inverted repeats and is poor if they are directly repeated.
We have isolated a series of different point mutations in gin which have a FIS independent phenotype. The most striking features of these mutations are effects on directionality and on supercoiling requirements which are both alleviated. We present an in vitro characterization of the mutant proteins and relate their properties to the role of the enhancer and FIS in the wildtype situation.

Initiation of DNA Replication: Prokaryotes

L 014 EARLY EVENTS IN THE ENZYMATIC REPLICATION OF PLASMID CONTAINING THE ORIGIN OF THE E.COLI CHROMOSOME. Tania A. Baker, Benjamin Y.-M. Yung and Arthur Kornberg, Stanford University School of Medicine, Stanford, CA 94305.
In the initiation of replication from the E.coli chromosomal origin by purified proteins dnaA protein (i) binds cooperatively to the four 9-bp "dnaA boxes" within oriC and forms a large initial complex, (ii) opens the DNA duplex in the 13-bp repeats at the left boundary of oriC to form the open complex, and (iii) enables the dnaB helicase to enter between these opened strands (by the transient action of the dnaC protein) forming the prepriming complex, and unwind the DNA duplex to create an initiation bubble. Origins from a wide variety of replicons resemble oriC in containing a distinct class of repeated sequence elements in an AT-rich region bordering the boxes recognized by the initiator protein. Binding tightly to one region and then opening the duplex in the other, likely represents a general mechanism by which initiator proteins direct the assembly of replication forks on duplex DNA (1).

Open complex formation requires that dnaA protein have a tightly bound ATP; the ADP-form generated by DNA dependent hydrolysis of the bound ATP, is inactive. ADP-dnaA protein fails to open the 13-mer repeats, although it binds effectively to the dnaA boxes within oriC (see abstract by Yung and Kornberg). Interaction with acidic phospholipid head-groups, in a fluid membrane, dislodges ADP from dnaA protein, and subsequent binding of ATP rejuvenates its replication activity. Duplex opening by dnaA protein is also extremely sensitive to DNA structure. Factors which stabilize the helix - binding proteins, low temperature and removal of negative supercoils - specifically inhibit this step. A transcript, hybridized to the template near the origin, facilitates duplex opening by dnaA protein on plasmids rendered inert by such forces. Thus the cell's membranes and transcriptional activity may trigger or postpone initiation at the replication origin.

1. Bramhill, D. and Kornberg, A. (1988) 54, 915-918.

L 015 **INITIATION OF BACTERIOPHAGE λ DNA REPLICATION, Roger McMacken, Christine Alfano, Kojo Mensa-Wilmot, Kathryn Stephens, Kathleen Carroll, Devanur Sampath, Robert Jordan, Soo-Jong Um, and Li Huang, Department of Biochemistry, The Johns Hopkins University, Baltimore, MD 21205**

The early stages in the initiation of bacteriophage λ DNA replication are characterized by sequential protein-DNA and protein-protein interactions that culminate in helicase-mediated unwinding of supercoiled *ori*λ plasmid DNA templates. We have developed a sensitive immunoblotting protocol that has permitted us to resolve the prepriming pathway into five separable stages. Binding of multiple copies of λ O initiator to *ori*λ leads to the formation of a nucleosomelike structure, the O-some (Stage I), that functions to localize subsequent events to the origin. In the second stage, formation of a tight complex between the λ P protein and the *E. coli* DnaB helicase activates the assembly of a stable *ori*λ:O-P-DnaB complex. This stage II nucleoprotein structure is specifically recognized by the *E. coli* DnaJ heat shock protein, which binds to form an *ori*λ:O-P-DnaB-DnaJ prepriming complex (Stage III). DnaK protein, the *E. coli* hsp70 heat shock protein homologue, interacts with each of the prepriming structures formed in the first three stages. In the absence of ATP a complete initiation complex (Stage IV) containing O, P, DnaB, DnaJ, and DnaK is formed at *ori*λ. The Stage I-III structures were isolated and shown to be functional intermediates in the initiation of λ DNA replication. Nevertheless, the known helicase activity of DnaB associated with the stage II-IV structures is completely suppressed, apparently as a result of strong protein interactions with λ P protein. In the presence of ATP, however, the DnaJ and DnaK heat shock proteins catalyze a series of protein disassembly reactions that results in the restoration of helicase activity to DnaB protein and the specific unwinding of *ori*λ plasmid templates to form an unwound complex (Stage V). Both P protein and DnaJ protein are efficiently removed from nucleoprotein structures during the disassembly reaction. GrpE protein, a third *E. coli* heat shock protein required for λ DNA replication *in vivo*, is not essential *in vitro*, but it does greatly stimulate λ DNA replication *in vitro* at low concentrations of DnaK. Prepriming structures I-IV are readily formed on relaxed templates, but initiation of DnaB helicase action absolutely depends on the presence of negative superhelicity in the *ori*λ template. In contrast, on relaxed templates DnaB is efficiently removed along with P and DnaJ from the stage IV structure when the heat shock proteins are activated by ATP.

L 016 PROTEIN-PRIMED REPLICATION OF BACTERIOPHAGE ø29 DNA. Margarita Salas, Angel Zaballos, Gil Martín, María J. Otero, Cristina Garmendia, Antonio Bernad, Manuel Serrano, José M. Lázaro, Maria A. Blasco, Julio Gutiérrez, Eulalia Parés, José M. Hermoso and Luis Blanco. Centro de Biología Molecular. Universidad Autónoma de Madrid. Canto Blanco, 28049 Madrid, Spain.

Phage ø29 has a linear, double-stranded DNA with a terminal protein covalently linked to the 5' ends through a phosphoester bond between the OH group of serine residue 232 and 5'-dAMP. Replication starts at both DNA ends by a protein-priming mechanism in which a free molecule of the terminal protein p3 forms a covalent complex with dAMP in a reaction catalyzed by the viral DNA polymerase p2, that further elongates each p3-dAMP complex to full-length ø29 DNA. The ø29 DNA polymerase is a highly processive enzyme able to produce strand-displacement in the absence of accessory proteins. By site-directed mutagenesis we have identified functional domains for initiation, elongation and 3'→5' exonuclease activity in the ø29 DNA polymerase. We have also constructed point and deletion mutants in the terminal protein that have allowed to determine functional domains for priming activity and binding to the DNA polymerase and DNA.
The products of the viral genes 5 and 6, required for in vivo ø29 DNA replication, stimulate in vitro ø29 DNA synthesis when added to the minimal system containing terminal protein and DNA polymerase. Protein p5 binds to single-stranded DNA, protects the latter against nuclease degradation and stimulates ø29 DNA replication, mainly at long incubation times. Protein p6, that stimulates the formation of the p3-dAMP complex and the transition to the first elongation products, binds to double-stranded DNA and specifically recognizes signals present at the ø29 DNA ends forming a nucleoprotein structure. By deletion analysis of both ø29 DNA terminal sequences and protein p6, a positive correlation has been found between the activity of protein p6 in the initiation of ø29 DNA replication and the formation of the nucleoprotein structure.

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L 017 METHYLATION OF THE REPLICATIVE ORIGIN OF *E. coli*: DOES IT DETERMINE THE TIMING OF CHROMOSOME SEGREGATION? IS DNA A PROTEIN INVOLVED?

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Bacteria do not have mitosis but their genome segregates with fidelity when they divide. This is though to take place by the attachment of DNA to the cell membrane. DNA from the *Escherichia coli* replicative origin binds with high affinity to outer membrane preparations[1]. Specific binding regions are contained within a 463 bp stretch of origin DNA between positions -46 and +417 on the *oriC* map. This region of DNA contains an unusually high number of GATC sites, the recognition sequence for the *E. coli* DNA adenine methylase. This laboratory has shown that *oriC* DNA binds to membrane only when it is hemimethylated[2]. The *E. coli* chromosomal origin is hemimethylated for 8-10 minutes after initiation of replication, and origin DNA binds to membranes only during this time period. Based on these results, a speculative model for chromosome segregation in *E. coli*. has been proposed[3].

DnaA, a protein required for initiation of DNA replication in *Escherichia coli*, binds to three of four DnaA binding sequences in the replicative origin *oriC* (boxes R1, R2, and R4). Protein-*oriC* DNA interactions in minichromosomes (origin containing *E. coli* sequences) carried by wild type and *dnaA* mutant strains were demonstrated by in vivo footprinting using dimethyl sulfate treatment of intact cells[4] The same characteristic enhancement/protection pattern was seen in wild type minichromosomes or mutants defective in *oriC* function but carrying the four DnaA boxes. Minichromosomes in *dnaA* (Ts) mutants showed no protein binding at non-permissive temperatures and reduced binding even at permissive temperatures. In vivo footprints of the wild type strain were identical to those obtained in vitro using purified DnaA protein and *oriC* DNA. Transcription into *oriC* affected the binding of DnaA protein to the DnaA boxes. These findings suggest that protein A causes the in vivo footprints at *oriC* .

1. Hendrickson WG, Kusano T, Yamaki H, Balakrishnan R, King M, Murchie J and Schaechter M. Cell 30:915, 1982.
2. Ogden GB, Pratt MJ and Schaechter M. Cell 54:127, 1988
3. Schaechter M. In: *The Bacterial Nucleoid*, Drlica and Riley (eds) (in press)
4. Samitt CE, Hansen FG, Miller JF and Schaechter M (EMBO J, in press)

L 018 CONTROL OF ColE1 PLASMID REPLICATION,

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RNA II of ColE1 forms a hybrid with the template DNA. The hybridized transcript either provides primer for synthesis leading strand DNA or activates synthesis of the lagging strand DNA by displacing nontranscribed strand. ColE1 DNA replication is regulated by inhibition of the hybrid formation by a plasmid-specified small RNA (RNA I). RNA I binds to RNA II and prevents it from formation of the hybrid. The binding procedes in a stepwise manner. Intermediates in the stepwise process of binding has been inferred by kinetic studies of inhibition of the binding by addition of a second, nonhomologous RNA I species; and finding of another intermediate that has been identified by an alteration in RNAase sensitivity. Comparison of results obtained with various pairs of RNA I and RNA II gives the following conclusions. Binding initiates by interaction at the loops and the tail of the folded structure of RNA I with the complementary sequences of RNA II. The product first made is very unstable; having only a limited number of interacting regions. As the number of interacting regions increases, the products become more stable. These interactions facilitate hybridization that starts near the 5' end of RNA I to generate the stable final product. ColE1 DNA replication is also regulated by another plasmid-specified product, Rom protein. This protein affects stability of intermediates in the process of binding of RNA I and RNA II.

Molecular Mechanisms in DNA Replication and Recombination

Protein-Protein Interactions at the Replication Fork

L 019 DNA REPLICATION IN *SACCHAROMYCES CEREVISIAE*. Judith L. Campbell, Martin E. Budd, Colin Gordon, Peter Rhode, and Karen C. Sitney, Divisions of Biology and Chemistry, California Institute of Technology, Pasadena, California 91125.

We have used yeast genetics and biochemistry to define the functions of the numerous DNA polymerases found in yeast. Many years ago we showed that DNA polymerase I, the analog of metazoan DNA polymerase α, was essential for DNA replication in yeast. More recently we have carried out structure function studies of DNA polymerase I. We have shown that a protein with a 155 amino acid deletion at the N-terminus of the protein is able to complement all functions of DNA polymerase I *in vivo*. By contrast, a 250 amino acid deletion from the C-terminus (nucleotide residues 1214-1468) results in a nonfunctional DNA polymerase. Five temperature sensitive mutations, described previously, all map to conserved regions II and III in the DNA polymerase.

We have recently found that there is a second essential DNA polymerase in yeast, DNA polymerase III. This was shown by demonstrating that yeast *cdc2* mutants, which are deficient in replication, are deficient in DNA polymerase III. We have cloned the *CDC2* gene and are studying the regulation of the polymerase.

We have purified DNA polymerase II to apparent homogeneity. The protein has a molecular weight of 170 kDa on activity gels. The polymerase activity copurifies with a 3′→5′ exonuclease through all purification steps. The preferred template is poly(dA):oligo(dT), suggesting that the enzyme is more processive than either DNA polymerase I or DNA polymerase III. DNA polymerase II is sensitive to aphidicolin and resistant to 100 μM BuPdGTP; thus it is not a member of the polymerase β family. Two proteins that stimulate DNA polymerase II have been identified, one of which increases the processivity of polymerase II. DNA polymerase II is not encoded by *POL1*, *CDC2*, or *REV3*, three genes that encode yeast DNA polymerases. On the basis of its biochemical characteristics, we propose that DNA polymerase II is analogous to the delta polymerases and may be involved in repair.

We have observed a fourth DNA polymerase. Since this species differs from polymerase II only in chromatographic properties, we have not yet given the enzyme a new designation.

In addition to our work with DNA polymerases, we have investigated protein DNA interactions at the yeast origin of replication, *ARS1*. We have purified ABF1, a protein that binds within 100 base pairs of the core consensus of at least seven different ARS elements. The protein shows two species of 132 and 135 kDa. Antibodies have been used to clone the *ABF1* gene and gene disruptions show that *ABF1* product is essential for the viability of yeast. The DNA binding domain has been identified by deletion analysis.

L 020 MOVEMENT AND CYCLING OF PROTEIN COMPLEXES PRESENT AT E. COLI REPLICATION FORKS

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Rolling-circle DNA replication supported by tailed form II DNA templates in the presence of the DNA polymerase III holoenzyme (Pol III HE) and the primosomal proteins has been used as a model system to examine mechanisms operating at the replication fork. The long double-stranded tails produced in this reaction are constituted of a long (>50 kb) leading-strand DNA and a family of short Okazaki fragments representing synthesis of the lagging strand. Factors influencing the cycling of the enzymatic machinery operating on the lagging-strand DNA template can be observed by determining their effect on the size and distribution of the Okazaki fragments produced.

At least three general parameters influence synthesis of the lagging strand: i) The frequency at which primers are synthesized and utilized, ii) the speed at which the replication fork moves and, iii) the distributive nature of the action of some of the enzymes, including the primase and some subunits of the Pol III HE.

Independent DNA helicase assays have demonstrated that there can be two distinct DNA translocation activities present on the lagging-strand template DNA, factor Y and the DNA B protein, both components of the primosome. Interestingly, these proteins move in opposite directions along the DNA strand. A possible consequence of this could be the generation of a primosome-anchored loop in the lagging-strand template DNA at the replication fork.

L 021 FORMATION AND PROPAGATION OF THE BACTERIOPHAGE T7 DNA REPLICATION FORK, Charles C. Richardson, Benjamin B. Beauchamp, Julie A. Bernstein, Yeon-Bo Chung, Hans E. Huber, Hiroshi Nakai, John Rush, Kathleen A. Ryan, Stanley Tabor, and Susannah M. Wurgler, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115

Four proteins account for the reactions at the replication fork of bacteriophage T7: T7 gene 5 protein, *E. coli* thioredoxin, T7 gene 4 protein, and T7 gene 2.5 protein. Gene 5 protein is a distributive DNA polymerase with a low macroscopic rate of synthesis and a 3' to 5' DNA exonuclease activity. Thioredoxin binds to gene 5 protein in a 1:1 complex increasing its affinity for a primer-template 50-fold. The complex is highly processive, polymerizing more than 10,000 nucleotides prior to dissociation. Thioredoxin also stimulates the double-stranded, but not the single-stranded DNA exonuclease activity, a result that reflects the increased binding to a primer-template structure. The redox capacity of thioredoxin is not required for its function as an accessory protein. Mutant thioredoxins lacking one or both cysteines at the active site have reduced affinity for gene 5 protein but still bestow processivity.

The exonuclease activities of T7 DNA polymerase can be inactivated by an oxidation reaction that requires oxygen, a reducing agent, and iron at a concentration equimolar to that of the gene 5 protein. Both exonuclease activities can be diminished by several thousandfold, with only a small decline in the polymerase activity. We propose that the exonuclease domain of gene 5 protein contains a metal binding site to which iron ions will bind. In the presence of oxygen, the bound iron generates reactive oxygen species that specifically modify amino acid residues in the exonuclease domain. We have used chemical reagents, known to react with specific amino acid residues, to identify amino acids in the gene 5 protein important for exonuclease activity. Such an identification is possible since modified T7 polymerase has properties that enable one to detect modified molecules when they are the minority in a population of wild-type gene 5 protein molecules. We have obtained, by *in vitro* mutagenesis, a collection of T7 DNA polymerases that contain from 20% to no detectable ($< 1 \times 10^{-5}$%) exonuclease activity.

Gene 4 protein binds to single-stranded DNA and translocates 5' to 3' using the energy of hydrolysis of NTPs. At the fork it interacts with T7 DNA polymerase and serves as a helicase for processive leading strand synthesis. The rate of fork movement is 300 nucleotides per sec at 30°C. On the lagging strand gene 4 protein acts as a primase, recognizing 3'-CTGGG/T-5' at which it synthesizes pppACCC/A primers. The primers are extended by T7 DNA polymerase to give Okazaki fragments. Lagging strand synthesis, in contrast to leading strand synthesis, is sensitive to dilution, indicating that recycling of the proteins includes a dissociation step. T7 gene 2.5 protein, a single-stranded DNA binding protein, stimulates primase activity by facilitating the rebinding of gene 4 protein to single-stranded DNA. The gene 4 protein exists in two mol. wt. species of 56- and 63 kDa. The small form arises as a result of a second initiation codon and ribosome binding site within gene 4 and thus lacks 63 amino acids at the amino terminus. The small form has been isolated from *E. coli* strains that overproduce only this species. The small form translocates on single-stranded DNA and has full helicase activity. However, the small form is unable to catalyze template-dependent primer synthesis. The amino terminus of the large form, not present on the small form, has a potential metal binding domain, cys-X_2-cys-X_{15}-cys-X_2-cys.

Eukaryotic Viral DNA Replication

L 022 CHARACTERIZATION OF THE HERPES SIMPLEX VIRUS PROTEINS INVOLVED IN DNA REPLICATION, Mark D. Challberg and Paul D. Olivo, Laboratory of Viral Diseases,National Institute of Allergy and Infectious Diseases, Bethesda, MD 20841

Using a transient complementation assay, we have shown that seven Herpes SimplexVirus (HSV) genes are both necessary and sufficient to support the replication viral DNA in infected cells. Two of these genes (pol and dbp) encode well-known DNA replication proteins (the DNA polymerase and the major single-stranded DNA binding protein, ICP8), Using the predicted amino acid sequences derived from DNA sequence analysis, we have raised rabbit antisera against the product of all seven genes, and have used these reagents to identify these proteins in infected cells. All seven proteins localize to the nucleus and are expressed in a manner consistent with the idea that they are the products of early genes. Various immunological assays suggest that four of these proteins (UL5, UL8, UL9 and UL52) are made in infected cells in very low abudance relative to the other three. In order to improve our ability to study these proteins, we have expressed UL5, UL8, UL9 and UL52 in insect cells using the baculovirus expression system. The HSV proteins made in insect cells are immunoprecipitable with the appropriate antisera and the size of each protein is indistinguishable from the corresponding protein made in HSV-infected cells.

Using these reagents, we have shown by an immunoassay for protein DNA interaction that the UL9 protein binds specifically the the HSV origins of replication. DNase I footprint analysis has shown that the UL9 protein interacts with two related sites within the origin, located on each arm of a nearly perfect palindrome.

Recombinant UL9 protein has been purified to homogeneity. The sedimentation coefficient of the purified protein in glycerol gradients (~7.5 S) suggests that UL9 exists predominately as a homodimeric structure. The interaction of purified UL9 with wild-type and mutated versions of the origin has been studied in some detail. Our results suggest that: 1) the binding of UL9 to site II (the right arm of the ori palindrome) correlates well with the ability of the origin to function in vivo, and 2) there is a cooperative interaction between UL9 bound at both arms of the origin palindrome

L 023 STUDIES ON THE REPLICATION OF DNA CONTAINING THE SV40 ORIGIN, P. Bullock, J. Borowiec, F. Dean, Y. Ishimi, M. Kenny, A. Kwong, S.H. Lee, Y.S. Seo and J. Hurwitz, Department of Molecular Biology and Virology Sloan-Kettering Institute New York, New York 10021

We have examined the action of SV40 T antigen in the replication of DNA containing the SV40 core origin (ori^+). In the presence of ATP, a multimeric T antigen complex is formed at the core origin which initially un lsts the DNA approximately two turns and leads to the melting of 8 bp of the duplex in the early palindrome domain and extensive distortion of the AT-rich region. In the presence of ATP, topo I and HeLa SSB, the intrinsic DNA helicase activity of T antigen unwinds ori^+ DNA bidirectionally. The further addition of dNTPs and purified DNA polymerase α-DNA primase complex results in the formation of the first labeled DNA species which migrates electrophoretically as unwound DNA. Further supplementation of reactions with topo II, DNA ligase, RNase H and a 5'-3 exonuclease, isolated from HeLa cells, leads to the formation of relaxed closed circu'ar products. Reactions carried out without the closing enzymes lead to the accumulation of short fragments, arising from the lagging strand, and longer DNA products from the leading strand.

DNA synthesis starts after a lag which is more pronounced with the purified system than with crude fractions. In addition, the pulse labeling of the unwound DNA and its chase into longer products is faster with cruder fractions than with the purified proteins. These results suggest that additional host protein(s) are required for efficient replication.

PCNA has been shown by Prelich et al. (Cell 53, 117 (1988)) to be essential for leading strand synthesis in the SV40 system. We have found that replication with the purified proteins is unaffected by PCNA or by antibodies against PCNA. However, replication with crude extracts was markedly inhibited (90%) by antibodies against PCNA and this inhibition was reversed (75%) by purified PCNA isolated from HeLa cells. These differences were traced to the presence of a protein inhibitor in crude fractions which binds to DNA chains and blocks the elongation reaction. In crude fractions, this inhibition is reversed by PCNA. The purification of the elongation inhibitor has revealed that the PCNA-mediated elongation reaction requires additional proteins distinct from the purified proteins described above. The isolation and characterization of the elongation inhibitor and activator proteins are now being pursued.

L 024 DNA REPLICATION *IN VITRO* FROM THE SV40 ORIGIN, Bruce Stillman, Micaela Fairman, Sulah-Ud Din, Toshiki Tsurimoto, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, New York 11724, U.S.A.

In the presence of SV40 T antigen, cell free extracts from human 293 cells support initiation and elongation of DNA replication on plasmids containing a functional SV40 origin of replication. Multiple stages of initiation and elongation have been characterized, as have the cellular proteins required with T antigen to completely replicate DNA. A cellular protein, called RF-A, is a multi-subunit protein containing subunits of 70,000, 34,000 and 11,000 daltons and cooperates with SV40 T antigen to unwind the origin of DNA replication. RF-A is a single strand DNA binding protein, but unlike prokaryotic analogues, can bind double strand DNA. The RF-A 34,000 dalton protein is phosphorylated and exists in multiple forms. Initiation of DNA replication can occur following unwinding of the origin by T antigen and RF-A by purified DNA polymerase α/DNA primase comlpex, however extensive and correct elongation does not occur. Recostitution of authentic elongation of replication requires other cellular proteins (PCNA, RF-C and others yet to be identified).

A cellular fraction (called SSI) is required for the replication of SV40 origin containing DNA in crude extracts, but not in a more purified system. This is due to the complete inhibition of the early stages of SV40 DNA replication by a protein derived from the cell extracts, and the SSI fraction antagonizes this inhibition.

Two cellular proteins, the proliferating cell nuclear antigen (PCNA, 36,000 daltons) and a multisubunit protein called RF-C (containing polypeptides of 37,000 - 41,000 daltons and polypeptides of 100,000 to 140,000 daltons) are both required for the elongation stage of DNA replication. In their absence, initiation at the origin occurs, but subsequent elongation of leading strands is absent and synthesis of lagging strands is abnormal. Both PCNA and RF-C are required after the synthesis of the first nascent strands at the origin and are required at a replication fork for coordinated leading and lagging strand synthesis. PCNA is a processivity factor for DNA polymerase δ, implicating this polymerase as the leading strand polymerase. The effects of these cellular proteins on DNA polymerase α activity will also be discussed.

Molecular Mechanisms in DNA Replication and Recombination

Initiation of DNA Replication: Eukaryotes

L 025 RNase MRP: A SITE-SPECIFIC ENDORIBONUCLEASE INVOLVED IN PRIMER RNA METABOLISM, David A. Clayton, Jeffrey L. Bennett, Robert Karwan, Emil Michelotti, Lori L. Stohl and James N. Topper, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5324.

RNase MRP (for Mitochondrial RNA Processing) is a site-specific, single-strand endonuclease involved in primer RNA metabolism in mammalian mitochondria. Cleavage by RNase MRP occurs at one of the transition sites of primer RNA synthesis to DNA synthesis, located adjacent to a sequence block (CSBII) that is evolutionally conserved in vertebrate mtDNA. The enzyme is a ribonucleoprotein complex. Based on physical copurification and a complementary oligonucleotide-directed inhibition assay, we have previously demonstrated that an RNA encoded in the nucleus is the RNA moiety of mouse RNase MRP; using complementary oligonucleotide probes we have identified and cloned a segment of mouse nuclear DNA that encodes this RNA (1). The primary transcription product is 275 nt in size. The 5′ end of the 275-nt RNA is not capped and retains a triphosphate group; the gene for this RNA contains features characteristic of both RNA polymerase II- and RNA polymerase III-dependent genes. The RNA can form an extensive secondary structure and contains a region that can potentially base pair with the substrate RNA at the evolutionally conserved sequence block immediately adjacent to the cleavage site. RNase MRP from human cells shows similar features and the corresponding human gene and its basic RNA products have been characterized; in this case the primary RNA is 266 nt. Site-directed mutagenesis has been used to demonstrate the requirement of the CSBII substrate sequence in the cleavage reaction. (Supported by NIH grant GM-33088 and ACS grant NP-9.)

1. Chang, D.D. and D.A. Clayton (1989) Cell 56, in press.

L 026 IDENTIFICATION OF REPLICATION ORIGINS IN THE AMPLIFIED DIHYDROFOLATE REDUCTASE DOMAIN IN METHOTREXATE-RESISTANT CHINESE HAMSTER OVARY CELLS. Joyce L. Hamlin, Tzeng-Horng Leu, Chi Ma, and James P. Vaughn, Department of Biochemistry and Cell and Molecular Biology Program, University of Virginia School of Medicine, Charlottesville, VA 22908.

Our laboratory is interested in the nature of origins of DNA replication in mammalian chromosomes, as well as the architectural arrangement of replicating units in the nucleus. As a model system, we have developed a methotrexate-resistant Chinese hamster ovary cell line (CHOC 400) that has amplified the dihydrofolate reductase (DHFR) gene and >220 kb of flanking sequence ~1,000 times. In previous in vivo labelling studies on synchronized cultures, we have been able to roughly localize three adjacent origins of replication within the DHFR amplicon that fire synchronously with respect to one another at the beginning of the S period; by utilizing a modification of an in-gel renaturation procedure to eliminate background labelling from single copy sequences, it was possible to localize these initiation sites within fragments less than 3 kb in length. The location of initiation sites in the DHFR amplicon has so far been confirmed for at least one origin by demonstrating the anomalous migration in agarose gels of selected restriction fragments due, apparently, to the presence of replication bubbles. The same results were obtained in cells that were synchronized or that were growing exponentially. Thus, the initiation sites that we have identified are not induced by the synchrony regimen. Our results also suggest that not all potential initiation sites in the multiple DHFR amplicons in the CHOC 400 cell line are used in a given S period. This result suggests that origins can be replicated passively by forks from adjacent active origins, and also suggests that there are no functional termini in the DHFR amplicons in this cell line. This result is also interesting because we have demonstrated the presence of a matrix attachment sequence that maps very near two of the initiation sites, but which appears to be attached to the matrix in less than 20% of amplicons. The sequence of at least two of the three initiation sites will be presented, as well as the distribution of matrix attachment regions in the amplicon.

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General and Site-Specific Recombination: Prokaryotes

L 027 MITOTIC RECOMBINATION IN THE rDNA OF S. CEREVISIAE IS SUPPRESSED BY THE COMBINED ACTION OF DNA TOPOISOMERASES I AND II, Michael F. Christman, Fred S. Dietrich, and Gerald R. Fink, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142 and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have found that mitotic recombination within the S. cerevisiae rDNA cluster (200 tandemly repeated 9.1 kb units) is strongly suppressed and that this suppression requires the combined action of DNA topoisomerases I and II. Strains with a null mutation in the TOP1 gene (encoding topoisomerase I) or a ts mutation in the TOP2 gene (encoding topoisomerase II) grown at a semi-permissive temperature show 50- to 200-fold higher frequencies of mitotic recombination in rDNA relative to TOP+ controls. Suppression of recombination is specific to the rDNA because the recombination frequency at another tandem array, the CUP1 locus, at a simple HIS4 duplication, or among dispersed repeats (MAT and HML or HMR) is not elevated in top1 or top2 mutants. The high frequency of mitotic recombination within the rDNA cluster in topoisomerase mutants shows that both TOP1 and TOP2 are required for suppression of recombination in this region of the genome.

L 028 BIOCHEMICAL ANALYSIS OF GENETIC RECOMBINATION IN *ESCHERICHIA COLI* AND *SACCHAROMYCES CEREVISIAE*, R. Kolodner, T. Griffin IV, W.-D. Heyer, S. Lovett, C. Luisi-Deluca, D. Norris, R. Reenan, and D. Tishkoff, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School and Department of Cellular and Molecular Biology, Dana-Farber Cancer Institute, Boston, MA 02115.

The major goal of our work is to reconstitute recombination reactions with purified proteins and study the mechanism of these reactions. In *E. coli* our work has concentrated on the proteins encoded by the RecF family of genes which include *recF*, *recJ*, *recN*, *recO*, *recQ* and *ruv*. Genetic analysis has demonstrated that recombination of a variety of substrates depends on different subsets of these genes depending on the genetic background studied. *E. coli* strains that overproduce many of these gene products have been constructed and used to characterize the proteins encoded by these genes. Induction of the *recJ* gene resulted in overproduction of a 60,000 Mr polypeptide and a concommitant increase in the levels of single-stranded DNA specific nuclease activity present in crude cell extracts. This nuclease was purified to homogeneity and found to reside in a 60,000 Mr polypeptide which had the N-terminal amino acid sequence predicted by the *recJ* gene sequence. The RecJ nuclease was greater than 10,000-fold more active on linear single-stranded DNA substrates than on linear double-stranded DNA and did not have detectable endonuclease activity. Analysis of the RecF protein is presented at an accompanying poster.

We have previously reported the purification of a 132,000 Mr protein from *S. cerevisiae* that catalyzes many of the same reactions that *E. coli* RecA protein catalyzes. In particular, the *S. cerevisiae* protein (SEP) catalyzes the homologous pairing and strand exchange of linear M13 double-stranded DNA and circular M13 single-stranded DNA with the same polarity as RecA protein. The reaction requires 1 SEP molecule per 12 nucleotides of single-stranded DNA (~600 molecules per M13 molecule). Two different protein factors have been purified that stimulate this reaction and reduce the amount of SEP that is required. The first protein binds tightly to single-stranded DNA and reduces the amount of SEP required for maximal activity about 3-fold. It can also substitute for *E. coli* SSB in reactions with RecA protein. The second stimulatory factor reduces the amount of SEP required for maximal activity 100- to 1000-fold so that significant activity can be observed with one SEP molecule per single-stranded substrate DNA molecule. The biological significance of these proteins is currently under investigation.

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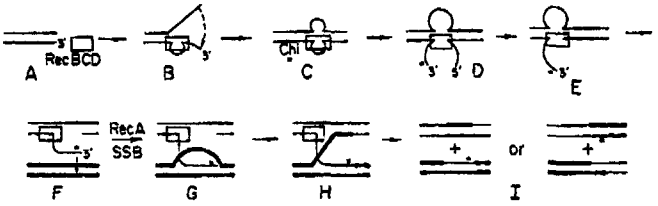
L 029 DYNAMIC, STRUCTURAL AND REGULATORY ASPECTS OF λ SITE-SPECIFIC RECOMBINATION, Arthur Landy, Lina Moitoso de Vargas, Sunghoon Kim, Ursula Snyder and Simone Nunes-Duby, Division of Biology and Medicine, Brown University, Providence, RI 02912

The λ site-specific recombination pathway consists of four different proteins and two DNA partners (att sites) that contain 15 protein binding sites. The recombinase, Int, is a monomer with two autonomous DNA binding domains of different specificity. The amino-terminal domain binds to a family of 5 "arm-type" sites distant from the region of strand exchange. The carboxy-terminal domain specifies topoisomerase function and binds to a family of "core-type" sites that comprise the sites for DNA cleavage and religation. The host-encoded protein, IHF, is required for both integrative and excisive recombination. However, under certain conditions high levels of IHF specifically inhibit the excision reaction. While all three IHF binding sites are occupied for integrative recombination one particular site must be vacant for excisive recombination. Upon binding, IHF induces an extreme bend in DNA of more than 140° at each site. Stimulation of Int binding and cleavage at the low affinity core-type DNA sites is shown to require interactions with the distal high affinity arm-type sites, and to depend upon simultaneous binding of the sequence-specific DNA-bending protein IHF. These interactions exhibit a helical phase dependence that correlates with competence for recombination. This mechanism allows a bivalent DNA binding protein to be "poised" at high affinity sites and directed, by a sequence-specific DNA bending protein, to interactions with lower affinity sites. Since the bending and bridging proteins do not have to interact in this complex, its assembly is independent of protein-protein recognition. Other features of the reaction will also be discussed.

L 030 THE RecBCD PATHWAY OF HOMOLOGOUS RECOMBINATION: ENZYMES AND CONTROLLING SITES, Gerald R. Smith, Susan K. Amundsen, Gary Braedt, Susan L. Holbeck, Nicole H. McKittrick, Aaron M. Neiman, Andrew F. Taylor, Stephanie M. Thibodeaux. Fred Hutchinson Cancer Research Center, Seattle WA 98104.

The RecBCD pathway is the principal route of homologous recombination following Escherichia coli conjugation or transduction and during lytic infection of λ Red⁻ (recombination-deficient) phage. RecBCD enzyme, RecA protein, SSB protein, DNA gyrase, DNA polymerase I, and DNA ligase have been shown or implicated to act in the RecBCD pathway. Chi sites, 5'G-C-T-G-G-T-G-G3', locally enhance recombination by the RecBCD pathway. We have previously proposed the model below for the RecBCD pathway.



We shall discuss genetic, enzymatic, and physical evidence for the following points: Purified RecBCD enzyme forms a loop (as observed by electron microscopy) on the strand with a (biotinylated) 3'-end at the entry site (steps A and B). During DNA unwinding, RecBCD enzyme cuts the Chi-containing strand a few nucleotides to its right (D). The resulting 3' end nick is proposed to initiate recombination (F). Two lines of evidence support this view: In λ crosses Chi stimulates recombination immediately to its right (within 430 bp), but not detectably farther to its right; stimulation falls exponentially (a factor of 2 each 2-3 kb) to Chi's left (I). Southern blot analysis of DNA extracted from λ infected cells suggests that RecA protein and Chi sites stimulate the formation of hybrid DNA (G and H). The properties of recently isolated recBCD mutants suggest that RecBCD enzyme may act at one or more additional steps, such as the cleavage of D-loops (G) or of Holliday junctions (H). Chi is a recombination hotspot recognized by RecBCD-like enzymes of the enteric bacteria but not other groups of bacteria, such as Pseudomonas.

Molecular Mechanisms in DNA Replication and Recombination

General and Site-Specific Recombination: Eukaryotes

L 031 NOVEL PRODUCTS OF THE IG/TCR GENE ASSEMBLY SYSTEM, Susanna Lewis, Joanne Hesse, Michael Lieber, Kiyoshi Mizuuchi and Martin Gellert, Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD 20892.

A rapid *in vivo* recombination assay has allowed isolation and quantitation of unusual products of site-specific V(D)J recombination. The characterization of these products as well as a comprehensive analysis of more standard V(D)J junctions highlights how little V(D)J joining may have in common with other types of site-specific recombination. 10% or more of the recombinants isolated using the extrachromosomal assay belong to a class of junction termed "hybrid junctions". In these joints, clearly the site-specificity of the standard joining reaction has been maintained, but the DNA elements have become connected in alternative configurations. This is in contrast to all other site-specific recombination systems described to date, in which fixed directionality is observed. "Open-and-shut" junctions, in which there is no net recombination and all elements become reconnected to their original partners, similarly suggest that the directionality of V(D)J joining is not stringently specified.

Another singular feature of V(D)J joining is the ubiquitous occurence of "N-regions". It is now clear that these non-templated inserts, previously thought to be limited to coding joints, can occur in all products of V(D)J recombination. We have found them in signal joints as well as in hybrid joints and open-and-shut joints. Rules governing the appearance and composition of N-regions emerge when these data are compiled. While correlative studies favor the view that the enzyme terminal deoxynucleotidyl transferase may be responsible for inserting nucleotides into V(D)J junctions, this explanation does not simply acccount for the patterns observed.

The expanded catalogue of V(D)J recombination products reveals a striking parallel between differences in germ-line gene segment organization at various Ig and TCR loci and the types of interconversions that can be acccomplished by V(D)J recombination. The variety of arrangements found at antigen receptor loci has long confounded attempts to design a straighforward evolutionary tree. The notion that the V(D)J recombination system may have been active in the germ line reduces the problem to much simpler terms. Several examples of structures that bear the imprint of V(D)J recombination activity will be presented.

L 032 PHAGE MU TRANSPOSITION AND RETROVIRAL DNA INTEGRATION, Kiyoshi Mizuuchi, Kenji Adzuma, Michiyo Mizuuchi, Tamio Fujiwara and Robert Craigie. Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, MD 20892

A DNA strand transfer reaction is the central step in transposition of Mu DNA. This reaction is comprised of several steps. MuA protein (Mu transposase) binds to the two ends of Mu DNA as well as to a third cis-acting DNA sequence which we call the IAS (internal activation sequence). In the presence of a host protein HU, MuA protein forms a specific DNA-protein complex which we call a plectosome complex involving the three DNA sequences mentioned above. This complex is formed only if a set of topological requirements are satisfied by the donor DNA (negatively supercoiled DNA with two Mu ends in inverted orientation with the IAS in between). Formation of this protein-DNA complex is followed by the first chemical reaction step, generation of single strand DNA cleavages at the ends of the Mu DNA sequence to expose the 3'-ends of the Mu DNA. The two Mu ends remain held together in a protein complex after the cleavage reaction.

This cleaved donor complex transfers the Mu 3'-ends to a target DNA which is bound by MuB protein. This reaction step comprises cutting the target and joining the resulting 5'-ends to the 3'-ends of the Mu DNA. MuB ATPase, in the presence of MuA protein, is involved in the selection of the target DNA to be used in the reaction. Mu repressor directly regulates the DNA strand transfer by competing with MuA protein for the binding to the IAS.

Recent studies of the retroviral DNA integration reaction in our group and by others point to the similarity of the chemical steps involved in this reaction to those of the Mu DNA strand transfer reaction. The retroviral "DNA integrase" seems to bind to the end sequences of the donor DNA and then introduces a pair of single strand cuts to expose the 3'-ends of the donor. The 3'-ends of the cleaved donor DNA are then transferred to the 5'-ends of a cut made in the target DNA.

Molecular Mechanisms in DNA Replication and Recombination

L 033 THE HUMAN RECOMBINATIONAL STRAND-EXCHANGE COMPLEX, Richard Fishel[1] and Alexander Rich[2], [1]Laboratory of Chromosome Biology, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701, [2]Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The initiation of general recombination is poorly understood. We have described a model for nuclease-free initiation of recombination that involves the generation of a four-strand paranemic joint in which topologically neutral left-handed heteroduplex Z-DNA adjacent to right-handed heteroduplex B-DNA is formed between homologous parent DNAs. The model predicts that recombination proteins may bind to left-handed Z-DNA. We have identified several DNA enzyme activities from a human T-lymphoblast cell line that bind to a Z-DNA chromatography matrix. These include topoisomerase I and II, DNA ligase, several DNA-dependent ATPases, and a recombinational strand-exchange protein. The human strand-exchange protein has been purified to near homogeneity (Moore and Fishel, submitted). The preparation contains a 120-kD peptide and promotes ATP-independent homology-dependent strand exchange between a single-strand circular substrate and a linear duplex substrate in a reaction that requires nearly stoichiometric amounts of protein. The peptide binds but apparently does not hydrolyze ATP. Additionally, a complex of five peptides can be isolated that promotes strand exchange and contains the 120-kD peptide plus a 130-kD, 85-kD, 70-kD, and 50-kD peptide(s). A blunt-end ligation activity appears to be associated with this complex. An ATPase that alters the catalytic nature of this complex appears weakly associated and is separated early in the purification procedure accounting for the ATP-dependent activity of the crude preparation. Several aspects of these Z-DNA binding peptides will be presented.

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Late Addition

L 034 RECONSTITUTION OF HOMOLOGOUS PAIRING ACTIVITY *IN VITRO* DEPENDENT UPON THE COMBINED ACTIVITIES OF PURIFIED *E. COLI* RECA, RECBCD, AND SSB PROTEINS. Stephen C. Kowalczykowski and Linda J. Roman, Department of Molecular Biology, Northwestern University Medical School, Chicago Il. 60611

The recA and recBCD proteins are essential components of the recBC-pathway of homologous recombination in *E. coli*. Using two different *in vitro* reactions, we can demonstrate homology-dependent DNA heteroduplex formation requiring the concerted action of recA protein, recBCD enzyme, and (depending on the DNA substrates used) SSB protein. DNA heteroduplex formation proceeds in three kinetically distinct steps. The first step is unwinding of the linear dsDNA by recBCD enzyme. The second step requires trapping of the unwound ssDNA by either recA or SSB proteins. The third step involves recA protein-dependent homologous pairing of the DNA intermediates: for reactions involving circular ssDNA and linear dsDNA, renaturation activity is required; for linear dsDNA and supercoiled dsDNA, joint molecule formation is required. These *in vitro* results suggest that the helicase activity of recBCD enzyme can initiate homologous pairing and are consistent with *in vivo* data which suggest that recBCD enzyme may act early (as well as late) in genetic recombination. Development of a novel assay to characterize the helicase activity of recBCD enzyme facilitated mechanistic analysis of the recA- and recBCD-dependent homologous pairing reaction. Under optimal conditions, recBCD enzyme can unwind dsDNA as fast as 930 bp/sec/functional recBCD enzyme; the K_m for dsDNA ends is approximately 1 nM; and 2-3 ATP molecules are hydrolyzed per bp unwound. The processivity of unwinding was also determined. An average of 25,000 bp are unwound before dissociation of the active recBCD enzyme; the relevance of this observation to activation at Chi site will be discussed. (Supported by AI-18987 & GM-41347)

Molecular Mechanisms in DNA Replication and Recombination

DNA Structure, Protein-DNA Interactions

L 100 Characterization and purification of a nuclear factor which binds to the recombination signal sequences of immunoglobulin (Ig) and T-cell receptor genes. Renato J. Aguilera* and Hitoshi Sakano, Department of Microbiology and Immunology, University of California at Berkeley, Berkeley, CA 94720 and *Department of Biology, University of California at Los Angeles, Los Angeles, CA 90024.

In an attempt to characterize the enzymatic machinery involved in antigen receptor gene recombination, we have searched for nuclear factors which specifically interact with the Ig recombination signals. A DNA-binding factor has been identified which footprints to the conserved heptamer sequence (GTGTCAC) found adjacent to all antigen receptor variable region genes. This factor has been purified close to homogeneity with the use of standard chromatographic techniques coupled with specific DNA affinity columns. Southwestern and silver stain analysis of fractions containing the specific binding activity reveal that the factor exhibits a molecular weight of ~100 kd on denaturing polyacrylamide gels. We expect to obtain sufficient amino acid sequence data from the purified material to be able to clone the gene for this DNA binding protein. Another approach which is currently being undertaken to clone this gene is the screening of λgt11 cDNA expression libraries of a recombination-active pre-B cell line with DNA probes containing the Ig recombination signal sequences. The gene for the heptamer binding protein as well as other nuclear factors which might specifically interact with the Ig recombination signals might be cloned with this approach.

L 101 INTERACTION OF *Rsr*I ENDONUCLEASE, AN ISOSCHIZOMER OF *Eco*RI, WITH OLIGODEOXYRIBONUCLEOTIDES CONTAINING BASE ANALOGUES, Christopher Aiken and R. I. Gumport, Department of Biochemistry, College of Medicine and School of Chemical Sciences, University of Illinois, Urbana, IL 61801

We have initiated a systematic kinetic study of the *Rsr*I endonuclease, an isoschizomer of *Eco*RI, using oligodeoxyribonucleotides containing base analogues as substrates. Several of these substrates were provided by Dr. L. McLaughlin, Boston College, who tested them with *Eco*RI. All of the base substitutions tested affect the interaction of both enzymes with DNA. In general, the effects with *Rsr*I are more pronounced than with *Eco*RI, as measured by the greater reduction in the specificity constant (k_{cat}/K_m) for a given substitution. For two substrates, the *Rsr*I-catalyzed reaction was very slow--less than one-tenth the rate of the corresponding *Eco*RI reaction. The lower tolerance of *Rsr*I for base analogues in its recognition sequence may reflect a difference in the mechanisms by which these two enzymes recognize the same DNA sequence and cut it at the same site. We are presently determining whether *Rsr*I kinks and unwinds DNA to the same extent that *Eco*RI does.

L 102 PROTEIN KINASE C-MEDIATED DOWN-RUGUALTION OF AN INTERFERON-INDUCIBLE GENE, Hiroaki Akai and Andrew Larner, Laboratory of Pathology, National Cancer Institute, Bethesda, MD. 20892 Interferons (IFNs) induce the expression of a variety of RNAs and inhibit phorbol ester induction of other genes. Recent results reported here indicate that phorbol esters can also specifically inhibit the expression of some IFN-induced RNAs (ie IFN-IND-1). Phorbol esters exert their effects by inhibiting IFN-induced transcription of the gene that encodes IFN-IND-1 (ISG-54K); the synthesis of new proteins is required for this inhibition to occur. The actions of phorbol esters are only seen in those types of cultured cells where cycloheximide in the presence of IFN prevents long-term IFN treatment of cells from inducing a "desensitized state." In desensitized cells, IFN is not able to reinduce the transcription of the RNA. Our results indicate that a protein kinase C-dependent pathway requiring protein synthesis may be one mechanism by which IFN is able to down-regulate the transcription of genes whose expression it initially induces.

L 103 REPAIR OF DOUBLE STRAND BREAKS IN DNA CAUSED BY TOPOISOMERASE II INHIBITORS.

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The xrs mutants of CHO cells are defective in the repair of double, but not single strand breaks introduced into their chromosomal DNA (1). They also have decreased frequencies of DNA mediated gene transfer compared with the parent strain. This is not a result of a decrease in DNA uptake. It is a deficiency in the subsequent integration of transfecting DNA into chromosomes by a non-homologous recombination mechanism (J. Smith-Raven and Penny A. Jeggo, in preparation). We have found that these xrs strains, unlike their parent, are particularly sensitive to drugs whose targets are DNA topoisomerase II, but not to a drug with topoisomerase I as its target. These results indicate a role for the xrs gene product in processing DNA-topoisomerase II-drug complexes or their derivatives. Recent work which investigates this role will be presented.

(1) Kemp, L.M., Sedgwick, S.G. and Jeggo, P.A. (1984) Mutation Res. 132: 189-196

L 104 DNA GYRASE CAN SUPERCOIL DNA CIRCLES AS SMALL AS 174 BASE-PAIRS.

Andrew D. Bates and Anthony Maxwell, Department of Biochemistry, University of Leicester, University Road, Leicester, LE1 7RH, UK.

DNA gyrase introduces negative supercoils into closed-circular DNA using the free energy derived from ATP hydrolysis. Consideration of steric and thermodynamic aspects of the supercoiling reaction indicates that there should be a lower limit to the size of DNA circle which can be supercoiled by gyrase. We have investigated the supercoiling reactions of circles from 116 to 427 base-pairs in size, prepared both by ligation of linear restriction fragments and directly, from *in vitro* constructs utilising the *lox*P-cre recombination system of bacteriophage P1, and have determined that gyrase can supercoil certain relaxed isomers of circles as small as 174 bp, dependent on the final superhelical density of the supercoiled product. Furthermore, this limiting superhelical density (-0.11) is the same as that determined for the supercoiling of plasmid pBR322, although the free energy associated with one turnover at this limit is at least twice as large for a small circle as for pBR322. This energetic paradox is currently under investigation. For circles below 174 bp in size, supercoiling of all relaxed isomers is thermodynamically impossible, although by carrying out the reactions in the presence of ethidium bromide to remove the thermodynamic constraint, we have shown that the conformational changes associated with supercoiling can be carried out in a circle as small as 116 bp. This finding strongly suggests that the segment of DNA translocated in the supercoiling reaction may comprise part of the approx. 120 bp wrapped around the enzyme, and that this wrap is not maintained throughout the supercoiling cycle.

L 105 NUCLEAR PROTEIN HMG1 SPECIFICALLY RECOGNIZES CRUCIFORM DNA

Marco E. Bianchi and Monica Beltrame, University of Pavia, via S.Epifanio 14, Pavia, Italy, and EMBL, Meyerhofstrasse 1, D-6900 Heidelberg, West Germany.

Cruciform DNA structures are formed *in vitro* and *in vivo* as a consequence of general and site-specific recombination, and of the supercoiling-induced transition from interstrand to intrastrand base pairing in palindromic DNA sequences. We have constructed small pseudo-cruciform DNAs by annealing synthetic oligonucleotides of appropriate base complementarity, and we have used them in a gel-retardation assay and as ligands for affinity chromatography. We have thus obtained from rat liver nuclear extracts an homogeneous preparation of a 23.5 kD protein, which binds to synthetic cruciforms, but not to control duplex or single-stranded molecules of identical sequence. Moreover, the protein binds to cruciform structures extruded by a supercoiled plasmid containing a 70 bp palindrome, but not to the same DNA linearized with a restriction nuclease. We sequenced four oligopeptides derived from the binding protein, and found that all of them correspond to the protein sequence previously deduced from rat HMG1 cDNA. Our 23.5 kD protein reacts with anti-HMG1 antibodies and appears to represent the central DNA-binding domain of HMG1 protein. Full-length HMG1 protein was synthesized *in vitro* from the cDNA clone, and was found to bind selectively to cruciform DNA. HMG1-like proteins are an abundant and essential component of eukaryotic nuclei, from yeast to mammals, but their function *in vivo* has not been identified unequivocally. Their interaction with cruciform DNA points to a critical role for such DNA conformations.

L 106 TETRACYCLINE PROMOTER MUTATIONS DECREASE NON-B DNA STRUCTURAL TRANSITIONS, LINKING NUMBERS, AND DELETIONS IN RECOMBINANT PLASMIDS IN ESCHERICHIA COLI

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The ability to clone sequences with varying capabilites of adopting non-B DNA structures (left-handed Z-DNA, cruciforms, or triplexes) into three loci of pBR322 was systematically studied. In general, the inserts were stable (non-deleted) in the EcoRI site (an untranslated region) of pBR322. However, sequences most likely to adopt left-handed Z-DNA or triplexes *in vivo* suffered deletions when cloned into the BamHI site, which is located in the tetracycline resistance structural gene (*tet*). Alternatively, when the promoter for the *tet* gene was altered by filling-in the unique HindIII or ClaI sites, the inserts were not deleted. Concomitantly, the linking numbers of the plasmids were reduced. Also, inserts with a high potential to adopt Z-DNA conformations were substantially deleted in the PvuII site of pBR322 (near the replication origin and the copy number control region), but were less deleted if the *tet* promoter was insertion-mutated. The deletion phenomena are due to the capacity of these sequences to adopt left-handed Z-DNA or triplexes *in vivo* since shorter inserts, less prone to form non-B DNA structures, or random sequences, did not exhibit this behavior. Potential cruciform sequences were stable in all sites under all conditions. These results reveal a complex interrelationship between insert deletions (apparently the result of genetic recombination), transcription, negative supercoiling, and the formation of non-B DNA structures in living *Escherichia coli* cells.

L 107 MONOMER -TETRAMER EQUILIBRIUM AND ssDNA BINDING PROPERTIES OF A MUTANT *E. coli* ssDNA-BINDING PROTEIN, SSB-1. Wlodzimierz Bujalowski and Timothy M. Lohman, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843.

The *E. coli* SSB protein is a stable homotetramer in solution; however the ssb-1 mutation (his to tyr 55) destabilizes this tetrameric structure with respect to monomers and we have undertaken a quantitative study of this equilibrium. The extent of tetramer formation was monitored by the increase in intrinsic protein fluorescence anisotropy accompanying the association. A comparison of the experimental isotherms with simulations for the general monomer ↔ dimer ↔ tetramer association indicates that the formation of the SSB-1 tetramer from the monomers can be described with high accuracy as a one step association of the four monomers indicating that dimers are not a highly populated species. In our standard conditions (25 °C, pH 8.1) an apparent aggregation constant, $K = 2 \times 10^{18}\ M^{-3}$, is obtained, although there is a strong temperature dependence of the SSB-1 tetramer formation with $\Delta H = -59 \pm 15$ kcal/mol (pH 8.1). There is no effect of monovalent salt concentration (NaCl) on the monomer ↔ tetramer association up to 1 M; however the presence of magnesium chloride at concentrations above 20 mM attenuates the formation of the SSB-1 tetramer. Characterization of the monomer ↔ tetramer equilibrium of the SSB-1 protein enabled us to determine, in binding studies with oligonucleotides, that the free SSB-1 monomer can bind ssDNA; however free monomer has a significantly lower association constant than a monomer that is part of an SSB-1 tetramer. Therefore, in the case of the SSB-1 mutant protein, the formation of the tetramer is not necessary in order to bind ssDNA.

L 108 EUKARYOTIC DNA TOPOISOMERASE I ACTIVITY IS INFLUENCED BY DNA CONFORMATION, G. Camilloni, M. Caserta, A. Amadei and E. Di Mauro, Centro Acidi Nucleici, Università di Roma "La Sapienza", Rome, Italy, The effects of supercoiling on the topoisomerization reaction by eukaryotic DNA topoisomerase I have been analyzed in vitro. Individually purified DNA topoisomers of various origin (mouse, yeast and E.coli) have been kinetically characterized for their behavior as substrates with topoisomerase I from different sources (calf thymus, chicken erythrocytes, wheat germ and yeast). In processive conditions, the production of the final Gaussian distribution of topoisomers, which provides a measure of enzyme activity, occurs much more rapidly on both positively and negatively supercoiled DNAs as compared to totally relaxed ones. This effect is visible only under limiting concentrations of enzyme. In the same conditions, the analysis of the nicking step of the reaction (i.e., of the DNA fragments produced by SDS treatment of topoisomerization mixtures containing or not the inhibitor camptothecin) reveals that topo I acts much faster on torsionally stressed molecules. The sites cleaved are qualitatively the same for different topoisomers: what vary are the kinetics of their appearance. The analysis of the binding step of the reaction is currently under investigation. We propose a model of topo I activity in which the enzyme binds rapidly to relaxed DNA molecules without reacting and, when a conformational stimulus (torsional stress due to modification of the physico-chemical environment, interactions with proteins, transcription) is propagated through the domain, it very quickly starts the catalysis.

L 109 MAPPING OF TOPOISOMERASE II CLEAVAGE SITES IN NUCLEOSOME RECONSTITUTED SV40 CHROMATIN. Giovanni Capranico, Kurt W. Kohn and Yves Pommier, Laboratory of Molecular Pharmacology, DTP, DCT, National Cancer Institute, NIH, Bethesda, MD 20892.
The effect of core histones upon the binding of mammalian topoisomerase II (topo II) to DNA was investigated by comparing the cleavage sites of purified mouse leukemia L1210 topo II induced by four antitumor topo II inhibitors (teniposide, amsacrine, 5-iminodaunorubicin, and 2-methyl-9-OH-ellipticinium) in purified and in nucleosome reconstituted SV40 DNA. SV40 chromatin was reconstituted by incubating [^{32}P]-end labeled linear DNA fragments with an excess of HeLa cell mononucleosomes in 0.7 M NaCl for 20 min at 37^{o}C, and by diluting slowly these mixtures in a step-wise manner to 70 mM NaCl. Chromatin reconstitution was checked by a gel shifting assay and nucleosome positioning by comparing micrococcal nuclease (M. nuclease) digestion patterns of chromatin with those of naked DNA. Three types of DNA regions were identified: 1) regions with 150 bp segments protected from M. nuclease digestion separated by sites of enhanced digestion, indicative of fixed nucleosome positioning, 2) regions of global protection without precise nucleosome positioning, and 3) one region of 200-300 bp around the origin of replication which did not seem reconstituted. Drug-induced topo II cleavage sites were markedly affected by nucleosomes. Most of the topo II cleavage sites were suppressed at sites of M. nuclease protection and other sites of cleavage appeared stronger at sites of M. nuclease enhancement. These results indicate that DNA bound to nuclesomes is not available for topo II cleavage and suggest that topo II is only active in the linker regions of chromatin and in the nucleosome free region of SV40.

L 110 DNA AND NUCLEOTIDE-INDUCED CONFORMATIONAL CHANGES IN THE *ESCHERICHIA COLI* REP AND uvrD (HELICASE II) PROTEINS, Kinlin L. Chao and Timothy M. Lohman, Department of Biochemistry and Biophysics, Texas A & M University, College Station, Tx 77843.
Proteolysis of the Rep and Helicase II proteins with trypsin and chymotrypsin has been used to probe the domain structure of the helicases and their ligand dependent conformational changes. Limited trypsin treatment of Rep protein (73 kDa) results in a 68 kDa Rep polypeptide which retains ssDNA binding, unwinding (helicase), and full ATPase activities. Trypsin cleavage of the Rep protein when bound to ssDNA yields a 58 kDa polypeptide which retains ssDNA binding and ATPase activities. Analysis of the amino-terminal sequences of the 68 kDa and 58 kDa Rep polypeptides show that both cleavages occur in the carboxyl-terminal end of the Rep protein. Both polypeptides are less susceptible to proteolysis in the presence of ATP, ADP, or ATPγS. Based on these and previous studies [Arai, N. & Kornberg, A. (1981) *J. Biol. Chem.* **256**, 5292], we conclude that the carboxyl-terminal (~44 amino acid fragment) end of Rep protein is not necessary for its helicase activity, but rather it is necessary for its interaction with the ΦX 174 cisA protein. Helicase II (82 kDa) is cleaved by chymotrypsin at Tyr_{254} to give 53 kDa carboxyl-terminal and 29 kDa amino-terminal polypeptides which remain associated under nondenaturing conditions. The chymotrypsin cleaved Helicase II has a low ssDNA binding activity compared to native Helicase II, and can no longer hydrolyze ATP. When ATP, ADP, ATPγS, and/or DNA are bound to Helicase II, it is protected from cleavage by chymotrypsin at Tyr_{254} and remains intact. Trypsin also cleaves Helicase II near the carboxyl-terminal to give a doublet of ~72 kDa Helicase II polypeptides which are stabilized upon ligand binding. This 72 kDa doublet retains full ssDNA-dependent ATPase and unwinding activities. Both the Rep and Helicase II proteins alter their structure upon binding ATP or DNA. These ligand induced conformational changes in the helicases are likely to reflect changes in the protein structure that accompany their ATPase and helicase activities.

L 111 SEQUENCE SPECIFIC ACTION OF DNA TOPOISOMERASE I.
Christiansen, K., Svejstrup, J., Andersen, A., H., Bonven, B., Nielsen, N.F. and Westergaard, O, Department of Molecular Biology, University of Aarhus, C. F. Møllers Alle 130, Aarhus, Denmark. Recently we have found that topo I is tightly associated with a hexadecameric sequence element occuring in double or triple repeats within DNase I hypersensitive sites located near the origen of replication as well as the regulartory sequences for initiation of transcription in Tetrahymena ribosomal chromatin. An identical sequence specifity is observed, when topo I purified from a variety of eukaryotes is reacted with naked DNA. The rate of topo I relaxation at the recognition sequence appears to be three orders of magnitude higher than at other sites. The specificity of this interaction is emphasized by the fact that a point mutation in the hexadecameric sequence abolish binding as well as enzyme activity at this site. The structure of hexadecameric binding appears to be tripartite in that a 6 bp. core matching the general topo I binding site consensus is flanked by two homopolymeric blocks of (dA)·(dT). These blocks are essential for topo I binding as deduced by footprinting and modification interference analyses. In another set of experiments where DNA fragments with a series of single-strand deletions created by DNA polymerase primer extension or 5'-exonuclease degradation were used as substrates the minimal duplex DNA sequence essentiel for topo I cleavage was found to lie within the region protected from nuclease digestion in presence of topo I. All together these observations raise the posibility that topo I play an impotant role in chromatin function through sequence specific regulation of DNA topology.

L 112 INTRAMOLECULAR TRIPLEX FORMATION IN PLASMIDS: EFFECT OF LENGTH, SUPERCOILING AND pH, D.A. Collier and Robert D. Wells, The University of Alabama at Birmingham, Department of Biochemistry, Birmingham, AL 35294 USA. Intramolecular DNA triplexes (H-DNA) form at short (<37-bp) oligopurine•oligopyrimidine (pur•pyr) mirror repeats. Triplex formation requires both low pH and negative supercoiling to form, in an interdependant manner. The structure can form in short pur•pyr segments at neutral pH at high (>σ -0.1) levels of negative supercoiling, but its existance under more physiological conditions has not yet been demonstrated. The structure(s) adopted by longer pur•pyr stretches is not well characterized, and it is not yet known if they can also form triplexes. In this study we have examined pur•pyr repeats of $(AGGAG)_n$ and $(GA)_n$ from 32- to 74-bp using 2-D gel electrophoresis and chemical probes. On formation of these structures a relaxation of ~1 turn per turn of primary helix is observed, consistent with the structure forming from a completely unwound stretch of DNA. Molecular modeling studies support the hypothesis that the triplex is a topologically unlinked structure. Chemical probing studies indicate that longer pur•pyr stretches also form triplexes, but at pH's closer to neutral than shorter stretches. At low pH they adopt a more complex structure(s) which may be a mixture of triplex isomers. If the pur•pyr stretch is of sufficient length, it can form a triplex at physiological pH's and at reasonable levels of supercoiling (~σ -0.06). Pur•pyr stretches in eukaryotic DNA are often very long (up to 370-bp of continuous purines). Thus, these results indicate the potential for triplex formation in vivo. In addition, the alternate structures formed by longer pur•pyr stretches can be transiently altered by modest changes in pH and superhelical density, consistent with their possible participation in genetic regulation in vivo. Supported by NSF and NIH.

L 113 **POST-TRANSLATIONAL MODIFICATION AND PROCESSING OF HUMAN DNA POLYMERASE α CATALYTIC POLYPEPTIDE.**
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Lab of Experimental Oncology, Dept. of Pathology, Stanford University School of Medicine, Stanford Ca 94303**

Biochemical and immunological studies have documented that the catalytic polypeptide of DNA polymerase α comprises a cluster of phosphopolypeptides predominantly of 180 and 165 kDa, determined by peptide mapping to be derivatives of the same primary structure (1,2). Deduced from the full-length cDNA of human DNA polymerase α, the estimated Mr of the recombinant human DNA polymerase α is 165 kDa (3). Two 20 amino acid peptides according to the very N- and C- terminus of the recombinant human DNA polymerase α were synthesized. Mono-specific polyclonal antibodies against these two peptides were produced. By immunoblot analysis, the presence of a cluster of polypeptides from 180 kDa to lower molecular weights of 165-125 kDa was due to processing from the N-terminus.

The detection of a 180 kDa polypeptide by the antibody specific for the very N-terminus of the 165 kDa polypeptide indicates the presence of 180 kDa protein is due to post-translational modification. The specific post-translational modification that causes the apparent molecular mass shift from 165 to 180 kDa is of the ConA interactive-type of glycosylation. The nature of the oligosaccharidic moiety, the linkage to polymerase α protein and the implications of this modification for mechanisms of DNA replication regulation will be investigated and discussed. (This study is supported by NIH Grant CA14835 and by gifts from Applied Biosystems Inc and Donald & Delia Baxter Fund)

L 114 MOLECULAR AND GENETIC ANALYSIS OF VACCINIA VIRUS TOPOISOMERASE MUTANTS,
Jill K. Countryman, Michele Fuortes, and Paula Traktman, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.
We have previously characterized a 32 kDa type I topoisomerase from vaccinia cores and shown that its relaxation of DNA involves a transient covalent linkage to the 3'-phosphoryl of the DNA (Shaffer and Traktman, JBC, 1987). Comparison of known genomic sequences with N-terminal amino acid sequence of the protein has led to the assignment of the H7 ORF of the viral genome as the topoisomerase gene (Shuman and Moss, PNAS, 1987; Shuman et al, JBC, 1988). We have cloned this ORF behind an inducible promoter, and inspection of plasmid DNA obtained from E. coli containing the topoisomerase gene showed the DNA to be markedly relaxed with topoisomers easily observable. Since bacterial protein extracts also relaxed exogeneous DNA, these simple assays of topoisomerase activity provide a quick and simple method for identification of mutations introduced into the topoisomerase gene. Both random (using nitrosoguanidine) and site-specific mutagenesis will allow us to identify protein domains involved in DNA relaxation. The sensitivity of altered topoisomerases to compounds known to inhibit the activity of the viral topoisomerase, such as two congeners of the epipodophyllotoxins and other classical type II topoisomerase inhibitors will also be evaluated (Shaffer and Traktman, JBC, 1987). Furthermore, the mutagenized gene can be introduced back into the viral genome via homologous recombination, and conditionally lethal topoisomerase mutants can be identified. The analysis of these mutants will facilitate the dissection of the complex role of this topoisomerase in the life cycle of vaccinia virus.

L 115 CHARACTERIZATION OF A PROTEIN-DNA INTERFACE: DNA SUBSTRATE FUNCTIONALITIES IMPORTANT FOR *Eco RI* METHYLASE SPECIFICITY, Michael J. Danzitz Jr. & N. O. Reich, Department of Chemistry, University of California, Santa Barbara, California 93106

Eco RI methylase is a monomeric 38,050 dalton enzyme requiring the cofactor S-adenosylmethionine (AdoMet) and its DNA substrate for activity. The methylase recognizes the double stranded DNA sequence 5'-GAATTC-3' and methylates the second adenine of each strand. Details about the DNA protein interface of a DNA methylase or any monomeric DNA binding protein have yet to be determined.

Hydroxyl radical footprinting experiments of recognition site containing oligonucleotides complexed with the methylase alone or with the methylase and sinefungin (an AdoMet analog and methylase inhibitor) have been carried out. These experiments have shown the sugar phosphate backbone regions in and around the recognition site which are protected by the methylase both with and without sinefungin present. Some differences in both the binding pattern and affinity have been observed between these complexes.

Specific functionalities have been removed from the substrate at defined locations in the recognition site by the incorporation of non-standard nucleotides, such as uracil. The kinetic parameters obtained with these substrates have allowed us to evaluate the importance of specific functional groups in substrate recognition (in the uracil example the methyl group of each of thymidine). The effect of single asymmetric substitutions on specificity and catalysis are being dissected out using this approach. The thermal denaturation characteristics of the modified substrates are being used to probe for additional substrate structural changes.

L 116 INHIBITION OF R6K PLASMID REPLICATION BY π PROTEIN IS HINDERED BY *ESCHERICHIA COLI* INTEGRATION HOST FACTOR (IHF) Stephanie Dellis, Julie Baskfield, and Marcin Filutowicz, Bacteriology Department, University of Wisconsin, Madison, WI, 53706

Plasmid R6K encodes π protein, which plays a dual function in the regulated initiation of replication from the α, β, and γ replication origins. The π protein is an origin specific DNA binding protein required for replication to occur, yet it inhibits replication in a concentration-dependent manner. It is unclear how π produces these two opposite effects, or which *Escherichia coli* proteins are involved in facilitating these processes.

In this study we show the involvement of *E. coli* Integration Host Factor (IHF) in the regulation of replication of the three R6K origins. Quantitative analysis of plasmid copy number of different R6K replicons in WT and *himA* or *hip* mutants has demonstrated that bacteria lacking functional IHF protein display enhanced sensitivity to replication inhibition by π. A minor increase in the intracellular π level which is still permissive for R6K derivatives containing all three replication origins (α, β, and γ) prevents plasmid replication in *E. coli* mutants lacking functional IHF. In examining the requirement for IHF protein in derivatives containing a minimal R6K replicon, we have found that the γ origin is most vulnerable to π concentration in the absence of IHF protein and is unable to replicate at normal π levels. Reducing π to 7% of the normal intracellular level allows replication of a γ origin plasmid in the absence of functional IHF protein. Furthermore, π variants present at normal levels but defective in replication inhibitor activity also permit replication in IHF deficient cells. Finally, we show IHF protein can bend γ origin DNA *in vitro*.

Thus, we propose that IHF protein is acting to hinder the π protein mediated inhibition of plasmid replication by altering the architecture of the origin.

L 117 **PURIFICATION, CHARACTERIZATION, AND MOLECULAR CLONING OF ESCHERICHIA COLI TOPOISOMERASE III,** Russell J. DiGate and Kenneth J. Marians, Sloan-Kettering Institute, New York, NY 10021. A potent decatenase has been purified from E. coli based solely on its ability to segregate pBR322 replication products in vitro. The purified decatenase, similar to other topoisomerases, catalyzed the relaxation of form I DNA; however, the relaxation properties of this enzyme were very temperature-dependent (occurring efficiently at only 52°). The decatenase-catalyzed decatenation of multiply interlinked form II:II DNA dimers, however, proceeded quite efficiently at 30°C. The decatenation of form II:II DNA dimers by this activity was dramatically stimulated by the presence of gaps on the substrate and exhibited a turnover number 70-fold greater than DNA gyrase and 10-fold greater than topoisomerase I on the same substrate. Analysis of topoisomerase-induced cleavage sites on a single-stranded DNA substrate and the apparent molecular weight of the polypeptide indicated that this enzyme was topoisomerase III (Topo III).

The gene encoding Topo III has been molecularly cloned and mapped in the E. coli genome. Details on the purification and cloning of the gene encoding Topo III will be presented.

L 118 **DNA-DIRECTED OLIGOMERIZATION OF THE MONOMERIC NER REPRESSORS FROM BACTERIOPHAGES Mu AND D108**, George Kukolj and Michael S. DuBow, Department of Microbiology and Immunology, McGill University, Montréal, Quebec, Canada H3A 2B4.
The 9 kDa ner gene products of the related transposable bacteriophages Mu and D108 (which are λ cro like proteins) function as negative regulators of transcription by binding to a DNA sequence which is specific for each phage. The ner gene DNA sequences are unrelated yet the proteins are approximately 50% homologous at the amino acid level and may share similar biochemical properties. Using operator-specific band retardation assays and crude extracts containing overexpressed Ner, the proteins from both phages were purified to homogeneity. DNase I footprinting shows the Mu ner-operator to contain two potential 12 bp Ner binding sites, each of which contains the consensus sequence (5' ANPyTAPuCTAAGT 3'), separated by a 6 bp AT-rich region. Moreover, regions corresponding to 3/4 of a turn of the DNA helix which flank these 12 bp repeats are protected by Ner. D108 Ner binding sites which display a similar DNase I protection pattern were further characterized by DMS protection and hydroxyl radical footprinting. The D108 ner-operator is uniquely organized as two apparent 11 bp (5' CCGTGAGCTAC 3') perfect inverted repeats separated by an 8 bp AT-rich region. D108 Ner protein makes base-specific contacts over this entire 11 bp sequence. Furthermore, the Mu and D108 Ner proteins chromatograph as monomers on Sephadex columns. Dimethyl suberimidate cross-linking indicates that the D108 Ner protein (at concentrations as high as 10 mM) is a monomer in solution, yet crosslinks as a dimer when bound to its operator site. As small (75 and 73 amino acids, respectively) monomeric proteins, Mu and D108 Ner do not display strong homology with the conserved helix-turn-helix regions of other procaryotic repressors and may constitute a new class of DNA-binding proteins.

L 119 **REVERSE-GYRASE PERFORMS UNWINDING OF THE DNA DOUBLE HELIX AND SINGLE-STRAND CLEAVAGE IN THE ABSENCE OF ATP**, Christine Jaxel*, Marc Nadal*, Gilles Mirambeau*, Patrick Forterre†, Miho Takahashi‡ and Michel Duguet*, Laboratoire d'Enzymologie des Acides Nucléiques, CNRS 96 Bd. Raspail 75006 Paris, France. †Institut de Microbiologie, Université Paris Sud, 91405 Orsay, France. ‡Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooa, Machida, Tokyo 194, Japan.
Stoechiometric amounts of pure reverse gyrase, a type I topoisomerase from the Archaebacterium *Sulfolobus acidocaldarius* were incubated at 75°C with circular DNA containing a single-chain scission. After covalent closure by a thermophilic ligase and removal of bound protein molecules, negatively supercoiled DNA was produced. This finding, obtained in the absence of ATP, contrasts with the ATP-dependent positive supercoiling catalyzed by reverse gyrase and is interpreted as the result of enzyme binding to DNA at high temperature. Another consequence of reverse gyrase stoechio- metric binding to DNA is the formation of a cleavable complex which results in the production of single-strand breaks in the presence of detergent. As eubacterial type I topoisomerase (protein ω), reverse gyrase is tightly attached to the 5'termini of the cleaved DNA.

L 120 THE ROLE of OBF1 - A S. CEREVISIAE ARS-BINDING PROTEIN - IN ORIGIN FUNCTION of ARS121 DNA, S. Eisenberg, S. C. Francesconi, S. S. Walker and C. Civalier, Department of Microbiology, The University of Connecticut Health Center, Farmington, Ct 06032. We have isolated a protein activity from S cerevisiae, OBF1, that binds specifically to a DNA element present in the telomeric, repetitive ARS120 and the single copy ARS121. Electrophoresis of the purified protein in SDS-acrylamide gels revealed the presence of two polypeptides. The major protein band had a denatured molecular weight of 123 kDa, and the minor protein band, which constituted only a small fraction of total protein, had a molecular weight of 127 kDa. Both polypeptides co-chromatographed with the specific ARS-binding activity and formed a stable protein-DNA complex, isolatable by sedimentation through sucrose gradients. Using antibodies, we have shown that both polypeptides are associated with the isolated protein-DNA complexes. The ARS DNA-binding activity has a Stoke's radius of 54 Å and a sedimentation coefficient of 4.28 S determined by gel filtration and sedimentation through glycerol gradients respectively. These physical parameters, together with the denatured molecular weight values, suggest that the proteins exist in solution as asymmetric monomers. Since both polypeptides recognize identical sequences and have similar physical properties, they might be related. In addition to ARS120, we found that the purified OBF1 binds to ARS121, ARS1 , and HMRE. To understand the relationship between OBF1 binding and ARS function we have subcloned ARS121 to a 500 bp fragment. This DNA fragment , with ARS activity, contains a high as well as a low affinity OBF1-binding site. Substitution mutants within either of the two sites obliterate detectable in vitro protein binding. Linker substitution (6bp) mutations within the high affinity site show effects identical to those of the complete substitution mutant, whereas substitutions outside the binding site bind OBF1 normally. We have also tested the mitotic stability of centromeric plasmids bearing wild type and mutagenized copies of ARS 121. Eighteen base pair substitutions within either of the two OBF1 binding sites reduces the percent of plasmid containing cells in the population from 88% to 63% under selective growth and from 46% to 20% under nonselective growth (for 10-12 generations). Furthermore, linker substitutions within the major binding site show similar defficiencies in plasmid stability. In contrast, plasmids containing linker substitutions in sequences adjacent to the high affinity site do not exhibit reduced stability. This tight, direct correlation between the ability of OBF1 to bind to ARS121 and plasmid stability in vivo suggest a role for OBF1 in origin function, possibly at the level of initiation of DNA replication.

L 121 MUTANTS OF PLASMID R6K π PROTEIN ALTERED IN DNA BINDING
Dona York and Marcin Filutowicz, Department of Bacteriology, University of Wisconsin, Madison WI 53706

A direct relationship between the activity of the R6K replication origin and the sequence-specific binding of π protein to the seven 22 bp repeats of the origin has been previously reported. Autogenous regulation of π protein synthesis is also related to a direct π-DNA interaction with the operator of the *pir* gene. In spite of the striking sequence homology in both π recognition sites it is intriguing that the origin repeats are tandemly aligned whereas the operator sequence is symmetrical. Furthermore, origin activation can occur at π levels as low as 1% of the normal value while the repression of the *pir* gene requires normal π levels. Thus, it is important to determine how the π-DNA complex involved in autoregulation differs from that which is involved in replication.

The binary plasmid system composed of a *pir*P-Cm fusion and a fusion of the coding segment of the *pir* gene with TacP has been utilized to obtain mutants of π protein altered in binding to the *pir* gene operator; selection based on chloramphenicol resistance. A π-immunoassay was used to identify clones which after IPTG induction produced full length π polypeptide at a level similar to the WT counterpart. Of six mutants with reduced ability to repress the *pir* p, five also demonstrated an inability to activate the R6K origin while one isolate retained initiator properties only when overproduced. We will present both the genetic analysis of these new mutants in the *pir* gene and the biochemical characterization of purified mutant π protein variants.

L 122 EUKARYOTIC AND EUBACTERIAL TYPE II DNA TOPOISOMERASE INHIBITORS (VP 16 AND CIPROFLOXACIN) INDUCE DNA CLEAVAGE AT THE SAME SITES IN AN ARCHAEBACTERIAL PLASMID. Patrick Forterre and Sioud Mouldy. *Institut de Microbiologie, Université* Paris-sud, 91405 Orsay, France. Archaebacteria contain a type II DNA topoisomerase in addition to the reverse gyrase discovered in thermophilic archaebacteria. Both inhibitors of eubacterial DNA gyrase (coumarines and quinolones) and of eukaryotic DNA topoisomerases II (several families of antitumoral drugs) inhibit the growth of several archaebacterial species. We have shown that the coumarin novobiocin and the antitumoral drug VP16 (an epipodophyllotoxin) induce topological changes in the plasmid pGRB-1 of the halophilic archaebacterium *Halobacterium* GRB similar to those produced by these drugs in eubacteria and eukaryotes, respectively. Novobiocin induces positive supercoiling (Sioud et al., NAR, 1988, 16, p1372) and VP16 induces DNA cleavage with a protein linked at the 5'ends of the breaks (Sioud et al., NAR, 1987, 15, p8217). Here we show that the fluoroquinolone ciprofloxacin which did not inhibit the growth of *Halobacterium* GRB nevertheless induces the cleavage of the pGRB-1 plasmid when added to the culture medium during magnesium-depletion. DNA cleavage induced by either VP16 or ciprofloxacin produce DNA fragments of similar length and relative abundance. This result suggests that the mode of interaction of both drugs with the DNA topoisomerase II-DNA complex is identical.

L 123 PROPERTIES OF THE P1 PLASMID PARTITION COMPLEX AT parS: BINDING OF ParB PROTEIN AND IHF. Barbara E. Funnell, Lab. of Biochemistry, NCI, NIH, Bethesda, MD 20892. Stable maintenance of low copy number plasmids, such as the P1 prophage, requires both proper DNA replication and subsequent chromosome segregation, or partition. The P1 par system encodes two proteins, ParA and ParB, and contains a cis-acting site, parS (a centromere analog). The proteins presumably function in concert with the host's own partition proteins, but as yet only one host factor is known to participate in P1 segregation, the E. coli integration host factor (IHF). This study describes the preliminary characterization of the partition complex at parS. Both ParB and IHF specifically recognize and bind to sequences at parS. The presence of IHF appears to greatly increase the affinity of ParB protein for this region. As measured by DNAseI protection experiments, IHF binds to a region approx. 30 bp long, including the sequence 5'-TAACTGACTGTTT, which deviates from the consensus (T/CAAnnnnTTGATT/A) in two positions. The IHF binding site is between two sets of ParB binding sites. IHF is known to bend DNA, and this property may explain the IHF stimulation of ParB activity at parS: tight ParB binding may require that the protein interacts with both sets of sites simultaneously. In addition, DNA topology profoundly influences the reaction. Supercoiled DNA is a much better substrate than linear DNA for ParB protein. Further characteristics of the ParB-IHF-parS complex will be presented.

L 124 MECHANISM OF THE TOPOISOMERASE II-MEDIATED DNA CLEAVAGE REACTION, Kevin C. Gale and Neil Osheroff, Dept. of Biochemistry, Vanderbilt University, Nashville, TN 37232
The ability to introduce transient double-stranded breaks in the DNA backbone is essential to the physiological functions of topoisomerase II. Despite the importance of this activity, the detailed mechanism of DNA cleavage is not completely understood. In order to monitor DNA cleavage in vitro, the enzyme is incubated with double-stranded DNA and allowed to establish an equilibrium. Cleaved DNA which is covalently linked to topoisomerase II via the newly generated 5'-termini is observed following the addition of a rapidly acting protein denaturant such as SDS. Due to the requirement for SDS in this assay, it is unclear whether DNA cleavage takes place prior to, or is induced by the addition of the denaturant. These two possibilities can be distinguished by determining whether enzyme-bound DNA contain 3'-OH termini prior to the addition of SDS. This was accomplished by using a circular single-stranded DNA substrate which could potentially generate 3'-termini with the ability to diffuse away from the active site of the enzyme. As previously observed for cleavage of duplex DNA, the enzyme was covalently linked to cleaved single-stranded molecules. Moreover, optimal buffer, salt and divalent cation concentrations were similar. In contrast, single-stranded DNA cleavage increased with time, showed little salt reversibility, and no longer required the presence of SDS. Furthermore, DNA products of the reaction which were generated in the absence of protein denaturants could be labeled at their 3'-termini upon incubation with terminal transferase and radioactive [α-^{32}P]-ddATP. This evidence strongly suggests that DNA is cleaved by topoisomerase II prior to the addition of SDS. Supported by NIH Grant GM-33944.

L 125 PALINDROMIC UNITS : A HIGHLY REPEATED DNA SEQUENCE IN ENTEROBACTERIA, Eric GILSON, David PERRIN and Maurice HOFNUNG, CNRS UA271 INSERM U163, INSTITUT PASTEUR, 75015, PARIS, FRANCE.

In bacteria, only a few highly repeated DNA sequence families have been described ; furthermore, very little is known about their functions. In our laboratory, we deal with the Palindromic Unit (PU) family (Gilson et al., 1984 *EMBO J.* 3, 1417-1421). This family is constituted of extragenic palindromic sequences (20-40 bp long) with an extremely high degree of sequence homogeneity. They may represent up to 1% of the chromosome of *E .coli* and *S.typhimurium*.

In several cases PU are known to affect gene expression (mRNA stability, transcription.....), but these properties cannot explain their high level of sequence homogeneity (reviewed in Gilson et al., 1987,*Trends in Genet.* , 3, 226-230)

We will present experimental data showing that :

- the PU consensus sequence and its genomic localizations exhibit species-specificity
- PU DNA sequences specifically bind nucleoid-associated protein(s); we have developped an *in vitro* assay to monitor the purification of the PU-binding protein(s).

Our present data on the PU are in agreement with the hypothesis that PU sequences constitute specific sites for functional and/or structural organization of the bacterial nucleoid, via DNA-protein interactions. One consequence of the PU sequence species-specificity is that these sites would not be interchangeable between species. This would tend to counterselect the insertion of a large segment of foreign chromosome devoid of specific PUs. We expect that the characterisation of the specific PU sequence-protein interactions and of its function will also bring new informations on the bacterial nucleoid.

L 126 CHARACTERIZATION OF BACTERIOPHAGE T4 GENES dda AND 59 AND THEIR PROTEIN PRODUCTS, Kevin Hacker, Jack Barry, and Bruce Alberts, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143
The bacteriophage T4 dda protein or "DNA-dependent ATPase" is a DNA helicase that has previously been shown in our laboratory to allow the T4 replication fork to move past DNA-bound RNA polymerase or lac repressor molecules. It is therefore presumed to play an important role in vivo where following infection, replication and transcription occur simultaneously. The dda protein also appears to play a role in T4 directed genetic recombination. In vitro the dda protein binds to the T4 uvs X protein (a rec A-type protein) and accelerates the rate of uvs X-protein catalyzed DNA branch migration. The dda sequence was determined by dideoxy sequence analysis of total genomic DNA or RNA and cloned DNA . The dda gene encodes a protein of 440 amino acids that contains an 8 amino acid sequence common to ATP-binding proteins. The dda gene's ribosomal binding site has been modified to overproduce active dda protein. The overproduced dda protein has been purified to homogeneity. In a wild-type background, the dda protein is nonessential, but in a gene 59$^-$ background, it becomes essential for DNA synthesis (Doherty et al. MGG 188, 77-90, 1982). Using the gene 59 sequence determined by Hahn, Kruse, and Ruger (NAR 14, 9311-9327, 1986), gene 59 was cloned into an expression vector, and the overproduced 59 protein was identified and purified to near homogeneity. It binds tightly to DNA and the gene 32 protein. Like dda, the gene 59 protein appears to help the replication fork to move pass DNA-bound RNA polymerase molecules; however unlike dda this activity requires the gene 41 DNA helicase. The gene 59 protein also stimulates T4 primosome activity on a single-stranded DNA template. The 59 protein appears to be an analog of the E. coli dnaC protein and the lamda P protein.

L 127 FUNCTIONAL SITES IMPORTANT FOR NUCLEOTIDE RECOGNITION AND SELECTION IN THE DNA POLYMERASE OF HERPES SIMPLEX VIRUS TYPE 1 (HSV-1), Jennifer D. Hall and Yi-sheng Wang, Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.

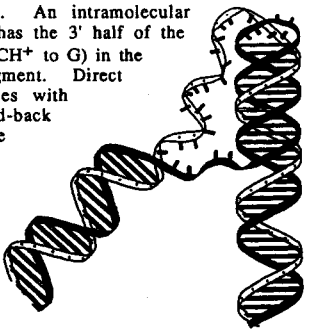
We are studying the HSV-1 DNA polymerase in order to identify the site(s) within this enzyme important for recognition and selection of dNTP substrates. We have employed computer and genetic analyses to define functional domains and to identify specific residues involved in substrate binding. Comparisons of the HSV-1 DNA polymerase amino acid sequence with other polymerase sequences have revealed striking similarities with polymerases from several animal viruses, bacteriophages, yeast, and human cells. The similarities suggest a common structural organization in these distantly related proteins and implicate the most highly conserved sites as being important for enzyme function. We have also attempted to identify amino acid residues which interact directly with dNTP substrates by isolating HSV-1 polymerase mutants with enhanced or impaired abilities to recognize correct dNTPs. These mutants are resistant or hypersensitive to nucleotide analogues and/or other inhibitors which interact at the dNTP binding site (e.g. aphidicolin). Some of the mutations also affect replication fidelity as indicated by changes in viral spontaneous mutation frequencies. DNA sequencing has located several mutations at or near the highly conserved sites in the polymerase sequences described above. These mutations, therefore, define a region of the HSV-1 polymerase involved in dNTP binding. The mutant phenotypes and the locations of the mutations are useful for suggesting what polymerase-dNTP interactions are altered in the mutants.

L 128 THE INITIATOR TITRATION MODEL FOR CONTROL OF BACTERIAL CHROMOSOME REPLICATION. Flemming G. Hansen and Tove Atlung, Department of Microbiology, Technical University of Denmark, DK-2800 Lyngby, Denmark.

Initiation of chromosome replication occurs at a characteristic time in the cell cycle depending on the growth rate. Chromosomal replication starts at the origin of replication, oriC, and proceeds at a constant speed to the terminus. Twenty min later the cell divides. Initiation takes place when the cell has obtained a certain mass per chromosomal origin. All origins present in a cell are initiated at this time. We present a specific model with the following elements: The DnaA protein. The DnaA-boxes, in oriC, in the dnaA promoter region, and at other positions on the chromosome. Activation of new origins. The model is base on three main assumptions. 1. The DnaA protein has a high affinity for the DnaA-boxes, which will titrate free DnaA protein. We assume that the DnaA-boxes are preferentially located close to oriC. Thus, after initiation of replication DnaA-box concentration will increase rapidly. 2. The DnaA protein has a lower affinity for the interaction in the initiation complex. This infers that formation of the initiation complex only occurs if the DnaA protein is present in excess of DnaA-boxes. During most of the cell cycle there are fewer DnaA proteins than DnaA-boxes. 3. A newly replicated origin is refractile to initiation for the period it takes to regain normal topology (supercoiling, methylation). A stochastic analysis of our model, using available data concerning the DnaA protein, the DnaA-boxes and the dnaA gene promoter, shows that it can mimick the observations on cell cycle control in E. coli.

L 129 INTRAMOLECULAR DNA TRIPLEXES IN SUPERCOILED PLASMIDS, Jeffery C. Hanvey, Mitsuhiro Shimizu, David A. Collier, Jan Klysik, and Robert D. Wells, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294

DNA sequences with primarily purines in one strand are abundant in eukaryotic genomes and often map near genes and recombination hot spots. These pur•pyr sequences form intramolecular triplexes and other unusual DNA structures in supercoiled plasmids. An intramolecular triplex, also called H-DNA, (see inset) is stabilized by acidic pH and has the 3' half of the pyrimidine strand folded back and Hoogsteen H-bonded (T to A and CH^+ to G) in the major groove to the purines in the opposite half of the pur•pyr segment. Direct evidence for the triple base interaction was obtained from sequences with site-specific interruptions in the pur•pyr insert. Due to the folded-back nature of the triplex, a single nucleotide change (A to T) within the 3' half of the purine strand caused a T 13 nucleotides away to become reactive to OsO_4, as this T does not Hoogsteen H-bond to the T in the purine strand. The triplex with an interruption in the stem is less thermostable and requires more supercoil energy for formation than one without an interruption. Also, triplex formation and stability as a function of loop size and G+C content, and the interconversion of duplex and triplex, has been studied. Supported by NIH and NSF.



L 130 TRIPLE-HELIX FORMATION, DNA BENDING, AND DNA UNWINDING IN SEQUENCES DERIVED FROM THE AMPLIFIED DHFR ORIGIN REGION, Nicholas H. Heintz, Richard H. Lussier, and Mark S. Caddle, Departments of Pathology and Biochemistry, University of Vermont, Burlington, VT 05405. The sequence of 5.9 kb of DNA thought to encompass the origin of replication associated with the amplified DHFR domains of CHOC 400 cells has been determined. Origin region DNA contains two Alu I repeats, a conserved repetitive element (termed ORR-1), numerous 10-12 bp repeats, and several repeating polymers and co-polymers, including poly (dA), poly (dA-dT), poly (dA-dC), poly (dG-dC), and poly (dC-dT). The poly (dA-dC) and poly (dA-dG) regions are organized in a block of 190 bp that contains poly $(dG\text{-}dC)_6$, poly $(dA\text{-}dC)_{19}$, poly $(dA\text{-}dG)_{20}$, $(dG)_9$, CACAC $(AGACAGACAGAGAGGG)_2$, poly $(dA\text{-}dG)_{27}$. Supercoiled plasmids containing origin region DNA were assayed for structural configurations that may function in origin activation. Origin region DNA contains a 280 bp Hae III fragment of stably bent DNA that contains 5 tracts of $(dA)_{3\text{-}6}$ that are phased 10 bp apart. Mungbean nuclease (MBN) sensitivity assays with supercoiled plasmids at pH 7.2 show that DNA unwinding occurs within a poly $(dA\text{-}dT)_{23}$ sequence prior to unwinding at poly $(dA)_{38}$, the bent DNA sequence, the poly (dA-dC) tracts, or vector sequences. At pH 5.2, prominent cleavage by MBN at the poly (dA-dG) tracts is observed. Intermolecular hybridization studies confirm that, at pH 5.2, the poly (dA-dG), poly (dT-dC) tracts in supercoiled plasmids form triple-stranded DNA. Thus, the DHFR origin region contains multiple sequence elements that are able to undergo structural perturbations consistent with DNA unwinding and origin activation.

L 131 FORMATION OF A FUNCTIONAL NUCLEOPROTEIN STRUCTURE AT THE PHAGE ø29 DNA REPLICATION ORIGINS. José M. Hermoso, Manuel Serrano, Julio Gutiérrez and Margarita Salas. Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain.
The B. subtilis phage ø29 protein p6 is needed for viral DNA synthesis in vivo and stimulates ø29 DNA initiation of replication in an in vitro system with purified proteins. Protein p6 binds specifically to right or left ø29 DNA terminal fragments containing the replication origins. DNase I footprint experiments show a regular pattern of hypersensitive bands spaced 24 nucleotides flanking protected regions that extends about 200 bp from each genome end. Hydroxyl radical footprints show protections of 3-4 nucleotides with a periodicity of 12. Protein p6 also binds to circular plasmid DNA inducing positive supercoiling. These results suggest the formation of a nucleoprotein structure in which the DNA forms a right-handed superhelix around a protein core. The formation of the nucleoprotein structure correlates with the protein p6 activity in the initiation of replication. Protein p6 recognition signals are present at multiple sites within 200 bp from each DNA end, a cluster of which has been mapped from nucleotides 62 to 125 and 46 to 68 from the right and left ø29 DNA ends, respectively. Protein p6 does not seem to recognize a sequence specific signal, but a structural feature that may be related to bendability.

L 132 IHF AND HU INTERACT IN VIVO AND ORGANIZE DNA IN VITRO INTO SPECIFIC STRUCTURES WHEN DNA IS TORSIONALLY STRAINED. Patrick Higgins and Victoria McGovern. Department of Biochemistry, University of Alabama at Birmingham, Schools of Medicine and Dentistry, Birmingham, AL 35294. In enteric bacteria a complex nucleoid structure is folded and maintained under negative superhelical tension by topoisomerases and small, heat-stable, acid soluble proteins that are often called "histone-like" proteins. The most abundant of these is HU, and a close relative is integration host factor (IHF). IHF alters transcription of bacteriophage Mu by binding a specific sequence in the operator and modulating transcription of two converging promoters. DNA sequences adjacent to the IHF binding site are flexible and altered by supercoiling. The effect of IHF on Mu transcription depends on DNA conformation. A topological model called a superloop explains the physical effect of IHF on supercoiled DNA during electrophoresis in acrylamide gels and during transcription. In a superloop IHF occupies a position at the apex of an interwound form of looped and supercoiled DNA. HU is related to IHF by protein sequence homology and recent evidence shows that plasmids expressing the HU-2 subunit cause growth arrest in strains with mutations in either the himA or hip subunits of IHF. Like IHF, HU forms supercoil-dependent structures with DNA in vitro. Thus, the ability of these proteins to organize supercoiled DNA may indicate that their role is punctuation for efficient reading of genetic information in the folded chromosome.

L 133 PROBING BIOPOLYMER INTERACTIONS WITH LASER CROSS-LINKING Joel W. Hockensmith, Theresa A. Bens and Larry D. Mesner, Department of Biochemistry, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908
We use a rapid laser cross-linking technique to investigate biopolymer interactions. We have been studying the equilibrium that exists between oligomers of (dT) bound to poly(dA) and poly(rA), determining the degree of cooperativity that exists between oligomers and calculating the free energy involved. The cooperativity and free energy of oligomer-oligomer interaction is much higher on poly(dA) than on poly(rA). We have also calculated the enthalpies and entropies involved. Our data provide fundamental information about (dA)·(dT) structure, and impact upon a host of other studies ranging from DNA polymerase mechanisms on oligo(dT)·poly(dA) to the structure of (dA)·(dT) tracts and their putative bifurcated hydrogen bonds and spine of hydration.

L 134 SPECIFIC CLEAVAGE AT DNA-SECONDARY STRUCTURES BY A CRUCIFORM RESOLVING ACTIVITY FROM YEAST. Frank Jensch, Nadrian C. Seeman*), Sven Pottmeyer, Hans Kosak, Patricia Solaro and Börries Kemper, Institut für Genetik, 5000 Köln 41, F.R.G.*)Department of Chemistry, New York University, New York, N.Y. 10003.

We have purified an endonuclease (Endo-Y3) more than 200 fold from crude extracts of mitotically grown cells of *S. cerevisiae*. The enzyme shows high specificity for DNAs with secondary structures and introduces characteristic pattern of 'nicks' in the immediate vicinity of each structure. Following substrates were analysed in detail: 1. natural cruciforms made from inverted repeats in supercoiled plasmid DNA, 2. synthetic cruciforms with short arms of 9 basepairs (bp), 3. heteroduplex loops with 8 nucleotides (nt) in the loop, 4. Y-junctions with 10bp in each arm, and 5. double-stranded DNA with single-stranded overhangs of 10nt. Nicks were exclusively found in the double-stranded part of the DNA and 3' of the respective structure. The Endo-Y3 induced cleavage pattern were exactly the same as those made by endonuclease VII, the cruciform resolving enzyme from phage T4. This suggests a functional relationship between the two enzymes, which is further supported by the finding that antibodies raised against pure protein of Endo VII inactivate endo-Y3 in functional assays with branched DNA.

L 135 **THE THREE-DIMENSIONAL STRUCTURE OF A DNA DUPLEX CONTAINING LOOPED-OUT BASES** Leemor Joshua-Tor*, Dov Rabinovich*, Hakon Hope*#, Felix Frolow*, Ettore Appella+ and Joel L. Sussman*

**Department of Structural Chemistry, The Weizmann Institute of Science, Rehovot 76100, ISRAEL; #Permanent address: Department of Chemistry, University of California, Davis, CA 95616 USA; +Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health Bethesda, MD 20205 USA.*

Unpaired bases (bulges) in DNA double helices are of interest because of their proposed role in frameshift mutagenesis. The introduction of an additional base in an otherwise fully complementary double helix could, in principle, produce several alternative structures. NMR studies of the tridecamer d(CGCAGAATTCGCG)$_2$ in solution have shown that the unpaired adenosines are *stacked into* the duplex.

Crystals of this tridecamer were grown, and X-ray data were collected at -150°C, using the shock-cooling techniques of Hope on a Rigaku AFC5-R Rotating Anode Diffractometer. Its structure was solved via ULTIMA, and refined at 2.8 Å resolution using CORELS and NUCLSQ. In marked contrast to the NMR results in solution, we find that the conformation of the tridecamer in the crystal is close to that of a B-DNA duplex, with the two additional adenosines *looped out* from the double helix and causing little disruption of the rest of the structure. This result may help clarify why certain sequences of DNA are less prone to correction by the repair mechanism than others.

L 136 THE SPECIFICITY DETERMINANTS OF 434 REPRESSORS AT 434 OPERATOR POSITIONS 3 & 4
Koudelka, G.B., SUNY at Buffalo, NY 14260, Ptashne, M. Harvard University 02138.
The repressor and Cro proteins encoded by bacteriophage 434 binds a partially rotationally symmetric 14 bp operator sites on phage chromosome. Crystallographic analysis shows that in both protein-DNA complexes, one "recognition" alpha helix of the bound protein dimer lies in each half-site of the operator. Each of these helicies is positioned in the major groove so that its side chains can contact the outermost 4 base pairs in one operator half-site. Repressor displays remarkable specificity for the outer four base pairs; mutating any of the highly conserved outer four base pairs decreases the affinity of the operator for repressor by at least 50-fold. Cro displays similar specificity at the outer 3 positions, but will accept both G:C and A:T base pairs at position 4. We have used site-directed mutagenesis of both the proteins and operator to investigate the molecular basis of specificity. X-ray results indicate that repressor's gln_{33} contacts operator position 4. Changing this residue to an alanine did not abolish the specificity of repressor for position 4. Mutating this residue to leu, the residue present at the homologous position of Cro, did not confer 'Cro-like' specificity at position 4 to repressor. These results indicate that other protein moeities in repressor, in addition to gln_{33}, contact position 4. Consistent with this conclusion, only the coordinated changes gln_{33}->ala, glu_{32}->gln, and thr_{27}->lys (the latter two being changes to residues present in homolgous positions in Cro) results in a repressor that has a loss of specificity at position 4. This result, together with others, allows us to elucidate the complicated network of side chain interactions that is resposible for generating repressor and Cro specificity at base pair 4. Substituting the A:T base pair at operator position 3 with either A:U or G:C decreased the affinity of the operator for repressor and Cro by >200-fold. Changing A:T at operator position 3 to G:^{5me}C decreased the affinity of operator for repressor by only 4-fold. Consistent with x-ray analysis, this result shows that repressor's specificity at operator position 3 is mediated exclusively through interactions with the thymine $-CH_3$ group. Interestingly, substitution of G:^{5me}C did not restore the high affinity of the operator for Cro. This suggests that Cro, despite its high homology to repressor, uses a different mechanism to recognize operator position 3.

L 137 **DNA-DIRECTED OLIGOMERIZATION OF THE MONOMERIC NER REPRESSORS FROM BACTERIOPHAGES Mu AND D108,** **George Kukolj and Michael S. DuBow, Department of Microbiology and Immunology, McGill University, Montréal, Quebec, Canada H3A 2B4.**
The 9 kDa ner gene products of the related transposable bacteriophages Mu and D108 (which are λ cro like proteins) function as negative regulators of transcription by binding to a DNA sequence which is specific for each phage. The ner gene DNA sequences are unrelated yet the proteins are approximately 50% homologous at the amino acid level and may share similar biochemical properties. Using operator-specific band retardation assays and crude extracts containing overexpressed Ner, the proteins from both phages were purified to homogeneity. DNase I footprinting shows the Mu ner-operator to contain two potential 12 bp Ner binding sites, each of which contains the consensus sequence (5' ANPyTAPuCTAAGT 3'), separated by a 6 bp AT-rich region. Moreover, regions corresponding to 3/4 of a turn of the DNA helix which flank these 12 bp repeats are protected by Ner. D108 Ner binding sites which display a similar DNase I protection pattern were further characterized by DMS protection and hydroxyl radical footprinting. The D108 ner-operator is uniquely organized as two apparent 11 bp (5' CCGTGAGCTAC 3') perfect inverted repeats separated by an 8 bp AT-rich region. D108 Ner protein makes base-specific contacts over this entire 11 bp sequence. Furthermore, the Mu and D108 Ner proteins chromatograph as monomers on Sephadex columns. Dimethyl suberimidate cross-linking indicates that the D108 Ner protein (at concentrations as high as 10 mM) is a monomer in solution, yet crosslinks as a dimer when bound to its operator site. As small (75 and 73 amino acids, respectively) monomeric proteins, Mu and D108 Ner do not display strong homology with the conserved helix-turn-helix regions of other procaryotic repressors and may constitute a new class of DNA-binding proteins.

L 138 IN VITRO CHARACTERIZATION OF THE N4 ENCODED DNA POLYMERASE AND SINGLE-STRANDED DNA BINDING PROTEIN,
G. Lindberg#, A. Sugino+, T. Kunkel+, S. Kowalczykowski=, and L. Rothman-Denes#, +NIEHS, NIH, Res. Triangle Park, N.C., =Northwestern U. Med. Sch. and #Univ. of Chicago, Chicago Ill.

The bacteriophage N4 encoded DNA polymerase and single-stranded DNA binding proteins have been purified to homogeneity. The DNA polymerase is a 87,000 dalton monomeric protein in solution and requires a template primer structure to initiate DNA synthesis. It has no strand displacement activity, cannot polymerize through regions of strong secondary structure, is non-processive and has no 5'-3' exonuclease activity. It does contain a strong 3'-5' exonuclease activity and synthesizes DNA with high fidelity.

The N4 single-stranded DNA binding protein, which aggregates in solution, has a monomeric molecular weight of 31,000 daltons. At 37 C in .22 M NaCl it has an intrinsic binding constant for M13 DNA of $3.4 \times 10^4 M^{-1}$, a cooperativity factor of 300 and binding site size of 11 nucleotides. In addition, it lowers the melting point of polydA:polydT but cannot denature natural DNA. N4 single-stranded DNA binding protein specifically enhances the rate of DNA synthesis by the N4 DNA polymerase. It does so by increasing processivity and melting out hairpin structures in natural DNA.

L 139 NEGATIVE COOPERATIVITY AMONG ssDNA BINDING SITES WITHIN the *E. coli* SINGLE STRAND BINDING (SSB) PROTEIN TETRAMER. Wlodzimierz Bujalowski and Timothy M. Lohman, Department of Biochemistry & Biophysics , Texas A&M University, College Station, TX77843

We have examined the salt and temperature dependences of the equilibrium binding of the *E. coli* SSB tetramer to a series of oligodeoxythymidylates, $dT(pT)_{N-1}$, with N=16, 28, 35, 56 and 70. Using a thermodynamically rigorous method (Lohman and Bujalowski, (1988), Biochemistry, 27, 2260), we determined stoichiometries of 4,2,2,1 and 1 per protein tetramer for N=16,28,35,56 and 70, respectively. Evidence will also be presented for the existence of a true negative cooperativity among the multiple ssDNA binding sites on the SSB tetramer. We have quantitatively analyzed the binding isotherms using a statistical thermodynamic ("square") model of the tetrameric protein to obtain the *intrinsic* binding constant, K_N, and the negative cooperativity constant, σ_N. For all oligonucleotides, we find that K_N decreases significantly with increasing monovalent salt concentration, indicating a large electrostatic component to the free energy of the interaction. There is a strong temperature dependence for the intrinsic binding of $dT(pT)_{15}$, such that $\Delta H° = -26 \pm 3$ kcal/mole $dT(pT)_{15}$. Negative cooperativity exists under all solution conditions tested and is independent of anion concentration and type; however, σ_{16} *decreases* with decreasing cation concentration. The negative cooperativity stabilizes the formation of the $(SSB)_{35}$ polynucleotide binding mode in which ssDNA interacts with only 2 subunits within each tetramer. This negative cooperativity may be important in regulating the switch between the high site size binding mode, possessing low, "limited " cooperativity , that may be used for recombination processes and the low site size binding mode, that may transiently form high "unlimited" cooperativity complexes.

L 140 THE DNA POLYMERASE I OF STREPTOCOCCUS PNEUMONIAE: FUNCTIONAL AND EVOLUTIVE FEATURES. **Paloma López, Asunción Díaz, M. Elena Pons, Susana Martínez and Sanford A. Lacks.** Centro de Investigaciones Biológicas, CSIC, Velázquez,144, 28006-Madrid, Spain and Brookhaven National Laboratory, Upton, NY 11973, USA. The polA gene of Streptococcus pneumoniae was cloned and its nucleotide sequence, determined. The gene encodes for the pneumococcal DNA polymerase I (99.5 kDa). Determination of the amino terminal amino acid sequence showed that the start site of the enzyme correspond to the polypeptide predicted from the nucleotide sequence. The pneumococcal gene is also expressed in Bacillus subtilis and in Escherichia coli and its mRNA start- and termination- sites were the same in the three hosts. Although the polA mRNA lacks a Shine-Dalgarno sequence, it can give rise up to 500 molecules of the protein per pneumococcal cell. S.pneumoniae Pol I (as the E.coli enzyme) has 5'and 3'exonuclease activities as well as polymerase activity, although the pneumococcal protein is 51 residues shorter than the E.coli Pol I. Comparison of the pneumococcal Pol I with other proteins encoded by bacteria or their viruses reveals blocks of homology, specially in the polymerase domains. These homologies indicate a common evolutionary origin for at least regions of the proteins.

L 141 A NOVEL A/G MISMATCH REPAIR IS CONTROLLED BY micA GENE FUNCTION IN Escherichia coli. **A-Lien Lu, Jyy-Jih Tsai, and Dau-Yin Chang, Department of Biological Chemistry, University of Maryland, Baltimore, MD 21201.**

A new mutator locus, micA, has been shown to be involved in the dam- and mutHLS-independent A/G mismatch repair pathway through an in vitro assay. This type of repair is specific for A/G mismatches, is unidirectional with dA to dC conversion and involves a short repair tract. The activity of the micA gene product can be monitored by complementation of micA mutant extracts to restore mismatch correction in vitro. An A/G mismatch binding protein and a tightly associated A/G endonuclease have been identified in E. coli cell extracts. The A/G endonuclease simultaneously makes incisions at the first phosphodiester bond 3' to and the second phosphodiester bond 5' to the dA of the A/G mismatch and has no incision site on the other DNA strand. Extracts prepared from micA mutants contain no A/G endonuclease activity, and can not form the A/G mismatch-specific binding complex. The A/G endonuclease and binding protein coelute with the gene product of micA during the phosphocellulose column step. Partially purified A/G endonuclease and binding protein restore repair activity in micA mutant extracts. When A/G binding protein is separated from the A/G endonuclease through an affinity column, it is unable to complement the mismatch correction of micA mutant extract. These results suggest that the micA gene encodes for A/G endonuclease.

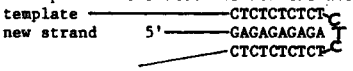
L 142 SEQUENCE HETEROGENEITY OF THE KINETOPLAST DNA MINICIRCLES OF LEISHMANIA DONOVANI, AN INDIAN STRAIN OF VISCERAL LEISHMANIASIS

Hemanta K. Majumder and Srimita Das Gupta, Indian Institute of Chemical Biology, Calcutta 700032, India.

The frequency distribution of various minicircle sequence classes of the kinetoplast DNA of Leishmania donovani UR6 was determined. Restriction enzyme analysis accounted for 70% of the network, comprising at least five minicircle sequence classes. A minicircle having a low copy number was cloned and characterized in detail. This minor sequence minicircle was found to share 230-290bp homology with major sequence class minicircles; this homologous region was found to be responsible for the anomalous gel migration of both classes of minicircles.

L 143 (dG-dA)n AND (dT-dC)n TRACTS ARREST DNA REPLICATION IN VITRO, Haim Manor, Aviva Lapidot and Nava Baran, Dept. of Biology, Technion, Haifa 32000, Israel.

Previous in vivo studies have indicated that (dG-dA)n.(dT-dC)n tracts (designated (GA)n.(TC)n), which are widely dispersed in mammalian genomes, may serve as pause or stop signals for DNA replication and amplification (Baran et al., Mol. cell. Biol. 7, 2636 (1987); Sridhara Rao et al., Nucleic Acids Res. 16, 8077 (1988)). To examine their ability to stop replication in vitro, (GA)n.(TC)n tracts were cloned into phage M13 vectors. Single stranded DNA molecules including either (TC)n, or (GA)n sequences, were prepared from the phages and replicated by extension of M13 primers, using the Klenow polymerase, or sequenase, or calf thymus DNA polymeraseα. The products were resolved on sequencing gels. In assays performed at relatively high polymerase/ template ratios, the replication was arrested around the middle of (TC)n and (GA)n tracts of n$\geq$16. At relatively low polymerase/ template ratios, periodical arrests were observed along these sequences. It is suggested that arrest of replication at (TC)n tracts was due to formation of triple helices (see Voloshin et al., Nature 333, 475 (1988), or Hanvey et al. PNAS 85, 6292 (1988), and the figure below), which cause dissociation of the enzyme. This suggestion is compatible with the observed positions of the arrest sites. It is also supported by our finding that in replication assays performed with 7 deaza dGTP, the polymerase was not arrested at the (TC)n tracts. Experiments are being currently performed to further test this hypothesis, and the possibility that formation of (GA)n.(TC)n.(GA)n triple helices accounts for the arrests at the (GA)n tracts.



L 144 HUMAN GENOMIC ORGANIZATION AND TELOMERIC LOCALIZATION OF A LOW COPY NUMBER SEQUENCE ASSOCIATED WITH MONKEY CENTROMERIC SATELLITE DNA. Antonella Maresca[1], Nicole Lemieux[2], Rosa Ferraiuolo[1] and Claude-Lise Richer[2]. [1]Laval University Cancer Research Center, L'Hôtel-Dieu de Québec, Québec, Canada, G1R 2J6, and [2]Dép. d'Anatomie, Faculté de Médecine, Université de Montréal, Montréal, Canada, H3C 3J7. The only known DNA that is specifically associated with centromeric regions is satellite DNA, which is composed of short tandem, highly reiterated repeats. A recombinant phage, λ MkA, previously isolated from the African green monkey genome, contains a 4.2 kb segment of a monkey centromeric satellite DNA, deca-satellite, bound on each side by two low copy number sequences, defined as LC-A left and LC-A right. Here, we present the genomic organization of the human homologous LC-A left sequences. Genomic southern analysis with the LC-A left monkey probe (1.4 kb) reveals the existence of only one major arrangement of sequences hybridizing to LC-A left under stringent conditions of hybridization. In moderate conditions of hybridization an additional pattern of faint bands is observed. The LC-A left probe hybridizes in situ to human prometaphase chromosomes, mainly to one of the telomeric regions of chromosomes 1 and 5. On other chromosomes a specific grain accumulation is observed around the telomeric regions, especially chromosomes 8, 16, 17 and 21. Several grains are also observed at the proximal region of the long arm of chromosome 11. Interestingly, independent cytological observations (T-banding) suggest that this location could be an inverted telomeric band. These data and other reports in the literature suggest a possible relationship between centromeric satellite DNAs and telomeres.

L 145 DNA-PROTEIN INTERACTION IN HUMAN CENTROMERE, Hiroshi Masumoto, Hisao Masukata, Yoshinao Muro*, Kinya Yoda, Tuneko Okazaki, Department of Molecular Biology, School of Science, and *Department of Dermatology, School of Medicine, Nagoya University, Nagoya, Japan 464-01

We have been studying DNA and protein elements involved in the structure and function of the centromere-kinetochore domain of human chromosomes. From a DNA library constructed from the centromere-enriched HeLa chromosomal segments, we have isolated a clone which specifically hybridized with centromere region of nine human chromosomes. A stretch of an alphoid dimer (340 bp) is responsible for the hybridization. The alphoid DNA sites in the chromosomes perfectly overlapped with the centromere antigen sites detected by anti-centromere serum obtained from a schleroderma patient throughout the cell cycle. We have developed an in vitro system to examine interaction between the alphoid DNA and centromere antigens. Particular types of alphoid DNA were immunoprecipitated by the anticentromere serum after incubation with a nuclear fraction containing 80 kD and 140 kD antigens. The region of the alphoid dimer required for the immunoprecipitation was localized in a 16 bp segment (h-CEN1 box), which is well conserved in the immunoprecipitable alphoid DNAs. When nuclear proteins bound to the immunoprecipitable alphoid DNA were affinity-purified and analyzed by immunoblotting with the anticentromere serum, 80 kD antigen was detected. These results indicate that 80 kD antigen is involved in the recognition of the specific alphoid DNA, which is probably the earliest stage of kinetochore formation.

L 146 DETECTION OF TEMPERATURE INDUCED PERTURBATIONS OF A-TRACT DNA.

James G. Mc Carthy, Loren D. Williams, and Alexander Rich. Department of Biology, MIT, Cambridge, MA, 02139. The structure of a 5 bp long A-tract present within an 81 bp fragment was studied using the chemical probes potassium permanganate (KMnO4), and diethylpyrocarbonate (DEPC). The reactivity of thymines to KMnO4 is a function of the accessability of the 5-6 double bond to attack from above or below the plane by this probe, and therefore can be used as a measure of the stacking interactions between a thymine, and its neighboring bases. Using this method we have found that at 23°C the thymines of the A-tract appear to be uniformly protected from KMnO4 attack. At low temperature (12°C), these thymines display a more normal reactivity to this probe (reactivity peaks at T3). The adenosines were less reactive to DEPC at 12°C versus 23°C, but the pattern of DEPC reactivity remained similar at both temperatures. This pattern is characterized by the increased reactivity of A4, and the decreased reactivity of A5, relative to the other adenosines in the tract. Similar reactivities have been observed for A-tracts present in other fragments. These results imply that A-tracts present in random sequence DNA have a rather uniform structure at 23°C in 50 mM salt/pH 7.0, with only one end displaying a significant junction. The potential relevance of these results to DNA bending will be discussed. We will also present arguments to suggest that the reactivity of purines to DEPC is not, as commonly accepted, exclusively due to the accessability of the N7 atom to attack, but may also depend on the exact configuration of the neighboring bases relative to this N7 atom.

L 147 COMPARISON BETWEEN DNA STABILITY AND DNA POLYMERASE FIDELITY.

John Petruska, Michael S. Boosalis and Myron F. Goodman, University of Southern California, Los Angeles, CA 90089-1340.

The relation between polymerase activity and stability of base pairs is investigated using primer-template duplexes having either matched (A:T) or mismatched (G:T, C:T, T:T) base pairs at the primer 3' end. Duplex melting studies and enzyme kinetic measurements are made for each kind of terminus. Melting data indicate only small differences in the free energy of dissociation for terminal A:T compared to G:T, C:T and T:T ($\Delta\Delta G^\circ$ = 0.2, 0.3 and 0.4 kcal/mole, respectively, at 37 deg. C). However, kinetic data for DNA polymerase α (*Drosophila*) show that the matched A:T terminus is extended faster than G:T, C:T and T:T by factors of 200, 1400 and 2500, respectively, mainly because of differences in Michaelis dissociation constant. The K_m values indicate $\Delta\Delta G^\circ$ = 2.6 to 3.7 kcal/mole in the polymerase active site, about 10 times that in aqueous medium. How does the active site cleft increase $\Delta\Delta G^\circ = \Delta\Delta H^\circ - T\Delta\Delta S^\circ$? We suggest that it restricts entropy differences between right and wrong base pairs while increasing enthalpy differences by excluding water. If $\Delta\Delta S^\circ$ is reduced to 0, then $\Delta\Delta G^\circ = \Delta\Delta H^\circ$. Since $\Delta\Delta H^\circ$ = 1-2 kcal/mole in aqueous medium, as found by melting studies, a two-fold increase is sufficient to make $\Delta\Delta G^\circ$ = 2-4 kcal/mole as indicated by K_m. Such an increase may occur if water is excluded when bases are bound (Petruska *et al.*, PNAS 83:1559-1562, 1986).

L 148 ALU-FAMILY REPEAT BINDING PROTEINS, Olga I.Podgornaya, Ludmila M. Perelygina, Nicolai V.Tomilin, Institute of Cytology, the Academy of Sciences of the USSR, Leningrad I94064, USSR. Using a gel retardation assay the proteins, which bind selectively to the Alu-repeat and BI-repeat have been identified and partially purified from human and mice cells nuclear extracts from some tissues and cell culture lines. The content of BI binding protein is maximal in mice liver, there is less of it in muscular tissue and we can't find any in heart, brain and spleen tissues. Alu-binding protein (ABP) was found in HeLa and A4 cell lines and much less is in cultured normal human lymphocytes. We have determined, that ABP protects I6-nucleotide sequence, which contains pentanucleotide dimer binding site II of T-antigen SV40. Competition experiments show, that ABP does not interact with poly(dA):poly(dT), with RNA polymerase III promotor and with TGGCA-sequence, but a high affinity binding site for ABP was found within a 660 bp restriction fragment, containing the SV40 promotor and replication origin. So pentanucleotide motif is important for the binding. It can be found in the Alu-repeat several times and the looping in RNA polymerase III promotor site, that is the result of ABP binding, may play a role in the regulation of transposition of Alu and BI-repeats. ABP and BI-binding proteins show the crossreactivity, so they may have the similar binding sites.

L 149 Structure and Function of HIV-1 Reverse Transcriptase Kenneth L. Powell, Denise M. Lowe, Alastair Aitken[+], Graham K. Darby, Brendan A. Larder, Dorothy J.M. Purifoy, Margaret Tisdale and David K. Stammers, Department of Molecular Sciences, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK and [+] National Institute for Medical Research, Mill Hill, London NW7 1AA, UK. The long term aim of our studies is to determine the three dimensional structure of the HIV reverse transcriptase (RT). To this end expression of the enzyme has been obtained in both prokaryotic and eukaryotic expression systems. These studies have supplied us with large amounts of starting material from which we can obtain pure RT by a simple two stage process. Expressed recombinant HIV-1 reverse transcriptase is active as both a homodimer of Mr 66,000 subunits and a heterodimer of Mr 66,000 and 51,000 subunits. The heterodimer is formed by cleavage of a C-terminal fragment from one 66,000 Mr polypeptide. The resistance of one subunit of the homodimer to proteolysis indicates that the subunits are not symmetrically related. The cleavage of the enzyme does not require the presence of the HIV protease and can be mimicked by chymotrypsin. Crystallisation of both forms of the enzyme has been possible and a variety of crystal forms have been observed. Such crystals have not proved to be sufficiently ordered for three dimensional studies. Further work has examined a variety of manipulations of the HIV enzyme designed to improve the structure of the crystals. The progress of this work will be discussed.

L 150 DIVERGENT TRANSCRIPTION UNITS, TRANSLATION, AND PLASMID DNA SUPERCOILING, Gail J. Pruss, Biology Dept, University of South Carolina, Columbia, SC 29208. pBR322 DNA isolated from topo I mutants of *E. coli* and *S. typhimurium* exhibits a distinctive supercoiling distribution characterized by a heterogeneous distribution of linking numbers that contains highly negatively supercoiled topoisomers (Pruss, JMB **185**, 51, 1985). Analysis of the supercoiling of deletion and insertion derivatives of pBR322 showed that transcription of the *tet* gene on pBR322 is responsible for this unusual distribution (Pruss & Drlica, PNAS **83**, 8952-8956, 1986). Liu and Wang (PNAS **84**, 7024, 1987) suggested that divergent transcription of the *bla* and *tet* genes is the basis of the effect, and Wu et al. (Cell **53**, 433, 1988) showed that inverting *tet* relative to *bla* reduced the negative supercoiling of the plasmid. However, the reduction was much less than that caused by inactivating the *tet* promoter. One possible reason for this discrepancy is the anti-tet promoter, which overlaps the *tet* promoter and initiates transcription towards *bla*. By starting with a pBR322 derivative lacking the anti-tet promoter and having *tet* expressed from the *trp* promoter, I have constructed plasmids which test this idea. Eliminating divergent transcription of the *bla* and *tet* genes in these plasmids reduced the amount of highly negatively supercoiled plasmid DNA, but still by much less than achieved by eliminating transcription of *tet*. In contrast, deleting the 5'-end of the *tet* gene (leaving an intact promoter) or inhibiting protein synthesis (for 10 min) has about the same effect on supercoiling as eliminating transcription of *tet*. Thus, synthesis of the *tet* protein appears to be of primary importance in generating high levels of supercoiling in pBR322, perhaps by anchoring the transcription ensemble to the cell membrane and thereby creating the twin-supercoiled domains proposed by Liu and Wang.

L 151 IDENTIFICATION OF A HIGH MOLECULAR WEIGHT TOPOISOMERASE I FROM *XENOPUS LAEVIS* OVARIES, Robert E. Richard and Daniel F. Bogenhagen, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY, 11794. DNA topoisomerase I has been purified from homogenates of mature *Xenopus laevis* ovaries. The initial stages in purification of the native enzyme employed a rapid series of three chromatographic steps, yielding an activity that sedimented in a glycerol gradient with a sedimentation coefficient of 4.3 S. The final steps in purification were performed in the presence of SDS. Polypeptides that might represent topoisomerase I were identified by specific labeling of the topoisomerase species with radioactive DNA. SDS polyacrylamide gel electrophoresis of topoisomerase I radiolabeled with DNA, identified three polypeptides with mobilities consistent with sizes of 165 kDa, 125 kDa, and 88 kDa. The 165 kDa polypeptide was found to be sensitive to camptothecin. All three polypeptides were found to possess topoisomerase activity following elution from the gel and renaturation. Partial proteolytic digestion of the radiolabeled polypeptides with S. aureus V8 endoproteinase resulted in identical autoradiographic patterns. This suggests that the 125 kDa and 88 kDa polypeptides may be degradation products of the 165 kDa species. An antibody raised against the 165 kDa species inhibits topoisomerase I activity.

L 152 BENZO[A]PYRENE-DNA ADDUCTS INHIBIT TRANSLOCATION BY THE GENE 4 PROTEIN OF BACTERIOPHAGE T7, Louis J. Romano and W. Clay Brown, Department of Chemistry, Wayne State University, Detroit, Michgan 48202. Bacteriophage T7 gene 4 protein is an essential component of the T7 DNA replication system. Gene 4 protein acts as a helicase and primase to facilitate simultaneous synthesis on both the leading and lagging strands at the replication fork. The binding of gene 4 protein to single-stranded DNA requires an NTP cofactor, preferably dTTP *in vitro*. After a stable complex is formed, gene 4 protein hydrolyzes the dTTP to dTDP and orthophosphate, presumably with the energy derived form this hydrolysis driving the observed 5'→3' movement of the gene 4 protein along the DNA. Thus, measuring the rate and extent of dTDP formation should provide a means to directly measure translocation. We have determined dTTP hydrolysis by the gene 4 protein is strongly inhibited by the presence of benzo[a]pyrene adducts on the DNA. This suggests that the benzo[a]pyrene adducts pose an impediment to the movement of the gene 4 protein on single-stranded DNA. Time course experiments on adduct-containing DNA show that after an initial burst of hydrolysis, that parallels what is observed on unmodified DNA, further hydrolysis abruptly ceases. Addition of excess unmodified DNA does not restore the hydrolysis activity, suggesting that the gene 4 protein is being sequestered on the DNA at the site of the adduct. This was confirmed by digestion/protection experiments in which gene 4 protein preferentially protected the radiolabeled adduct-containing DNA as opposed to randomly labelled M13 DNA. The gene 4 protein bound to the B[a]P-modified DNA was isolated and the complex found only to contain dTTP. These results, taken together, have been used to formulate a model for gene 4 protein translocation in which the power stroke for unidirectional movement along the single-stranded DNA is the displacement of dTDP by dTTP.

L 153 DNA SEQUENCE OF LOW COPY NUMBER SEQUENCES ASSOCIATED WITH A MONKEY CENTROMERIC SATELLITE. Sylvie Roy[1], Ronald E. Thayer[2] and Antonella Maresca[1], [1]Laval University Cancer Research Center, L'Hôtel-Dieu de Québec, Québec, Canada, G1R 2J6, and [2]Lab. of Biochemistry, N.I.H., Bethesda, MD 20892. The centromeric regions of higher eukaryote chromosomes are characterized by the presence of a large amount of tandemly repeated sequences known as satellite DNAs. The molecular mechanisms that amplify satellite DNAs at the centromere are unknown. We are interested in understanding the structural and functional relationship between centromeric satellite DNAs and low copy number sequences. A previously described monkey DNA segment, cloned in the recombinant phage λMkA, contains the monkey species-specific deca satellite enclosed by two low copy number sequences (LC-A right and LC-A left). In the major genomic organization and in another recombinant phage (λMk8) LC-A right is contiguous to another low copy number sequence (subcloned in p8). LC-A right, LC-A left and p8 contain sequences that are evolutionary conserved. Previous experiments suggested that the chromosomal region detected by probe p8 and LC-A right is susceptible to recombination in the monkey genome. We determined the nucleotide sequences of p8 (2.2 kb), LC-A left (1.4 kb) and relevant regions from LC-A right. None of the nucleotide sequences determined code for proteins. The DNA sequence suggests that the LC sequences are conserved for structural reasons. Many DNA sequence motifs found in these DNA fragments have been implicated in recombination and theoretically can assume alternating DNA conformation.

L 154 MULTIPLE DNA UNWINDING ACTIVITIES OF E. coli HELICASE II (UvrD) PROTEIN, **Greg Runyon and Timothy M. Lohman, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843**

The *E. coli* Helicase II (UvrD) protein has been shown to unwind duplex DNA in a 3' to 5' direction, *in vitro*, (Matson, S. 1986 *JBC* 261, 10169). The directionality assay monitors the preferential displacement of either a 143 base (3' to 5' unwinding) or a 202 base (5' to 3' unwinding) DNA fragment from either end of a linear M13 single stranded (ss) DNA. We show that the 143 base DNA fragment is preferentially displaced at low helicase II concentrations, but that displacement of both fragments can occur at low [$MgCl_2$] and/or high Helicase II concentrations. We have also shown that the ATP-dependent displacement of the 202 base DNA fragment does not require a ss DNA leader, since Helicase II protein can unwind "blunt-ended" duplex DNA. Furthermore, the extent of "blunt-end" unwinding quantitatively follows the extent of displacement of the 202 base DNA fragment from the DNA unwinding substrate. We have furthermore shown that an ATPase activity of helicase II can be activated in the presence of blunt-ended duplex DNA fragments in buffer conditions identical to those used in the unwinding assays, which likely reflects the subsequent interaction of the helicase with ss DNA after unwinding regions of the duplex DNA. The duplex DNA length dependence of this apparent duplex DNA-dependent ATPase activity suggests that initiation of unwinding of "blunt-ended" DNA occurs from the ends of the DNA, although it does not rule out the possibility of some initiation of unwinding from internal regions of the duplex DNA.

L 155 TELOMERIC G-RICH STRANDS FORM PARALLEL, QUADRUPLE-STRANDED AGGREGATES (G4-DNA): IMPLICATIONS FOR CHROMOSOMAL SYNAPSIS, Dipankar Sen and Walter Gilbert, Department of Cellular & Developmental Biology, Harvard University, Cambridge, MA 02138. All nuclear telomeres sequenced so far share a structural theme of distinctly G-rich and C-rich complementary strands with simple, repeating sequences. In the case of ciliates, the 3' portion of the G-rich strand protrudes beyond its complementary strand, and such telomeric oligonucleotides have recently been shown by Henderson et al. to form novel hairpin structures containing G-G base pairs. We, however, have found that G-rich motifs in single-stranded DNA (including the above telomeric sequences) are capable at physiological salt of forming parallel, quadruple-stranded complexes ('G4-DNA'), that are thermally stable. Such quadruple-strands (excluding the G4-forming G-rich motifs themselves) are able to bind their complementary strands to give eight-strand structures, effectively four identical duplexes synapsed together via a recognition of 'self'. We postulate that G4-DNA structures may be important in chromosomal recognition processes in vivo, particularly in the recognition and synapsis of the four chromatids of a homologous chromosome pair at meiosis. Synapsis could begin at the telomeres by G4-DNA formation ('telomeric bouquets') or elsewhere in selected G-rich sequences along the chromosomes, and would proceed to completion by correct 'self' recognition of defined G4-forming sequences along the entire lengths of the homologues.

L 156 SITE-SPECIFIC MUTAGENESIS OF T4 GENE 32: THE ROLE OF TYROSINE RESIDUES IN PROTEIN: NUCLEIC ACID INTERACTIONS, Y. Shamoo, L.R. Ghosaini, J.M. Sturtevant, K.R. Williams and W.H. Konigsberg, Yale University School of Medicine, New Haven, CT 06510.

Bacteriophage T4 gene 32 encodes a single-stranded DNA (ssDNA) binding protein (gp32) required for T4 DNA replication, recombination and repair. Previous physicochemical studies on gp32 and other ssDNA binding proteins have suggested that binding may involve hydrophobic interactions that result from the close approach of several aromatic amino acid sidechains with the nucleic acid bases. In the case of gp32, five tyrosines and two phenylalanines have been implicated in gp32:ssDNA complex formation. Site directed mutagenesis of T4 gene 32 was employed to produce a set of eight gp32 mutants, each of which contained a single substitution at one of the eight tyrosine residues within gp32. The mutant gp32s were then subjected to physicochemical analysis in order to evaluate the role of each tyrosine residue in gp32 structure and function. Oligonucleotide binding studies suggest that tyrosine residues 84, 99, 106, 115 and 186 contribute from 0.31 to 0.66 Kcal/mole to ssDNA binding and corresponds to 2 to 10% of the overall binding energy for gp32:ssDNA complex formation. Replacement of tyrosine residues 73 and 92 appears to lead to large structural changes that may be the result of disrupting the zinc binding subdomain within gp32.

L 157 PARTIAL CHARACTERIZATION OF THE *IN VITRO* CLEAVAGE OF 5-METHYLCYTOSINE MODIFIED DNA BY THE E.COLI K-12. MCRB ACTIVITY. Ellen Sutherland and Elizabeth Raleigh, New England Biolabs, 32 Tozer Rd., Beverly, MA 01915.
DNA containing the modified base 5-methylcytosine is biologically restricted in many laboratory strains of E.coli K-12. This restriction is due to at least two genetically distinct and sequence specific systems, *mcrA* and *mcrB*. We have developed an *in vitro* assay to detect the the restriction activity of McrB. The activity we have characterized reflects the *in vivo* behavior of the McrB system in two ways. First, the activity is found only in extracts from cells carrying an *mcrB+* allele. Second, the activity acts only on substrates that are restriction targets *in vivo*. Our *in vitro* studies suggests that McrB activity makes a double-strand cut in DNA, is inhibited by high salt concentrations and exhibits nucleotide co-factor dependence. Purification of the activity and further characterization of its co-factor requirements are underway.

L 158 GENETIC ANALYSIS OF THE BINDING OF *E. coli* LexA PROTEIN TO DNA, Andrew T. Thliveris, L. Kevin Lewis and David W. Mount. Department of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.

Dominant negative mutants in the *E. coli lexA* gene have been used to define amino acids important for DNA binding. The mutations lie in an amino acid sequence which is similar to that found in repressors which bind to DNA by means of a helix-turn-helix structural motif. This region was strongly mutagenized using highly substituted oligonucleotides. Mutant LexA proteins which bound to symmetrically altered *recA* operators were obtained using a new method of genetic selection. Mutants which have altered DNA binding specificity i.e. bound to mutant but not wildtype operator or which have relaxed specificity i.e. bound to both mutant and wildtype operators were isolated. These results suggest possible interactions between amino acids in LexA protein and *recA* operator DNA.

In a separate line of investigation, we have devised a genetic screen for *E. coli* promoters which are controlled by the SOS regulatory system. New genes in the SOS regulon have thus been identified. These promoters have been sequenced and physically mapped on the *E. coli* genome using the Kohara phage library. We are presently attempting to identify these new genes and to define their function.

L 159 THE STRUCTURE AND PROPERTIES OF THE RIGHT END OF THE RAT L1 ELEMENT AND ITS TARGET SITES. K.Usdin and Anthony V.Furano, Building 8, Room 203, N.I.H., Bethesda, MD, 20892.The L1 element homopurine:homopyrimidine sequence, an evolutionarily conserved feature of this class of mobile, repetitive elements, induces the unpairing of contiguous DNA sequences allowing uptake of oligmers which can then serve as primers for DNA synthesis. We have examined the structure of this region in order to understand the basis of this phenomenon. Our data indicates that at least two different non-B DNA structures are present. One is a simple G·G·C triplex, and the other a C·G·C triplex with a hairpin loop formed by the resultant free fourth strand. The presence of a 24bp homopurine:homopyrimidine sequence contiguous to these structures greatly enhances the amount of the triplexes formed. Three L1 element target sites picked at random, themselves adopt various non-B DNA structures. When the L1 homopurine:homopyrimidine sequence is present on a supercoiled molecule together with its target site, both the L1 and the target site non-B DNA structures exist in dynamic equilibrium. Conditions that favor triplex formation shift the equilibrium towards the L1 non-B DNA structure. Triplex formation by L1 DNA may account for the recombinogenic properties of this element. In addition, insertion of an L1 element into a region whose properties are supercoil dependent, might affect the behavior of the target site by competing for the available free energy.

L 160 AN AUTONOMOUSLY REPLICATING SEQUENCE FROM HELA DNA SHOWS A SIMILAR ORGANIZATION AS THE YEAST ARS1 ELEMENT, Manuel S. Valenzuela, Chakradhari Sharan*, Deena Kegler and Maria del Pilar Aguinaga*, Division of Biomedical Sciences and Department of Genetics*, Meharry Medical College, Nashville, TN 37208.
Dissection of a 3.0 kb HeLa DNA fragment capable of functioning as an autonomously replicating sequence (ARS) in the yeast Saccharomyces cerevisiae, indicates that the ARS activity resides on a 0.45 kb region which also shows a retarded mobility on polyacrylamide gels. Electron microscopy studies have confirmed that the retarded mobility is due to the presence of bent DNA, and that this fragment also contains an A+T rich region. The position of these features has been unambiguously determined by DNA sequencing analysis. The results show an strikingly similar organization to that found in the yeast ARS1 element. Although it is not yet clear the significance of this relationship, nor whether this sequence plays any role in the replication of human DNA, a protein factor present in a HeLa cell nuclear extract appears to bind to this DNA fragment.
This work was supported by NSF grant DCB-8502681 to M.S.V.; C.S. and M.d.P.A. were supported by grants TDHE-Z31512 and DHHHS-MCJ440-21 to D. Singh.

L 161 REPLICATION OF PSORALEN ADDUCTS ON POL I AND POL II TRANSCRIBED MAMMALIAN GENES, Jean-Michel H. Vos, Lineberger Cancer Research Center and Department of Biochemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.
Although the majority of genetic alterations induced by carcinogens are suggesting the essential role played by DNA replication, the mechanism of lesion tolerance in mammalian cells is poorly understood. Because carcinogenic adducts are known to mutate genes and activate protooncogenes through sequence specific events, it is important to understand the relationship between DNA replication and transcription. By improving a recently developed procedure (Vos and Hanawalt, Cell 50:789, 1987), we have been able to demonstrate efficient replication of DNA monoadducts induced by psoralen + UVA in two housekeeping genes of human fibroblasts: the dihydrofolate reductase (DHFR) and the ribosomal RNA (rRNA) genes. Thus, such bulky adducts do not greatly interfere with the replication of genes transcribed by either RNA Polymerase I or RNA Polymerase II. Furthermore, this was observed in both human and Chinese hamster cells and in cells either proficient or deficient in excision repair. It is proposed that the toxic effect of blocks to RNA polymerase progression posed by inefficiently repaired DNA lesions from active transcription units can be relieved through translesion replication of the damaged genes. Possible mechanism(s) will be discussed.

L 162 CRYSTALLOGRAPHIC STRUCTURE OF T7 RNA POLYMERASE AT A RESOLUTION OF 4.0 Å, B. C. Wang[1,2], E. M. Lafer[2], R. Sousa[2], Y. J. Chung[1] and J. Rose[1], Departments of [1]Crystallography and [2]Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

T7 RNA polymerase (RNAP) is a single chain protein with molecular weight of 98,856 Daltons. It is among the smallest of the RNA polymerases that are known. Despite its size, the enzyme transcripts genetic information with a remarkable speed of 200-300 base pairs per second, which is almost 10 times faster than the *E.coli* enzyme. T7 RNAP performs promoter recognition, initiates an RNA chain, extends the chain, and finally terminates the chain and is released from the DNA template. This enzyme is highly specific and a detailed study of its three-dimensional structure should yield significant information on the nature of transcription. T7 RNAP has been crystallized from samples prepared from an *E.coli* strain overexpressing system, and the crystals diffract to at least 3.0 Å. The general shape of the molecule resembles that of a kidney with overall dimensions 75 x 50 x 50 Å. The molecule consists of a small and a large domain. A large groove 18-22 Å wide, 18 Å deep and 40 Å long is formed along one side of the molecule between the two domains,

L 163 DNA POLYMERASES α AND δ ARE IMMUNOLOGICALLY AND STRUCTURALLY DISTINCT

Scott W. Wong[1], Juhani Syvaoja[2], Cheng-Keat Tan[3], Kathleen M. Downey[3], Antero G. So[3], Stuart Linn[2] and Teresa S.-F. Wang[1]
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The relationship between DNA polymerases α and δ are evaluated immunologically by monoclonal antibody specific for DNA polymerase α and murine polyclonal antiserum against calf thymus DNA polymerase δ. DNA polymerases α and δ are found to be immunologically distinct. The structural relationship between the proliferating cell nuclear antigen (PCNA)-dependent calf DNA polymerase δ and DNA polymerase α from human and calf was analyzed by two dimensional tryptic peptide mapping of the catalytic polypeptides. The results demonstrate the catalytic polypeptides of the PCNA-dependent calf polymerase δ and DNA polymerase α are distinct, unrelated and do not share any common structural determinants.

The immunological and structural relationship between a recently identified, PCNA-independent form of DNA polymerase δ from Hela cells was also assessed. This PCNA-independent human polymerase δ was found to be immunologically unrelated to human polymerase α, but to share some immunological and structural determinants with the PCNA-dependent calf thymus polymerase δ. [This research was supported by grants from the National Institute of Health (CA 14835) to T.S.F.W.; (DK26206) to A.G. So and (GM30415) to S. Linn; S.W.W. is a postdoctoral fellow supported by NIH training grant (CA09151)].

L 164 DNA/Intercalator Complexes: Chemical and Structural Heterogeneity

L.D. Williams, G. Ughetto, A.H-J. Wang, G.J. Quigley, A. Rich, and C.A. Frederick, Department of Biology, M.I.T., Cambridge, MA 02139

The daunomycin family of anthracyclines, which acts by inhibiting DNA transcription/replication processes, is clinically useful in treating human carcinomas. We have solved the structures of a series of daunomycin/oligodeoxynucleotide complexes. The complexes differ in modification of the daunomycin, in DNA sequence, and in modification of the DNA. Daunomycin derivatives [daunomycin, 14-hydroxy-daunomycin (adriamycin), 11-deoxy-daunomycin and 4'-deoxy-daunomycin] were co-crystallized with several deoxynucleotide sequences [d(CGATCG), d(CGTACG), d(CGTsACG) and d(^{m}CGAT^{m}CG), where s = thiol ester]. The interactions between the DNA and the planer intercalative moiety of this drug family are strictly conserved. In contrast, the binding mode of the minor groove binding amino-sugar moiety of the drugs is heterogeneous. In addition, we are studying complexes formed when intercalators bind specifically at a DNA lesion created by intercalation (i.e., at a single base bulge). We hope to understand the molecular basis for the frameshift mutagenic properties of intercalatoring agents.

L 165 ALTERNATE STRUCTURES ADOPTED BY THE DR2 REPEATS OF THE SEGMENT INVERSION SITE OF HERPES SIMPLEX VIRUS TYPE 1. Franz Wohlrab and Robert D. Wells, Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, AL 35294.

The genome of herpes simplex virus type 1 (HSV-1) contains a set of tandem repeats (DR2) of the dodecamer GCGAGGAGGGGG capable of adopting a non-B DNA structure under a variety of conditions. These repeats are present at the site of segment inversion as well as at the termini of the viral DNA and represent a recombinationally active region. Plasmids carrying the DR2 sequences can undergo a series of supercoil-driven conformational transitions resulting in different extents of relaxation for molecules with identical linking numbers. The final extent of relaxation is determined by the location of a nucleation site. The transitions depend on the presence of divalent metal ions, negative supercoiling, and pH, as well as the length of the participating sequence. At neutral pH and physiological salt concentrations, the transition to a non-B form occurs at negative superhelical densities of 0.035 or less. The data strongly support the formation of a multi-stranded structure by the DR2 repeats under these conditions. Because of the absence of long single-stranded regions, the structure is different from the triplexes adopted by shorter inserts, and presumably is a type of four-stranded conformation. A protein fraction of uninfected cell nuclei interacts specifically with the DR2 repeats and similar sequences. Supported by NSF and NIH.

L 166 KINETIC PATHWAY OF THE DNA CLEAVAGE/RELIGATION REACTION OF EUKARYOTIC TOPOISOMERASE II, E. Lynn Zechiedrich, Kent Christiansen, Anni H. Andersen, Ole Westergaard, and Neil Osheroff, Dept. of Biochem., Vanderbilt Univ., Nashville, TN 37232 and Dept. of Mol. Biol., Univ. of Aarhus, DK-8000 Aarhus C, Denmark

Central to the catalytic mechanism of topoisomerase II is its ability to cleave and religate duplex DNA. In addition, the DNA cleavage reaction is the physiological target for many clinically relevant antineoplastic drugs. Therefore, the kinetic pathway of the cleavage reaction of Drosophila topoisomerase II was examined. Conditions were defined in which topoisomerase II created predominantly (>90%) single-stranded breaks in duplex DNA. This enzyme-mediated DNA nicking possessed all the characteristics of the DNA double-stranded cleavage reaction. The enzyme was covalently bound to nicked DNA products and nicking was salt reversible. Furthermore, sites of single-stranded DNA breaks were identical to those mapped for double-stranded breaks. In a novel DNA religation assay, nicked DNA was demonstrated to be an obligatory kinetic intermediate in the topoisomerase II-mediated reunion of double-stranded breaks. The first order rate constant for the religation of the first break was approximately 6-fold faster than that for the religation of the second break. Finally, electron microscopy studies of topoisomerase II-DNA cleavage complexes revealed that each end of doubly-cut linear DNA was complexed with a separate polypeptide of the enzyme. Therefore, topoisomerase II cleaves/religates double-stranded DNA by making two coordinated sequential single-stranded breaks in the nucleic acid backbone, each of which is mediated by a separate subunit of the homodimeric enzyme. Supported by NIH grant GM-33944 and contract BI-6-0170-DK with EURATOM, CEC, Brussels.

L 167 INITIATION OF λ DNA REPLICATION WITH PURIFIED HOST- AND BACTERIOPHAGE-ENCODED PROTEINS: THE ROLE OF THE DNAK, DNAJ AND GRPE HEAT SHOCK PROTEINS, Maciej Zylicz*, Debbie Ang[1], Krzysztof Liberek* and Costa Georgopoulos[1], *Division of Biophysics, Department of Molecular Biology, University of Gdansk, 80-822 Kladki, Gdansk, Poland, [1]Department of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City, Utah 84132

Based on previous *in vivo* genetic analysis of bacteriophage λ growth, we have developed two *in vitro* λ DNA replication systems composed entirely of purified proteins. One is termed "grpE-independent" and consists of supercoiled λdv plasmid DNA, the λO and λP proteins, as well as the *E. coli* dnaK, dnaJ, dnaB, dnaG, ssb, DNA gyrase and DNA polymerase III holoenzyme proteins. The second system includes the *E. coli* grpE protein and is termed "grpE-dependent." Both systems are specific for plasmid molecules carrying the *ori*λ DNA initiation site. The major difference in the two systems is that the "grpE-independent" system requires at least a ten-fold higher level of dnaK protein compared to the grpE-dependent one. The λ DNA replication process may be divided into several discernible steps, some of which are defined by the isolation of stable intermediates. The first is the formation of a stable *ori*λ-λO structure. The second is the assembly of a stable *ori*λ-λO-λP-dnaB complex. The addition of dnaJ to this complex also results in an isolatable intermediate. The dnaK, dnaJ, and grpE proteins, with the help of ATP, destabilize the λP-dnaB interaction, thus liberating dnaB's helicase activity, resulting in unwinding of the DNA template. At this stage, a stable DNA replication intermediate can be isolated, provided that the grpE protein has acted and/or is present. Following this, the dnaG primase enzyme recognizes the single-stranded DNA-dnaB complex and synthesizes RNA primers. Subsequently, the RNA primers are extended into DNA by DNA polymerase III holoenzyme. Many protein-protein interactions have been defected between various parts of purified proteins, following cross-linking, affinity chromatography or gel filtration. These protein interactions substantiate the models presented above.

Recombination, Transposition, Enzymes

L 200 SELF-ASSEMBLY OF RecA PROTEIN STUDIED BY ANALYTICAL ULTRACENTRIFUGATION.

Stephen L. Brenner*, Adam Zlotnick*, and Walter F. Stafford+, *E. I. du Pont de Nemours & Co., Inc., Experimental Station, Central Research & Development Dept., P. O. Box 80328, Wilmington, DE 19880-0328; +Boston Biomedical Research Institute, 20 Staniford St., Boston, MA 01810 and Dept. of Neurology, Harvard Medical School, Boston, MA. We have investigated the self-association of RecA protein by equilibrium ultracentrifugation. Monomeric recA (M_r = 37,842) could be observed in reversible equilibrium with dimers and hexamers at high salt in the presence of 1.5M KCl, 5mM HEPES, 1mM EDTA, 2mM ATP, pH 7.0 at 1°C. Polymerization increased with increasing temperature and was reversible with respect to temperature. At lower salt concentrations, only small amounts of monomeric RecA could be detected in either 10mM TRIS-acetate, 10% glycerol, pH 7.5, 4°C or 50mM citrate, 5mM ADP, 5% glycerol, pH 7.0, 4°C. The smallest species observed in significant amounts under these conditions was the dimer (M_r = 75,684). At pH 7.5, higher aggregation states were also observed with molecular weights between 228,000 and 456,000, suggesting a stoichiometry of 6 to 12 monomers per oligomer. At pH 6.0, in the absence of ADP, oligomers containing at least 24 monomers also appeared to be present. The data are consistent with a mixture of monomers, dimers, hexamers, dodecamers, 24-mers and higher oligomers with the distribution of oligomers dependent on solution conditions. The thermodynamic analysis indicates that these oligomeric species are in equilibrium with each other. It is not certain whether dimers assemble directly into hexamers, or whether disassembly into monomers is a prerequisite for the formation of higher oligomers.

L 201 RecA RECOMBINASE RECOGNIZES AND PAIRS REGIONS OF HOMOLOGY SHORTER THAN A HELICAL TURN OF DNA, R. Daniel Camerini-Otero, Carol S. Camerini-Otero and Peggy Hsieh, Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD 20892.

We have devised a novel assay to detect homology-dependent ternary interactions between single strand DNA, double strand DNA and the *E. coli* RecA recombinase protein. In the presence of ATP-γ-S and ADP the ternary complexes formed by coincubating RecA, single strand oligonucleotides and either supercoiled or linear duplex DNA are stable. Their formation can be monitored by using either enzymatic or chemical probes. For example, the complexes protect the duplex DNA from restriction endonuclease cleavage. We do not observe this protection when the duplex DNA is incubated with either oligonucleotides or RecA alone. Surprisingly, although we have not observed complexes with non-homologous oligonucleotides, stable ternary complexes can be efficiently formed with oligonucleotides that are homologous to as little as 8 base pairs of the duplex. When these complexes are probed with restriction endonucleases their footprint extends for only 12 or 13 pase pairs from the 5' or 3' end of the paired oligonucleotide. We are investigating whether this extension of the footprint is merely a reflection of the large size of the probe we used(the restriction endonuclease) or, more interestingly, is due to either a perturbation of the DNA structure in this region or an extension of the protein lattice beyond the edges of the third strand.

Delimiting the structural domains of homologous pairing to such small regions should allow us to extend the use of the full armamentarium of probes that have been used in the study of DNA-protein and DNA-DNA interactions in site-specific contexts to an analysis of these interactions in homologous recombination.

L 202 INTRAPLASMIDIC RECOMBINATION BY COPY-CHOICE IN VITRO: THE ROLE OF RecA PROTEIN AND HELICASE II. Danielle Canceill and Era Cassuto, Departement de Génétique Microbienne, Institut des Biotechnologies, CRJJ, 78350 Jouy-en-Josas, FRANCE.

Genetic data from E.coli indicate that intraplasmidic recombination between duplicated sequences, can proceed through a copy-choice mechanism instead of breakage-reunion.

To study this slippage mechanism in detail, we have designed an in vitro replication system with purified DNA Polymerase III and a primed single-stranded circular template. RecA protein and Helicase II (UvrD) were then added to this reaction. The use of templates that differ by the length of the duplicated sequences and by the presence of inverted repeats allowed us, after analysis of the products, to gain insight into the mechanism and the role of the enzymes involved in the recombinational process.

L 203 RecA - DEPENDENT TRANSLESION SYNTHESIS IN E. COLI. Era Cassuto, Departement de Génétique Microbienne, Institut des Biotechnologies, CRJJ, 78350 Jouy- en- Josas, FRANCE.

Bulky base adducts inhibit DNA replication in vitro as well as in vivo. The gaps created by temporary arrest and reinitiation of DNA synthesis are thought to be filled in by transfer of a complementary strand from an undamaged duplex mediated by RecA protein.

We have used duplex circular gapped DNA containing at least one pyrimidine dimer in the single- stranded region as a substrate for DNA Polymerase III. As expected, DNA synthesis stopped when the growing end reached a dimer. However, when RecA protein, other E. Coli proteins and a second undamaged homologous substrate were added to the reaction, DNA synthesis could proceed past the dimer, suggesting that the DNA complementary to the damaged region had been synthesized on the undamaged template by a RecA- dependent copy- choice mechanism.

Molecular Mechanisms in DNA Replication and Recombination

L 204 NOVEL MUTANTS OF RecA PROTEIN THAT OVERCOME THE ACTION OF PsiB, A PROTEIN THAT INHIBITS RecA PROTEIN ACTIVATION, Adriana Bailone, Suzanne Sommer and Raymond Devoret, GEMC, Enzymology, CNRS, F-91198 Gif-sur-Yvette, France.

PsiB protein is encoded by about half of the naturally-occurring conjugative plasmids. Gene *psiB* is located in the leading region, that is, in the portion of DNA injected first upon conjugation. PsiB protein has been demonstrated to prevent activation of RecA protein *in vivo*. The protein inhibits prophage λ induction as well as induction of genes under the control of LexA protein. We have recently proved that PsiB protein inhibits cleavage of LexA protein *in vivo*.

We have looked for mutants of RecA protein overcoming the inhibition of PsiB protein. The mutants obtained fall into different categories that will be described in this presentation. Some of the mutants display novel phenotypes that show the dissociation of various activities of RecA protein.

L 205 RECOMBINATION OF KNOTTED SUBSTRATES BY TN3-RESOLVASE Peter Dröge and Nicholas R. Cozzarelli, Department of Molecular Biology, University of California, Berkeley, California 94720.

TN3 resolvase, like other site-specific recombination enzymes, shows a marked specificity for the orientation of its recombination sites in the primary sequence. Efficient strand exchange in vivo and in vitro requires that the two recombination sites (res) must be directly repeated (head-to-tail) in a negatively supercoiled DNA substrate, whereas DNA molecules with inverted (head-to-head) res sites are poor substrates. We examined the influence of the global structure of the substrate on site-orientation specificity by resolvase and found that i) knotted negatively supercoiled DNA molecules with two res sites in inverted orientation are excellent substrates for resolvase; ii) the analysis of the topology of the recombination products shows that the sign of nodes in the synaptosome and the one introduced by exchange are reversed from that of the standard substrate; iii) knotted DNA molecules lacking negative supercoiling can be recombined by resolvase under permissive conditions but with reduced efficiency. However, the sign of the node introduced remains unchanged in the absence of negative supercoiling. The implications of these findings are discussed.

L 206 In vitro STUDIES ON THE E. coli RecBCD PROTEIN AND THE RecD SUBUNIT, Peter T Emmerson, Subhendu Chaudhuri, Paul E Boehmer and Ian D Hickson[1], Department of Biochemistry and Genetics, and Department of Clinical Oncology[1], The Medical School, The University, Newcastle upon Tyne, NE2 4HH, UK

We have purified the RecBCD enzyme to homogeneity from a strain harbouring the runaway copy number plasmid pPF307 (Finch et al., NAR 14 8583-8594, 1986) by a three-column procedure and begun to study its action on substrates made from synthetic oligonucleotides of defined sequence and secondary structure. The M_r of the holoenzyme was determined to be 345,000 by gel-permiation chromatography and 320,000 by gel electrophoresis. This indicates a 1:1:1 association of the subunits which would give an M_r of 330,000 based on DNA sequence. We have also determined the N-terminal sequences of the three subunits and these confirm the proposed start positions on which these M_rs were based.

We have fused the recD gene to the tac promoter in the expression vector pKK223-3. Upon induction, strains harbouring this plasmid produce RecD protein amounting to about 10 per cent of total protein. The RecD protein accumulates as insoluble inclusion bodies in these cells. The protein aggregate was solubilised in 6 M guanidinium chloride and purified to homogeneity by differential centrifugation and FPLC (Superose 6). RecD protein was bound to Q-sepharose and then renatured by exposure to decreasing concentrations of denaturing agent.

We have used site directed mutagenesis to convert Lys_{177} to Gln at the proposed ATP binding site of the recD gene and are studying the ability of plasmids carrying this mutated gene to complement the deficiency in ATP-dependent DNase activity of recD mutants.

L 207 **NUCLEOTIDE SEQUENCE, DISTRIBUTION, AND TRANSPOSITION ACTIVITY OF A NEW INSERTION SEQUENCE FROM SHIGELLA DYSENTERIAE.** Olivier Fayet, Marie-Francoise Prère, and Michael Chandler, C.R.B.G.C.-C.N.R.S., 118 route de Narbonne, 31062 Toulouse, France.

Three independant insertions into the cI gene of phage Lambda were isolated from a lysogenic derivative of a Shigella dysenteriae strain. Two of these are almost identical to the recently described IS600. The third one is a 1250 bp insertion sequence generating a 3 bp duplication in the target DNA and belonging to the IS3-IS600 family as shown by its degree of homology (around 30%-40%) at the DNA level. Sequences hybridizing to this new IS are present in the chromosome of strains of the four Shigella species (4 to 30 copies) and of E. coli K12 (2 copies) but are absent in E. coli W. Its transpositional activity was found to be rather high (around 10^{-5} in a mating out assay with an F plasmid derivative), the final products being both direct transpositions and cointegrates.

L 208 ANALYSIS OF BASE PAIRS IN THE TRANSPOSASE BINDING SITE OF THE INVERTED REPEATS OF Tn3. Dwight Nissley, Frank Lindh and Michael Fennewald, Department of Microbiology and Immunology, University of Health Sciences/The Chicago Medical School, 3333 Green Bay Road, North Chicago, Illinois 60064

The transposon Tn3 encodes a transposase which binds to terminal 38 base pair inverted repeats (IR's). Using a nitrocellulose filter binding assay and in vivo transposition assay we have measured the effects of base pair changes on binding and transposition. The inside 21 base pairs of the IR are sufficient for binding at transposase. We have identified two smaller regions necessary for binding and transposition. Changing base pairs 4-8 or 16, 17, 19 and 21 from the inside end of the IR greatly diminishes binding and abolishes transposition. We are currently investigating the effects of single base pair changes within these two regions.

L 209 A GRADIENT OF RECOMBINATION IN THE HYPERELEVATED AND COMPARTIMENTALIZED RECOMBINATION ASSOCIATED WITH THE INTEGRATION/TRANSFORMATION PATHWAY OF POLYOMAVIRUS. Michele M. Fluck and David Hacker. Microbiology, Michigan State University. East Lansing, Mi.48824-1101.

Hyperelevated levels of interviral recombination appear to be associated with the integration of the polyoma virusgenome into the host genome during normal viral infections of rat cells (Hacker and Fluck, Mol. Cell. Biol. in press). The high rates of interviral recombination appear to affect only those genomes which end up integrated in the genome of transformed cells, since within the same population of infected cells from which the transformants are isolated, the unintegrated replicated viral genomes do not undergo detectable recombination. Among the integrated viral genomes, recombination occurs at a rate of 38% in an interval of 350 bp.. In the all recombinant transformants, recombination has occurred in multiple intervals and most recombinant transformants contain evidence for 2 or 3 recombination events, which define at least 2 alternative recombination events in the same overall interval. When the number of recombination events are compared in 3 adjacent intervals spanning from the nucleosome-free region on the late side of the origin (from nucleotide 4634), through the origin, to a location dowstream from the early acceptor splice sites (through nucleotide 1018), a 6-fold gradient is found with its minimum in the nucleosome-free region and its maximum in between nucleotides 659 and 1018.

Molecular Mechanisms in DNA Replication and Recombination

L 210 PURIFICATION AND CHARACTERIZATION OF RECF PROTEIN, Thomas J. Griffin IV and Richard D. Kolodner, Department of Cellular and Molecular Biology, Dana-Farber Cancer Institute, Boston, MA 02115

The *recF* gene of *Escherichia coli* encodes a 40kD protein that is involved in DNA recombination and post-replication DNA repair. *recF* has also been shown to affect the expression of a number of genes under SOS control. These genes include *recA, recN,* and *uvrA*. To further characterize the role of the *recF* gene product in these processes, the *recF* gene was cloned downstream of a *tac* promoter to facilatate overproduction of the *recF* gene product. The RecF protein was overproduced and purified to near homogeneity. N-terminal protein sequence analysis demonstrated that the purified protein had the sequence that was predicted from the DNA sequence of the *recF* gene except that the N-terminal met was not present. The RecF protein was found to bind to single-stranded oligonucleotides using both filter binding assays and gel retardation assays. The binding occurred at temperatures ranging from 23° C to 58° C and maximal binding required 2-5 minutes of incubation. The binding reaction had a pH optimum of 7.0, did not require divalent cations and was inhibited by NaCl concentrations of greater than 250 mM. The relationship between this DNA binding reaction and *recF*-dependent metabolic processes is presently under investigation.

Supported by NIH grant GM26017 and American Cancer Faculty Research Award FRA-271.

L 211 SITE-SPECIFIC INSERTION OF ANTIBIOTIC RESISTANCE GENES

Ruth M. Hall and Cassandra Vockler, CSIRO Division of Biotechnology, PO Box 184 North Ryde, NSW 2113, Australia

A wide variety of antibiotic resistance genes are found located at a specific site within a common DNA segment. This region is found associated with different resistance genes in several unrelated plasmids (eg R46, R388) and transposons (eg Tn*21*, Tn*2620*, Tn*1696*). The common segment 5'- to the resistance genes encodes an integrase which catalyses site-specific co-integration of plasmids (1). We have sequenced this region, and found three open reading frames (2). One of these encodes a protein with 45% identity to an ORF in Tn*7*, and 22% identity to a 215aa segment of the P22 int protein. In Tn*21* the *int* gene is adjacent to the *res* gene and in R46 adjacent to a protein with significant homology to known resolvase proteins. The R46 protein differs at two positions from the the amino acids conserved in all known resolvases, and may not be functional.

1.Martinez and de la Cruz, Molec. Gen. Genet. 211, 321 (1988).
2.Hall and Vockler, Nucleic Acids Res. 15, 7491 (1987)

L 212 HEAD TO HEAD DIMER MODEL FOR THE FUNCTION OF RECA PROTEIN. Toshihiro Horii Tomoko Ogawa and Hideyuki Ogawa. Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan.

The structural information of RecA protein is much less than enough to discuss its functions. Some efforts were paid to address the functional domains previously by identifying the mutational changes of amino acid on mutant RecA proteins. However, it was not conclusive because of the possibility of allosteric effects. Although the structural analysis is difficult, the understanding of RecA protein structure is very critical for the elucidation of strand transfer mechanism. For this purpose, we are trying to identify the structural and functional domains by several lines of experiments including analyses of internal suppressors, biochemical analysis of truncated recA gene products and also site directed mutagenesis. Currently we postulate four domains in the RecA protein for ATPase, DNA binding and two interaction sites with other RecA molecules. Based on the structural information we obtained, we constructed a model of strand transfer mechanism by RecA protein. The model was designed to be compatible with almost all of the biochemical characteristis so far known and the electron microscopic observations, including an explanation for the mechanism of polar strand transfer.

Molecular Mechanisms in DNA Replication and Recombination

L 213 MINIMUM HOMOLOGY REQUIRED FOR STABLE JOINT MOLECULE FORMATION BY RECOMBINASES, Peggy Hsieh, Carol S. Camerini-Otero and R. Daniel Camerini-Otero, Genetics and Biochemistry Branch, NIDDK, NIH, Bethesda, MD 20892

We have previously described the partial purification and characterization of DNA strand exchange activities, recombinases, from HeLa cells and from *Drosophila melanogaster* embryos. We have determined the minimum length of homology required for the formation of stable joint molecules by these recombinases and by purified *E. coli* recA protein. As few as 13 bp of homology is recognized by the human and *Drosophila* recombinases in forming stable, deproteinized joint molecules between a linear duplex and a homologous circular single-strand DNA. RecA protein formed stable joint molecules with as few as 38 bp of homology. Electron microsocopy of joint molecules confirmed that the linear duplex was joined at one end to the single-strand DNA. In a novel assay, human recombinase formed stable, deproteinized joint molecules between a linear duplex and a homologous single-strand oligonucleotide sharing 17 bases of homology.

The unusual stability of joint molecules having very short paired regions prompted us to examine their relative thermal stabilities compared to nonenzymatically formed structures that undergo branch migration. We conclude that these joint molecules are stable because the third strand is not available to participate in branch migration. We also demonstrate that these joint molecules consist of three intact strands that remain closely associated in the absence of any detectable recombinase protein. Our results suggest that recombinases can form structures that, when deproteinized, are triple helices; further experiments are required to determine whether a triple helix is the structure that mediates DNA homology recognition during strand exchange.

L 214 THE Flp RECOMBINASE: STEP-ARREST MUTANTS AND INTERMEDIATES IN RECOMBINATION, Makkuni Jayaram, Ronald Parsons, and Barbara Evans. Research Institute of Scripps Clinic, La Jolla CA 92037.

The *Flp* recombinase of yeast, like other members of the *Int* family, contain the invariant family triad -- His, Arg, and Tyr at positions 305, 308 and 343, respectively. *Flp* mutants altered at these positions have shed light on the possible roles of these amino acids in substrate recognition and catalysis. Mutations of Arg308 result in loss of tight substrate binding by *Flp*. Arg308 could potentially play a direct role in catalysis as well. Mutations of Tyr343 have little effect on substrate binding; however, such mutants fail to execute the strand cleavage step. The formation of a transient DNA-protein link through a phosphotyrosine by *Flp* and other well-studied members of the *Int* family, together with the invariance of a single tyrosine within the family, suggests that Tyr343 is the active residue in strand cleavage and protein attachment to DNA. We have devised methods to obtain sufficient amounts of the phosphoprotein to verify this inference. For the lambda *Int* protein, covalent attachment to DNA has been unequivocally mapped to the invariant Tyr. His305 mutants of *Flp* bind DNA and catalyze strand cleavage, but they cannot bring about strand exchange and reunion. These step-arrest mutants allow a genetic dissection of the biochemical mechanism of recombination. There is good evidence to suggest that *Flp* recombination proceeds through two steps of single strand exchange with a Holliday junction as an intermediate. Reactions with substrates in which one of the two strands is eliminated from exchange indicate that strand exchange during recombination may be asymmetric.

L 215 CLEAVAGE OF CRUCIFORM STRUCTURES BY HUMAN PLACENTAL ENDONUCLEASE. R.Jeyaseelan and G.Shanmugam. Cancer Biology Division, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India.

We have partially purified an endonuclease from human placenta which cleaves the cruciform structures. This is the first report of an enzyme activity involved in resolution of recombination intermediates in higher eukaryotes. The placental enzyme is active both on extruded cruciform structures from negatively supercoiled covalently closed circular plasmid DNA and on synthetic X-junctions formed by reannealing short oligonucleotides. The plasmid pBR322 which has a small natural palindrome gave rise to more relaxed circular and less linear forms upon action by the endonuclease and the plasmid pHD101-3 which has a bigger cloned palindrome than the pBR322 palindrome, gave rise to more linear and less relaxed circular forms. The synthetic X-junction tetramer was converted into linear dimer form by the enzyme. The enzyme has a molecular weight of 34,000 D under native and denaturing conditions.

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L 216 **A Highly Inducible Host-Vector System for Expression of Foreign Genes in Chinese Hamster Ovary Cells.** R.J. Kaufman and D.I. Israel. Genetics Institute, 87 Cambridge Park Dr. Cambridge MA 02140.

A tightly regulatable expression system for heterologous genes has been developed. Chinese hamster ovary cell lines that stably express high levels of the rat glucocorticoid receptor protein (GR) were obtained by transfection with a GR cDNA expression vector. In transient DNA transfection assays, expression from a vector containing the adenovirus major late promoter and multiple copies of the glucocorticoid responsive element from the mouse mammary tumor virus (MMTV) enhancer is not detectable in the absence of hormone. Dexamethasone (Dex) induces a 1000-fold increase in the transient expression from this vector in cell lines that express GR but not in the parental CHO cells. By addition of multiple glucocorticoid responsive elements, the induced expression is 10-fold greater than that obtained from an efficient expression vector utilizing the adenovirus major late promoter and the SV40 enhancer. Inducible expression is maintained when these vectors are stably integrated in the CHO genome. This host-vector system should be of great utility to identify the role(s) of specific gene products in replication and recombination within intact cells.

L 217 Purification and characterization of an recombinase from calf thymus. Ichiro Kawasaki, Shoji Sugano, and Hideo Ikeda. Department of Molecular Biology, The Institute of Medical Science, The University of Tokyo, P. O. Takanawa, Tokyo 108, Japan. An activity that catalyzes the strand transfer from linear double-stranded tet^r DNA to circular M13mp8-tet^r viral DNA was detected in crude extract from calf thymus. This activity was purified by batchwise treatment with hydroxyapatite, phosphocellulose column, single-stranded DNA column, hydroxyapatite column, and glycerol gradient centrifugation. The purified enzyme fraction exhibited a single band in SDS polyacrylamide gel, the apparent molecular mass of which is 31 kDa. The reaction required homologous substrates, but neither Mg^{++} nor ATP. The reaction also required stoichiometric amounts of protein. The purified protein lacked detectable exo- and endonuclease activities, which might mimick the strand transfer reaction.

L 218 RecA PROTEIN BINDING TO Z DNA, Jong-Il Kim and Michael M. Cox, Department of Biochemistry, University of Wisconsin, Madison, WI 53706

RecA protein binding to duplex DNA is enhanced when a B form DNA substrate is replaced with a left-handed Z form helix. This represents a kinetic rather than an equilibrium effect. Binding to Z DNA is much faster than binding to B DNA. In other respects, binding to the two DNA forms is quite similar. RecA protein binds to B or Z DNA with a stoichiometry of 1 monomer per 4 base pairs. The final protein filament exhibits a right-handed helical structure when either B or Z form DNAs are bound. There are only two evident differences: the k_{cat} for ATP hydrolysis is reduced 3-4 fold when Z DNA is bound, and recA binding at equilibrium is less stable on Z DNA than on B DNA. At steady state, the binding favors B DNA in competition experiments. The results indicate that Z DNA binding by recA protein follows the same pathway as for recA binding to B DNA, but that the nucleation step is faster on the Z form helix.

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L 220 BIOCHEMICAL AND GENETIC ANALYSIS OF THE RECBCD ENZYME OF ESCHERICHIA COLI EMPLOYING TEMPERATURE SENSITIVE MUTATIONS IN THE RECB AND RECC SUBUNITS, Sidney R. Kushner, Miroslawa Wlodarczyk and Kristine M. Palas, Department of Genetics, University of Georgia, Athens, GA 30605

The RecBCD enzyme (exonuclease V) of E. coli is a multi-subunit enzyme that is required for both genetic recombination and DNA repair. While recB and/or recC mutants are recombination deficient, sensitive to DNA damaging agents and have reduced viability compared to a wild type strain, recD single mutants are recombination proficient and resistant to ultraviolet light. The catalytic activities of the enzyme include DNA-dependent exonucleolytic activity on single- and double-stranded DNA, ATP-stimulated endonucleolytic activity on single-stranded DNA, DNA-dependent ATPase activity and ATP-dependent DNA helicase activity. Using a series of temperature sensitive recB and recC alleles, we have been able to dissociate the recombination, repair and SOS induction activities of the RecBCD enzyme. By comparing the in vitro activities of the purified mutant enzymes with in vivo phenotypic characteristics, we have developed the following hypothesis. The ATP-dependent exonucleolytic activity is required for the repair of DNA damage induced by MMS but not UV light or mitomycin C. It is not essential for homologous recombination. In contrast, the ATP-stimulated endonucleolytic activity is absolutely required for both homologous recombination and DNA repair. Neither activity is required, however, for SOS induction following treatment of cells with nalidixic acid. We hypothesis that the DNA helicase activity of the RecBCD enzyme is important for SOS induction. (This work was supported by NIH grant GM27997 to S.R.K.)

L 221 INHIBITION OF recA PROTEIN-PROMOTED ATP HYDROLYSIS, Jongwon Lee and Michael M. Cox, Department of Biochemistry, University of Wisconsin, Madison, WI 53706

RecA protein is a DNA-dependent ATPase. High concentrations (~2 M) of salt can also stimulate ATP hydrolysis in the absence of DNA. ADP and ATPγs inhibit recA-mediated ATP hydrolysis. The kinetics of inhibition are complex. ATPγs acts as a purely competitive inhibitor at high ATP concentration under high salt conditions. However, ATPγs can increase the rate of ATP hydrolysis by recA protein when both ATP and ATPγs concentrations are relatively low. ATPγs and ADP act as antagonists in the inhibition of high salt-activated ATP hydrolysis. These results indicate that recA protein is an allosteric enzyme. ADP also inhibits recA protein-promoted ATP hydrolysis in the presence of high salt or poly dT. The extent of ATP hydrolysis promoted by recA protein in high salt is 100%, whereas it is only about 60% when poly dT or nicked duplex DNA are present in low salt. Cessation of ATP hydrolysis at 60% for nicked duplex DNA-dependent ATP hydrolysis is due to dissociation of recA protein from the DNA. Small amounts of ATPγs increase the extent of ATP hydrolysis to 100% for the DNA-dependent reaction. This is due to stabilization of recA filament on the nicked duplex DNA. These results show that ADP has dual effects on the DNA-dependent ATP hydrolysis by recA protein: inhibition by competitive binding of ADP to recA protein and a second effect that tends to dissociation of recA protein from DNA. Finally, inhibition of recA protein-promoted ATP hydrolysis by ADP for poly dT-dependent ATP hydrolysis shows hysteresis. Burst or lag type kinetics occur depending on the order of addition of components, i.e., recA protein, poly dT, ATP and ADP.

L 222 EPISOMAL ELEMENTS IN TRANSGENIC MICE, Pierre Léopold, J. Vailly, A. Blangy, F. Cuzin and M. Rassoulzadegan, Unité 273 de l'INSERM, Centre de Biochimie, Université de Nice, 06034 Nice, France

We reported previously the establishment of a series of transgenic mouse strains which stably maintained autonomous circular DNA molecules in low copy numbers. Their presence in the germ line and high frequency of transmission indicated that they segregate efficiently at meiosis. These effects were the result of the coinjection of plasmid pPyLT1, encoding the large T protein of polyoma virus, and of a minute amount of another plasmid, p12B1, which carries a 345 BP mouse DNA sequence with a binding site for large T. Five of these twelve transgenic strains maintain unrearranged p12B1 DNA molecules, while the other plasmids appear as 'patchwork' structures with a juxtaposition of parts from p12B1 and from pPyLT1, and of mouse sequences of unkown origin. The mouse DNA in p12B1 and in one of the patchwork plasmids which was completely sequenced, p12B2, are not homologous, but they both include nucleotide boxes corresponding to the consensus for murine ARS sequences and to the consensus for yeast centromere elements CDE1 and CDE3. Gel retardation assays evidenced a binding site for a protein present in nuclear estracts of cultivated mouse cells, corresponding to, or overlapping with the CDE1 element. In addition, both the p12B1 and p12B2 mouse sequences exhibit inverted repeats of various lengths. Transient replication of p12B1 DNA could be evidenced upon transfection in cultivated mouse cells. Depending on a series of experimental conditions which are presently under study, injection in mouse eggs of p12B1 and of combinations of p12B1 and pPyLT1 DNA led either to the maintenance of free circular molecules or to a highly efficient integration into host sequences. The latter phenomenon was also observed in cell culture upon selection for a linked neo resistance gene. The occurence of non-random recombination events was suggested by the observation that in independent sub-lineages of one of the plasmid-bearing transgenic families, apparently identical and complex rearrangements of the transgene occured after 5 to 6 generations of purely autonomous transmission.

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L 223 NUCLEOTIDE SPECIFICITY OF THE recA PROTEIN DNA PAIRING ACTIVITIES. K.L. Menge and F.R. Bryant. The Department of Biochemistry, The Johns Hopkins School of Hygiene and Public Health, Baltimore MD., 21205.

We have found that the DNA pairing activities of the recA protein are sensitive to changes in the purine ring structure of the nucleoside triphosphate cofactor. Although ATP, purine riboside triphosphate (PTP) and ITP are hydrolyzed by the recA protein, only ATP and PTP support the three-strand exchange reaction. This suggests that interactions between the 6-keto group of the purine ring and the recA protein are responsible for the inability of ITP to act as a cofactor in the three-strand exchange reaction. Experiments examining the molecular basis for these observations will be discussed.

L 224 RecA NUCLEOPROTEIN FILAMENTS CAN CONTAIN TWO MOLECULES OF ssDNA: BINDING AFFINITIES ARE DIFFERENTIALLY REGULATED BY NUCLEOTIDES. A. Zlotnick, R. S. Mitchell and S. L. Brenner, E. I. du Pont de Nemours & Co., Inc., Central Research and Development Department, Experimental Station, P. O. Box 80328, Wilmington, DE 19880-0328.

Three different assays have been used to determine the number of bases of ssDNA bound per recA protomer in a recA nucleoprotein filament. The DNA-dependent ATPase of recA protein yields a site size of 3.2+/-0.4 bases per recA. An alternative assay which monitors the binding of fluorescent etheno-ssDNA gives a site size of 7.0+/-0.6 bases in the presence of ATP, ADP, or ATPγS. If a fixed concentration of ssDNA is titrated with recA protein and the ATPase is monitored, the complex is kinetically trapped in the 7:1 stoichiometry; the smaller stoichiometry is only observed when all of the recA is added in one portion rather than by titration. To account for these results, we propose that each recA nucleoprotein filament can contain two ssDNA molecules, each contributing ca 3.5 bases per recA, with full ATPase activity activated by binding only one strand. Using a third assay, the cosedimentation of 5'-$^{32}PO_4$-poly(dT) with recA at 100,000xg, we find a stoichiometry of ~6 bases sedimented per recA in the presence of ATP; only half of the labeled poly(dT) is rapidly displaced by added unlabeled DNA, indicating that one of the two strands is more weakly bound. When ADP or no nucleotide is present, only ~3 bases sediment per recA, indicating that the second filament is so weakly bound it is lost during sedimentation. In the absence of nucleotide, the remaining bound ssDNA (3 bases/recA) does not readily exchange with unlabeled DNA, while in ADP this second strand rapidly exchanges. The apparent affinity of the recA filament for the two strands (A and B) is modulated by nucleotide with one strand always more tightly bound than the second, i.e. $A_{ATP} \sim A_{no\ nuc} > A_{ADP} \sim B_{ATP} > B_{no\ nuc} \sim B_{ADP}$.

L 225 RecA PROTEIN-PROMOTED CLEAVAGE OF LexA REPRESSOR IN THE PRESENCE OF ADP AND STRUCTURAL ANALOGUES OF INORGANIC PHOSPHATE, THE FLUORIDE COMPLEXES OF ALUMINUM AND BERYLLIUM,

Patrice L. Moreau and Marie-France Carlier, Laboratoire d'Enzymologie, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France.

Complexes formed from Al^{3+} or Be^{2+} and fluoride inhibit the single-stranded DNA-dependent ATPase activity of RecA protein. In contrast, poly(dT)-RecA-ADP complexes, which are inactive for cleavage of LexA protein, become fully active in the presence of AlF_4^- and BeF_3^- ions. These data suggest that fluoride complexes of aluminum and beryllium (called herein X) convert RecA-ADP complexes, which bind weakly to single-stranded DNA, into RecA-ADP-X complexes, which bind tightly to single-stranded DNA, the ADP-X moiety behaving as a nonhydrolysable analogue of ATP. We propose that AlF_4^- and BeF_3^- ions act as analogues of inorganic phosphate by binding to the site of the γ-phosphate of ATP on RecA-ADP complexes, hence mimicking the single-stranded DNA-RecA-ADP-Pi transition state. We conclude that the elementary reaction that switches RecA protein from a high affinity single-stranded DNA binding state to a low affinity single-stranded DNA binding state is not ATP hydrolysis *per se* but Pi release.

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L 226 ALTERATION OF THE CATALYTIC PROPERTIES OF THE E.COLI recA PROTEIN BY SITE DIRECTED MUTAGENESIS, K.A. Muench and F.R. Bryant, Department of Biochemistry, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD 21205

RecA protein promotes the ATP-dependent pairing of DNA strands during homologous recombination in E. coli. In order to determine the mechanistic role of ATP hydrolysis in recA protein-promoted DNA pairing reactions, we are using oligonucleotide-directed mutagenesis to construct new mutant recA proteins with altered catalytic activities. In particular, we have recently prepared a new mutant recA protein in which the histidine residue at position-163 of the recA polypeptide has been replaced with an alanine residue. The [ala-163]recA protein has a ssDNA-dependent ATPase activity that is similar (kinetically) to that of the wild type recA protein. In addition, the [ala-163]recA protein is able to promote the ATP-independent renaturation of complementary DNA strands. However, the [ala-163]recA protein is unable to promote the ATP-dependent three-strand exchange reaction. Thus, the [ala-163]recA protein is functionally similar to our previously reported [asn-160]recA protein (F.R. Bryant, J. Biol. Chem. 263, 8716 (1988)). Additional experiments with these recombinase-deficient recA proteins indicate that the mutations interfere with ATP-induced conformational changes of the recA protein that are essential to the DNA pairing process.

L 227 ANALYSIS OF BASE PAIRS IN THE TRANSPOSASE BINDING SITE OF THE INVERTED REPEATS OF Tn3. Dwight Nissley, Frank Lindh and Michael Fennewald, Department of Microbiology and Immunology, University of Health Sciences/The Chicago Medical School, 3333 Green Bay Road, North Chicago, Illinois 60064

The transposon Tn3 encodes a transposase which binds to terminal 38 base pair inverted repeats (IR's). Using a nitrocellulose filter binding assay and in vivo transposition assay we have measured the effects of base pair changes on binding and transposition. The inside 21 base pairs of the IR are sufficient for binding at transposase. We have identified two smaller regions necessary for binding and transposition. Changing base pairs 4-8 or 16, 17, 19 and 21 from the inside end of the IR greatly diminishes binding and abolishes transposition. We are currently investigating the effects of single base pair changes within these two regions.

L 228 Length dependency of replicative transposition of Bacteriophage Mu. Martin L. Pato. Department of Molecular and Cellular Biology, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206. Replicative transposition of bacteriophage Mu is markedly decreased by the insertion of foreign DNA which increases the length of the genome. The frequency of transposition of a Mu derivative twice the length of the normal genome is reduced about 10^4, and replication is undetectable following induction whether the Mu genome is located in the host chromosome or on an F plasmid. Replication is also affected by decreasing the size of the genome - replication of a mini-Mu containing the entire Mu early region, but deleted for most of the remainder of the genome, is delayed, following induction, relative to full-length Mu. Experiments will be presented addressing two questions: 1) Is the effect of inserted DNA due solely to the length of the DNA, or also to the nature of the inserted DNA; 2) Why is replication impaired both by increasing and by decreasing the length of the Mu genome.

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L 229 PURIFICATION OF A PROTEIN FRACTION WITH rec-A LIKE ACTIVITY FROM HIGHER PLANTS, Alex Peterhans, Ingo Potrykus and Jerzy Paszkowski, Institute of Plant Sciences, Swiss Institute of Technology - ETH Zürich, CH 8092, Switzerland.

In vitro recombination studies of the mechanism of general recombination were performed in both procaryotic and eucaryotic systems. DNA strand transferases which promote homologous by forming a complex between double stranded and homologous single stranded DNA have been purified from Escherichia coli (Rec-A) and Ustilago maydis (Rec-1). Similar activities have also been found in yeast, lily, Drosophila, mouse and human cells.

We report experiments leading towards purification and characterization of a rec-A like activity present in germinating seedlings of higher plants.

L 230 PURIFICATION AND CHARACTERIZATION OF UMUD, UMUD', AND UMUC: PROTEINS REQUIRED FOR SOS MUTAGENESIS IN *ESCHERICHIA COLI*

Roger Woodgate, Malini Rajagopalan, Chi Lu and Harrison Echols.
Department of Molecular Biology, University of California, Berkeley CA94720

The mutation rate of *Escherichia coli* increases 100-fold after treatment with replication-inhibiting agents such as UV light. This enhanced mutation rate requires the action of the UmuD and UmuC proteins, which are induced as part of the SOS response to DNA damage. We have previously reported the overproduction and purification of the UmuD protein and shown that UmuD undergoes proteolytic processing by a similar mechanism to that previously described for LexA protein, the repressor of the SOS genes (Burckhardt et al (1988) Proc. Natl. Acad. Sci. USA 85: 1811-1815). We report here that the amino-terminal sequence of the larger cleavage fragment, which we term UmuD', agrees with that predicted by the DNA sequence if UmuD were cleaved at the cys-gly site proposed on the basis of homology with LexA (Perry et al (1985) Proc. Natl. Acad. Sci. USA 82: 4331-4335). To purify substantial quantities of UmuD', we treated cells with Mitomycin C prior to induction of the overproducing UmuD,C vector. We have purified UmuD' protein and a mixture of UmuD/UmuD' proteins. Genetic evidence has suggested that UmuD (and/or UmuD') interact with UmuC. We have coupled the mixture of UmuD/UmuD' to affi-gel support beads and constructed a UmuD/UmuD' protein affinity column. We have used the UmuD/UmuD' affinity column to purify UmuC protein. UmuC was specifically retained on the affinity column and was not dissociated by 2M NaCl. UmuC protein was eluted from the column in the presence of 2M Urea or 1.5M KSCN. These data suggest a strong protein-protein interaction between UmuD/UmuD' and UmuC.

L 231 A POTENTIAL RECOMBINASE FOR SITE-SPECIFIC REARRANGEMENTS OF ANTIBIOTIC RESISTANCE GENES, Paul H. Roy, Marc Ouellette and Luc Bissonnette, Département de Biochimie, Université Laval, Québec PQ G1K 7P4, Canada.

Plasmid-specified antibiotic resistance genes are often found in clusters. In Tn21-like transposons, and in a group of plasmids (R46, R388, etc.) which contain a 4-kb region from Tn21, the streptomycin-sulfonamide (Sm-Su) resistance operon may be altered by insertion of an antibiotic resistance structural gene between the Sm promoter and its structural gene or between the Sm and Su genes. Alternatively, a resistance structural gene may substitute for the Sm gene. These insertions and substitutions take place at specific sites flanking the Sm gene. In Tn2603, in which the OXA-1 Beta-lactamase structural gene is inserted 5' to the Sm gene, our sequencing data reveals an ORF, adjacent to the resistance operon but on the other strand, potentially coding for a protein with significant homology to site-specific recombinases: the phage integrases, the fimB and E proteins of E. coli, and the tnpA and tnpB genes of Tn554. This putative recombinase may be involved in the resistance gene rearrangements at the nearby specific sites. Experiments are in progress to isolate the product of this ORF and investigate its binding to DNA.

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L 232 DNA STRAND TRANSFER AND STRAND INVASION CATALYZED BY A DROSOPHILA MELANOGASTER STRAND TRANSFERASE, Miriam Sander, Ky Lowenhaupt, Charlotte Hauser and Alexander Rich, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. A Drosophila melanogaster strand transferase (STase) has recently been purified (Lowenhaupt, K., Sander M., Hauser, C. and A. Rich, manuscript submitted). The activity copurifies with a 105,000 dalton polypeptide in the last two chromatographic steps of the purification.This STase is capable of promoting strand transfer between circular ssDNA and homologous dsDNA at low protein:ssDNA ratios. Neither ATP nor exogenous ssb protein are required for activity; however, under suboptimal conditions, E. coli ssb protein will stimulate the Drosophila activity. The Drosophila strand transfer protein is capable of carrying out a "D-loop" reaction in which short ssDNA fragments invade ds circular plasmid DNA. This reaction occurs to a limited extent relative to the strand transfer reaction. D-loop and strand transfer reactions both occur with rapid kinetics at 37°C.

L 233 DNA BENDING IN FLP-MEDIATED SITE-SPECIFIC RECOMBINATION: AN IN VITRO ANALYSIS, Carol J.E. Schwartz and Paul D. Sadowski, Department of Medical Genetics, University of Toronto, Toronto, Ontario, M5S 1A8, Canada. The FLP recombinase is encoded by the 2μ plasmid of S. cerevisiae. FLP interacts with two inverted target sites on the plasmid to invert one unique region of the plasmid with respect to the other. The FLP recombination target site (FRT) is composed of three 13 bp symmetry elements; two in direct orientation and a third in inverted orientation on the opposite side of an 8 bp core. A single symmetry element is sufficient for FLP to bind to DNA and binding to the FRT site produces three discrete complexes. When FLP is bound to the two symmetry elements surrounding the 8 bp core, bending of the DNA and strand cleavage are observed. Mutants of the FLP recombinase have been isolated which can bind to the FRT site but are unable to bend it. We are studying the effects of changes in the base composition of the core region on the FLP-induced bending and cleavage of the FRT site.

Supported by the Medical Research Council of Canada.

L 234 **CLONING AND ANALYSIS OF SCHIZOSACCHAROMYCES POMBE RAD GENES** Per Sunnerhagen, Brent L. Seaton, Anwar Nasim* and Suresh Subramani. Dept. Biology, Univ. of California, San Diego and *National Research Council Canada, Div. of Biological Sciences

In S. pombe, mutants sensitive to UV and/or gamma irradiation, representing 22 distinct loci have been identified. By screening for UV resistance, we have attempted to complement the rad1 and rad3 mutations with a S. pombe genomic library. The rad1 and rad3 mutants are characterized by decreased mutability, and so the affected gene products are believed to be involved in recombinational repair of DNA. DNA clones have been isolated that restore rad1 or rad3, respectively, to wild-type levels of resistance. The complementing activities have been sublocalized to a 2.2 kb fragment for the putative rad1-containing clone, and to a 4 kb fragment for the putative rad3 clone. Integration studies are under way to establish whether the actual rad genes or suppressors thereof have been cloned. We are also using insertional mutagenesis to identify open reading frames that encode proteins that complement these rad mutants.

Molecular Mechanisms in DNA Replication and Recombination

L 235 CHARACTERIZATION OF BACTERIOPHAGE Mu TRANSPOSASE.Robert Alazard[1], Mireille Bétermier[1], Mik Chandler[1], Lucie Desmet[2], M. Faelen[2], Rafor Fehrat[2], Marie-José Gama[2], Valérie Lefrère[1] and Ariane Toussaint[2] (1) Centre de Recherche en Biologie et Génétique Cellulaire, CNRS, 118 route de Narbonne, Toulouse , France. (2) Laboratoire de Génétique, ULB, 67 rue des chevaux, B1640 Rhode St Genèse, Belgium;
Through the isolation and characterization of a large number of mutations (am and C-terminal deletions) in the A gene of phage Mu, which encodes the phage transposase (pA), we were able to define several functional domains in the protein. Deletion from the C-terminal end of 58 aa abolishes phage growth and leaves residual global transposase activity in vivo whithout affecting specific binding to the ends of the phage genome in vitro. Deletion of 71 aa removes all transposase activity but leaves binding unaffected. Deletion of 249 aa eliminates even specific binding. Overproduction of the truncated pA's which retain binding activity strongly inhibits the developement of a superinfecting Mu phage. These proteins may interfere with one of the early steps of the phage lytic cycle, since an N-terminal fragment of 95 aa of pA which displays strong homology with the Mu repressor protein can block Mucts62 thermal induction in some conditions. Western blot analysis of the transposase synthesized by induced wild type and mutant prophages revealed that the enzyme is synthesized in constant amount throughout the phage lytic cycle and that the protein is unstable, though at different levels in different media. Moreover plasmid vectors expressing the different A genes form either a p_{KM} or a λ_L promotor produce several proteins which are translated in the same frame as the fullsize transposase and whose role in phage development remains to be elucidated.

L 236 RECOMBINATION AND REPAIR IN Escherichia coli BY recBCD ENZYMES FROM Proteus mirabilis AND Serratia marcescens: FUNCTIONAL ANALYSIS OF HETEROLOGEOUS, OF HYBRID, AND OF MUTANT ENZYMES, Wilfried Wackernagel, Regina Rinken and Dieter Weichenhan, Arbeitsgruppe Genetik, Universität Oldenburg, D-2900 Oldenburg, F.R.G.
The recBCD gene regions from P. mirabilis and S. marcescens were cloned in E. coli in order to study the functions of other recBCD enzymes using the molecular recombination and repair assays available in E. coli. The regions show similar arrangements of genes thyA, recC, recB, recD, and argA as in E. coli. The enzymes of both species are functional in E. coli deleted for recBCD genes in (i) recombination of E. coli DNA during conjugation, in (ii) Chi-stimulated recombination of λ DNA, in (iii) repair of UV-damage and in (iv) restoration of normal cell viability. Chi-activation was lower by the P. mirabilis enzyme (2.9) than with the enzymes from E. coli (6.0) and S. marcescens (5.9). By combination of structural genes located on the chromosome and on plasmids we achieved the assembly of hybrid recBCD enzymes with subunits from different species. Their functional capacity ranged from zero (not formed?) to fully active. The studies indicated that Chi-activation is determined by recBD and recC subunits. The functional similarity of enzyme subunits supported the DNA sequence relatedness seen in cross hybridization (Southern blots) under low stringency. The enzyme from a P. mirabilis exoV mutant was also studied in E. coli. The mutation was ascribed to the recB gene. It abolished Chi-independent recombination in λ crosses, repair of UV-damage and normal cell viability, but partly retained Chi-stimulated recombination, recombination in conjugation, and ATP-dependent duplex DNA exonuclease activity. The phenotype supports the notion of two pathways of recBCD-promoted recombination.

L 237 RecA PROTEIN PROMOTES HOMOLOGOUS PAIRING BETWEEN REGIONS OF DUPLEX DNA, Stephen C. West and Edward C. Conley, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts EN6 3LD, U.K.
The RecA protein from *E. coli* gains access to duplex DNA by nucleation from a short single-stranded gap, to form a spiral nucleoprotein filament which is capable of interaction with homologous duplex DNA. Homologous pairing can occur at any point along the nucleoprotein filament, at sites that contain either single- or double-stranded DNA. Duplex-duplex pairing has been demonstrated in three ways: (i) the rate and final extent of pairing was dependent on the length of homologous duplex DNA, (ii) inhibition of pairing occurred when competitor duplex DNAs effectively blocked potential sites of contact between duplexes, and (iii) unwinding of form I chimeric DNA, which occurred as a consequence of homologous pairing, provided a direct visualization of the pairing reaction.

The results indicate that the additional local interactions available in a long duplex molecule facilitate homologous pairing and the formation of a nascent synaptic intermediate. Pairing involves underwinding of both DNA helices and the intermediate structure is likely to take the form of a nucleoprotein filament in which four DNA strands are aligned and intertwined. Conversion of the intermediate into a true heteroduplex joint may require longitudinal rotation of the filament, such that the four-stranded joint is extended to the point of the gap where strands are free to interwind.

Molecular Mechanisms in DNA Replication and Recombination

L 238 ANALYSIS OF ISl ENCODED PROTEINS REQUIRED FOR TRANSPOSITION. ZERBIB[1] D., GAMAS[1] P., JACQWEK[2] M., PRENTKI[2] P., GALAS[2] D. and CHANDLER[1] M. (CRBGC du CNRS TOULOUSE-FRANCE)[1] (Molecular Biology, University of southern California-LOS ANGELES)[2].

The insertion sequence *ISl* exhibits a complex genetic organisation. Out of the eight major open reading frames (ORF), two ; *insA* and *insB* are essential for transposition. We have cloned these two frames under strong initiation signals for transcription and translation.

The InsA protein (91 aa) is strongly basic and has an helix/turn/helix motif in its C-terminal end. By using crude extracts we have shown that InsA is a DNA binding protein specific for the 24 terminal bp of both inverted repeats of *ISl*. InsA could then provide the specificity for strand cleavage at the ends during the transposition event.

A direct comparison of the transposition activity in vivo of synthetic mutant ends with their capacity to be recognised and bound by InsA suggests that the ends of IS1 are organised in two separate domains : one for binding and the other for activity.

Moreover, the InsA binding sites are superimposed on sites for the binding of a host factor : the histone like protein IHF. Both InsA and IHF bend the DNA on binding.

We are investigating the properties of InsA and a collection of site directed mutant proteins to define the DNA-protein interactions and the potential protein-protein interactions between InsA and other proteins involved in transposition.

Using *insA*, *insB* and *insA+B* clones, we have undertaken the study of the regulation of their expression. The role played by the corresponding protein in transposition is also under investigation.

L 239 AN ALPHA-HELICAL PEPTIDE MODEL FOR NON-SPECIFIC PROTEIN-DNA INTERACTIONS,

Adam Zlotnick and Stephen L. Brenner, E. I. du Pont de Nemours & Co., Inc., Experimental Station, Central Research and Development Department, P. O. Box 80328, Wilmington, DE 19880-0328.

The sequence-independent binding of proteins to DNA involves electrostatic interactions with the DNA phosphate backbone and/or stacking interactions between aromatic amino acids and nucleotides. Several proteins that bind to single-stranded DNA contain amino acid sequences are predicted to form structurally similar motifs: alpha-helices in which amino acids are segregated by charge to opposite faces of the helix. We propose that the positively charged face of such helices interacts with the phosphate backbone of DNA. The recA protein of *E. coli* provides one model system. It is known that protein-phosphate interactions are involved in recA-DNA binding and genetic evidence suggests that the DNA-binding domain is at the N-terminus. At neutral pH, the N-terminal 23 amino acids are predicted to be in an α-helical conformation with +4 charge on one face of the helix and -3 on the opposite face. We have synthesized a peptide based on the recA N-terminus, NH_2-AIDENKQKALAAALGQIEKQFGKG-$CONH_2$, and studied its binding to DNA. As determined by circular dichroism (CD) in water at 25°C, the peptide helicity increases from ca. 0% to 40% upon addition of poly d(T). As predicted by modeling, the site size determined by CD is 5 bases per peptide. Binding is very salt sensitive, consistent with an electrostatic interaction. We have found a surprising dependance of the observed helicity of DNA-bound peptide on the fraction of the DNA occupied, suggesting a peptide-peptide interaction that stabilizes the α-helical conformation.

Prokaryotic Replication -I

L 300 ROLLING CIRCLE PLASMID REPLICATION MODES IN *Bacillus subtilis*,

Alicia Bravo, Heinrich Leonhardt and Juan C. Alonso, Max-Planck-Institut für Molekulare Genetik, Ihnestr. 73, D-1000 Berlin 33, Germany.

Most of the *Staphylococcus aureus/Bacillus subtilis* plasmids replicate via different rolling circle mechanisms. In the first mode a plasmid encoded protein introduces a single strand nick into a double-stranded DNA molecule and replication proceeds via an asymmetric rolling circle replication mechanism. Circular single-stranded unit length DNA molecules are intermediates of this mode of replication.

The second and third mode of replication are independent of plasmid encoded products but recombination-dependent. In the second mode linear single-, double-stranded and double-stranded DNA molecules with single-stranded tails, with a mean size of 40 Kb, are identified. In the third mode, observed in phage SPP1 infected cells, linear double-stranded multigenome-length plasmid molecules of at least one phage genome equivalent are generated. Differences and similarities between these modes will be discussed.

Molecular Mechanisms in DNA Replication and Recombination

L 301 THE PARTITION OF P1 PLASMID DNA: A CENTROMERE-LIKE SITE IN BACTERIA, Stuart Austin, Michael Davis, and Kathy Martin, Laboratory of Chromosome Biology, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

The prophage of bacteriophage P1 is maintained as a unit-copy plasmid, and yet is lost less than once in a million cell division events. In addition to a sophisticated replication control system, the plasmid employs a partition mechanism that actively segregates the daughter copies to daughter cells. We find that two plasmid-encoded proteins and a small cis-acting site are required for partition. One of the proteins (ParB) binds specifically to the site (parS), perhaps promoting a specific pairing event. Fine-structure analysis of the wild-type partition site and its activities reveals that it is a complex structure with at least three ParB binding regions flanking a site that binds a host protein (IHF). Much of this information is not essential for partition: a core ParB binding region of just 23 base pairs that lacks an IHF site and 2 of the parB binding sites promotes partition but demonstrates an altered specificity. We propose that the wild-type structure "presents" this core recognition sequence at the surface of the folded DNA. The minimal site can still function (although perhaps with a somewhat reduced efficiency) but is not "presented" in the same way. This change in presentation is reflected in a change in the recognition of plasmids as "self" in the subsequent steps of the partition process. Research sponsored by the National Cancer Institute, DHHS, under contract NO. NO1-CO-74101 with Bionetics Research, Inc.

L 302 DAM METHYLATION IS REQUIRED FOR PRECISE TIMING BETWEEN DNA REPLICATION INITIATION EVENTS IN *Escherichia coli*, Andreas Bakker and Douglas W. Smith, Department of Biology, B-022, and Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093

The *E. coli* origin of DNA replication, *oriC*, contains eleven GATC sites within the minimal 245 bp, 8 of which are positionally conserved in six *E. coli*-type bacterial origins. Adenines in GATC sites are methylated by the *dam* gene product, a Dam methylase. Evidence has been presented that state of methylation of one or more GATC sites in or near *oriC* is involved in initiation, both in timing between initiation events and in segregation of daughter chromosomes. Mutants in the *dam* gene are mainly point mutants. We have constructed a chromosomal deletion mutation, *dam-100*, a total deletion of the *dam* gene, and an insertion mutation, *dam-101*, using recombinant DNA procedures which precisely define the mutations. The resulting *dam* mutant cells are viable but sick; the *dam* gene is thus not an essential gene for cell viability. Most properties of these mutants are similar to those of other *dam* mutants. We have examined timing between initiation events in these and other *dam* mutants using a CsCl density transfer approach. Results show that initiation timing is severely altered in these mutants, approximating that of random timing. These experiments indicate that a hemimethylated state following initiation is required for precise timing between initiation events, and that precise timing between such events is not required for cell viability.

L 303 MODIFICATION OF pT181 PLASMID DNA 2° STRUCTURE AFFECTS THE EFFICIENCY OF ORIGIN ACTIVATION BY THE PLASMID-CODED INITIATOR PROTEIN

Jill Bargonetti and Richard P. Novick, Department of Plasmid Biology, Public Health Research Institute New York, N.Y. 10016.

Plasmid pT181 of *Staphylococcus aureus* specifically requires the plasmid-coded RepC protein for initiation of replication. It has been shown that RepC is rate limiting for replication. We have constructed a gene fusion system in which the *S. aureus* β-lactamase gene has been translationally coupled to the RepC gene of pT181 and various derivative plasmids. The rate of RepC gene expression *in vivo* for pT181 derivatives has been determined by measuring *blaZ* production from strains containing these fusion plasmids. The rate of RepC production was generally proportional to the plasmid copy number for pT181 wt and several copy mutant derivatives; however there were several notable exceptions to this general rule. In the most striking case a strain containing a mutant with a copy number of 400 produced twice as much RepC as a strain containing a pT181 mutant with a copy number of 800. In an independent study (P. Noirot and R. Novick, in preparation) it has been shown that these two plasmids differ significantly in secondary structure; it is suggested that secondary structure may affect the efficiency with which RepC is utilized and that the efficiency of origin activation by RepC is an important feature of the plasmid replication control system. Correlations between RepC production, replication frequency and plasmid DNA secondary structure will be presented.

L 304 REPLICATION CONTROL IN INC Q PLASMIDS, Peter T. Barth, Patrick Costello and Stephen Burgess, Biotechnology Department, ICI Pharmaceuticals, Macclesfield, Cheshire SK10 4TG, UK.

IncQ plasmids (prototype: R300B) have a broad maintenance host-range amongst Gram-negative bacteria. Their mechanism of replication control in diverse species is of both academic and industrial interest. We have isolated temperature-sensitive and constitutive copy number mutants of a derivative of R300B carrying the bla gene using selection of high ampicillin resistance. The mutations were approximately located by restriction fragment exchange experiments and then precisely located by plasmid sequencing. They fall outside the minimal oriV region into the adjacent mobilization region. The three temperature-sensitive mutants all carry the same mis-sense mutation in the coding region designated ORF E (Derbyshire, Hatfull & Willetts, 1987, MGG 206:161) or K (Haring, Scholz & Scherzinger, 1987 EMBO Workshop, Birmingham). We designate this gene: repK. One of the constitutive cop plasmids has a mutation that reduces the similarity of the repK promoter to the consensus sequence. (It also has a synergistic mutation in repA or repC.) It appears, therefore, that the RepK protein may act as a negative regulator of replication. The repK gene contains 3 and 2 copies respectively of unique 9 and 10 base pair sequences. The latter is closely related to the 20 base pair repeats upstream of oriV which have previously been shown to be involved in replication control and incompatibility. The RepK protein may bind at the sites within its own coding sequence to prevent the RepC protein forming its initiation complex by looping out the oriV region (Haring et al, 1987).

L 305 INSERTION AND PROOFREADING FIDELITY OF THE LARGE FRAGMENT OF *E. coli* POLYMERASE I. Katarzyna Bebenek, Catherine M. Joyce*, Mary P. Fitzgerald and Thomas A. Kunkel, Lab of Mol. Genetics, NIEHS, Research Triangle Park, NC 27709 and *Yale University, New Haven, Conn.

We are using the Klenow fragment of *E. coli* DNA polymerase I as a model polymerase to examine the mechanisms and protein-DNA interactions that are important for determining the fidelity of DNA synthesis. This enzyme contains both a polymerase activity in a large protein domain and a proofreading exonuclease activity in a smaller domain of the same polypeptide. We have determined the fidelity of DNA synthesis catalyzed by the wild-type polymerase, by two mutant derivatives lacking the exonuclease activity but containing both domains and a normal protein structure, and by a protein that contains only the large polymerase domain. All of these polymerases produce a variety of different errors at a number of different positions in the mutational target sequence. The rate of committing mistakes depends on the error, the site and the polymerase. The fidelity results obtained permit 1) a determination of the contribution of base selectivity and proofreading to both base substitution and frameshift fidelity, 2) the examination of a model to explain the production of minus-one-base frameshift errors at non-reiterated base sequences and 3) an examination of the effect of the small domain on the fidelity of polymerization by the large domain.

L 306 HIGHLY CONSERVED DOMAINS IN α-LIKE DNA POLYMERASES ARE INVOLVED BOTH IN THE INITIATION AND POLYMERIZATION ACTIVITIES OF THE Ø29 DNA POLYMERASE. Antonio Bernad, Eulalia Parés, María A. Blasco, José M. Lázaro, Margarita Salas and Luis Blanco. Centro de Biología Molecular, Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain.

The Ø29 DNA polymerase is a single polypeptide (66.5 KDa) that has two enzymatic activities, DNA polymerase and 3'→5' exonuclease, common to many other DNA polymerases, and a third one that catalyzes the formation of a primer protein-5'dAMP initiation complex, probably specific of DNA polymerases from terminal protein- containing genomes. Structural and functional studies have shown a close relationship between the Ø29 DNA polymerase and other prokaryotic and eukaryotic α-like DNA polymerases. These studies have revealed highly conserved domains in the C-terminal region, probably involved in catalysis and/or in dNTP substrate recognition. The catalytic significance of these homologous regions is being studied in the Ø29 DNA polymerase by site-directed mutagenesis. The mutants were purified, and the effect of each mutation on the different activities of the Ø29 DNA polymerase was studied. The results obtained indicate that these conserved regions are not involved in the 3'→5' exonuclease activity whereas they are essential for the polymerization activity. Direct evidence for the 3'→5' exonuclease active site location is presented by Blanco et al. (this volume). Data suggesting that general polymerization domains are involved in the initiation reaction will be also presented.

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L 307 INVOLVEMENT OF IHF IN THE MAINTENANCE OF PLASMID pSC101 IN *E. coli*, Donald Biek[1] and Stanley N. Cohen, Department of Genetics, Stanford University School of Medicine, Stanford, CA, [1]present address: Department of Microbiology & Immunology, University of Kentucky, Lexington, KY. One class of *E. coli* chromosomal mutations that affect the maintenance of plasmid pSC101 is located in the *him* genes, which encode the subunits of integration host factor (IHF). IHF is required for pSC101 replication. Compensating mutants that no longer required IHF for pSC101 replication were isolated and could be divided into two classes: (1) mutations in the pSC101 replication initiator *rep* protein, and (2) mutations in the chromosomal gene *topA*, which encodes topoisomerase I. Plasmid DNA isolated from the *topA* mutant was more highly supercoiled than DNA from *top*$^+$ strains. We have examined plasmid DNA isolated from *him* mutants and compared it to DNA from *him*$^+$ strains and found that DNA from *him* mutants is under-supercoiled. These findings suggest that IHF may be required for the maintenance of a proper degree of pSC101 supercoiling. We speculate that *rep* protein may fail to act in replication initiation as a result of the lowered superhelicity that accompanies the absence of IHF. The mutational alteration in the *rep* protin might allow it to productively interact with under-supercoiled plasmid DNA in the absence of IHF, while increasing superhelicity through a decrease in topoisomerase I activity would allow interaction of wild type *rep* protein.

L 308 CHARACTERIZATION OF THE 3'→5' EXONUCLEASE ACTIVE SITE IN THE Ø29 DNA POLYMERASE. Luis Blanco, Antonio Bernad, José M. Lázaro and Margarita Salas. Centro de Biología Molecular, Universidad Autónoma de Madrid, Canto Blanco, 28049 Madrid, Spain.
The Ø29 DNA polymerase, active as a single subunit of 66.5 KDa, has three enzymatic activities: an initiation activity, that catalyzes the covalent linkage of dAMP to a specific seryl residue in the Ø29 terminal protein, a DNA polymerase, and a 3'→5' exonuclease. The DNA pol I (Klenow fragment), similar to the Ø29 DNA polymerase both in size and catalytic activities except initiation has two separate domains: the C-terminal region (~400 aa) contains the polymerase active site, whereas the N-terminal region (~200 aa) contains the 3'→5' exonuclease active site. Direct evidence of the polymerase active site location in the Ø29 DNA polymerase is presented by Bernad et al. (this volume). Comparison of the N-terminal region of several prokaryotic and eukaryotic DNA polymerases, including the Ø29 DNA polymerase showed the existence of a conserved domain of amino acid homology, that is also present in the E. coli DNA pol I and contains its 3'→5' exonuclease active site. Ø29 DNA polymerase mutants obtained by site-directed mutagenesis in the most conserved residues had the wild-type level of initiation and polymerase activities but essentially no 3'→5' exonuclease activity. These results, probably extrapolable to the other DNA polymerases compared, reflect a high evolutionary conservation of this general catalytic domain.

L 309 LOCALIZED MUTAGENESIS OF THE SITE AT WHICH THE E. COLI DNA POLYMERASE τ SUBUNIT IS CLEAVED TO GENERATE THE γ SUBUNIT, Aleksandra Blinkowa and James R. Walker, Department of Microbiology, University of Texas, Austin, TX 78712.
DNA polymerase III contains two subunits, τ and γ, which are encoded by one gene, dnaX. γ is identical to the first 498 amino acids of the 643 residue τ. In vitro, τ (as a τ'-'LacZ fusion protein) is cleaved between two lysine residues at positions 498 and 499 to generate γ. We investigated in vivo processing by mutagenizing the 498th and 499th codons of the dnaX gene. Each lysine was altered to a threonine. The effects of these mutations on τ processing and γ formation will be determined by radioimmune precipitation of the mutated proteins.

L 310 *ESCHERICHIA COLI* DNA POLYMERASE II: CHARACTERIZATION OF AN SOS-INDUCIBLE POLYMERASE CAPABLE OF INSERTION AND BYPASS AT ABASIC LESIONS IN DNA, Cynthia A. Bonner, Sandra K. Randall, Kevin McEntee, and Myron F. Goodman, Department of Biological Sciences, Molecular Biology Section, University of Southern California, Los Angeles, Ca. 90089-1340. DNA polymerases purified from SOS-induced and uninduced *E. coli* were investigated for their ability to incorporate nucleotides opposite and extend past single abasic lesions in DNA using polyacrylamide gel electrophoresis. Standard tritium assays demonstrated a 2-fold increase in polymerase activity for DNA polymerase III and a 7-fold increase for DNA polymerase II by nalidixate treatment. DNA pol II was able to efficiently incorporate opposite the abasic template and to extend past the lesion. In contrast, various forms of DNA pol III lacked the ability to incorporate at or bypass abasic lesions. Induction of DNA pol II is under control of the SOS regulon since an increase in polymerase activity was not observed in strains containing a noncleavable form of the Lex A repressor (lnd-). No cross reactivity of DNA pol II with polyclonal antibody against DNA pol III was observed; however, DNA pol II interacts with the B-subunit of DNA polymerase III holoenzyme in the presence of SSB to increase its activity. A potential role for DNA polymerase II in DNA repair may be demonstrated in its ability to bypass noncoding lesions in times of severe DNA damage (SOS) and to increase its activity by interacting with the B-subunit. Currently, we are attempting to clone the gene for DNA polymerase II.

L 311 THE dnaA PROTEIN DETERMINES THE TIME OF INITIATION OF DNA REPLICATION IN Escherichia coli. Erik Boye, Kirsten Skarstad and Anders Løbner-Olesen[1], Institute of Cancer Research, 0310 Oslo 3, Norway, and [1]Technical University of Denmark, 2800 Lyngby, Denmark.

By placing the dnaA gene under control of different inducible promoters we have been able to vary the transcription rate and the dnaA protein concentration from severalfold below to severalfold above the wild type level. When a plac-dnaA$^+$ construct was introduced into a dnaA46 strain growth at 42^{0}C was totally dependent upon addition of the inducer IPTG. Flow cytometry revealed that at very low IPTG concentrations (<0.07mM) the DNA/mass ratio was low and the cells were filamentous. At 0.09mM IPTG the growth rate, DNA content, cell mass, and timing and synchrony of initiations were the same as in wild type control cells. Progressively higher IPTG concentrations caused the cells to initiate earlier in the cell cycle and at a correspondingly lower initiation mass. A sudden increase to high expression levels (from λpL at 42^{0}C) resulted in a rapid twofold increase in the number of origins and a 40% increase in the DNA content. The data show that at a defined, constant level of dnaA gene expression normal control of DNA replication is maintained and also suggest that the dnaA protein is limiting initiation under normal physiological conditions.

L 312 DNA POLYMERASE ACTIVITIES AND A dnaA GENE IN RHIZOBIUM MELILOTI, David Bramhill and Sharon R. Long, Dept. Biological Sciences, Stanford University, Stanford, CA 94305 The gram-negative bacterium R. meliloti (Rm) is unusual in that it contains three replicons with chromosomal properties. It provides a model system to investigate the co-ordinated regulation of initiation at chromosomal origins. Our studies of the replication enzymes of Rm have begun with its polymerases and the identification of Rm initiation factors. Two distinct DNA polymerase activities are present in Rm extracts. A processive polymerase, capable of efficiently replicating primed M13 viral DNA, is precipitated at low ammonium sulphate (0.2 g/ml) concentrations. This enzyme resembles the multisubunit E. coli DNA polymerase III holoenzyme in its activity, stimulation by E. coli SSB and its sensitivity to dilution. Concentrations of ammonium sulphate above 0.26 g/ml precipitate a second polymerase, which is most active on multiply nicked/gapped duplex DNA substrates. This latter activity is not stimulated by SSB, nor is it sensitive to dilution. Many prokaryotes carry a gene closely homologous to the essential initiation factor of E. coli, dnaA. A gene resembling dnaA gene has been cloned from Rm. The dnaA sequences of E. coli, P. putida and B. subilis contain conserved regions. The least degenerate of these was selected, and a 38 base oligonucleotide probe designed to match the Rm preferred codon usage. This probe selectively hybridizes to a sequence present in Rm DNA but not to E. coli DNA at 63°C in 6 x SSC. The 38-mer was used to identify three independent clones of the Rm gene. Restriction analysis shows that the clones carry overlapping regions of Rm DNA. These fragments have been subcloned for sequence determination and analysis of encoded proteins.

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L 313 CHROMOSOME REPLICATION IN *E. coli* INDUCED BY OVERSUPPLY OF DnaA, H. Bremer and Y.-C. Xu, Biology Programs, University of Texas at Dallas, P.O. Box 688, Richardson, TX 75080.

Replication of the bacterial chromosome was studied in a *dnaA*$^+$ strain carrying extra copies of wild-type *dnaA* genes expressed from the inducible *lacUV5* promoter on a multicopy plasmid. At 42°C in minimal medium, overexpression of DnaA led to the initiation of one extra round of replication in every cell cycle. This effect was not observed in rich (LB) medium or at lower temperature (30°C). It is suggested that the timing of initiation depends on a step preceding the DnaA step, and that, under conditions of increased temperature and reduced protein synthesis (minimal medium), unphysiologically high levels of DnaA produce a single "hitchhiked" round of replication at the normal time. This extra round does not trigger an extra cell division after termination, resulting in diploid offspring. Once the chromosome copy number has doubled in this manner, the extra chromosome continues to replicate once every generation, presumably in synchrony with the other chromosome like an *oriC* plasmid. The result indicates that the concept of a constant mass per origin (initiation mass) and origin counting models of replication control need to be reevaluated.

L 314 **INITIATION REQUIREMENTS FOR P1 PLASMID REPLICATION**, **Therese Brendler, Ann Abeles, Lucretia Reaves and Stuart Austin, Laboratory of Chromosome Biology, BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, Maryland 21701**

Our goal is to determine the precise replication controls which ensure that P1 DNA is replicated only once during the cell cycle. The P1 plasmid replicon encompasses the replication origin, the gene for the essential P1 replication protein (RepA) and a copy control element. The origin contains 2 dnaA boxes where presumably the required dnaA protein binds during initiation. Adjacent are 5 GATC sites for the dam adenine methylase. Four of these sites are nested in iterated repeats of 7 base pairs. Distal to the dam methylation sites are the RepA protein bindng sites with 5 repeats of 19 base pairs. Also located in these repeats is the promoter of the repA gene which is autoregulated by the RepA binding. Deletion mutations generated by Bal 31 and insertion of synthetic oligonucleotides demonstrate that P1 DNA replication in vivo requires the dnaA boxes and multiple RepA binding sites. Insertion mutations between the dam methylation sites and the RepA binding domain block P1 replication, indicating that spacing of the origin cis elements is critical. Dam methylation of the origin is also required for P1 replication. Plasmids driven by the P1 origin cannot be established in dam strains of E. Coli. In vitro, only methylated and hemimethylated P1 DNA replicates. We have constructed various point mutations in the region of dam methylation in the P1 origin. So far, mutations in the GATC sites or surrounding repeats block P1 replication in vivo, whereas mutations outside these regions have little effect. Experiments are underway to identify host factors which recognize the region of dam methylation. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with Bionetics Research, Inc.

L 315 CLONING OF THE *pcbA1* MUTATION OF Escherichia coli BY GENE EVICTION. Sharon K. Bryan and Robb E. Moses, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The *pcbA1* mutation in *E. coli* allows DNA polymerase I to function in replication when DNA polymerase III is non-active. We have postulated that there are two replisomes in *E. coli*, Rep-E when the *dnaE* product is the synthesis component for replication and Rep-A when the *polA* product is the synthesis component for replication. The *pcbA1* allele is located close to *dnaN* and *gyrB*. We have cloned a 12 kb Hind III fragment from a strain containing *pcbA1* into pUC18. With this plasmid we can show *trans*-complementation of *dnaN*ts and *gyrB*ts strains. We can also show restoration of temperature-resistant phenotype in *polA*$^+$ *polC*ts strains by using gene eviction, indicating that *pcbA1* is carried on this 12 kb DNA fragment. For gene replacement we used the plasmid pPH1, which is incompatible with the colE1 origin. Subcloning indicates that *pcbA1* is very tightly linked to *gyrB*. Transformation with purified plasmid suggests *pcbA1* acts weakly in *trans* as assessed by complementation testing. The *pcbA1* mutation is also tightly linked to *hisU*, an allele of *gyrB* which does not complement in *trans*. This work was supported by NIH grant GM19122.

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L 316 The RATE OF DNA ADENINE METHYLATION IN E. COLI, Joseph L. Campbell and Nancy Kleckner, Department of Biochemistry and Molecular Biology, MA 02138. The dam methylase of E. coli methylates the N-6 position of adenine on both strands of the symmetrical sequence 5' GATC. We have measured the rate of re-methylation of individual sites in the E. coli chromosome after passage of the replication fork in a synchronized culture. We find that sites in the chromosomal origin of replication take significantly longer to re-methylate than sites located elsewhere in the genome. The abundance of GATC sites at the origin (11 in the 245bp "minimal origin", 8 of which are conserved in six different bacterial origins.) has led to speculation that origin function is regulated by methylation. (Reviewed in Zyskind and Smith, Cell 46, 489-490, 1986) Recently it has been shown that an E. coli membrane fraction binds hemi-methylated origins. (Ogden et al., Cell 54, 127-135, 1988) Such binding could sequester the origin from dam methylase, and play a role in chromosome segregation. We are analyzing dam mutants to see if they have defects in chromosome segregation, or in initiation of DNA replication from the normal origin.

L 317 P1 PLASMID REPLICATION: CHARACTERIZATION OF AN INHIBITORY ACTIVITY ASSOCIATED WITH THE INITIATOR GENE, Kanae Muraiso and Dhruba K. Chattoraj, Laboratory of Biochemistry, NCI, NIH, Bethesda, MD 20892

The initiator protein, RepA, binds to its own promoter and represses transcription efficiently. There are only about 20 RepA dimers present per repA gene. However, when repA expression is artificially increased only five-fold the replication rate begins to decrease. With 40-fold over-expression the replication is abolished completely. The inhibitory activity is P1-specific: growth of E. coli and replication of pBR322, pSC101, R6K and miniF plasmids are not affected. The inhibitor is apparently not RepA itself. Excess of purified RepA does not inhibit replication in vitro. Mutating repA initiation codon ATG to ATA abolishes synthesis of the initiator but not of the inhibitor. Deletion from both the N- and C- terminal ends of repA(28 and 69 codons respectively out of the 286 codon ORF) affects the initiator but not the inhibitor. Further deletions affect both the activities. The identity of the inhibitor remains to be determined. Since the ATA mutant did not show any protein that reacts with RepA antibody, we are currently exploring the possibility of an RNA inhibitor.

L 318 CLONING OF THE *polB* GENE OF Escherichia coli BY MINI-MU TRANSPORTATION, H. Chen*, S.K. Bryan and R.E. Moses, Department of Cell Biology and *Institute of Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

polB is the gene coding for DNA polymerase II of *E. coli*. We have cloned *polB* using the mini-Mu cloning system of Casadaban with *leu* as selectable marker (2% co-transduction by P1). The activity of DNA polymerase II in polA12 cells was assayed by a rapid, modified procedure and monitored by its sensitivity to NEM. Cells containing the multiple-copy plasmid with the subcloned *polB* gene overproduce DNA polymerase II 10-15 fold above the normal level and grow normally. The restriction pattern of *polB* does not match either *polA* or *polC*. We have used an antibody prepared against purified DNA polymerase II for inhibition studies. The overproduced activity of DNA polymerase II is inhibited by pol II antibody, but not by pol I antibody. The anti-polymerase II antibody does not inhibit DNA polymerase I or DNA polymerase III, nor does it cross-react or immunoblot with these enzymes. Thus, *polB* appears to be a separate DNA polymerase and does not seem to be part of a complex. This work was supported by USPHS grant GM 19122.

L 319 COUPLING OF GROWTH RATE AND DNA REPLICATION: THE ROLES OF THE *mioC* TRANSCRIPT AND DnaA PROTEIN, Anne E. Chiaramello, Martine N. Uittenbogaard, Amy L. Svitil, and Judith W. Zyskind, Department of Biology and Molecular Biology Institute, San Diego State University, San Diego, CA 92182

DNA replication from *oriC* in the *E. coli* chromosome probably requires synthesis of an RNA transcript. One possible source of this transcript may be the *mioC* promoter. Transcripts coming from the *mioC* promoter may act as primers of DNA replication at *oriC* because the 3' ends of these transcripts are located at and near the RNA:DNA transitions detected in vivo. The expression of these transcripts has been investigated under conditions that affect DNA replication and growth rate. We have found that the *mioC* promoter is stringently controlled, growth rate regulated, and much less susceptible to repression by DnaA protein when located in the chromosome than when in a plasmid. Effects of deletions and insertions involving *mioC* and other sequences adjacent to *oriC* will be presented. DnaA protein, which is required for initiation from *oriC*, is autoregulated but the concentration increases as the growth rate increases. It binds to the sequence TTATCCACA and related sequences, with four such sequences located in *oriC* and one in the *mioC* promoter. We find that the presence of *oriC* together with *mioC* in plasmids with very high copy numbers, such as pUC19, inhibits cell growth, presumably by titrating all of the available DnaA protein.

L 320 Two pairs of replication origins in chloroplast DNA of Oenothera Wan-Ling Chiu and Barbara Sears, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48824

In Oenothera (the evening primrose), plastids are transmitted from both parents in sexual crosses. In a constant nuclear background, the extent of biparental plastid transmission depends on the types of plastid genomes (plastomes) involved in the crosses due to differential efficiencies of plastid multiplication (Chiu et al., Curr. Genet. 13:181-189, 1988). Since cpDNA replication is an essential process in plastid multiplication, we chose to investigate whether DNA sequence variations in the cpDNA origin of replication are responsible for differences in the efficiency of cpDNA replication, which might result in differential plastid multiplication. Two pairs of displacement loop replication origins, each lying within a large inverted repeat, were mapped to the Oenothera cpDNA molecule by electron microscopic analysis of cpDNA restriction fragments. The starting points of the two adjacent D-loops are approximately 4 kb apart, with one located inside the spacer region between the 16S and 23S rRNA genes and the other 1 kb upstream from the rRNA operon. Within each pair, replication proceeds unidirectionally from one D-loop towards the other. The current study is designed to analyze the replication origins of Oenothera plastomes to see if there are structural differences that might lead to different efficiencies in initiating DNA replication. Complementing this are genetic studies to examine the transmisison abilities of mutants having deletions near the D-loop region.

L 321 P4 PHAGE DNA REPLICATION, Rosemarie Christian and Richard Calendar, Department of Molecular Biology, University of California Berkeley, Berkeley, CA 94720

Bacteriophage P4 requires two cis-actng sequences for DNA replication; the origin of replication, ori and the cis replication region, crr. The ori and crr are situated approximately 4 kb apart in the P4 genome. The crr and ori have a common octameric sequence called ori type 1 repeat: GGTGAACA, which is repeated 8 times in crr and 6 times in ori. The crr is comprised of two 120 bp direct repeats separated by a 60 bp spacer region. Either crr direct repeat alone is sufficient for P4 specific replication, and inversion of the region relative to the origin does not affect function (1). The importance of the spacing and orientation between these essential cis-acting replication regions has been further investigated. In addition to these cis-acting sequences, P4 requires the P4 α protein for DNA replication. The α protein gene was cloned into a plasmid under the control of λ pL promoter and λ cI857 repressor. The overproduced α protein has been purified and the specificity of this protein for a P4 template investigated. (1) J. Flensburg and R. Calendar. J. Mol. Biol. 195:439, 1987.

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L 322 SYNTHESIS ON DISCONTINUOUS TEMPLATES CATALYZED BY DNA POLYMERASE I OF E. COLI, James M. Clark, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

A novel, nucleotide addition reaction catalyzed by an exonuclease-deficient form of E. coli DNA polymerase I (Klenow fragment) was characterized using a synthetic substrate consisting of two complementary pentadecanucleotides annealed to form a blunt-ended duplex. Addition of nucleotides to the 3' hydroxyl terminus of a 5' labeled strand of the duplex was monitored electrophoretically. When unlinked, single-stranded oligonucleotides of mixed sequence were included in synthesis reactions containing all four dNTPs, templated addition of nucleotides to the 3' end of the blunt-end duplex was observed. In control experiments, no addition of nucleotides to a single-stranded primer (corresponding to the labeled strand of the blunt-end duplex) was observed in the presence or absence of the unlinked oligonucleotides. Therefore, annealing in situ of the mixed sequence oligonucleotides to the labeled strand of the duplex to form substrates with recessed 3' ends cannot account for the observed synthesis. We conclude that continuity of the phosphodiester backbone of the template strand is not absolutely required for template-directed synthesis by the mutant Klenow enzyme. The ability of an exonuclease-deficient DNA polymerase to use unlinked, single-stranded DNA as a template suggests a novel mechanism by which linear genomes may have increased in size and diversity during evolution.

L 323 THE *uvsW* GENE OF BACTERIOPHAGE T4: A POSSIBLE LINK BETWEEN REPLICATION AND RECOMBINATION. Leslie K. Derr[1,2], John W. Drake[1] and Kenneth N. Kreuzer[2], [1]Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC and [2]Genetics Program, Duke University, Durham, NC.

The *uvsW* gene of bacteriophage T4 is involved in many aspects of phage DNA metabolism, including replication, recombination, recombinational repair and error-prone repair. Analysis of gene transcripts has demonstrated that *uvsW* is a late gene and a deletion mutation of *uvsW* has been constructed. The role of *uvsW* in phage DNA replication was investigated using plasmid model systems for secondary (recombination-dependent) and tertiary (origin-dependent) replication initiation. The *uvsW* deletion had no detectable effect on phage or plasmid DNA replication. However, a *uvsW* mutation has been reported to suppress the arrest in DNA synthesis caused by mutations (*e.g.*, in *uvsY*) that block secondary initiation. Therefore, the double mutant *uvsW uvsY* was constructed to explore the mechanism of this suppression. The low level of replicated phage DNA from a *uvsY*-mutant infection was restored to the wild-type level in a double-mutant infection. Based on the plasmid model systems, this increased synthesis is not simply due to restored secondary initiation, but may be due to increased tertiary initiation. Alternatively, a novel mode of initiation may increase replication in the double mutant. Understanding suppression by a *uvsW* mutation may provide insights into the relations between replication and recombination.

L 324 INTERACTION OF ColE1 ROM PROTEIN WITH RNA I AND RNA II, Yutaka Eguchi and Jun-ichi Tomizawa, Laboratory of Molecular Biology, NIDDKD, NIH, Bethesda, MD 20892

Initiation of ColE1 DNA replication depends on hybridization of RNA II to the template DNA. Hybridized RNA II is cleaved by RNase H to be used as primer or displaces the nontranscribed strand that is used as the template for DNA synthesis. Hybridization of RNA II to the template DNA is inhibited by binding to RNA II of RNA I that is antisense to RNA II. Binding of RNA I to RNA II is modulated by plasmid-specified Rom protein. To study how Rom protein modulates the binding of two RNAs, binding of small derivatives of RNA I and RNA II with single stem-loop structure was examined. Rom protein enhances the binding of these two RNAs. In the presence of both RNA I and Rom protein, RNA II forms a product that moves faster than the product formed by binding of RNA I and RNA II in gel filtration. These complexes are different also in sensitivities to RNases. Kinetic studies on binding suggest that the Rom protein binds to the RNA I - RNA II complex. We also examined effects of Rom protein on binding of other pairs of short complementary RNAs.

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L 326 THERMOPHILIC DNA POLYMERASES FROM ARCHAEBACTERIA, Christiane Elie, Samia Salhi*, Abdellah Hamal, Jean-Michel Rossignol*, Anne-Marie De Recondo* and Patrick Forterre, Institut de Microbiologie, Université Paris-sud, 91405 Orsay, France and *IRSC, CNRS, 94800 Villejuif, France. Archaebacteria are a group of prokaryotes as distantly related to eubacteria as to eukaryotes. Consequently, analysis of their DNA polymerases should yield information about the evolution of these enzymes during the eukaryote-prokaryote transition. We have previously described a thermophilic and thermostable DNA polymerase of 100 kDa devoid of exonuclease activity in *Sulfolobus acidocaldarius* (Elie et al, Eur. J. Biochem. 1988, in press). We show here that this enzyme can perform DNA amplification by the PCR method like the *Thermus aquaticus* DNA polymerase. We also present the purification of another thermophilic DNA polymerase isolated from *Thermoplasma acidophilum*, a representative of the other subgroup of archaebacteria. This DNA polymerase is a monomer of 85kDa with an associated 3'-5' exonuclease activity. Antibodies against *S. acidocaldarius* DNA polymerase did not recognize the *T. acidophilum* enzyme by immunoblotting.

L 327 IDENTIFICATION OF A NEW BACTERIOPHAGE T4 GENE IN THE REGION OF THE PHAGE GENES uvsX (RECOMBINATION PROTEIN) AND 41 (PRIMASE-HELICASE), Richard L. Ellis and Deborah M. Hinton, National Institutes of Health, Bethesda, MD 20892
The bacteriophage T4 genes βgt (β-glucosyl transferase), uvsX (recombination protein, phage analog of recA), 40 (stimulates head formation), and 41 (component of the primase-helicase) are located together on the T4 genome (5'→3' βgt, uvsX, 40, 41). In between βgt and uvsX lie 840 bp of DNA which is genetically unmapped, but which is expressed prior to DNA replication along with the downstream genes uvsX, 40, and 41. In order to investigate the protein-encoding capacity of this region, we have sequenced plasmids containing DNA from the end of βgt to the beginning of uvsX. Our sequencing reveals an open reading frame (ORF) starting 165 bp downstream of βgt and ending just before the start of uvsX. The ORF predicts a basic protein of 221 amino acids, which shares homology with the T4 gene 69 protein, a product needed for normal DNA replication [MacDonald and Mosig (1984) EMBO J. 3, 2863] and with a putative T4 protein (ORF5) encoded upstream of the tRNA arginine [Broida and Abelson (1985) J. Mol. Biol. 185, 545]. To ask whether the DNA upstream of uvsX actually expresses protein, plasmids and restriction fragments containing all or part of the region were transcribed/translated in vitro. This analysis reveals two proteins whose expression is dependent on the DNA upstream of uvsX. How these proteins are related to the determined ORF and possible functions for these proteins are presently being investigated.

L 328 ROLES OF T4 PROTEINS IN RECOMBINATION-DEPENDENT DNA REPLICATION, H.W. Engman and K.N. Kreuzer, Department of Microbiology, Duke University, Durham, NC 27710
The majority of late DNA replication in phage T4 is dependent upon the phage recombination genes 46/47, uvsX, uvsY, and 59. The products of these genes apparently participate in "secondary initiation," a novel mode of initiating DNA synthesis. We have shown that plasmids bearing homology to the phage chromosome, but no particular T4 sequence, are replicated during T4 infection. This plasmid replication is dependent upon the same gene products as phage late replication and therefore provides a simple system for studying secondary initiation. Mosig's model for secondary initiation involves the generation of replication forks in a two-step process: (1) formation of a recombination intermediate, and (2) conversion of that intermediate into a replication fork. We have also developed a plasmid integration assay to measure plasmid-phage recombination, allowing a direct comparison with the results of plasmid replication assays. The products of phage genes uvsX and uvsY are strictly required for both plasmid-phage recombination and plasmid replication. Thus, the uvsX and uvsY proteins carry out a function common to replication and recombination (e.g., step 1 above). In contrast, gene 59 is apparently required for plasmid replication but not plasmid-phage recombination, suggesting a role in step 2 above. The plasmid integration data also implies multiple pathways of recombination in T4. Mutations in uvsX or uvsY reduce phage-phage recombination by a few-fold, but essentially abolish plasmid integration. The phage must therefore use at least one uvsX-independent recombination pathway that is distinct from the uvsX-dependent pathway used to integrate plasmids.

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L 329 **DNA REPLICATION AND DNA-PROTEIN INTERACTIONS IN THE BROAD-HOST-RANGE PLASMID pLS1.** **Manuel Espinosa, Gloria H. del Solar, José Pérez-Martín and Adela G. de la Campa.** Centro de Investigaciones Biológicas, CSIC, Velázquez 144, 28006-Madrid, Spain.
Plasmid pLS1 is a 4408-bp streptococcal replicon that is able of autonomous replication in a variety of hosts, Gram-positive and Gram-negative. The structure of the plasmid is singular in the sense that it shows similarities with some Gram-positive replicons (replication through asymmetric rolling circle) and with Gram-negative plasmids (three 11-bp iterons nearby the plus origin of replication). Replication of pLS1 is initiated by the plasmid-encoded protein RepB through binding and nicking to the plasmid ori(+). Upon nicking, the leading strand peels off, generating single-stranded plasmid intermediates which are afterwards converted to double-stranded plasmid forms. This second step is initiated at the plasmid ori(-). A second plasmid gene, repA is placed upstream of repB and both genes constitute a transcriptional unit. RepA is a 5.1 kDa-repressor that binds to the repA repB promoter, thus controlling its own synthesis and the rate of synthesis of RepB. A complex intrinsic DNA bending has been located in a region of pLS1 that contains the plasmid ori(+) and the rep promoter. Centered at this promoter, a strong bend induced by RepA has been detected.

L 330 **REPLICATION OF A COLE1-TYPE PLASMID IN rnh polA MUTANTS REQUIRES DnaA PROTEIN,** [1]Edwina Fuge* and [1,2]Tokio Kogoma, Departments of [1]Biology and [2]Cell Biology, University of New Mexico, Albuquerque, NM 87131.
DnaA protein, encoded by the dnaA gene of E. coli, is essential for initiation of chromosome replication at oriC. The protein, which binds to a 9 base pair sequence (DnaA box) found four times in the oriC region, has been shown also to be required for replication of certain plasmids. ColE1-type plasmids initiate replication by three different mechanisms depending upon the presence or absence of RNase H and DNA Polymerase I. These plasmids contain a single DnaA box near the origin of replication. Recent in vitro work suggests that the DnaA protein may be involved in successful primosome assembly in the absence of the n' site. However, whether the DnaA protein plays an essential role in the initiation of replication in vivo remains uncertain. We have measured the plasmid copy number in rnh⁻ polA⁻ dnaA⁻ cells of several derivatives of pBR322 (a ColE1-type plasmid) that lack both the DnaA box and/or n' sites. Our results indicate that the copy number is reduced, in the absence of the DnaA box on the plasmid or in the absence of the DnaA protein, only in rnh- polA- cells. This effect is not compensated by the presence of the n' site on the lagging strand. Insertion of a DNA fragment with strong transcriptional stop signals between the promoter for tet and the origin of replication appears to alleviate the requirement for DnaA protein. The possibility that DnaA protein acts as an inhibitor of the tet gene transcription or as an effector that overcomes the topological constraints placed on plasmid replication by the transcription is being investigated.

L 331 HOST-ENCODED FACTOR(S) SPECIFICALLY BIND TO *cmp*, THE REPLICATION ENHANCER IN PLASMID pT181. Maria Laura Gennaro, Public Health Research Institute, New York, N.Y. 10016.

Replication of the staphylococcal plasmid pT181 is initiated at the origin by the plasmid-encoded initiator protein RepC. pT181 also contains a replication enhancer, called *cmp*, which exerts a direct stimulatory effect on origin function. Previous work has shown that *cmp* increases the ability of a plasmid to compete with a coresident plasmid, containing the same origin of replication, for the RepC protein and that *cmp* increases plasmid replication rates under derepressed conditions.

The present work describes the partial purification of host-encoded factor(s) that specifically bind to the *cmp* sequence. Crude cell-free extracts were loaded onto a heparin-agarose column which was eluted with a KCl gradient. Active fractions were identified by a gel retardation assay using a 5'-end labelled 250-base-pair DNA fragment containing the *cmp* sequence. SDS-polyacrylamide gel analysis of the pooled active fractions showed multiple bands; the band (or bands) associated with the binding activity have not been yet identified. Binding of the pooled active fractions to *cmp* was still detectable in the presence of a 100-fold excess of unlabelled competitor DNA (salmon sperm DNA, poly[d(I-C)], linear pBR322 DNA). Unlabelled cmp^+ plasmid DNA was 10 times more effective than cmp^- plasmid DNA in competing for binding by the active fractions.

L 332 **POSTSEGREGATIONAL KILLING OF PLASMID FREE CELLS: THE hok mRNA IS EXTREMELY STABLE AND INTERACTS REVERSIBLY WITH ITS ANTISENSE, THE sok RNA.** Kenn Gerdes, Dept. of Molecular Biology, Odense University, Campusvej 55, 5230 Odense M, Denmark. The parB (hok/sok) system of plasmid R1 stabilizes plasmids via postsegregational killing, that is, cells which lose a parB carrying plasmid are rapidly killed (Gerdes et al. 1986a). The killing is mediated via the regulated expression of a small gene, hok, the product of which causes a dramatic change in cell morphology and cell death (Gerdes et al. 1986b). The regulation of the hok gene was recently elucidated (Gerdes et al. 1988): The hok mRNA is extremely stable, while the suppressor of hok expression, the sok antisense RNA, is unstable. The differential decay of the two RNAs explains the molecular mechanism behind the postsegregational killing phenomenon: Cells which lose a hok/sok carrying plasmid will experience a gradual derepression of hok mRNA translation due to decay of the sok RNA. Consequently, the Hok protein is synthesized, and killing of the plasmid free cells ensues. We have recently attempted to measure the stability of the hok mRNA: Even 80 minutes after addition of transcriptional inhibitors (rifampicin or streptolygidin), we could detect no measurable decay of the hok mRNA. Additionally, by comparing RNA fractionated under denaturing and non-denaturing conditions, it seems that the sok antisense RNA interacts reversibly with the hok mRNA. Presently, we are investigating the determinants responsible for the extreme stability of the hok mRNA.

References: Gerdes et al. 1986a. Proc. Natl. Acad. Sci. USA 83, 3116-3120. Gerdes et al. 1986b. EMBO J 5, 2023-2029. Gerdes et al. 1988. J. Mol.Biol., 203, 119-129.

L 333 **MOLECULAR INTERACTIONS OF A NATURAL REPLICATIVE COMPLEX: SALT AND FLUORESCENCE STUDIES ON DNA POLYMERASE III HOLOENZYME AND ITS β SUBUNIT,** **Mark A. Griep and Charles S. McHenry,** Department of Biochemistry, University of Colorado Health Sciences Center, Denver, CO 80262. Holoenzyme is responsible for the majority of *E. coli* replicative DNA synthesis. However, even though *E. coli* can grow in media containing in excess of 1M NaCl, as low as 200mM NaCl completely inhibits holoenzyme activity *in vitro*. Recent work from Record's lab showed that *E. coli* uses potassium and glutamate as primary intracellular osmolytes (Richey et al., 1987, *J. Biol. Chem.* **262**, 7157-64). Inspired, we found that glutamate was 7 to 10 times less inhibitory in the holoenzyme assay than chloride. The molecular basis for the apparent salt inhibition was anion-specific changes in the holoenzyme-DNA binding constant. The β subunit was labeled with fluorescein maleimide (FM) generating one label per subunit with full retention of activity. When FM-β was titrated with $MgCl_2$, a saturable increase in fluorescence was observed; the K_D for Mg^{2+} was 2mM. Gel filtration of β demonstrated that the β dimer dissociates to monomers over a physiologically significant range and that Mg^{2+} drives β toward dissociation. In addition, pH studies indicated that Mg^{2+} binding to β shifts the pK of FM from 6.8 to 6.7. Thus the fluorescence enhancement caused by dimer dissociation was due to a subtle change in the environment surrounding the fluorophore. Fluorescence energy transfer and hydrodynamic measurements indicated that the fluorophores on the dimer's roughly spherical halves are located on the furthest opposing sides. We are currently attempting to establish the aggregation state of β when it is bound to holoenzyme.

L 334 TERMINATION OF ROLLING CIRCLE REPLICATION OF PLASMID pC194. Marie F. Gros, Hein te Riele and S. Dusko Ehrlich. Lab Génétique Microbienne, INRA, Domaine de Vilvert, 78350 Jouy en Josas, France.
We previously showed that pC194, a small staphylococcal plasmid, replicates via a rolling-circle mechanism. Replication is initiated by the plasmid initiator protein RepA, that introduces a nick in the (+) strand origin, providing a primer for displacement synthesis. After one round of replication, RepA recognizes a sequence within the origin, called the termination signal, that triggers its nicking-closing activity, thus releasing the displaced strand as a single-stranded circle. To analyse sequence requirement for termination of pC194 replication, we constructed a system in which RepA synthesis was inducible. A pC194 origin was carried by a plasmid in which the sequences to be tested for termination activity were inserted. Termination in this insert would lead to the production of single-stranded molecules of smaller size than the parental. Using this assay, we showed that, 1) sequences necessary for termination are less stringent than for initiation of replication. 2) pC194 replication is not reinitiated after termination. This is an important functional difference between single-stranded plasmids and phages. Unlike phages, plasmids regulate their copy number, generally by controlling the Rep protein synthesis. Reinitiation of successive rounds of replication by a single protein molecule would make such a control impossible.

L 335 MUTATIONAL ANALYSIS OF PHAGE G4 ORIGIN OF THE COMPLEMENTARY DNA STRAND SYNTHESIS, Hiroshi HIASA, Hiroshi SAKAI, Tohru KOMANO and G. Nigel GODSON,* Department of Agricultural Chemistry, Kyoto University, Kyoto 606, JAPAN and *Department of Biochemistry, New York University Medical Center, New York, NY 10016

The replication origin for the complementary DNA strand of phage G4 ($\underline{Gori}_c$) is located in the intercistronic region between genes F and G. Mutational analysis have showed structural features essential for the $\underline{Gori}_c$ functional activity. The $\underline{Gori}_c$ region contains three potential stem-loop structures, I, II, and III, which might be involved in the functioning of $\underline{Gori}_c$. Some nucleotide substitutions and insertions in loop II did not seriously affect the $\underline{Gori}_c$ activity, but deletion of four or more nucleotides there almost abolished the activity. Thus, perhaps a minimum essential length of this region irrespective of base composition is needed for the $\underline{Gori}_c$ functional activity. The length and base composition of the spacer region between the stem-loop structures II and III is crucial for the $\underline{Gori}_c$ activity. Seven nucleotides, rather than more or fewer, and guanine and cytosine, rather than adenine and thymine, were advantageous for the functional activity. The lowermost GC base pair at the bottom of the stem III was needed for the $\underline{Gori}_c$ activity. Another important region was the one containing the starting position for primer RNA synthesis. Deletion of this domain destroyed the $\underline{Gori}_c$ functional activity. We concluded that the $\underline{Gori}_c$ determinant sequence contained some domains with structural features different from each other, which possibly make distinct functional contributions to the $\underline{Gori}_c$ activity.

L 336 TERMINATION OF DNA REPLICATION IN ESCHERICHIA COLI REQUIRES BINDING OF THE TUS PROTEIN TO THE TERMINATOR SEQUENCES, Thomas M. Hill[1] and Peter L. Kuempel[2] ; [1]Department of Biosciences and Biotechnology, Drexel University, Philadelphia, PA 19104 and [2]Department MCD Biology, University of Colorado, Boulder, CO 80309-0347. The *Escherichia coli* chromosome contains specific sites at which replication forks are halted. These sites, called terminators, are located in the terminus region of the chromosome and are polar in function, allowing replication forks to enter but not exit that region. We have reported the identification of a 23 basepair sequence, 5′-AATNAGTATGTTGTAACTAAAGT -3′ (*Cell* **55:459, 1988**), which is common to the chromosomal terminators and which is sufficient to halt replication forks. A similar sequence is also found in the terminus region of the plasmid R6K. In addition, we have identified a *trans*-acting factor called *tus* that is required for function of the terminator sequences. Using crude cellular extracts, we have demonstrated that the *tus* gene product is a DNA-binding protein which interacts directly at the terminator sequences. We have determined the sequence of the *tus* gene and constructed a plasmid which overproduces the Tus protein. Tus has been partially purified and used in a filter binding assay to determine the binding constant and dissociation rate of Tus on various terminator sequences. We predict that the affinity of the Tus protein for different terminator sequences determines the efficiency of replication fork inhibition at a particular terminator site.

L 337 INTEGRATION HOST FACTOR ENHANCES PHAGE f1 DNA REPLICATION.
Kensuke Horiuchi and David Greenstein, Rockefeller University, New York, NY 10021

The replication origin of the filamentous phages (f1, M13, and fd) consists of two adjacent domains: core origin and replication enhancer. The core origin (about 50 bp) contains inverted repeats which serve as the binding site for the phage-encoded initiator protein (gene II protein or gpII). GpII binds to the core origin in two steps and introduces a specific nick in the plus strand which serves as the primer for rolling-circle replication. The replication enhancer (about 100 bp) is AT-rich, and is located immediately downstream of the core origin.

We present data which show that the $\underline{E.\ coli}$ integration host factor (IHF) activates f1 DNA replication through its action on the replication enhancer. Phage f1 poorly infects bacterial strains lacking IHF because IHF is required for efficient expression of F-pili, the receptor for f1. However, even when cells are transfected with f1 DNA the phage replicates in IHF mutants ($\underline{himA}$, $\underline{himD}$, or $\underline{himA}$ $\underline{himD}$) at a rate of only 3% of that in wild-type bacteria. Plasmids dependent on f1 replication system fail to replicate in IHF mutants, indicating that the growth defect of f1 in IHF mutants is at the level of DNA replication. DNaseI footprints show that IHF specifically binds to multiple sites within the replication enhancer. The effect of IHF mutations on f1 growth is suppressed by mutations in phage gene II that restore efficient replication from origins lacking a functional replication enhancer. Both gpII and IHF bend the DNA upon binding, suggesting that bent/wound DNA-protein complexes play a role in initiation at the f1 origin.

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L 338 MEMBRANE VESICLE ASSOCIATION OF A MINI-F PLASMID IN ESCHERICHIA COLI. Shelley M. Horne and Kevin D. Young, Department of Microbiology and Immunology, University of North Dakota, Grand Forks, ND 58202. Association of the mini-F plasmid, pMF45 (1-2 copies per cell) with membrane vesicles of Escherichia coli was followed to determine whether distinct functional domains exist in bacterial membranes which participate in accurate plasmid segregation. Vesicles were fractionated through sucrose density gradients (30% to 65%, wt/wt). The plasmid was found to be associated with vesicles that have a density of about 1.2 g/ml which corresponds to the density of outer membrane vesicles. Since it seems unlikely that the plasmid is physically attached to the bacterial outer membrane, membrane association of the plasmid was also determined by two other techniques developed to separate membrane vesicles: electrophoresis through dilute agarose and Sephacryl S-1000 sizing chromatography. These techniques reveal vesicle subpopulations beyond the classical inner membrane-outer membrane dichotomy, and therefore show promise in identifying specific vesicles potentially involved in plasmid partition.

L 339 *STAPHYLOCOCCUS AUREUS* MUTATIONS LEADING TO THE MAINTENANCE OF pT181 AND RELATED PLASMIDS AT A LOWER COPY NUMBER, Serban Iordanescu, The Public Health Research Institute, New York, N.Y. 10016.

Staphylococcus aureus chromosomal mutants which maintain plasmid pT181 and its copy number (Cop) derivatives at a copy number significantly lower than that observed in a wild type host have been isolated. The reduction in copy number differed for the same plasmid in different mutants, and between different pT181 derivatives in the same mutant. Four other plasmids closely related to pT181 were also affected in these mutants, while there was no effect on all other staphylococcal plasmids tested. The mutations, named *pcr* (plasmid copy number reduction) did not affect the growth rate of the cells. The mechanism by which the *pcr* mutations decrease the copy number of pT181 has been explored and the results obtained can be summarized as follows: (i) The replication of pT181 is controlled at the level of the synthesis of a protein, RepC, essential and limiting for its replication. Using *repC-bla* (β-lactamase) translational and trancriptional fusions it was found that the *pcr* mutations do not interfere with the control of RepC synthesis; (ii) The reduction in copy number in the *pcr* mutants was also observed for constructs carrying the replication origin of plasmid pT181 when their replication was dependent upon the RepC protein provided by another hybrid. Since the vector used to clone the pT181 origin was not affected by the *pcr* mutations, these results suggest that the response to the *pcr* mutations depends only upon the replication origin and the Rep protein of the affected plasmids; (iii) The *pcr* mutations might determine the reduction in plasmid copy number by affecting a host factor which becomes limiting for plasmid replication. The lack of competition between two compatible plasmids which both respond to the *pcr* effect makes this possibility unlikely. Taken together these results suggest that the *pcr* mutations lead to a less efficient Rep utilization in plasmid replication, by a mechanism which is not yet known.

L 340 REPLICATION OF COLE2 AND COLE3 DNA AND ITS REGULATION. Tateo Itoh. Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, JAPAN.

The basic replicon of ColE2 and ColE3 is about 1.3-kb long and consists of three functional portions: a gene for the plasmid-specific Rep protein (35kd); an origin region (shorter than 50bp) capable of initiating replication in the presence of the Rep protein; a gene for an anti-sense IncA RNA (115n) complementary to the 5' untranslated region of the Rep mRNA and regulating initiation of replication. A region about 100 bp away from the origin region shows an extensive homology with the n' protein-dependent priming signal and a region adjacent to the incA region is involved in the plasmid stability. We have identified the nucleotides within the origin region of each plasmid which are responsible for determination of the plasmid-specificity in interaction with the Rep protein.

In vitro replication of ColE2 and ColE3 DNA starts at a fixed position within the cloned origin region and proceeds unidirectionally. The newly synthesized leading strand contains a few ribonucleotide residues at its 5' end. However, initiation of replication apparently does not require RNA synthesis by the host RNA polymerase and dnaG primase.

In vitro binding studies have shown rapid and specific formation of a complex between the IncA RNA and the Rep mRNA. The second order rate constant for the binding is affected by single base changes in the incA region and correlates well with the extent of inhibition of expression of the Rep protein by the IncA RNA. The IncA RNA inhibits expression of the Rep protein by binding to the Rep mRNA and thereby controls plasmid copy number.

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L 341 ENZYMATIC AND STRUCTURAL CHARACTERIZATION OF THE T4 DNA POLYMERASE ACCESSORY PROTEINS, Thale C. Jarvis, Leland S. Paul, Joel W. Hockensmith and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

In vitro studies of the bacteriophage T4 DNA polymerase accessory proteins, coded by T4 genes 44, 62 and 45, show that together they modulate the activity of T4 DNA polymerase (gene 43 protein), increasing its rate, processivity and fidelity. We have characterized the physical association states of the proteins, and find that the gene 45 protein self-associates to form a trimer; molecular weight determination and subunit composition studies indicate that the gene 44/62 protein complex consists of four gene 44 protein subunits and one gene 62 protein subunit. The accessory protein stimulation of polymerase enzymatic activity requires ATP hydrolysis; the gene 44/62 protein complex has a DNA-dependent ATPase activity that is stimulated by gene 45 protein. In order to understand the underlying mechanism of accessory protein function, we have examined this ATPase activity in detail. We find that the primer-template junction with a recessed 3' configuration (such as would be found at a replication fork) is the optimal DNA cofactor for the accessory protein ATPase activity. Gene 45 protein confers the cofactor specificity, causing the accessory proteins to bind to primer-template junction sites 2 to 3 orders of magnitude tighter than to single-stranded sites. The binding preference for primer-template sites is enhanced at physiological salt concentrations and cannot be competed by gene 32 protein. Recognition of the primer-template site does not involve specific recognition of the recessed 3'-OH group. Therefore specific cofactor recognition by the accessory proteins must involve both a double-stranded and a single-stranded binding site in a suitable spacial orientation for binding of the primer-template junction. Occupation of the single-stranded site is required for activation of the ATPase. These results, in conjunction with current studies on the accessory protein stimulation of polymerase, provide insight into the role of ATP hydrolysis in T4 DNA replication.

L 342 FACTORS AFFECTING OKAZAKI FRAGMENT SYNTHESIS AT REPLICATION FORKS FORMED WITH THE E. COLI PRIMOSOME AND DNA POLYMERASE III HOLOENZYME, Ellen Johnson, Carol Wu, Minsen Mok and K. J. Marians, Sloan-Kettering Institute, New York, NY 10021. Rolling-circle DNA replication supported by tailed form II DNA templates in the presence of the DNA polymerase III holoenzyme (Pol III HE) and the primosomal proteins has been used as a model system to examine mechanisms operating at the replication fork. The long double-stranded tails produced in this reaction are constituted of a long (>50 kb) leading-strand DNA and a family of short Okazaki fragments representing synthesis of the lagging strand. Factors influencing the cycling of the enzymatic machinery operating on the lagging-strand DNA template can be observed by determining their effect on the size and distribution of the Okazaki fragments produced.

At least three general parameters influence synthesis of the lagging strand: i) The frequency at which primers are synthesized and utilized, ii) the speed at which the replication fork moves and, iii) the distributive nature of the action of some of the enzymes, including the primase and some subunits of the Pol III HE.

L 343 A RECOMBINATION HOTSPOT FROM THE MOUSE MHC LOCUS CONTAINING THE SEQUENCE $(CAGG)_{17}$ FORMS Z-DNA DESPITE THE ABSENCE OF STRICT PURINE-PYRIMIDINE ALTERNATION. Brian H. Johnston and Alexander Rich, Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

A recombinational hotspot within the Eβ gene from the mouse MHC locus has been localized to a DNA fragment containing the sequence element CAGG repeated 17 times, except that two of the repeats are CATG (Steinmetz et al., Cell 44, 895; 1986). Using chemical probes for Z-DNA (Johnston and Rich, Cell 42, 713-724; 1985), we have found that this repeated sequence forms Z-DNA under a negative superhelical density $-\sigma$ of 0.051 or greater. Results of 2-D gel electrophoresis experiments indicate that at $-\sigma = 0.051$, less than half of the sequence is in the Z form. However, hyper-reactivity of alternate purines toward diethyl-pyrocarbonate is seen over the entire sequence, indicating that the Z-DNA region can be found at every position within the repeated sequence (Johnston et al., JBC 263, 4512-4515; 1988). With increases in negative supercoiling beyond 0.051, the proportion of the sequence which adopts the Z confomation increases, until at $-\sigma = 0.08$ the transition is largely or entirely complete. This is the first sequence of the pattern $(YRRR)_n$ [Y=pyrimidine, R=purine] to be shown to form Z-DNA, although this possibility has been suggested on energetic grounds (Ellison et al., PNAS 82, 8320-8324; 1985)). It is not known whether the sequence adopts the Z conformation *in vivo*, but the likelihood of generating negative supercoiling behind a transcription complex moving through the Eβ gene (Liu and Wang, PNAS 84, 7024-7027; 1987) suggests that this is a real possibility, and might be involved in the enhanced level of recombination at this site.

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L 344 THE MINI-F PLASMID ccd OPERON IS AUTOREGULATED BY A COMPLEX OF ccdA and ccdB GENE PRODUCTS, Jeffrey E. Tam[1] and Bruce C. Kline, Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC 27599[1], and Department of Biochemistry and Molecular Biology, Mayo Graduate School of Medicine, Rochester, MN 55905. The Escherichia coli mini-F plasmid encodes for a cytotoxin which becomes active in cells which have lost mini-F due to irregularities in plasmid replication or segregation. The cytotoxin is a 11,700 Mr protein designated CcdB. The F plasmid also neutralizes the CcdB protein by production of a second protein, CcdA, 8,300 Mr. The ccdA, ccdB, and repD genes are contiguous, have the order specified, and have a regulated promoter next to the ccdA gene. Transcriptional fusion of lacZ to the ccd promoter is repressed in vivo when complemented with both CcdA and CcdB protein but not by CcdA alone. These results indicate a role for the CcdB protein in autorepression but give no indication if CcdA protein has any role. In the present work we found that all detectable CcdB protein purified as a complex with the CcdA protein. We also found that CcdA was made in excess of CcdB. Furthermore, we found using gel retardation assays that neither CcdA alone nor CcdB alone specifically bound to plasmid DNA carrying the ccd operator. In contrast, the CcdA:B complex, native or reconstituted, specifically bound to operator DNA. Thus, our in vitro results indicated that ccd transcription is autoregulated by a complex of CcdA and CcdB. Our results are consistent with the suggestion that an in vivo CcdA:B complex is integral to autorepression and may be the basis for neutralization of ccdB cytotoxicity. In earlier literature, ccdA was designated letA or H and ccdB was designated letD or G.

L 345 CLONING OF NEW ORIGINS OF DNA REPLICATION (oriK) THAT FUNCTION SPECIFICALLY IN E. coli rnh MUTANTS, Tokio Kogoma, Departments of Cell Biology and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

In E. coli rnh mutants lacking RNase H activity, DNA replication can begin at several sites (collectively termed oriK) other than oriC. The initiation from oriK is independent of DnaA protein and does not require protein synthesis (i.e., stable DNA replication). Previous attempts to clone oriK failed mainly because of the inefficiency of initiation from an isolated oriK site. Recently, we made an observation that an initiation structure (R-loop) that forms at the replication origin of pBR322 plasmid specifically in rnh mutants can become a hotspot for homologous recombination (Fuge and Kogoma, submitted). We considered that if initiation at oriK also involves a similar R-loop structure as previously proposed, such an initiation structure should stimulate homologous recombination. Accordingly, we screened for E. coli sequences that stimulate integration of a mini-F plasmid into the chromosome when the plasmid replication is prevented at high temperature in dnaA(Ts) mutants. We have obtained several such sequences. Preliminary characterizations of one of these sequences (oriK2) have revealed the following. 1) It stimulates plasmid integration by about 10-fold in rnh mutants. 2) It elevates the copy number of mini-F by about 3-fold and that of mini-pSC101 by about 6-fold in rnh mutants. 3) It allows mini-F to replicate in the absence of protein synthesis albeit very slowly. 4) It maps at 44.3 minutes on the E. coli chromosome, near one of the previously mapped oriK sites. These activities have been localized on a 1.3-Kb DNA fragment.

L 346 THE DNA UNWINDING ELEMENT: AN ESSENTIAL, CIS-ACTING COMPONENT OF THE *E. COLI* REPLICATION ORIGIN, David Kowalski and Martha J. Eddy, Molecular & Cellular Biology Department, Roswell Park Memorial Institute, Buffalo, NY 14263

Interaction of the *E. coli* DNA replication origin (*oriC*) with the dnaA initiator protein is known to induce the specific duplex opening of a tandemly repeated 13-mer sequence essential for origin function. We have discovered that the same DNA sequence is induced to unwind by negative supercoiling alone in the absence of initiator protein. The 13-mer repeats have a low free energy requirement for unwinding in naked supercoiled DNA as revealed by 1) their hypersensitivity to single-strand-specific nucleases and 2) detection of thermodynamically-stable origin unwinding using 2D gel electrophoresis of topoisomers. A replication-defective *oriC* mutant lacking one 13-mer is not nuclease hypersensitive in the two remaining 13-mers and exhibits an increased free energy requirement for origin unwinding. Origin function is reactivated in the *oriC* mutant after inserting an easily-unwound pBR322 sequence which restores both the hypersensitivity of the remaining 13-mers and the ease of origin unwinding. The inserted sequence is a poor match to the 13-mer consensus. Therefore, the "unwindability" of the 13-mer sequence, not simply the DNA primary structure, appears crucial for *oriC* function in living cells. We call this novel, cis-acting sequence a "DNA unwinding element" (*DUE*). The *DUE* is highly conserved in the chromosomal origins of other enterobacteria. Our studies on eukaryotic DNA replication indicate that a *DUE* is essential in yeast origins [*Cell* **52**, 559 (1988)]. We propose that, in addition to initiator protein recognition elements, a *DUE* is an essential component of replication origins.

L 347 IDENTIFICATION AND CHARACTERIZATION OF THE MINIMAL REPLICATION DETERMINANTS OF THE ANTIBIOTIC RESISTANT BACTERIAL PLASMID pCU1, B.Rajendra Krishnan and V.N.Iyer, Biology Department and Institute of Biochemistry, Carleton University, Ottawa, Ont., K1S 5B6, Canada.

The minimal replicon of a 39kb broad host-range E.coli incompatibility N group plasmid pCU1 was shown to be contained within a contiguous 2kb PvuII DNA fragment of the plasmid. *The nucleotide sequence (Gene, 58:217-228, 1987) of this replicon* exhibits several interesting features. Among the most notable are the presence of three ORFs: ORF48, ORF57 and ORF207. ORF207 encompasses thirteen 37bp tandem iterons. ORFs 48 and 57 encode putative low MW polypeptides. The organization of this mini-replicon is unique among the broad host-range plasmids. Subcloning and deletion analysis revealed that the replication and incompatibility determinants of pCU1 are localized in a 1.2kb DNA segment. The cis-acting elements of replication are within the tandem iterons and the DNA sequences (0.1kb) adjacent to it. Replication from this region is dependent on a plasmid function-(s) provided in trans by the 1.2kb DNA segment. Genetic evidence employing site-directed mutagenesis suggests a role for ORFs 48 and 57 in plasmid replication. Protein analysis of this replicon in E.coli minicells demonstrated a polypeptide of MW~25,000. This could be a product of ORF207. Replication of pCU1 was also shown to require the E.coli dnaA gene function.

L 348 Ap4A AND ITS BINDING PROTEINS REGULATE THE TOPOLOGICAL CONVERSION OF oriC-CONTAINING pSY317 AND SV40 DNAs, K. Kuratomi and Y. Kobayashi, Department of Biochemistry, Tokyo Medical College, Tokyo 160, JAPAN.

Since DNA replication is an essential event for cell proliferation, we studied the effects of diadenosine 5',5'''-P^1,P^4-tetraphosphate(Ap4A) and its binding proteins on the initiation of DNA replication on oriC-containing plasmid pSY317. Three Ap4A-binding proteins, fraction A(64 KDa), B(40 KDa) and C(14 KDa) were purified from E. coli. (a) As the binding proteins have hydrophobic nature, the effects of phospholipids which constitute a part of cell membrane on the Ap4A-binding activity were studied. This activity of crude preparation was enhanced in the presence of CL, PC and PE, while PI, PG and PA were inhibitory. For the purified fraction B or C, the inhibiton of Ap4A-binding activity was observed in the presence of CL. (b) On the other hand, it is interesting that dnaA protein, an essential *factor concerning in the initiation of DNA replication from oriC* (1) significantly enhanced the Ap4A-binding activity. (c) The both fraction B and C stimulated the topoisomerase I activities on pSY317 and SV40 DNA and coexistence of Ap4A further enhanced the stimulatory effects, when the proteins were preincubated with manganese ion, DTT and Ap4A, whereas the inhibition of the enzyme activities were observed without the preincubation. Moreover, the binding proteins also stimulated gyrase activities on pSY317 without the above preincubation. From the results of both (b) and (c), the Ap4A-binding proteins might regulate the initiation of DNA replication from oriC through the interactions with dnaA protein, topoisomerase I (2) and gyrase. (1) Fuller & Kornberg (1983) PNAS 80, 5817. (2) Fuller, et al. (1983) Mechanisms of DNA Replication and Recombination, p.275, ARL, Inc. NY.

L 349 CONTROL OF MINI-F REPLICATION : ENHANCED REPRESSION OF repE GENE EXPRESSION BY THE incC REPEAT SEQUENCES. Leon Huynen Thierry Lints and David Lane, Department of Cellular and Molecular Biology, University of Auckland Auckland, New Zealand.

Mini-F copy number is regulated by restricting the amount of the essential initiator protein, RepE, that is available for binding to the origin of replication. This restriction is exerted by autoregulatory control of *rep*E gene transcription and by binding to a set of repeated sequences, *inc*C, similar to those present in the origin. To test the idea that autoregulation of RepE production and *inc*C-mediated titration of RepE protein could be accomodated in a self-consistent control model by prposing that each process involves the binding of distinct forms (repressor and initiator) of RepE protein, we attempted to determine whether the *inc*C repeats could relieve the inhibition of expression of a *repE-lac*Z fusion gene by RepE protein. Contrary to expectation we found that the *inc*C repeats, *in trans* or *in cis*, intensified the inhibition of expression. This result implies that RepE protein binds more strongly to the *rep*E gene promoter-operator when simultaneously bound to *inc*C, and suggests an alternative to titration for the role of *inc*C in mini-F copy number control.

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L 350 INITIATION OF IncP CONJUGATIVE PLASMID TRANSFER: ASSOCIATION OF A TRANSFER GENE PRODUCT WITH THE 5'-TERMINAL NUCLEOTIDE AT THE RELAXATION SITE, Erich Lanka, Werner Pansegrau and Günter Ziegelin, Abteilung Schuster, D-1000 Berlin 33, Germany. Initiation of transfer DNA replication during bacterial conjugation requires assembly of nucleoprotein complexes (relaxosomes) and subsequent site and strand specific cleavage of a single phosphodiester bond at the *nic* site within a conjugative plasmid's transfer origin (*oriT*). To characterize protein-DNA interactions involved in the formation of relaxosomes, we isolated and dissected *oriT* as well as the adjacent transfer genes of the sequence related promiscuous plasmids RP4 and R751. Essential features of the transfer origins (ca. 350 bp) are conserved: symmetric sequence repeats, the *nic* site and a pair of potential promoters that allow for divergent transcription of two *tra* operons encoding relaxosome components. The corresponding genes *traH*, *traI*, *traO*, *traJ*, and *traK* map on both sides next to *oriT*. Proteins TraJ and TraK are the only components of the RP4 and R751 transfer machineries which confer specificity to their homologous transferorigins exclusively. The products of genes *traI*, *traJ* and probably *traO* are required for specific relaxation of *oriT* DNA; *traK*, although not essential for the relaxation process affects the number of relaxed DNA molecules indicating a functional interaction with the relaxosome. Purified TraJ (13.3 kDa) and TraK (14.7 kDa) proteins bind specifically to their homologous *oriT* DNA by recognizing different defined nucleotide sequences. The TraJ binding site contains a palindromic sequence located within the right arm of a 19 bp inverted repeat. The relaxation nick and the end of the 19 bp repeat are interspaced by eight base pairs. TraJ binding and nicking in the relaxosome occur on the same side of the double helix. We presume that the TraJ-*oriT* nucleoprotein structure is the initial complex in the pathway of the assembly of functional relaxosomes. The 3'-terminal nucleotide of the nick is accessible to extension by DNA Polymerase I. The 5'-terminal nucleotide of the nicked strand is attached to the *traI* product via an alkali resistant covalent linkage suggesting that it might consist of a nucleoside 5'-phospho O^4-tyrosylresidue.

L 351 DNA HELICASE ACTIVITY OF THE E. COLI PRIMOSOME, Myung Sue Lee and K. J. Marians, Sloan-Kettering Institute, New York, NY 10021. Two of the protein components of the primosome have intrinsic DNA helicase activity. The DNA B protein acts in the 5' →3' direction, whereas factor Y acts in the 3' →5' direction. The primosome complex has DNA helicase activity when present at a replication fork in conjunction with the DNA polymerase III holoenzyme. In this report, evidence is presented that the multiprotein primosome per se can act as a DNA helicase in the absence of the DNA polymerase III holoenzyme. The primosome DNA helicase activity can be manifested in either direction along the DNA strand. The directionality of the primosome DNA helicase activity is modulated by the concentration and type of nucleoside triphosphate present in the reaction mixture. This DNA helicase activity requires all the preprimosomal proteins (the primosomal proteins minus the dnaG-encoded primase). Preprimosome complexes must assemble at a primosome assembly site in order to be loaded onto the single-stranded DNA to act subsequently as a DNA helicase. The 5' →3' primosome DNA helicase activity requires a 3' single-stranded tail on the fragment to be displaced, while the 3' →5' activity does not require a 5' single-stranded tail on the fragment to be displaced. Multienzyme preprimosomes moving in either direction are capable of associating with the primase to form complete primosomes that can synthesize RNA primers.

L 352 SOS IS INDUCED BY THE DELETION OF *DIF*, A *CIS*-ACTING LOCUS IN THE TERMINUS REGION OF THE *ESCHERICHIA COLI* CHROMOSOME, D.F. Lim and P.L. Kuempel, Dept. of MCD Biology, University of Colorado, Boulder, CO, 80309-0347.

We have identified a locus in the terminus region of the *Escherichia coli* chromosome, called *dif* (*d*eletion *i*nduced *f*ilamentation), which results in the induction of the SOS system of DNA repair and produces a filamentous phenotype. *dif* is *cis*-acting and maps at minute 33.5.

About 5% of the cells in strains carrying a *dif* deletion are filamentous and can be greater than 60 μm long. When stained with DAPI, about 10% of the cells lack DNA, and filaments exhibit aberrant chromosome distribution. Nucleoids form a solid mass in the center of the filaments, rather than individual nucleoids along the length of the filaments.

Mutations that block SOS induction, such as *rec*A56 and *lex*A3, suppress filamentation. *rec*BC mutations also suppress the *dif* phenotype, whereas it is intensified by *pol*A mutations. The *pol*A1 Δ*dif* double mutant is barely viable, while the *pol*A12ts Δ*dif* combination is lethal at 42°C. Presumeably, the repair functions of Polymerase I are necessary in the absence of *dif*.

Complementation studies indicate that *dif* is a *cis*-acting locus. Using subcloned fragments from the surrounding area, we have determined that *dif* maps to a 530 bp *Cla*I/*Bam*HI fragment located at kb 346.5 on the Bouche terminus map. *dif*$^+$ plasmids are able to complement Δ*dif* only if they are able to cross into the chromosome via homologous recombination. Homology is provided by a plasmid-borne *km*^{r} gene and a chromosomal kanamycin resistance (*km*^{r}) gene inserted at the *dif* deletion. If there is no *km*^{r} gene on the plasmid, then filamentation and the temperature-sensitive lethality exhibited by the *pol*A12ts Δ*dif* double mutant are not suppressed.

L 353 EFFECT OF RNaseE ON THE REPLICATION AND RNAI STABILITY OF ColE1-TYPE PLASMIDS IN E. coli, Sue Lin-Chao and Stanley N. Cohen, Departments of Genetics and Medicine, Stanford University, Stanford, CA 94305. Inactivation of the RNA processing enzyme, ribonuclease E (RNaseE), in a temperature sensitive mutant inhibits the rate of replication and decreases the concentration of DNA of plasmids ColE1, pBR322 and pACYC184. The inhibition of plasmid DNA replication does not occur in the isogenic rne+ strain under the same growth conditions. In addition, the inactivation of RNaseE results in an increase in the half-life (from 1-2 min to 6.5-8 min) of RNAI, which regulates the initiation of replication of ColE1-type plasmids. This effect of RNaseE inactivation on the half-life of intact RNAI is independent of the presence of the Rom (or Rop) protein indicating that Rom protein does not affect the processing rate of RNAI by RNaseE. Our results show that RNaseE recognizes and cleaves both the sequences 5' ACAGUAUUUG at the 5'-end of RNAI of pBR322 and ColE1 and 5' ACAAGUUUUG at the 5'-end of RNAI of pACYC184. Inactivation of RNaseE increases the half-life of intact RNAI. The consequent increase in steady state levels of intact RNAI results in inhibition of replication of plasmid DNA. Thus the stability of RNAI plays an important role in control of plasmid replication.

L 354 THE ROLE OF β SUBUNIT OF DNA POLYMERASE III HOLOENZYME IN TRANS-LESION DNA REPLICATION, Zvi Livneh, Orna Shavitt, Hasia Shwartz, Yaakov Tadmor and Orna Cohen, Department of Biochemistry, The Weizmann Institute of Science, Rehovot 76100, Israel.
The sequence of events occurring following the encounter of the replisome with UV-lesions in DNA during replication is believed to be of major importance in mutagenesis and carcinogenesis caused by UV-light. We have used purified in vitro replication systems to investigate bypass and termination at UV-lesions during replication with DNA polymerase III holoenzyme, the multi-subunit major polymerizing complex in E. coli. Our results show that the polymerase has the intrinsic capability to bypass pyrimidine photodimers even in the absence of SOS-induced proteins, although at a low frequency. The major important factor in bypass is most likely the strong polymerase-DNA association, reflected in the very high processivity of the polymerase. Inhibition of the 3'-5' exonuclease proof-reading activity of the polymerase is not essential for bypass. In fact, under in vitro conditions, 3'-5' exonucleolytic processing can lead to an increased extent of bypass. The beta subunit of the polymerase was found to modulate the frequency of bypass. The polymerase bypassed photodimers at low beta concentrations, whereas at high beta concentrations, equal to the intracellular concentration in E. coli, little, if any, bypass occurred. Evidence that the β subunit is involved in UV-mutagenesis will be presented.

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L 355 METHYLATION OF THE ORIGIN IS NECESSARY FOR COORDINATION OF INITIATION OF DNA REPLICATION IN ESCHERICHIA COLI. Anders Løbner-Olesen and Erik Boye , Dept of Microbiology, The Techn. Univ of Denmark , Copenhagen Denmark and Dept. of Biophysics, The Norwegian Radium Hospital, Oslo Norway.
The Escherichia coli dam gene codes for a methylase which methylates -GATC- sequences in double stranded DNA. Within the 245 bp replication origin this sequence is found eleven times. The fact that minichromosomes transform dam strains poorly raised the possibility that methylation of the -GATC- sequences within oriC is a prerequisite for normal initiation of DNA replication. Flow Cytometric studies of DNA replication in strains where the level of dam methylase could be controlled exogenously showed that initiation was coordinated only within a narrow range of dam methylase concentrations. Both decreased and increased levels of dam methylase resulted in asynchrony of initiation within the single cell. When dam methylase was expressed at high levels, the cells contained more origins. We therefore suggest that the time required to prepare the origin for a new round of initiation, is at least partly due to the time required for methylation of the origin -GATC- sequences.

L 356 INITIATION OF INDUCED STABLE DNA REPLICATION IN E. coli OCCURS AT oriC AND ANOTHER INDUCIBLE ORIGIN WITHOUT THE PARTICIPATION OF RNA POLYMERASE OR THE DnaA PROTEIN, [1]Thomas R. Magee and [1,2]Tokio Kogoma, [1]Departments of Biology and [2]Cell Biology, University of New Mexico, Albuquerque, NM 87131

Following induction of the SOS response, DNA replication no longer requires RNA or protein synthesis. This mode of DNA replication has been termed induced stable DNA replication (iSDR). Initiation of iSDR occurs at two origins, one located at or very near oriC and the other in the terminus region of the chromosome at approximately 31.5 minutes as determined by the marker frequency assay. Following thymine starvation, minichromosomes initiate DNA replication without the requirement for protein synthesis, the activity of RNA polymerase or the DnaA protein. The inducible initiation activity has been localized to the oriC region between -178 and +425 bp. The two AT-rich 13-mer sequences in the minimal oriC sequence appear not to be required since a BamHI-BamHI deletion (+1 to +92 bp) of the minimal oriC sequence does not prevent initiation of stable DNA replication from this region. The inducible origin located in the terminus region also does not require protein synthesis or the activity of RNA polymerase or the DnaA protein. This origin is not associated with the cryptic prophage rac or kim known to exist in this region of the chromosome because strains deleted for these prophages still exhibit origin activity. It appears that an early step of DNA initiation following SOS induction such as the initial melting and subsequent unwinding of the DNA template is substantially different from that found for normal oriC-based initiation of DNA replication.

L 357 MECHANISMS OF PRIMING FOR LEADING AND LAGGING STRAND SYNTHESES IN INITIATION OF DNA REPLICATION, Hisao Masai, Nobuo Nomura*, and Ken-ichi Arai, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304; *Nippon Medical School, Tokyo, Japan. Initiation of DNA replication in prokaryotic replicons could be classified into three groups in terms of mode of priming for leading and lagging strand syntheses. In oriC or λ, dnaB helicase, loaded through an initiation complex, migrates toward both directions on the two lagging strand templates generating primers for Okazaki fragments at multiple sites in conjunction with primase. Primers for the leading strand synthesis would be provided by those for the first Okazaki fragments synthesized near the replication origin. In replicons replicating unidirectionally, such as R1 or pSC101 plasmid, dnaB protein, which is loaded through an initiation complex onto the lagging strand, may migrate only in one direction and primes the lagging strand synthesis at multiple sites. In R1 plasmid, a primer RNA for the leading strand is synthesized by primase alone at a specific site downstream of oriR. In ColE1-type plasmids, after initiation of leading strand synthesis by RNA polymerase and DNA polymerase I, dnaB protein may be loaded at n' protein recognition site on a lagging strand template located downstream of the origin[1]. A novel priming system on R6K plasmid-derived single-stranded DNA, which requires dnaA, dnaB and dnaC proteins in the prepriming stage, can replace the function of the n' site and the primosome for efficient lagging strand synthesis of pBR322, supporting the importance of loading of dnaB helicase but not other prepriming proteins.

[1] Masai, H. and Arai, K. (1988) J.B.C. 263, 15016.

L 358 INVOLVEMENT OF THE TEMPLATE STRAND DNA FOR RNA II TRANSCRIPTION IN INITIATION OF PLASMID ColE1 REPLICATION, Hisao Masukata* and Jun-ichi Tomizawa, Laboratory of Molecular Biology, NIDDKD, NIH, Bethesda, MD 20892 and *Department of Molecular Biology, School of Science, Nagoya University, Nagoya 464-01, Japan

Transcription of RNA II starts 555 bp upstream of the replication origin. Nascent RNA II transcripts form a hybrid with the template DNA and the hybridized RNA II is cleaved by RNAase H at the origin to serve as a primer for DNA synthesis by DNA polymerase I.

We have isolated ColE1 mutants deleting various segments including the replication origin. The native ColE1 sequence in the region upto 6 bp upstream from the origin is not required for hybrid formation, while base substitutions extended beyond this point affect hybridization. A stretch of dT's in the template strand at the replication origin is not required for hybridization but necessary for utilization of RNase H-cleaved RNA II as primer. Results of transcription on heteroduplex templates carrying various deletions in either strand show that the nontemplate strand for RNA II transcription upto 60 nucleotides upstream of the origin is not required for hybrid formation. Oligodeoxyribonucleotides corresponding to the segment of the template strand from 9 to 24 nucleotide upstream of the origin carrying a stretch of 6C's bind to RNA II in the region consisting of a stretch of 6G's 265 nucleotide upstream of the origin and inhibit the hybrid formation. Several other sequences rich in dC's showed no effect. These and other results suggest that the interaction of the template strand DNA near the replication origin with RNA II in its far upstream region is involved in the mechanism of hybrid formation.

Molecular Mechanisms in DNA Replication and Recombination

L 359 MINI-P1 REPLICATION IN VITRO: LIMITS OF THE ORIGIN AND REQUIREMENT FOR dnaA, dnaB, dnaC, dnaJ AND dnaK PROTEINS, Keith McKenney[1], Joel Hoskins[1], and Sue Wickner[2], [1]CARB, NIST, Gaithersburg, MD 20899; [2]LMB, NCI, NIH, Bethesda, MD 20892.

The P1 origin is contained on a 245 bp DNA fragment with 5 repeats of a 19 bp sequence to which the P1 initiator protein, RepA, binds. About 100 bp to the left there are two direct repeats of the 9 bp dnaA protein binding site. We have developed an in vitro system that replicates plasmid DNA containing the P1 origin. To define the limits of the origin we cloned the region in M13, constructed and sequenced deletions of the ends, and tested RF DNA of these deletion phage for their ability to support RepA-dependent replication in vitro. The minimal origin that is functional in vitro includes only one of the two dnaA binding sites and extends through the first two and one-half RepA binding sites. Furthermore the dnaA binding site can be inverted without loss of origin activity. We have also been studying the proteins involved in initiation. Two origin specific DNA binding proteins are required, dnaA and RepA. Unlike oriC replication, the ADP form of dnaA is active for mini-P1 replication, suggesting the function of dnaA in these two systems may be different. In addition, dnaB and dnaC are required. The E. coli heat shock proteins, dnaJ and dnaK, are also essential. We have found that dnaJ and RepA form a protein complex. Thus this system resembles oriC in some of its properties, (the requirement for dnaA, B and C), and oriλ in others (the requirement for a dnaB, J and K and a phage encoded origin binding protein). It provides a new system for studying the functions of these proteins in replication.

L 360 THE ROLE OF HEAT-SHOCK PROTEINS IN SUPPRESSION OF THE TEMPERATURE-SENSITIVE *ssb-1* MUTATION OF *E. COLI* Ralph R. Meyer, Phyllis S. Laine and Diane C. Rein. Dept. of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221.

In *E. coli*, exposure of cells to elevated temperatures results in the induction of at least 17 different polypeptides. This "heat-shock response" protects the cells from thermal killing. We have recently demonstrated a relationship between the major heat-shock protein *groEL* and DNA replication. We have isolated a revertant to *ssb-1*, and by cloning have found an allele of *groEL* (*groEL411*) that suppresses the temperature-sensitive (ts) phenotype of cells carrying the DNA-binding protein mutation (Ruben *et al., Proc. Nat. Acad. Sci., USA* 85:3767, 1988). In examining DNA synthesis in these strains, an interesting observation was made. Upon shift to 42.5°C, there is a transient temperature-sensitivity of DNA synthesis: within one minute DNA synthesis stops, but then resumes again at its normal rate within five minutes. In investigating the mechanism of suppression, we have now shown that heat-shock induction is required, since blocking RNA or protein synthesis just prior to temperature-shift abolishes the transient response, and the cells remain ts for DNA synthesis. Moreover, this recovery is not solely dependent upon induction of the *groEL411* suppressor, since strains carrying a *groEL411* plasmid and already over-producing GROEL411 protein still cannot restore DNA synthesis at 42.5°C if heat-shock induction is blocked. This has prompted us to search for additional genes required for suppression of *ssb-1*. At the same time we have been unable to transduce the *groEL411* allele in a single-copy number into other genetic backgrounds containing *ssb-1*. This has led to the discovery of a second suppressor, currently named *sup304*, which appears to be required along with *groEL411* to suppress *ssb-1* when both of these suppressors are present in a single chromosome copy. When overproduced, however, GROEL411 protein alone is sufficient for suppression. The identity of this second suppressor and whether it is also a heat-shock protein have not as yet been determined.

L 361 GENETIC CONTROL OF F1 REPLICATION PROTEINS. Benedicte MICHEL, Norton D.ZINDER The Rockefeller University, 1230 York Avenue New-York NY 10021.

Two filamentous phage gene products are required for the replication of phage DNA. One of these, the gene II protein (gpII), is a site-specific endonuclease. The other one, the gene V protein (gpV), is a single-stranded DNA binding protein. Gene V specifically represses the translation of gpII's m-RNA. The operator and the repressor of this system are defined by mutations which overproduce gpII: geneV is the repressor while the leader sequence of gene II m-RNA appears to be the operator. By an in vitro assay gpV's affinity for different in vitro synthesized RNAs was measured. Different portions of the gene II leader sequence were fused to lac Z sequences. A 19 base long sequence was found to be sufficient to increase 5 to 10 fold the affinity of gpV for a 210 nucleotide long RNA. This higher affinity is probably sufficient for repression. Binding experiments with DNA sequences analogous to these RNA regions have shown that gpV also have a 5 to 10 fold higher affinity for the same 19 DNA nucleotides. The possible role of this gpV preferen--tial binding for DNA replication is under investigation. Also with the use of a gene II-lac Z translational fusion and a gene V under the control of an inducible promoter, we found , in vivo, a ten fold repression of gene II in the presence of gpV. Operator mutants are under investigation.

Molecular Mechanisms in DNA Replication and Recombination

L 362 CYTOSINE ARABINOSIDE(AraC) IN TEMPLATE DNA: MECHANISM OF POLYMERASE ARREST. Thomas Mikita and G. Peter Beardsley. Departments of Pediatrics and Pharmacology, Yale University School of Medicine. New Haven, CT. 06510.
We have used a chemical synthetic method (Nucleic Acids Res. 16, 9165-9176(1988)) to site specifically introduce an araC residue into the template overhang of synthetic DNA primed-templates. We have previously shown that various DNA polymerases weakly or strongly arrest after inserting G opposite araC, and that the strength of the arrest seemed to correlate with the presence or absence of an associated exonuclease (Biochemistry 27, 4698-4705(1988)). The present studies were undertaken to elucidate factors which affect the degree of replicative bypass of araC in template DNA. We have measured nucleotide turnover at the site of arrest in order to more clearly define the role of 3'-5'exonuclease activity on lesion bypass. Both the Klenow Fragment and T4 polymerase rapidly turnover dNMPs at the site of arrest. Differences in the site of arrest were seen depending on polymerase and local sequence context of the lesion. The Klenow Fragment partially arrests opposite araC and turns over dGMP. T4 polymerase arrests primarily one nucleotide before the araC site and rapidly turns over dAMP when the base 3' to araC is T. Changing the 3' neighbor of araC to A,C or G results in two arrest sites for the T4 polymerase: opposite the lesion, and its 3' neighbor. The local sequence 3'AaraCA 5' results in the weakest arrest. The Klenow Fragment is largely insensitive to the above sequence context changes. Interestingly, a mutant form of the Klenow Fragment which lacks an exonuclease also exhibits a partial arrest opposite araC although no turnover of dGMP occurs. This suggests that polymerase-associated exonuclease activity may not be a major determinant in limiting trans-lesion bypass.

L 363 STABILITY OF pSC101 AND *par* DERIVATIVES IN BACKGROUNDS OF ALTERED SUPERHELICAL DENSITIES, Christine A. Miller and Stanley N. Cohen, Dept. of Genetics, Stanford University School of Medicine, Stanford, California, 94305. The plasmid pSC101 is stably maintained in wild-type genetic backgrounds, and also in mutant backgrounds having decreased gyrase. Previously we have identified mutations in the partition (*par*) locus of pSC101 showing effects on stability ranging from mild (the Cmp$^-$ phenotype; stable alone but unable to be maintained with a related *par*$^-$ replicon) to severe (the Super-par$^-$phenotype; very unstable alone) (Tucker, *et al.*, 1984, *Cell*, *38*, 191-201). The marked instability of Super-par$^-$ plasmids in wild-type cells can be reversed in topoisomerase mutant strains; and stable Cmp$^-$ pSC101 derivatives become unstable alone when gyrase is limited. In *top*$^-$ mutants, wild-type plasmids and all *par* mutants show comparable increases in supercoiling. Similarly, the decreased levels of supercoiling in gyrase mutants is comparable for wild-type plasmids and those mutated in the *par* region. Thus, differential effects on the supercoiling for wild-type and mutant plasmids were not observed in either *top*$^-$ or *gyr*$^-$ mutants despite the effects of the mutations on plasmid stability. Wahle and Kornberg (EMBO J., 1988, *7*, 1889-1895) have shown that the *par* region of pSC101 includes a site that binds gyrase strongly *in vitro*. However, while the sites involved in partitioning and gyrase binding are overlapping, they are not congruent since gyrase binding and partitioning can be separated by mutation. Instability in *gyr*$^-$ mutants and stabilization of otherwise unstable plasmids in *top*$^-$ hosts is not specific to pSC101 replicons, but was observed also for ColE1 type and F derived plasmids. Collectively, these findings suggest that the superhelical density of the DNA is a factor that influences plasmid maintainence.

L 364 TRANSCRIPTION OF THE *STB* OPERON OF INCFII PLASMID NR1, You-nong Min, David D. Womble, and Robert H. Rownd, Department of Molecular Biology, The Medical and Dental Schools, Northwestern University, Chicago, IL 60611. In addition to replication during the cell division cycle, stable maintenance of low copy number bacterial plasmids requires proper partitioning of plasmid molecules to daughter cells at the time of cell division. For plasmid NR1, this second function is provided by the stability (*stb*) locus. The *stb* locus is composed of about 1500 bp that contain an operon with two tandem genes, *stbA* and *stbB*. These genes encode *trans*-acting proteins that are essential for stability. The third essential component of *stb* is a *cis*-acting DNA site located in the upstream part of the operon. There is a third open reading frame, *stbC*, within *stbB* in the opposite DNA strand. There is no evidence available concerning the role, if any, that *stbC* might play in stable inheritance. *In vitro* transcription experiments with *stb* DNA have revealed several transcripts of different lengths that hybridize with strand specificity to both *stbA* and *stbB* or only to *stbA*. No *in vitro* generated transcripts were found to hybridize with *stbC*. Primer extension experiments with both *in vitro* and *in vivo* generated transcripts identified the start site for *stbAB* mRNA that corresponds to the transcription promoter site P_{AB} previously identified in the nucleotide sequence. Several weak "internal" start sites were identified upstream of *stbB* from the *in vitro* transcripts, but corresponding RNA species were not observed *in vivo*. No start sites that would correspond to *stbC* mRNA were found for either *in vitro* or *in vivo* transcripts. The rate of *in vivo* transcription of the *stb* locus was measured by pulse labeling and quantitative RNA/DNA hybridization. Deletion of the P_{AB} promoter resulted in a 10-fold decrease in the measured rate of transcription. Deletion of *stbB* and the 3' end of *stbA* resulted in a 5 to 10-fold increase in the measured rate of transcription. These data are consistent with previous suggestions that *stbA* and *stbB* are cotranscribed as an operon and that transcription is autoregulated by StbA and/or StbB.

L 365 THE T4 uvsY PROTEIN MODULATES RECOMBINATION-DEPENDENT DNA SYNTHESIS IN VITRO. Scott W. Morrical and Bruce M. Alberts, Department of Biochemistry and Biophysics, School of Medicine, University of California - San Francisco, San Francisco, CA 94143. The rate of DNA synthesis in a T4 recombination-dependent in vitro system is highly dependent on the concentration of the uvsX recombinase. The uvsY recombination accessory protein dramatically alters this uvsX concentration dependence. Low concentrations of uvsY *stimulate DNA synthesis at sub-critical concentrations of uvsX, but inhibit the reaction at high* uvsX concentrations. As a result, the observed critical concentration of uvsX for the reaction is lowered 8-fold, and the uvsX concentration for maximum activity is lowered 4-fold, in the presence of small amounts of uvsY. Higher concentrations of both proteins inhibit DNA synthesis. An examination of product distributions in reaction mixtures containing uvsY demonstrates that this protein affects DNA synthesis during both the initiation and elongation phases. At high uvsX concentrations (where uvsY inhibits synthesis), the average length of daughter molecules is shorter with increasing uvsY concentration. This indicates that uvsY somehow interferes with the elongation phase of synthesis under these conditions, perhaps by binding to the template and blocking movement of the replication fork. In contrast, at low uvsX concentrations (where uvsY stimulates synthesis), the distribution of products is independent of uvsY concentration. Instead, the frequency of initiation varies with uvsY concentration. This is probably a result of uvsY stimulation of synapsis catalyzed by the uvsX protein. Work in progress will help to better define the relationship between uvsY, uvsX, and recombination-dependent DNA synthesis.

L 366 INITIATION AND PAUSING OF DNA REPLICATION AT THREE DIFFERENT ORIGINS OF BACTERIOPHAGE T4, Gisela Mosig, Gene Lin, Chi Chen, Michael Gruidl, and Robert Thompson , Department of Molecular Biology, Vanderbilt University, Nashville,TN 37235

Phage T4 can initiate DNA replication from primary or tertiary origins (and from recombinational intermediates). We have sequenced and characterized three T4 origins (A, E, and F) and compared their use under different growth conditions. These origins can function in the primary (RNA polymerase- dependent) mode. Ken Kreuzer's lab has shown that oriF functions also in the tertiary (RNA polymerase-independent) mode. - Our recent results show that oriE is preferred when host gyrase is inhibited or when the DNA is heavily labeled with ^{32}P, suggesting that it functions better when the DNA is more relaxed. OriE maps between late genes in an intercistronic region which is transcribed from early (prereplicative) promoters. Unlike oriA or oriF, it contains multiple repeat sequences at precise 24bp distances. To investigate why this origin is preferentially used in relaxed DNA, we are testing the idea that binding of proteins to these sequences can introduce localized torsional stress into relaxed templates.

When phage T4 topoisomerase and host gyrase are defective, origin initiation is unidirectional and replication pauses or stalls at certain distances which are different for each of the origins. Pausing at these sites results in apparent preferential replication of certain T4 regions, (also observed by Kozinski's group when T4 protein synthesis was inhibited with chloramphenicol). Electronmicrographs and other experiments indicate that under these conditions, lagging strand DNA synthesis is anomalous. We are testing the idea that topoisomerases facilitate the precise assembly of the T4 priming proteins and their association with the replisome and that these associations are important for bidirectional replication and for processivity beyond natural pause sites in DNA. - This requirement for topoisomerase activities to overcome pausing, appears not unique to T4. When a type II topoisomerase that we found in the alga Chlamydomonas, is inhibited with novobiocin, certain chloroplast DNA segments are preferentially underreplicated, perhaps due to pausing.

Prokaryotic Replication -II; Eukaryotic Replication-I

L 367 P1 PLASMID REPLICATION: CHARACTERIZATION OF AN INHIBITORY ACTIVITY ASSOCIATED WITH THE INITIATOR GENE, Kanae Muraiso and Dhruba K. Chattoraj, Laboratory of Biochemistry, NCI, NIH, Bethesda, MD 20892

The initiator protein, RepA, binds to its own promoter and represses transcription efficiently. There are only about 20 RepA dimers present per repA gene. However, when repA expression is artificially increased only five-fold the replication rate begins to decrease. With 40-fold over-expression the replication is abolished completely. The inhibitory activity is P1-specific: growth of E. coli and replication of pBR322, pSC101, R6K and miniF plasmids are not affected. The inhibitor is apparently not RepA itself. Excess of purified RepA does not inhibit replication in vitro. Mutating repA initiation codon ATG to ATA abolishes synthesis of the initiator but not of the inhibitor. Deletion from both the N- and C- terminal ends of repA(28 and 69 codons respectively out of the 286 codon ORF) affects the initiator but not the inhibitor. Further deletions affect both the activities. The identity of the inhibitor remains to be determined. Since the ATA mutant did not show any protein that reacts with RepA antibody, we are currently exploring the possibility of an RNA inhibitor.

L 368 PROTEIN-PROTEIN INTERACTIONS WITHIN THE BACTERIOPHAGE T4 DNA REPLICATION COMPLEX, Ross W. Richardson, Richard L. Ellis and Nancy G. Nossal, N.I.H., Bethesda, MD 20892

Seven T4 encoded proteins (DNA polymerase, the 44/62 and 45 polymerase accessory proteins, 32 ssDNA binding protein, and the 61/41 primase-helicase) are required for leading and lagging strand synthesis at a replication fork in vitro. Polymerase and the 44/62 protein complex each form a filterable complex with a 32 protein covered primer-template in the absence of ATP, while 45 protein binds only if polymerase, 44/62 protein and ATP are present. Polymerase, the accessory proteins, 32 protein and the 41 protein helicase carry out rapid, synchronous strand displacement synthesis on forked DNA templates, as required for leading strand synthesis, and fail to displace an unforked complementary strand, as required to avoid displacing Okazaki fragments on the lagging strand. Tryptic cleavage 17 or 20 amino acids from the C-terminus of 41 protein does not alter the helicase or primase activities of the 61/41 complex alone, but does prevent 41 protein from acting as a helicase or primase component in conjunction with the other replication proteins. Our results suggest that 41 protein interacts with the polymerase accessory proteins to facilitate primer synthesis on 32 protein covered DNA, and that alteration of the C-terminus prevents this essential interaction.

L 369 RECENT STUDIES ON PT181 REPLICATION, R. Novick, P. Noirot, S. Iordanescu, S.J. Projan, Public Health Research Institute, 455 First Avenue, New York, NY 10016

pT181 is a 4.5 kb multicopy plasmid from *Staphylococcus aureus* that replicates by an asymmetric rolling circle initiated by a plasmid-coded protein, RepC. Its replication is controlled by anti-sense RNAs that regulate the rate of initiator protein synthesis. New data to be presented involve the mechanisms of leading strand initiation and of countertranscript-mediated inhibition of initiator synthesis.

The pT181 leading strand replication origin contains a strong potential hairpin with the initiation nick site located in the loop region. We have demonstrated that this loop is sensitive to nuclease S1 *in vitro* and is also sensitive to bromoacetaldelyde (BAA) *in vivo* and *in vitro*. Its formation is a function of superhelix density, and, remarkably, is enhanced by RepC binding; at a relatively low superhelix density, where very few, if any, molecules spontaneously extrude the hairpin, its formation is dramatically increased by the protein. It is suggested that the resulting open complex is the immediate substrate for polymerase III-catalyzed initiation of 3' extension.

The pT181 countertranscripts are thought to act by complexing the *repC* mRNA leader and causing the formation of a stem-loop immediately 5' to the protein start. This stem-loop structure could block translation by sequestering the SD site or it could serve as a termination signal. We have recently been able to show that premature termination (attenuation) is the primary consequence of the countertranscript-target interaction. Supporting data include a demonstration of the attenuated transcript and the stimulation of its formation by the countertranscripts in *trans*, plus a demonstration by gene fusion analysis that termination occurs at the predicted site and is enhanced by the countertranscripts. Additionally, mutation of an upstream sequence that is thought to block formation of the attenuator stem in the absence of the countertranscripts causes an absolute requirement for RepC *in trans*. This mutation is suppressed by a second mutation that greatly weakens the attenuator stem.

L 370 PROTEIN PRIMED REPLICATION OF ESCHERICHIA COLI BACTERIOPHAGE PRD1, Tiina M. PAKULA, Harri Savilahti, Javier Caldentey and Dennis H. Bamford, Department of Genetics, University of Helsinki, Arkadiankatu 7, 00100 Helsinki, Finland

PRD1 is a lipid-containing dsDNA bacteriophage infecting a variety of Gram-negative bacteria harbouring P, N or W incompatibility group plasmids. Among the hosts are E. coli and S.typhimurium. The phage DNA replication is primed by covalently linked proteins at the 5'-ends of the genome. We are studying this phage system in order to understand the function of phage and possibly host encoded replication regulation proteins. The phage encodes four early proteins. At the genome left end there are the genes for the genome terminal protein (VIII) and the polymerase (I). The right genome end contains genes for two small regulation proteins (XII and XIX). The nucleotide sequence of all these genes as well as the inverted terminal repeats at the genome ends has been determined. The genes have been separately cloned into expression vectors leading to overproduction of corresponding gene products. Both replication initiation and elongation can be followed using crude extracts containing the phage replication proteins. Partially purified PRD1 replication proteins are available. We are currently purifying these proteins to homogenity in order to set up in vitro replication system for PRD1.

Molecular Mechanisms in DNA Replication and Recombination

L 371 **PRIMOSOME-CATALYZED DNA UNWINDING FOLLOWS TRANSCRIPTIONAL ACTIVATION OF pBR322 DNA,** Camilo Parada, and K.J. Marians, Sloan-Kettering Institute, New York, NY 10021. Mechanisms that could operate to initiate pBR322 DNA replication in the absence of RNase H and DNA polymerase I are described. Two different pathways leading to extensive unwinding of pBR322 DNA have observed under DNA replication reaction conditions. In the presence of only RNA polymerase holoenzyme and DNA gyrase, specifically initiated RNA II can form an RNA-DNA hybrid with the template that starts just upstream of the origin of DNA replication and continues for almost 3 kb. This can be detected by subsequently digesting the RNA, the product, form I^{*} DNA, has a specific linking number of about -0.7. Extensive hybrid formation is inhibited by both RNA I and concentrations of RNase H sufficient for the catalytic processing of the RNA I required during normal leading-strand DNA synthesis.

Omission of DNA gyrase during the transcription step leads to the formation of a stable RNA II-DNA hybrid extending about 250 nucleotides downstream of the origin of DNA replication. This transcriptionally activates the primosome assembly site on the lagging-strand DNA template. Subsequent addition of the preprimosomal proteins, the single-stranded DNA binding protein and DNA gyrase leads to the assembly of a preprimosome that can then catalyze extensive unwinding of the DNA. The product of this reaction, form I^{**}, has a specific linking number of about -0.8. Formation of form I^{**} is also inhibited by RNA I and catalytic concentrations of RNase H.

L 372 KINETIC MECHANISM OF T7 DNA POLYMERASE, Smita S. Patel & Kenneth A. Johnson, Molecular and Cell Biology, Pennsylvania State University, University Park, PA 16802

T7 DNA polymerase is a complex of two proteins: the 80 kDa T7 gene 5 protein and the 12 kDa *E. coli* thioredoxin protein. The complex catalyzes highly processive $5' \rightarrow 3'$ polymerization of DNA primer-templates; in addition, it also contains a nonprocessive $3' \rightarrow 5'$ exonuclease activity, which is believed to be important in maintaining replication fidelity. We are interested in studying the kinetic mechanism of T7 DNA polymerase in order to elucidate the dynamics of DNA replication and understand the mechanism of its fidelity. The various elementary steps of the polymerase and exonuclease activities have been studied by presteady state kinetic methods. Synthetic DNA primer-templates of well defined sequences have been successfully employed to quantitate the elementary steps of the polymerase and the exonuclease activities. The binding of T7 DNA polymerase to the DNA occurs at diffusion limited rate and the resulting E·DNA complex has a very low dissociation constant. Under conditions of single nucleotide incorporation, the enzyme displays burst kinetics with a single nucleotide incorporation rate of around 200 s^{-1}. The E·DNA off rate is measured to be around 0.1 s^{-1}. In the absence of nucleotides or the next correct nucleotide, the 3′-bases of the primer are nonprocessively hydrolyzed by the exonuclease activity of the enzyme. The enzyme hydrolyzes single-stranded and melted 3′-bases of duplex DNAs at a rate greater than 150 s^{-1}. The excision rate of a matched 3′-base is considerably slower (1 s^{-1}) compared to a mismatched 3′-base (> 150 s^{-1}), consistant with its role in proofreading mechanism. Further, we have kinetic evidence for existence of two separate sites and a rapid switch of the 3′-end of the primer-template between the two sites.

L 373 REPLICATION-INDUCED DNA AMPLIFICATION IN THE BACTERIAL CHROMOSOME. M.A. Petit, Juan M. Mesas, Philippe Noirot, S. Dusko Ehrlich. Laboratoire de Génétique Microbienne, INRA - Domaine de Vilvert, 78350 Jouy en Josas, France.

Mechanisms of DNA amplification are difficult to study since the frequency of the process is relatively low. We describe a system that circumvents this problem since it allows to induce DNA amplification at a high frequency. It was constructed by inserting in the Bacillus subtilis chromosome a plasmid thermosensitive replicon at the vicinity of a duplication. Within three hours of activation of the plasmid replication, DNA amplification reached an average level of 20 copies of the duplicated sequences (composing an amplification unit, A.U.) per chromosome. DNA anlalysis of the amplified structures showed that these were composed of tandemly arrayed units which were unevenly distributed in the cell population, and that the first detectable molecules were large (more than 20 A.U., and possibly up to 300 A.U.). Intermediate-size structures, between 1 and 20 A.U. associated in tandem, were absent. This argues in favour of a rapid, rolling-circle like mechanism, rather than a step by step, unequal recombination process.

L 374 BIOCHEMICAL CHARACTERIZATION OF MUTATIONS WHICH ALTER THE POLYMERASE ACTIVITY OF KLENOW FRAGMENT. Andrea H. Polesky, Catherine M. Joyce, and Nigel D. F. Grindley. Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

The Klenow fragment structure, together with many biochemical experiments has suggested a region of the protein that may contain the polymerase active site.[1,2] To determine which amino acids are involved in the substrate binding and catalytic steps of the polymerase reaction, we have changed amino acid residues within this region of the polymerase domain by site-directed mutagenesis. Ten mutant proteins have been analyzed with the following in vitro assays: steady state kinetics to measure K_m(dNTP) and k_{cat}, and DNAse I footprinting to measure K_d(DNA) and footprint size. The major effect of mutations at positions 668 (R668A) and 882 (D882A, D882S) is a decrease in k_{cat} of at least 1000 fold. More conservative amino acid substitutions at residues 668 and 882 are currently being studied. A second cluster of mutations at positions 776, 841, and 845, which are close to positions 668 and 882 on the three-dimensional structure, cause an increase in K_m(dNTP) suggesting that contacts with the incoming dNTP are made in this region.

1. Ollis, D. L. et al. (1985) Nature 313:762-766.
2. Joyce, C.M. and Steitz, T.A. (1987) Trends Biochem. Sci. 12:288-292.

L 375 *CIS*-INHIBITORY ELEMENTS IN THE pT181 REPLICATION SYSTEM
Steven J. Projan and Richard P. Novick. The Public Research Insitute, New York, N.Y. 10016

The naturally occurring *Staphylococcus aureus* plasmid pT181 has been used as a prototype for the study of replication in gram positive bacteria. Replication from the pT181 origin requires RepC, a pT181 encoded protein that acts in *trans* to initiate plasmid replication. It has previously been shown that the pT181 origin can be complemented in *trans* when it is cloned onto a temperature sensitive vector. However, when cointegrate plasmids are constructed with pT181 and an unrelated, compatible plasmid, pE194, replication from the pT181 origin is inhibited even though the origin sequence has not been disrupted and RepC is supplied by a donor plasmid in *trans*. We have identified two loci on the pT181-pE194 cointegrates that are responsible for the observed *cis*-inhibition. One is the pE194 *palA* sequence, which has been identified as a lagging strand origin of replication for plasmids in *S. aureus*. The second inhibitory locus maps to the promoter region for the pT181 countertranscripts (PrCt), small antisense RNAs that regulate pT181 replication by controlling the amount of RepC produced. *Cis*-inhibition requires both *palA* and PrCt, since deletion or mutation of either locus restores the ability of the pT181 origin to initiate replication when RepC is supplied in *trans*. *Cis*-inhibition is apparently unrelated to the function of the pE194 *palA* sequence as a lagging strand origin but probably occurs at a stage after the binding of RepC to the pT181 origin.

L 376 ABSENCE OF COPY NUMBER CONTROL OF MINICHROMOSOMES, M. Roland Jensen, A. Lobner-Olesen and K. V. Rasmussen, Institute of Microbiology, University of Copenhagen, 1353 Copenhagen K, Denmark

As model systems for bacterial chromosome replication minichromosomes, i. e. plasmids that can replicate from an integrated *oriC*, have been puzzling because of (1) their high copy number compared to that of the chromosomal *oriC*, (2) the absence of incompatibility with the chromosome, and (3) their high loss frequencies . We have followed the development of minichromosome copy number distributions in E. coli cells that have been transformed with minichromosomes and are then allowed to grow towards the steady state. This was done using single cell resistance to tetracycline or ampicillin as an indicator of copy numbers. We show that these cells must grow for a large number of generations before the very broad minichromosome copy number distribution characteristic of steady state growth is established. If the minichromosome carries the *sop* genes from plasmid F, the development of the copy number distribution is further drastically delayed.

All the observations mentioned, old as well new ones, can be understood on the assumption that E. coli cells have no function that directly controls chromosomal or minichromosomal copy numbers.

L 377 STRUCTURAL AND FUNCTIONAL DISSECTION OF THE EXONUCLEASE ACTIVITY OF T4 DNA POLYMERASE, Mary Kay Dolejsi, Michael Reddy, Mary Dasso, John Newport and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

In an ongoing attempt to understand the molecular bases of processive DNA synthesis and editing, we have investigated the physical interactions between T4 DNA polymerase and its DNA substrates by kinetic and thermodynamic means. We have concentrated on characterizing the exonucleolytic digestion of various DNA substrates as an approach by which to determine the molecular details of processivity. Under conditions of single-hit kinetics, we have measured the processivity of the T4 DNA polymerase while in its exonuclease mode. The exonuclease has a probability of 0.85 for continuing exonucleolytic degradation of a single-stranded DNA substrate that is greater than 20 nucleotide residues in length. Processivity drops as the substrate length decreases, and the exonuclease becomes non-processive at lengths shorter than 15 residues, suggesting that this represents a minimal functional site size.

The number of electrostatic interactions between the polymerase and various DNA substrates have been elucidated. Binding to single-stranded DNA (in the absence of Mg^{2+}) involves 3-4 electrostatic interactions. However, binding of polymerase to its functional substrate, a primer-template, appears to involve only 1-2 electrostatic interactions. An extrapolated binding affinity (K_{1M}), representative of the non-electrostatic contribution to the binding free energy, is approximately 70 M^{-1} for single-stranded internal DNA; and for the the primer-template structures approximately 4 x 10^4 M^{-1}. This suggests that the polymerase has an inherent specificity for primer-template structures that is masked at typical salt concentrations of our *in vitro* reactions. Currently we are probing the structure of the polymerase bound to the primer-template structures by various methods, and examining further its proccessivity (as an exonuclease) on such structures.

L 378 **IDENTIFICATION OF PHAGE T4 DNA POLYMERASE FUNCTIONAL DOMAINS**, Linda J. Reha-Krantz, Shelley Stocki, Randy Nonay and Cheryl Maughan, Department of Genetics, University of Alberta, Edmonton, Alberta T6G 2E9, CANADA. We have developed a new approach to identify T4 DNA polymerase functional domains that is based on the *optA*-sensitive (*optA^S*) phenotype of certain T4 DNA polymerase mutant strains. The *E. coli* mutant strain, *optA1*, restricts growth of phage T7*1.2* (one point two) mutants because of decreased levels of dGTP due to increased amounts of a novel dGTPase (Seto, D. *et al.* (1988) *J. Biol. Chem.* **263**, 1494-1499, Beauchamp, B. B. & Richardson, C. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 2563-2567). Certain T4 DNA polymerase mutant strains are also restricted on the *optA1* host, presumably because of decreased dGTP. One type of T4 DNA polymerase mutant that is restricted on the *optA1* strain is exemplified by the antimutator mutants, *tsL141* and *tsL42*. The antimutator phenotype, i.e. increased accuracy of DNA replication, is due, in part, to the high levels of 3' -> 5' exonucleolytic-proofreading activity in the mutant DNA polymerases (Gillen, F. D. & Nossal, N. G. (1976) *J. Biol. Chem.* **252**, 5219-5224). Increased 3' -> 5' exonuclease activity results in excessive hydrolysis of dNTPs; the depletion of dNTP pools, especially dGTP, is presumed to account for the inability of the antimutator DNA polymerase strains to grow on the *optA1* host. We have obtained evidence that supports the hypothesis that the proofreading activity is responsible for the *optA^S* phenotype. Second-site compensating mutants have been isolated that suppress both the *optA^S* and antimutator phenotypes of the *tsL141* and *tsL42* strains. Most of the compensating mutants produce amino acid substitutions near the *tsL141* and *tsL42* sites and, thus, may define interacting protein regions. We believe that this region may be important for DNA binding.

The *optA^S* phenotype can also be used to identify amino acids that participate in dNTP binding. A mutant DNA polymerase with decreased affinity for dNTPs may also have an *optA^S* phenotype. We have identified putative dNTP-binding amino acids in a region of the T4 DNA polymerase that contains a highly conserved amino acid sequence, S *LYPSI *I. An *I->V substitution produces *optA^S* that can be suppressed by a second mutation, *L->M, within the conserved sequence. These strains and our overall strategy to use *optA^S* to probe T4 DNA polymerase functional domains will be discussed.

L 379 THE ROLE OF HEAT-SHOCK PROTEINS IN SUPPRESSION OF THE TEMPERATURE-SENSITIVE *ssb-1* MUTATION OF *E. COLI* Ralph R. Meyer, Phyllis S. Laine and Diane C. Rein. Dept. of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221.

In *E. coli*, exposure of cells to elevated temperatures results in the induction of at least 17 different polypeptides. This "heat-shock response" protects the cells from thermal killing. We have recently demonstrated a relationship between the major heat-shock protein *groEL* and DNA replication. We have isolated a revertant to *ssb-1*, and by cloning have found an allele of *groEL* (*groEL411*) that suppresses the temperature-sensitive (ts) phenotype of cells carrying the DNA-binding protein mutation (Ruben *et al., Proc. Nat. Acad. Sci., USA* 85:3767, 1988). In examining DNA synthesis in these strains, an interesting observation was made. Upon shift to 42.5°C, there is a transient temperature-sensitivity of DNA synthesis: within one minute DNA synthesis stops, but then resumes again at its normal rate within five minutes. In investigating the mechanism of suppression, we have now shown that heat-shock induction is required, since blocking RNA or protein synthesis just prior to temperature-shift abolishes the transient response, and the cells remain ts for DNA synthesis. Moreover, this recovery is not solely dependent upon induction of the *groEL411* suppressor, since strains carrying a *groEL411* plasmid and already over-producing GROEL411 protein still cannot restore DNA synthesis at 42.5°C if heat-shock induction is blocked. This has prompted us to search for additional genes required for suppression of *ssb-1*. At the same time we have been unable to transduce the *groEL411* allele in a single-copy number into other genetic backgrounds containing *ssb-1*. This has led to the discovery of a second suppressor, currently named *sup304*, which appears to be required along with *groEL411* to suppress *ssb-1* when both of these suppressors are present in a single chromosome copy. When overproduced, however, GROEL411 protein alone is sufficient for suppression. The identity of this second suppressor and whether it is also a heat-shock protein have not as yet been determined.

Molecular Mechanisms in DNA Replication and Recombination

L 380 COMPLEX FRAMESHIFTS PRODUCED IN VITRO BY DNA POLYMERASE I AND ITS KLENOW FRAGMENT: THE MOLECULAR BASIS OF MUTAGENIC SPECIFICITY,

Lynn S. Ripley, Department of Microbiology and Molecular Genetics, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

Complex frameshift mutations that have changes in DNA sequence as well as numbers of nucleotides occur at significant frequencies during in vitro elongation reactions catalyzed by E. coli DNA polymerase I or its Klenow fragment. The DNA sequences of the mutations are accounted for by the misalignment of the primer terminus to alternative positions on the newly synthesized strand, followed by a brief synthesis reaction, followed by realignment of this DNA to the original template. Some of these in vitro mutations arise by a strand-switch mechanism resembling that previously hypothesized to account for complex frameshift mutations arising in vivo (Ripley, Proc. Natl. Acad. Sci. 79:4128, 1982).

Polymerase I and its Klenow fragment differ in their mutational specificity, and these differences suggest that there are polymerase-specific preferences for creating or utilizing misalignments in particular sequences. The molecular basis for these specificity differences is under investigation. The results presently suggest that DNA polymerase is a major determinant of specificity and that DNA sequence homologies, while necessary, are not sufficient for the definition of the major hotspots for mutation.

L 381 rip, A GENE THAT SUBSTITUTES FOR RecA IN STABLE DNA REPLICATION IN E. coli rnh MUTANTS, Raymond R. R. Rowland and Tokio Kogoma, Departments of Cell Biology and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

dnaA::Tn10 rnh double mutants are viable because of the presence of an alternative DNA replication pathway (i.e., constitutive stable DNA replication; cSDR) activated by the rnh mutation. cSDR requires a functional RecA protein and thus dnaA::Tn10 rnh recA200(Ts) mutants are temperature-sensitive for cSDR and growth. Previously, we reported that lexA(Def) mutations suppress the Ts phenotype of the triple mutant (Torrey and Kogoma, Mol. Gen. Genet. 208:420-427, 1987). Thus, dnaA::Tn10 rnh recA(Ts) lexA(Def) mutants can grow at 42 °C. This suggests that a gene product, under LexA control, can substitute for $RecA^+$ in this process. In order to identify the hypothetical gene, rip (for recA-independent process), a dnaA::Tn10 rnh recA(Ts) lexA(Def) strain was mutagenized with a mini-Tn10(Spc^r) hopper, and the Spc^r mutants were screened for Ts growth. Ts mutants were found at approximately 3 x 10^{-4} among the Spc^r mutants. One such Ts mutant, rip-25::Spc^r, was characterized. The mutant cannot perform cSDR at 42 °C and is extremely sensitive to UV irradiation regardless of the temperature. rip-25::Spc^r is not a recA mutation because the insertional inactivation of recA would have been lethal to the strain at any temperature. Yet, these phenotypes can be complemented by the presence of a plasmid bearing $recA^+$. The rip-25 mutant allele has been cloned.

L 382 MutT-INDUCED DNA REPLICATION ERRORS IN E. COLI.

Roel M. Schaaper , Ronnie L. Dunn, Robin Cornachio, and Robert G. Fowler*. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and * Department of Biological Sciences, San Jose State University, San Jose, CA 95111

E. coli mutT is a strong mutator producing exclusively A•T->C•G transversions. We show that DNA replication in mutT-derived cell-free extracts (M13mp2 ss->RF conversion) is less accurate than in wild-type extracts (as measured by reversion of mp2 lacZα nonsense codons) due to increased (template A)•G mispairings. Mismatch repair of heteroduplexes containing A•G or other mispairs is normal in mutT. These combined suggest that the $mutT^+$ gene product works directly during DNA replication, most likely by preventing the formation of A•G mispairs. A•T->C•G mutagenesis in mutTmutL mutators is not increased over the single mutT alone, indicating that mutT-induced A•G mispairs may not be subject to mismatch repair. This is not necessarily true for A•G mispairs generated in other backgrounds. These findings have implications for the nature of A•G replication errors in the various backgrounds.

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L 383 A POST-INITIATION CONTROL OF CHROMOSOMAL REPLICATION IN *B.SUBTILIS*, Simone J. Séror, Gilles Henckes, Alain Levine and F. Vannier, Institut de Microbiologie, Bât. 409, Université Paris XI, 91405 Orsay, Cedex 05, France.

DnaB is a DNA-binding protein specifically required for the initiation of DNA replication from two closely linked origins on the *B.subtilis* chromosome. When the *dnaB* 37 mutant is returned to permissive temperature, after accumulation of initiation proteins at 45°C, we have shown, using extensive DNA-DNA hybridization analysis, that the ori-region is specifically over-replicated (approximately two fold). This replication is however limited to a region of about 150 kb either side of the origin region. This has been confirmed by autoradiographic analysis of the amplified region. During the second round of synchronized replication at 30°C, replication in fact appears to resume from the stalled forks flanking the origin. We conclude that, in *B. subtilis*, in addition to a first level at the origin, a second level of control exists outside the origin in order to limit over-replication of the chromosome. These two controls might normally be tightly coupled. We have previously shown that initiation, in *B. subtilis*, is subject to control by the RelA system (Séror et al, 1986, Nature *321*, 709). Interestingly, this control acts, not at the origin, but approximately 150 kb outside the origin. When the Stringent response is removed, DNA replication resumes from the stalled forks. Resumption of replication in this case, is independent of protein synthesis and DnaB. We suggest that a specific control region, either side of oriC, may facilitate, under certain conditions, the reversible inhibition of fork movement via the modification of a replisome component.

L 384 COMPARISON OF THE *Proteus mirabilis* AND *Escherichia coli dnaA* GENES. **Ole Skovgaard, Institute of Life Sciences and Chemistry, University of Roskilde, DK-4000 Roskilde, Denmark.**

The DnaA protein is essential for, and has a regulatory role in, the initiation of DNA replication from *oriC* in *E. coli*. The protein binds to: i) itself, forming an oligomeric complex, ii) DNA at recognition sequences known as DnaA-boxes and iii) ATP. Binding to other proteins has been suggested. I have cloned a 6.2 kb. DNA fragment containing the *P. mirabilis dnaA* gene and surrounding genes, in order to investigate the relationship between these functions and specific domains of the DnaA protein. The *P. mirabilis dnaA* gene complements the *dnaA46* mutation of *E. coli*. The *P. mirabilis dnaA* gene was sequenced and the aminoacid sequence of the DnaA protein was compared with the *E. coli* DnaA protein. A region with only 50 % conserved aminoacids coincides with the variable region defined previously. This region separates two highly conserved regions. There are two new sites with three aminoacids out of six and seven aminoacids, respectively, exchanged in the large conserved region. The variable region and these sites may limit some functional regions of the DnaA protein.

L 385 SITE-SPECIFIC MUTAGENESIS OF BACTERIOPHAGE T4 DNA POLYMERASE, Donald O'Hearn, Eloisa DiMayuga, Tsung-chung Lin, William H. Konigsberg, and Eleanor K. Spicer, Department of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510.

T4 DNA polymerase has at least five regions of amino acid sequence that are similar to corresponding regions in both procaryotic and eucaryotic DNA polymerases. To evaluate the role of conserved residues in the 3'-5' exonuclease and polymerase reactions, we have introduced specific mutations into the T4 DNA polymerase gene (g43) carried on an inducible overexpression vector (pTL43Q). Mutations were introduced at codons corresponding to residues D189 and E191A to test their possible involvement in the 3'-5' exonuclease activity of the enzyme. In vitro assays indicate both D189A and E191A derivatives have wild type levels of polymerase and 3'-5'exonuclease activity. However, in vivo studies by L. Reha-Krantz (University of Alberta) demonstrate that E191A polymerase has a low-level mutator phenotype that is elevated in the presence of 2-amino purine. An insertion mutation at residue 678 had a more dramatic effect, producing an enzyme lacking polymerase but retaining 3'-5' exonuclease activity. Truncation of the structural gene to give a protein of 650 rather than 898 residues resulted in a derivative that produced an enzyme that retained 3'-5'exonuclease activity but lacks polymerase activity. These results are consistent with the hypothesis that T4 DNA polymerase, like E. coli polymerase I, has separate functional domains for 3'-5' exonuclease and polymerase activities, and that the C-terminal third of the enzyme contains some of the amino acid residues required for DNA synthesis.

L 386 EFFECT OF λ REPLICATION PROTEINS ON DnaB HELICASE ACTION, Kathryn Stephens and Roger McMacken, Department of Biochemistry, The Johns Hopkins University, Baltimore, MD 21205

Using purified λ and *E. coli* replication proteins, we have established an *in vitro* system that supports the replication of *oriλ* plasmid templates. We had earlier reported the reconstitution of a similar system that replicates single-stranded circular chromosomes in a reaction that is dependent on the λ O and P replication proteins. Biochemical studies indicate that in both of these systems the λ O and P initiators promote the assembly of nucleoprotein structures containing the *E. coli* DnaB helicase. These prepriming complexes are functionally inert, however, since the helicase and ATPase activities of DnaB are completely suppressed by a tight association with the λ P protein. In each system the *E. coli* DnaJ and DnaK heat shock proteins act to restore DnaB function by catalyzing the removal of P protein from the nucleoprotein complexes. We have constructed an artificial rolling-circle substrate so that the effects of the λ initiators and the *E. coli* DnaJ and DnaK heat shock proteins on DnaB helicase function could be assessed. In this system DnaB helicase unwinding promotes DNA chain elongation by the DNA polymerase III holoenzyme. Our preliminary results indicate that the presence of O, P, DnaJ and DnaK greatly increases the efficiency of initiation of DNA unwinding and rolling circle DNA synthesis, but the rate of unwinding (~ 500 bp/sec at 30°C) is not affected. Once DNA unwinding is initiated by DnaB, helicase movement is not sensitive to inhibition by added λ P protein. However, P protein rapidly blocks DnaB from initiating unwinding when P is added in the absence of O, DnaJ and DnaK.

L 387 CYCLING OF DNA POLYMERASE III HOLOENZYME APPEARS TO BE MEDIATED BY DIRECT INTERACTION BETWEEN ACCESSORY PROTEINS. Patricia S. Studwell and Mike O'Donnell. Microbiology Department, Cornell University Medical College NY, NY 10021.

DNA polymerase III holoenzyme (pol III H.E.) is the multiprotein replicase of E.coli and is composed of 10 different subunits. Pol III H.E. can be reconstituted in two parts: the polymerase core subassembly which contains the polymerase (α), the 3'-5' exonuclease (ϵ) and θ; and the accessory proteins, β and the γ complex ($\gamma\delta\delta'\chi\psi$), which form an ATP-activated "preinitiation complex" (PIC) with a primed DNA template. The remaining subunit, τ, is not absolutely required in the reconstitution assay but stimulates the reaction. We have constituted and purified an $\alpha\epsilon$ complex which was compared to α and core to determine the minimum core requirements for for rapid and processive synthesis characteristic of the holoenzyme. We extended the study to include the minimum subunit requirements for polymerase cycling from a completed template to another PIC on an acceptor primed template. Although the α subunit was stimulated many fold by the PIC it was only processive for 1-2 kb. The $\alpha\epsilon$ complex was as efficient as core in reconstituting a fully processive polymerase and they both cycled to a new template within 20s, hence θ is not required for these activities. We were also able to isolate an $\alpha\epsilon\tau$ complex. Although $\alpha\epsilon\tau$ and $\alpha\epsilon$ showed similar speed, processivity and cycling rates, they had strikingly different rates of binding a PIC (> 2min for $\alpha\epsilon$ vs. 5s for $\alpha\epsilon\tau$). Interestingly, $\alpha\epsilon$ is able to cycle to a new template in much less time than $\alpha\epsilon$ in free solution can find a PIC. This indicates that the polymerase is handed directly from the accessory proteins on the completed template to the PIC on an acceptor template. On the basis of this data we discuss models for the cycling of pol III H.E. to multiple primers on the lagging strand of a moving replication fork.

L 388 DNA POLYMERASE III HOLOENZYME ACCESSORY PROTEINS - MECHANISM OF PREINITIATION COMPLEX FORMATION

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The multiprotein replicase of Escherichia coli, DNA polymerase III holoenzyme (Pol III H.E.), can be reconstituted onto a primed DNA by mixing the polymerase core ($\alpha\epsilon\theta$) and 6 of the 7 accessory proteins. During the reconstitution process the accessory proteins β and the γ-complex ($\gamma\delta\delta'X\psi$) form a preinitiation complex on the primed and SSB "coated" ϕX ssDNA (5.4kb) in a reaction that depends on ATP. The polymerase core then binds tightly to the ATP-activated preinitiation complex to form the rapid and processive polIII H.E. Addition of dNTPs results in rapid and fully processive replication of the large circular DNA after which the polymerase quickly dissociates from the RFII circle and cycles to another preinitiation complex on a new primed template. Hence, the accessory proteins confer onto the polymerase two seemingly opposite properties: tight association to the DNA primer terminus for greater processivity and rapid dissociation of polymerase from the DNA product. With the eventual goal of understanding how the accessory proteins work in both of these reactions we have been exploring the assembly mechanism of the preinitiation complex and its fate after DNA replication. The minimal subunit requirements for the preinitiation complex, order of steps in its assembly, the rate limiting step in its formation, and identity of subunits bound to DNA before and after replication will be presented.

Molecular Mechanisms in DNA Replication and Recombination

L 389 TWO TYPES OF MEMBRANE BINDING FOR BOTH THE ORIGIN AREA OF THE CHROMOSOME AND A PLASMID pUB110 IN *BACILLUS SUBTILIS*, Noboru Sueoka, Timothy McKenzie, Marianne McCollum and Gale Etherton --- Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309 In the past, we have recognized two types of membrane binding of a plasmid pUB110 in *Bacillus subtilis* (type-I and type-II). Type-I binding is salt resistant, its binding site is near the plasmid replication origin. Previously, we had also recognized type-I membrane binding of the oriC region of the *B. subtilis* chromosome. Type-I binding may be an essential part of the initiation of replication. An initiation mutant *dnaB* at nonpermissive temperature loses both replication and membrane binding and the ability to initiate DNA replication in pUB110 and in the *B. subtilis* chromosome. Cloning of the *dnaB* gene of *B. subtilis* revealed that the *dnaB* locus is the first of 3 or 4 polycistronically arranged genes (Hoshino et al., 1987; Ogasawara et al., 1986). Type-II binding of pUB110 was discovered by *in vitro* membrane binding experiments with pUB110 and the membrane fraction from *B. subtilis* that did not harbor the plasmid. Type-II binding is salt sensitive and independent of the *dnaB* function. Recently, we identified an area close to the *purA* locus of the *B. subtilis* chromosome; the *purA* locus is located about 20 to 50 Kb from the replication origin. We have examined and found that the *purA* area indeed contains the major site for the type-II binding site(s). Currently, we do not know the functional role of type-II membrane binding for chromosome replication. It is likely, however, that type-II binding plays an important role in either partition or replication initiation of the chromosome in conjunction with type-I binding. The properties deduced from the DNA sequence of the genes of the *dnaB* operon and the two types of membrane binding led us to propose an existence of a super-structure in the membrane which holds replicons of *B. subtilis* and provides structure essential for their initiation of replication. From the DNA sequence of the regions of oriC, type-II binding and pUB110, the state of DNA methylation does not seem critical for those areas to bind to membrane in *B. subtilis*.

L 390 THE STBB PRODUCT OF THE STABILITY LOCUS OF INCFII PLASMID NR1 MAY BE AN AUTOREPRESSOR OF THE STB OPERON, Akira Tabuchi, You-nong Min, Choon Kwang Kim, Xiao Buo Tang, David D. Womble, and Robert H. Rownd, Department of Molecular Biology, The Medical and Dental Schools, Northwestern University, Chicago, IL 60611. The stability (stb) locus of plasmid NR1 is located within a 1691 bp NaeI-TaqI restriction fragment. Miniplasmids composed of the repA replicon and the stb locus of NR1 are inherited stably in both PolA$^+$ and PolA$^-$ host cells. According to nucleotide sequence analysis of the stb locus, there are 3 open reading frames: stbA, stbB, and stbC. stbA and stbB are arranged tandemly and share a common transcription promoter, P_{AB}, to form the stbAB operon. stbC is contained within stbB in the opposite DNA strand. In minicell experiments, protein products of 36,000 M_r and 13,000 M_r were identified as products of stbA and stbB, respectively. No protein corresponding to a stbC product was detected. In similar experiments, mutants that have deleted stbB and the 3' end of stbA produce large amounts of truncated StbA protein. Although we have cloned the stbB gene by itself as well as the entire stbAB operon, it has so far not been possible to clone an intact stbA gene in the absence of stbB. Several P_{AB}-lacZ transcriptional fusions were constructed to test the repressor function of StbA and StbB. The β-galactosidase activity from these lac-fusions was repressed by the presence in trans of stbB and also by stbA plus stbB. The trans repressor activity of either stbA$^+$ stbB$^-$ or stbA$^-$ stbB$^+$ point mutant loci was less than that of a wild-type stbA$^+$ stbB$^+$ locus. These results suggest that StbB protein by itself has autorepressor activity, and that StbA protein may also contribute to repression.

L 391 DNA INTERACTIONS OF THE τ SUBUNIT OF DNA POLYMERASE III HOLOENZYME, Zenta Tsuchihashi and Arthur Kornberg, Department of Biochemistry, Stanford University, Stanford, CA 94305

The τ and γ subunits of DNA polymerase III holoenzyme of E. coli were purified separately from a novel strain overproducing the dnaZX gene. Each binds one ATP but only τ showed a DNA-dependent ATPase activity (similar to that found in a τ/β-gal fusion by J. R. Walker *et al.*). A striking feature of the τ-ATPase is a requirement for single-stranded DNA not supplied by homopolymers.

	Relative rates of ATP hydrolysis		
φX174 ssDNA	(100)	poly(dT)	3
M13Gori1 ssDNA	115	poly(dA) · poly(dT)	8
Linearized plasmid DNA	15	poly(dA-dT)	11
Heat denatured linearized plasmid DNA	95	poly(dI-dC)	8
Poly(dA)	2	no DNA	1

We conclude that some form of secondary structure is needed. Were a hairpin region the true effector for τ-ATPase activity, the τ subunit might change the conformation of hairpin DNA regions in the template (using the energy derived from ATP hydrolysis) to prevent pausing or dissociation of DNA polymerase during replication.

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L 392 MINI-P1 DNA REPLICATION IN VITRO: ROLE OF CONTROL LOCUS, Sue Wickner[1], Dhruba Chattoraj[2], Joel Hoskins[3], Keith McKenney[3], [1]LMB, NCI, NIH, Bethesda, MD 20892; [2]LB, NCI, NIH, Bethesda, MD 20892; [3]CARB, NIST, Gaithersburg, MD 20899.

The mini-P1 replicon consists of an origin of replication, a replicon specific initiator protein, RepA, and a control locus. The origin contains 5 direct repeats of a 19 bp sequence to which the RepA protein binds. The control locus, located 1 kb to the right of the origin, is required to maintain mini-P1 plasmids at about one copy per chromosome. This region contains nine repeats of the 19 bp RepA binding site. We have cloned the origin with and without the control region in M13 and tested the RF DNA for RepA-dependent replication in vitro. Using reaction conditions that allow replication of DNA carrying the origin region, plasmid DNAs carrying in addition the control region are not replicated even when RepA is added in excess relative to binding sites. In vitro, we have seen by electron microscopy that RepA binds to the origin and control region simultaneously, causing the intervening DNA to loop. Our interpretation is that this looping inhibits initiation. Replication in vitro is restored when six or more of the nine RepA binding sites are deleted from the control region. Replication is also restored when wild-type RepA protein is substituted with RepA protein isolated from a repA mutant that has an increased copy number in vivo.

L 393 THE REPA REPLICON OF INCFII PLASMID NR1: ELEMENTS AFFECTING INITIATION OF REPLICATION AT THE ORIGIN, David D. Womble, Xinnian Dong, Xiao Buo Tang, You-nong Min, Branko Latinkic, and Robert H. Rownd, Department of Molecular Biology, The Medical and Dental Schools, Northwestern University, Chicago, IL 60611. The repA1 gene of plasmid NR1 encodes the plasmid origin-specific replication initiation protein. The RepA1 protein interacts in cis with the replication origin located 187 bp downstream from the 3' end of the repA1 gene. When the RepA1 binding sites are deleted from the origin locataed downstream from a repA1 gene cloned in a high expression vector plasmid, then RepA1 protein can act in trans. If the binding sites are deleted, in vivo activity of RepA1 provided in trans can be detected by an elevation of copy number of coresident miniplasmids that contain the NR1 origin. The presence of the origin sequences downstream from repA1 also affects the yield of RepA1 protein detectable by SDS-PAGE during overexpression from a high copy number vector. To obtain high protein yield, either the origin sequences must be deleted or the initiation protein must be inactivated by mutation. The DNA sequences in the 187 bp between the 3' end of repA1 and the replication origin contain static DNA bending loci. DNA bending in this intervening region may serve to align the origin in a favorable orientation with respect to repA1. This could influence the transfer in cis of nascent RepA1 initiation protein to the origin and enhance the efficiency of initiation of replication. The effects on plasmid copy number of deletion or insertion mutations within the intervening region may also be consistent with this hypothesis. There is also a DnaA box located immediately upstream from the minimal replication origin. Plasmids from which the DnaA box has been deleted can still replicate. Also, NR1-derived miniplasmids can replicate in dnaA null mutant host cells. However, both plasmid copy number and stable inheritance are reduced in the absence of DnaA. Therefore, DnaA may play an auxiliary role in NR1 replication initiation. Plasmid mutants with deletions or insertions downstream from the minimal origin also exhibit reduced stability of inheritance, even when such plasmids carry the stb locus for plasmid partition. This region downstream from the origin contains the repA4 gene of unknown function and also contains homology to the terminus of E. coli chromosomal replication.

L 394 **PURIFICATION AND CHARACTERIZATION OF UMUD, UMUD', AND UMUC: PROTEINS REQUIRED FOR SOS MUTAGENESIS IN *ESCHERICHIA COLI***

Roger Woodgate, Malini Rajagopalan, Chi Lu and Harrison Echols.
Department of Molecular Biology, University of California, Berkeley CA94720

The mutation rate of *Escherichia coli* increases 100-fold after treatment with replication-inhibiting agents such as UV light. This enhanced mutation rate requires the action of the UmuD and UmuC proteins, which are induced as part of the SOS response to DNA damage. We have previously reported the overproduction and purification of the UmuD protein and shown that UmuD undergoes proteolytic processing by a similar mechanism to that previously described for LexA protein, the repressor of the SOS genes (Burckhardt et al (1988) Proc. Natl. Acad. Sci. USA 85: 1811-1815). We report here that the amino-terminal sequence of the larger cleavage fragment, which we term UmuD', agrees with that predicted by the DNA sequence if UmuD were cleaved at the cys-gly site proposed on the basis of homology with LexA (Perry et al (1985) Proc. Natl. Acad. Sci. USA 82: 4331-4335). To purify substantial quantities of UmuD', we treated cells with Mitomycin C prior to induction of the overproducing UmuD,C vector. We have purified UmuD' protein and a mixture of UmuD/UmuD' proteins. Genetic evidence has suggested that UmuD (and/or UmuD') interact with UmuC. We have coupled the mixture of UmuD/UmuD' to affi-gel support beads and constructed a UmuD/UmuD' protein affinity column. We have used the UmuD/UmuD' affinity column to purify UmuC protein. UmuC was specifically retained on the affinity column and was not dissociated by 2M NaCl. UmuC protein was eluted from the column in the presence of 2M Urea or 1.5M KSCN. These data suggest a strong protein-protein interaction between UmuD/UmuD' and UmuC.

Molecular Mechanisms in DNA Replication and Recombination

L 395 **FACTORS AFFECTING OKAZAKI FRAGMENT SYNTHESIS AT REPLICATION FORKS FORMED WITH THE E. COLI PRIMOSOME AND DNA POLYMERASE III HOLOENZYME,** Ellen Johnson, Carol Wu, Minsen Mok and K. J. Marians, Sloan-Kettering Institute, New York, NY 10021. Rolling-circle DNA replication supported by tailed form II DNA templates in the presence of the DNA polymerase III holoenzyme (Pol III HE) and the primosomal proteins has been used as a model system to examine mechanisms operating at the replication fork. The long double-stranded tails produced in this reaction are constituted of a long (>50 kb) leading-strand DNA and a family of short Okazaki fragments representing synthesis of the lagging strand. Factors influencing the cycling of the enzymatic machinery operating on the lagging-strand DNA template can be observed by determining their effect on the size and distribution of the Okazaki fragments produced.

At least three general parameters influence synthesis of the lagging strand: i) The frequency at which primers are synthesized and utilized, ii) the speed at which the replication fork moves and, iii) the distributive nature of the action of some of the enzymes, including the primase and some subunits of the Pol III HE.

L 396 CHARACTERIZATION OF A PROTEIN REQUIRED FOR SIGNIFICANT UNWINDING BY REP HELICASE, J.E. Yancey, K.R. Smith, K.A. Kaiser-Rogers and S.W. Matson, Dept. of Biology, Univ. of North Carolina, Chapel Hill, N.C. 27599. Rep helicase catalyzes a limited unwinding reaction *in vitro*. The low extent of this reaction led to the search for factors which could enhance the helicase activity of Rep protein. The reaction is not stimulated by *E. coli* SSB, however, a 15 kDa protein which does stimulate the unwinding activity was isolated and purified. Rep helicase stimulatory protein (RHSP) exhibits no helicase or ATPase activity alone. Immunoblot analysis demonstrates that RHSP is not SSB. It does not stimulate the reaction catalyzed by helicase I or helicase IV, but specifically enhances the unwinding reaction catalyzed by Rep helicase and helicase II up to 20-fold with little effect on ATPase activity. The DNA helicase reaction kinetics indicate that RHSP increases the extent of DNA unwinding by Rep protein. The reaction rate remains dependent on Rep helicase concentration. The stimulation may result from an interaction between RHSP and ssDNA. Filter binding experiments and RHSP-DNA substrate preincubation experiments are consistent with this hypothesis. High concentrations of RHSP inhibit the helicase and ATPase activities of Rep protein. Excess RHSP may coat the substrate molecule such that the helicase cannot enter the duplex region. A specific interaction between the helicase and RHSP cannot be ruled out. Preliminary amino terminal sequence analysis suggests that RHSP may be *E. coli* ribosomal protein L14.

L 397 FUNCTIONS OF T4 TERTIARY ORIGINS IN THE PHAGE LIFE CYCLE, W.Y. Yap and K.N. Kreuzer, Department of Microbiology, Duke University, Durham, NC 27710 Bacteriophage T4 utilizes at least three modes of replication initiation. Primary initiation is rifampicin sensitive, secondary initiation requires phage recombination proteins (e.g., gp46/47), and tertiary initiation is both rifampicin resistant and independent of gp46/47. We have developed plasmid model systems to study replication via the secondary and tertiary modes. Plasmids that replicate via the secondary mode do so only in the presence of phage recombination proteins and must also bear homology to the infecting phage DNA. Plasmids that replicate via the tertiary mode require specific replication origin DNA sequences. Each tertiary origin contains a T4 middle mode promoter, however, not all T4 middle promoters function as tertiary origins. The two known tertiary origins map to recombination hotspots, suggesting a relationship between replication and localized homologous recombination. A minimal tertiary origin has been inserted into a region of the T4 genome that is not a recombination hotspot, and studies are under way to determine if this inserted origin stimulates recombination. A T4 mutant lacking one of the tertiary origins was constructed to assess the importance of tertiary origins in the phage life cycle. Comparisons of this deletion mutant and a control phage strongly suggest that tertiary origins are important for T4 DNA replication, especially at elevated temperatures.

L 398 STUDIES ON THE SINGLE-STRANDED-DNA-STIMULATED NTPASE ACTIVITY OF GENE 41 PROTEIN OF PHAGE T4, Mark Young, Derk Schultz, Sigrid Kuhl, and Peter H. von Hippel, Institute of Molecular Biology, The University of Oregon, Eugene, Oregon, 97403.

The gene 41 protein (g41p) of bacteriophage T4 is the helicase component of the phage replication apparatus. The helicase also has a single-stranded-DNA(SS-DNA)-stimulated NTPase activity. This activity was used as a probe of DNA-gene-41-protein interactions, and protein-protein interactions with another component of the T4 replication complex, the gene 61 protein (g61p). Various SS-DNA effectors, mostly homopolymers, were assayed for ability to stimulate the NTPase of g41p. Both ATPase and GTPase activities of g41p are stimulated by oligomers of length 8 and larger. Both K_m and V_{max} depend on the length of polymer used. There is also a sequence dependence of NTPase activation; pyrimidine homopolymers are more effective than purine homopolymers. Pyrimidine homopolymers of average length ~800 bases are as effective activators as SS circular M13 DNA. Implications of this length and sequence dependence for the mechanism of interaction of the protein with DNA are discussed. Interactions of g41p with the g61p are studied by examination of the effect of g61p on the g41p NTPase rate in the absence and presence of DNA. Addition of g61p removes the length dependence of the NTPase of g41p on dT oligomers, indicating an interaction of the two proteins on SS-DNA.

L 399 DnaA PROTEIN IN INITIATION OF REPLICATION FROM THE ORIGIN (oriC) OF THE E. COLI CHROMOSOME, Benjamin Y.-M. Yung and Arthur Kornberg, Stanford University School of Medicine, Stanford, CA 94305.

The dnaA initiator protein in its operation on oriC successively (i) binds to its four 9-mer boxes to form an initial complex, (ii) binds and subsequently melts the three tandem 13-mers in an adjacent AT-rich region to form an open complex, and (iii) enables dnaB protein to enter this melted region to form a prepriming complex, from which the helicase actions of dnaB protein generate the future forks of bidirectional replication. ATP, tightly bound to dnaA protein, is required for its actions; the ADP form is inactive. HU protein and an adequate temperature (>25°) stabilize the interaction of ATP-dnaA protein with the 13-mer sequence; ADP-dnaA protein binds but rapidly dissociates from the 13-mer region. Whether binding of the 13-mers depends on their sequence (unrelated to the 9-mers) or their inherent "unwindability" remains uncertain. Binding of dnaA protein to the head group of acidic phospholipids in a fluid bilayer destabilizes a tightly bound nucleotide and can rejuvenate the inactive ADP-form. Controlled proteolysis of dnaA protein results in a prompt loss of oriC binding; an NH_2-terminal, 30-kDa peptide contains the domain that binds ATP and phospholipids known to destabilize the tightly bound ATP.

L 400 MECHANISM OF DNA POLYMERASE DELTA STIMULATION BY PCNA

David M. Andrews, Cheng-Keat Tan, Kathleen M. Downey, Antero G. So, Departments of Medicine and Biochemistry/Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101. The proliferating cell nuclear antigen (PCNA) is a specific accessory protein of DNA polymerase ∂ that dramatically increases the enzymes processivity and macroscopic activity on primer/templates containing long regions of single-stranded DNA. The processivity of DNA polymerase ∂ core enzyme is stimulated by PCNA on both homopolymeric and natural DNA templates, replicating singly primed M13mp19 DNA (7.2kb) in a single processive cycle under physiological conditions. In the absence of PCNA, DNA polymerase ∂ dissociates freely from primer terminus whereas in the presence of PCNA, a stable *PCNA-polymerase-primer/template* ternary complex can be isolated by gel filtration chromatography. On poly(dA)/oligo(dT), KCl inhibits the macroscopic activity of DNA polymerase ∂/PCNA by disrupting the interaction of PCNA with the enzyme. The data suggest that PCNA affects the processivity of DNA polymerase ∂ by stabilizing the association of the core enzyme with primer terminus. The functional properties of the DNA polymerase delta/PCNA complex at the primer terminus are consistent with the proposed role as a processive leading strand polymerase at the DNA replication fork.

L 401 SUBSTRATE BINDING SITE OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) REVERSE TRANSCRIPTASE: AN ANALYSIS OF PYRIDOXAL 5' PHOSPHATE SENSITIVITY AND IDENTIFICATION OF LYSINE 263 AS A SUBSTRATE dNTP BINDING SITE RESIDUE. Amaresh Basu, Radhakrishna S. Tirumalai, and Munkund J. Modak, Department of Biochemistry and Molecular Biology, New Jersey Medical School, Newark, NJ 07103. Human immunodeficiency virus (HIV) reverse transcriptase exhibits a strong sensitivity to pyridoxal 5' phosphate (PLP), a substrate binding site directed reagent for DNA polymerases [Modak, M. J. (1976) Biochemistry, 15, 3620]. Treatment of HIV-RT with PLP followed by sodium borohydride reduction of the enzyme-PLP adduct results in irreversible inactivation of polymerase activity while RNase H activity is minimally affected. Investigation of PLP mediated inactivation clearly indicated that PLP reacted at the deoxynucleoside triphosphate (dNTP) binding site in HIV-RT as judged by a) competitive mode of inhibition with respect to substrate dNTPs and b) the observation that protection from PLP inactivation can be achieved only by addition of substrate dNTPs and appropriate template primer but not by template primer alone. Stoichiometric studies further indicated that only one mole of PLP is incorporated per mole of enzyme, implying only a single site of reactivity. Comparative tryptic peptide mapping of enzyme treated with PLP under a variety of conditions revealed the presence of a PLP reactive peptide which eluted at 96 min from C-18 reverse phase column. Further purification of the peptide followed by amino acid composition and sequence analyses clearly showed that lysine 263 is the site of PLP reactivity. We therefore, conclude that the tryptic peptide spanning 260-275 constitutes a part of dNTP binding domain and lys-263 serves as a principal residue in this domain.

L 402 **INTERACTION OF YEAST DNA POLYMERASE III WITH YEAST AND MAMMALIAN PROLIFERATING CELL NUCLEAR ANTIGEN/CYCLIN**, Glenn A. Bauer and Peter M.J. Burgers, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110

We have previously reported the purification and characterization of yeast DNA polymerase III and yeast proliferating cell nuclear antigen/cyclin, analogs to mammalian DNA polymerase δ and proliferating cell nuclear antigen, respectively. Through the use of Biogel A-5m gel filtration chromatography, we have studied the interaction of the model template-primer system poly(dA)·$(dT)_{16}$ (40:1) with yeast DNA polymerase III and PCNAs. Yeast DNA polymerase III binds to the DNA in the absence of yeast proliferating cell nuclear antigen/cyclin, but comigration of either yeast or calf thymus proliferating cell nuclear antigen/cyclin with the DNA requires the additional presence of yeast DNA polymerase III. The 3'→5' exonuclease associated with yeast DNA polymerase III acts in a distributive manner on poly(dA)·$(pT)_{16}$ and dissociates from the DNA when addition of dTTP allows switching from the exonuclease to the polymerase mode. Addition of proliferating cell nuclear antigen/cyclin had no effect on these activities. From these data, we propose that proliferating cell nuclear antigen/cyclin is not involved in the binding step of the polymerase to the template-primer nor in proofreading, but primarily in the elongation step of DNA synthesis.

L 403 AUTONOMOUS REPLICATION OF PLASMIDS CONTAINING MAMMALIAN DNA SEQUENCES ISOLATED FROM EARLY S PHASE, A.W. Braithwaite, W. Court* and J. Jenkins*, Marie Curie Research Institute, The Chart, Oxted, Surrey, UK* and John Curtin School of Medical Research, Australian National University, Canberra, ACT Australia 2601. Cells were pulse-labelled with methyl deoxyadenosine in early S phase following 2 successive hydroxyurea induced G1/S blocks. This procedure generates recognition sites for the restriction endonuclease Dpn 1 which cleaves at GmATC on both strands. Methylation on dA is normally absent from higher eukaryote DNA. Genomic DNA so labelled was restricted with Dpn 1 and the fragments cloned into bacterial plasmids (mostly pUC 19). 80 clones were screened (in pools of 8) for the ability to replicate autonomously in a transient assay in monkey COS cells; input and replicated daughter DNA molecules being distinguished using Dpn 1 (1,2). Clones which gave Dpn 1 resistant colonies by transformation of DH5 were then examined further. 8 clones were finally selected as containing potential 'ars' regions. 1 clone was found to be SV40 origin-like by hybridization to SV40 DNA and by suppression of replication by mouse p53 (2). The remaining clones were negative for SV40 origin-like DNA by hybridization and 2 clones were negative by mouse p53 suppression. These 2 clones were studied further. They replicated in HeLa as well as in COS cells and in both cell types the proportion of plasmids which replicated was variable in different experiments, in contrast to SV40 origin plasmids. These clones and others that replicated all appear to contain deletions in the bacterial vector.

Refs:
1. Vasavada et al. (1987) Gene 55: 29-40. 2. Braithwaite et al. (1987) Nature329:458-460

L 404 DNA POLYMERASE I AND THE ASSOCIATED 86KD SUBUNIT OF S. CEREVISIAE, R. Glenn Brooke and Lawrence B. Dumas, Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60208 and Rakesh Singhal and David C. Hinkle, Department of Biology, University of Rochester, Rochester NY 14627 Plasmids have been constructed in the Hinkle laboratory to overproduce in yeast the α (catalytic) and β (86kD) subunits of the *S. cerevisiae* DNA polymerase I. The Dumas laboratory has generated monoclonal antibodies directed against the subunits of the DNA polymerase. Immunoaffinity chromatography with several of these antibodies has been used to purify the 180 kD DNA polymerase and the 86kD subunit to homogeneity from the overproducing strains of yeast. The 180kD and 86kD subunits can be reconstituted *in vitro* to form a stable complex. The 86kD subunit stimulates the DNA polymerase activity in the 180kD subunit 2-fold on poly(dAdT) template, although it has no detectable DNA polymerase activity itself. We are examining the processivity and potential exonuclease activities of the isolated 180kD subunit and the reconstituted p180-p86 complex.

L 405 Saccharomyces cerevisiae cdc2 MUTANTS ARE DEFICIENT FOR DNA POLYMERASE III, Peter M.J. Burgers and Glenn A. Bauer, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110
Yeast DNA polymerase III consists of two subunits of 125 and 55 kDa. The enzyme has a proofreading 3′-5′ exonuclease activity and is with regard to its sensitivity to various inhibitors and its interaction with proliferating cell nuclear antigen (PCNA) an analog of the mammalian DNA polymerase δ. Two alleles of cdc2, cdc2-1 and cdc2-2, lacked DNA polymerase III activity after cells had been shifted to the non-permissive temperature and extracts from these cells were fractionated by DEAE silica gel HPLC. Fractions corresponding to the elution position of DNA polymerase III were also deficient for the 3′-5′ exonuclease activity. These experiments suggest that both the polymerase and proofreading exonuclease activity of DNA polymerase III are contained in the cdc2 encoded protein.

L 406 DNA POLYMERASE COFACTOR FROM RABBIT BONE MARROW, John Byrnes, Richard Cowman, Department of Medicine, Miami Veterans Administration Hospital and Center for Blood Disease, University of Miami School of Medicine, Miami, FL 33125.
DNA polymerases alpha and delta are involved in eukaryotic cell DNA replication (Hammond et.al. Biochem. 26:6817). DNA polymerase alpha from rabbit bone marrow is associated with a primase activity. The catalytic core of alpha is a 135 KDa polypeptide. DNA polymerase delta is not associate with a primase. DNA polymerase delta is a 122 KDa polypeptide which also contains a 3′-5′ exonuclease activity. The first column chromatographic step in our purification of the DNA polymerases, phosphocellulose, separates a factor which allows either alpha or delta to synthesize poly dA on the template poly dT. The factor also greatly stimulates DNA synthesis by alpha "core" and delta on all template/primer combinations tested. Analysis of the reaction requirements with the template/primer, poly dA/oligo dT reveals a more than 10 fold decrease in K_m for primer and a greater than 5 fold increase in V_{max}. There is no change in the K_m for deoxynucleotide triphosphates or other reaction requirements. The DNA polymerase cofactor has been purified more than 1000 fold by chromatography on DE cellulose, hydroxylapatite and Ultrogel AcA 34. The factor sediments about 7 S in glycerol gradient. DNA polymerase delta cofactor from calf thymus (from A.G. So) does not cause the same effect. While similar in effect to that described for factors C_1 C_2, (Prichard et al., J. Biol. Chem. 258:9810), the column chromatographic behavior is substantially different and the protein involved has an acidic rather than basic isoelectric point.

L 407 FIDELITY OF HELA CELL DNA POLYMERASE ALPHA IN A RECONSTITUTED IN VITRO REPLICATION SYSTEM, M.P. Carty,[1] Y. Ishimi,[2] J. Hurwitz,[2] A.S. Levine,[1] and K. Dixon[1] NICHD, National Institutes of Health, Bethesda, MD 20892[1] and Dept. of Molecular Biology, Sloan-Kettering Cancer Center, New York, NY 10021[2]

Cellular DNA is replicated in vivo with very high fidelity: the average error frequency is estimated to be about 1 per 10^{10} bases polymerized. To investigate whether factors in addition to DNA polymerases contribute to DNA replication fidelity, we have replicated the SV40-based mutagenesis test vector, pZ189, in crude extracts of human (HeLa) cells, and in a reconstituted replication system containing purified HeLa cell polymerase alpha/primase, topoisomerase II, DNA ligase, RNase H, single-stranded binding protein, a 5'->3' exonuclease, and SV40 large T-antigen. The pZ189 shuttle vector contains the SV40 early region including the origin of replication, the origin of replication of pBR322, the beta-lactamase gene, and the supF gene which serves as a selectable mutagenesis marker. After treatment of replicated DNA with DpnI to remove unreplicated DNA and remethylation with dam methylase, E. coli carrying a suppressable nonsense mutation in the beta-galactosidase gene were transfected and mutations scored after plating on indicator agar. The results indicate: (i) purified HeLa cell polymerase alpha is 10-20 fold more accurate when involved in a DNA replication reaction in vitro, than when carrying out gap-filling DNA synthesis in the absence of other proteins, and (ii) crude HeLa cell extracts appear to contain additional factors not present in the reconstituted system that further enhance fidelity (about 5 fold), leading to an error rate of about 1 per 400,000 nucleotides polymerized. This system should be useful in identifying these factors.

L 408 **A Eukaryotic Trans-activator Nuclear Factor I (NF-I) Stimulates The Replication Of SV40 DNA Minichromosome: *in vivo* and *in vitro* Study** Linzhao Cheng and Thomas J. Kelly, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medine, Baltimore, MD 21205 It has been demonstrated that the cis-acting sequence elements involved in transcription of the SV40 genome can significantly increase the efficiency of SV40 DNA replication *in vivo*. These stimulatory elements include the 21 bp GC-rich repeat which binds the transcription factor SP-1, and the 72 bp repeats identified as the transcriptional enhancers. Recently we have found that the enhancer element of the human papovavirus BK can also stimulate the replication of plasmid DNA containing the minimal SV40 origin of replication. Upon further characterizating we found that an 18 bp segment from BKV enhancer was suffcient for this stimulation. This segment contains a binding site for Nuclear Factor I (NF-I), which is a cellular protein required for Adenovirus DNA replication and functionally identical to the transcription factor CTF (CCAAT Transcription Factor). We have investigated this phenomenon *in vitro* using a system which coupled chromatin assembly and DNA replication. Nucleosomes were assembled on plasmid DNA *in vitro* using Xenopus oocyte extracts. We found that DNA replication was gradually inhibited as nuclesome formation occured; however, this inhibition could be prevented by the pre-incubation of the DNA which contained a NF-I binding site with purified NF-I protein. These experiments suggest a simple model for one level of regulation of DNA replication: DNA binding proteins like NF-I may stimulate DNA replication by disrupting the formation of nuclesomes around replication origins and making the origins accessible to initiation proteins.

L 409 CLONING OF GENES RESPONSIBLE FOR RNase H ACTIVITY IN THE YEAST *SACCHAROMYCES CEREVISIAE*, Alan B. Clark and Akio Sugino, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709.

The enzyme RNase H specifically degrades the RNA strand of a DNA-RNA hybrid molecule. One possible role for the enzyme in the cell is the removal of the RNA primer during DNA replication. Various polypeptides having RNase H activity have been purified. We have raised polyclonal antibodies and isolated recombinants from a λgt11 fusion library. We are currently using these recombinants to investigate the relationships between the different RNase H activities and determine their roles *in vivo* through both bichemical and genetic analyses.

L 410 DNA ATTACHMENT PROTEINS AND CHARACTERIZATION OF THE ATTACHED DNA FRAGMENTS, Anne E. Cress and Kirsten M. Kurath, Department of Radiation Oncology, The Arizona Cancer Center, Tucson, AZ 85724.
We have previously described the isolation of three proteins which were found to be attached to DNA in Chinese Hamster Ovary cells (Cress, A.E. and Kurath, K.M., J. Biol. Chem. 263, in press 1988). The proteins were identified as containing attached DNA by detection of the nucleic acid co-migrating with the protein within a denaturing two dimensional polyacrylamide gel with ethidium bromide, amido black, and silver staining. In vitro labelling of the fragments attached to the proteins with terminal deoxynucleotidyl transferase, DNA polymerase I (Klenow) and T4 polynucleotidyl kinase indicates that one protein contains double stranded DNA fragments, two proteins contain single stranded DNA stretches with accessible 3'OH termini and one protein contains DNA with 5'OH termini which are inaccessible to labelling by polynucleotide kinase. DNA isolated from the individual proteins are fragments whose size are less than 100 base pairs as judged by migration on a 20% polyacrylamide gel containing 8M Urea. Antibodies specific for the three proteins of interest have been produced and cross-react with antigens isolated from human, hamster, mouse and E. coli cells. These data suggest that the DNA attachment proteins which we have isolated are nuclear proteins present in a variety of organisms and that the attached DNA bound to the individual proteins can be isolated as distinct sized fragments.

L 411 THE HERPES SIMPLEX VIRUS 1 HELICASE-PRIMASE: A COMPLEX OF THREE HERPES ENCODED GENE PRODUCTS, James J. Crute, Edward S. Mocarski and I. Robert Lehman, Stanford University School of Medicine, Stanford, CA 94305.
In an earlier report, we described a DNA helicase that was specifically induced upon infection of Vero cells with herpes simplex virus 1 [Crute, J. J., Mocarski, E. S.and Lehman, I. R. (1988) Nucleic Acids Res. 16, 6585-6596]. We have purified this enzyme to near homogeneity and found it to consist of three polypeptides of M_r 120,000, 97,000 and 70,000. Immunochemical analysis has shown these polypeptides to be the products of three of the genes, UL52, UL5 and UL8, that are required for replication of a plasmid containing a herpes simplex 1 origin (ori$_S$). In addition to helicase activity, the enzyme contains a tightly associated DNA primase. Thus, the three-subunit enzyme is a helicase-primase complex that may prime lagging strand synthesis as it unwinds DNA at the viral replication fork.

L 412 ANALYSIS OF ORI-S SEQUENCE OF HSV-1 : IDENTIFICATION OF ONE DNA BINDING DOMAIN, Sumitra Deb and Swati P. Deb, Department of Microbiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226
Using gel retardation assays, we have detected an Ori-S binding activity in the nuclear extract of HSV-1 infected Vero cells. The sequence-specific DNA binding activity seems to be identical to "Origin Binding Protein", described initially by Elias et al. (Proc. Natl. Acad. Sci. USA 83: 6322-6326, 1986). This activity fails to retard a mutant origin DNA that has a 5 bp deletion in the reported protein binding site along with an A to T substitution at a position 16 base-pairs away from the site. Significantly, this mutant fails to replicate in an in vivo transfection/infection assay in which replication depends on the presence of Ori-S. This directly correlates OBP binding to the Ori-S with the origin function. Using crude nuclear extracts as the source of the factor and through footprint analyses, we confirmed that protection is only observed on the preferred site of binding on and near the left arm of the Ori-S palindrome. The 67 base-pair origin fragment containing the left half of the palindrome up to the end of the AT stretch is retarded in the native polyacrylamide gel. In order to analyze the sequence specificity of the binding we have generated a set of binding site substitution mutants. Competition experiments with these mutant origins indicate that the sequence 5'-TTCGCACTT-3' is crucial for binding. This domain includes 9 out of 11 base-pairs of the sequence 5'-CGTTCGCACTT-3' found also in the Ori-S region of varicella zoster virus.

Molecular Mechanisms in DNA Replication and Recombination

L 413 **Origin Binding Proteins from Yeast,** John F.X. Diffley and Bruce Stillman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

In the yeast *Saccharomyces cerevisiae*, specific DNA sequences (ARS) are required to function as origins of DNA replication. Cellular factors which interact with these sequences, therefore, are likely to be important in mediating the initiation of DNA replication. We have identified two such yeast proteins, ARS binding factors I and II (ABFI and II). ABFI binds to a single site within an important, though non-essential, functional domain of ARS1. Interestingly, although ABFI is not a universal ARS binding factor, it binds to three of the four ARSs required for the silencing of mating type transcription at HMR and HML and under certain conditions, silencing is dependent upon the ABFI site. These data suggest that ABFI functions in both initiation of DNA replication and repression of transcription. We have purified ABFI to apparent homogeneity and have isolated a full length copy of the gene encoding this protein. The amino acid sequence and *in vivo* requirements of this multifunctional protein will be discussed.

Yeast ARSs are composed of an essential exact match (11/11) to the 11bp ARS consensus sequence (ACS) and important flanking sequences which often contain near matches (9/11) to the ACS. Moreover, an efficient ARS can be constructed simply from certain reiterations of the ACS. Therefore, proteins whose binding correlates with the ACS are likely to have a critical role in ARS function. ABFII is a small protein with a number of unusual biochemical properties which will be described. Most importantly, ABFII binds randomly to non-specific DNA but binds at multiple discrete sites within the several ARSs tested. This binding is not random and corresponds exactly with the locations of the 11/11 and 9/11 ACSs. Instead of ABFII binding directly to the ACS, however, the ACSs are located precisely at the borders of ABFII binding sites suggesting that ABFII is specifically excluded by the ACS, thus providing a potential biochemical function for this sequence. Models for origin function based on ABFII binding will be presented.

L 414 MONOCLONAL ANTIBODIES AGAINST DNA POLYMERASE α PREFERENTIALLY INHIBIT THE DISCONTINUOUS COMPONENT OF DNA REPLICATION IN HUMAN CELLS, Steven L. Dresler and Mark G. Frattini, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110

Two monoclonal antibodies (SJK287-38 and SJK132-20), which specifically inhibit DNA polymerase α, were found to inhibit replicative DNA synthesis in permeable diploid human fibroblasts, however, neither antibody would inhibit the process completely, even when used at concentrations over 25 times greater than those required to completely inhibit isolated polymerase α. Maximal levels of inhibition of DNA replication by SJK287-38 and SJK132-20 were 50% and 20%, respectively. The same maximal level of inhibition by SJK287-38 was seen in permeable human fibroblasts of two types (AG1518 and IMR-90), in fibroblasts which had been fragmented by sonication, and in permeable HeLa (human epithelial tumor) cells. Electrophoretic analysis of denatured products of replication revealed that, for both antibodies, the discontinuous ("lagging strand" or "Okazaki fragment") component of replicative synthesis was inhibited more strongly than the continuous ("leading strand") component. In multiple experiments, SJK287-38 inhibited lagging strand synthesis 1.9- to 4.4-fold more strongly than it inhibited leading strand synthesis. Antibody SJK132-20 inhibited lagging strand synthesis 2.5- to 4.0-fold more strongly. Our data indicate that the macromolecular complexes responsible for leading and lagging strand DNA synthesis in mammalian cells are asymmetric and suggest that DNA polymerase α may be primarily involved in the lagging strand component of DNA replication in human cells.

L 415 RAT LIVER DNA LIGASES, Rhoderick H. Elder and Jean-Michel Rossignol, Biol.& Génét.Mol.IRSC, BP 8, 94800, Villejuif, France.

Two immunologically distinct forms of DNA ligase have been identified and extensively purifed from rat liver. DNA ligase I is the principal activity in normal liver and it is this activity uniquely which is increased more than 10 fold during liver regeneration, while the activity of DNA ligase II remains at a constant low level. These enzymes can be differentiated by their ability to ligate blunt ends (DNA ligase I), or oligo (dT) monomers hydrogen bonded to poly (rA) (DNA ligase II). In the presence of Mg++ and ATP both enzymes can form a stable ligase-AMP intermediate. These intermediates, labeled after incubation with ^{32}P-α-ATP and separated by SDS-PAGE, correspond to polypeptides of 130 kDa for DNA ligase I and to 100 kDa for DNA ligase II. That these bands are indeed due to DNA ligase is shown by the disappearance of the radiolabeling, only after incubation with an appropriate substrate and also by specific immunolabeling. In contrast to other mammalian ligases, rat DNA ligase II has a lower Km for ATP (12μM) than ligase I (60 μM). Furthermore, only DNA ligase II can use ATP-α-S as a cofactor. The combination of the specific assay for DNA ligase II (oligo dT.poly rA) and the direct labeling of the ligase polypeptides has enabled us to detect and characterize unambigously for the first time, these two molecular forms of DNA ligase from this tissue.

L 416 A REPETITIVE MOUSE DNA SEQUENCE INHIBITS SV40 DNA REPLICATION IN VIVO, E. Fanning and M. Hartl, Institute for Biochemistry, University of Munich, Karlstr. 23, 8000 Munich 2, West Germany. The SV40-transformed mouse cell line VLM was fused with COS1 cells, which supply wildtype T antigen in trans, or with uninfected TC7 monkey cells. Supercoiled SV40 DNA of discrete sizes was obtained from the COS1 fusions, but little viral DNA was rescued from TC7 fusions, suggesting that the VLM T antigen was replication-defective. To confirm this interpretation and localize the mutation responsible for the defect, viral DNA rescued from VLM-COS1 fusions was isolated, cloned and characterized. Unexpectedly, the early region of a prototype clone pV4 encoded a T antigen competent for replication when reconstructed into a wildtype background. However, the same T antigen gene in the pV4 background induced only poor replication, despite a fully competent pV4 origin region. Analysis of the pV4 late region revealed that about 2 kb of viral DNA was replaced by mouse DNA sequences. Hybridization of the cellular pV4 sequences against genomic DNA and DNA sequencing revealed repetitive DNA bearing numerous direct and inverted repeats and $(dT\text{-}dG)_{17}$ and $(dA\text{-}dC)_{25}$ stretches. Transfection into TC7 cells of wildtype SV40 plasmid DNA carrying a 0.4 kb insertion of this cellular DNA demonstrated that this sequence was sufficient to inhibit SV40 replication almost completely. The mouse DNA sequences did not inhibit SV40 DNA replication in trans: on the contrary, cotransfection of plasmid DNA bearing the inhibitory mouse sequences relieved the cis-active inhibition of SV40 replication in a dose-dependent manner. Possible mechanisms for the replication inhibition are under investigation and will be discussed.

L 417 AFFINITY ISOLATION OF EUKARYOTIC DNA REPLICATION COMPLEXES. Barbara Fenn and Timothy Herman, Medical College of Wisconsin, Milwaukee, WI 53226. An affinity isolation system is being used to isolate proteins involved in the replication of eukaryotic DNA. Replication forks in lysolecithin-permeabilized cells are labeled with the chemically-cleavable biotinylated nucleotide, Bio-19-SS-dUTP. Newly replicated chromatin is then isolated by streptavidin/biotin-cellulose chromatography and eluted by cleavage of the disulfide bond in the linker arm of the biotinylated nucleotide. This procedure results in the enrichment of newly replicated chromatin and, therefore, should result in the recovery of protein complexes that had assembled at replication forks initiated in vivo.

DNA synthesis in the permeabilized cell system has been shown to be normal, semiconservative replication by its sensitivity to aphidicolin, the density of DNA labeled with IdUr and the sensitivity of newly replicated chromatin to micrococcal nuclease digestion. Following solubilization of the chromatin, 50-60% of the newly replicated chromatin can be bound to and eluted from the streptavidin affinity column at a 120-fold enrichment of newly replicated chromatin over mature chromatin. We are developing a polyclonal antisera against the polymerase alpha catalytic subunit. This antisera will be used along with monoclonal antibodies against polymerase alpha, and polyclonal antisera against topoisomerase II, a single-stranded binding protein, and PCNA (polymerase delta auxiliary protein) to immunologically characterize the proteins in the affinity-purified fraction of chromatin.

L 418 AN EXAMINATION OF DNA-BINDING PROTEINS FROM THE YEAST SACCHAROMYCES CEREVISIAE, Tim Formosa and Leland H. Hartwell, Department of Genetics, University of Washington, Seattle, WA 98195. We have tried to identify proteins that function in DNA metabolism in a eukaryotic cell and are amenable to both biochemical and genetic characterization . Extracts of yeast nuclei were chromatographed on DNA cellulose and the complex binding fractions were used to generate antibodies. An expression vector library of yeast genomic sequences was screened with these antisera, yielding 23 different DNA-binding protein genes. This collection included the genes encoding the yeast poly-A binding protein and the translation elongation factor EF-1alpha--proteins expected to be in a collection of DNA-binding proteins based on their abundance and physical properties. Antibodies were purified from the original antisera using individual antigen-producing clones; these were used to determine the size and subcellular localization of the antigens in yeast cells. Disruptions along the length of the DNA inserts were generated using a modified Tn3 element, and the effects of these disruptions on viability, chromosome loss and recombination were tested in haploid cells after transplacement into the yeast genome. Seven of the clones encode proteins localized to the nuclear fraction. Disruptions of 4 of these have no apparent effect on haploid yeast cells, while one appears to cause increased sensitivity to DNA damage.

L 419 PURIFICATION OF THE EPSTEIN-BARR VIRUS ORIGIN BINDING PROTEIN (EBNA1) FROM AN E.COLI OVERPRODUCING STRAIN, Lori Frappier and Michael E. O'Donnell, Department of Microbiology, Cornell University Medical College, New York, NY 10021

Epstein-Barr virus nuclear antigen 1 (EBNA1) interacts with the oriP sequence of Epstein-Barr virus (EBV) and enables the initiation of DNA replication on plasmids containing oriP. The precise role of EBNA1 in replication, however, has not been determined, and the low abundance of EBNA1 in EBV-infected cells has hampered the purification of the protein. To facilitate the purification of EBNA1, we have used the T7 expression system to overproduce EBNA1 in E.coli. The entire gene for EBNA1 was cloned under the control of the T7 gene 10 promoter, and recombinant plasmids were used to transform BL21(DE3)pLysS cells, which contain an integrated copy of the T7 RNA polymerase gene under control of the lac UV5 promoter. After induction of the T7 RNA polymerase gene with IPTG, EBNA1 was detected in cell lysates by the specific retention of oriP DNA on nitrocellulose filters. Gel retention assays showed that, at low EBNA1 concentrations, only the 30 bp repeated sequence of oriP was bound by EBNA1, whereas at higher concentrations, the fragment of oriP containing the 65 bp dyad symmetry element was also bound. A scheme for the purification of EBNA1 will be presented.

L 420 REPLICATION TIMING OF THE EPSTEIN-BARR VIRUS (EBV) GENOME AND EBV PLASMIDS IN LATENTLY INFECTED CELLS, Toni A. Gahn and Carl L. Schildkraut, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, N.Y. 10461

Replication of eukaryotic genes proceeds in a defined temporal order depending on cell type. The mechanism by which this order is determined and controlled is unknown. To develop a model by which to study this process, we examined the time of replication of the EBV genome in latently infected cells and of plasmids containing the EBV replication origin in transfected cells. Neither EBV nor the plasmids integrate into the host cell genome but remain extrachromosomal. Like chromosomal DNA, EBV replicates only during S phase of the cell cycle.

We found that in an EBV transformed lymphoblastoid cell line the EBV genomes replicate during the first half of S phase. However, in a lymphoma cell line transfected with an EBV plasmid, the plasmid replicates throughout S phase. In an EBV containing Burkitt lymphoma line that has been transfected with an EBV plasmid, EBV replicates early in S phase while the plasmid replicates throughout S. We conclude that either EBV does not produce a trans-acting factor responsible for its early time of replication or that the plasmids lack the cis-acting sequence upon which such a factor acts.

A genetically defined EBV origin of replication, oriP, has been identified by others. We examined whether oriP is acting as the site of initiation of DNA replication using a recently described two-dimensional gel electrophoresis technique. We found that oriP is the primary origin used in the EBV plasmids. It appears that the same origin, EBV oriP, can display different temporal patterns of replication.

L 421 GENE EXPRESSION OF HUMAN DNA POLYMERASE α IN TRANSFORMATION, AGING AND TERMINAL DIFFERENTIATION

Amy M. Geis, Alan F. Wahl, and Teresa S.-F. Wang
Department of Pathology, Stanford University School of Medicine, Stanford, Ca. 94305

Gene expression of human DNA polymerase α was investigated utilizing the full-length cDNA and specific monoclonal antibodies. DNA polymerase α gene expression is found to be 10 to 40 fold amplified in transformed cells and is positively induced during activation of cell proliferation, but is constitutive through the cell cycle (Wahl et al, 1988). The mechanism responsible for the amplification of expression in transformed cells is investigated at three levels: (1) an increase in transcription rate by nuclear run-off assay; (2) greater message stability; (3) amplification of polymerase α gene.

The expression of this major replicative DNA polymerase gene is also investigated in cells which have permanently exited the cell cycle, i.e. in cells which are senescent and terminally differentiated. A normal human fibroblast cell line, IMR-90, which undergoes senescence with a predictable timecourse in tissue culture, and a human promeyelocytic leukemic cell line, HL60, induced to differentiate in culture by DMSO, are utilized as models for aging and terminal differentiation, respectively. A pure population of HL60 cells which is terminally differentiated, (as assayed by loss of capacity for ^{3}H-thymidine incorporation) is obtained by the technique of centrifugal elutriation. A dramatic decline in the level of polymerase α steady state mRNA in nondividing, terminally differentiated cells has been found. The mechanism for the down-regulation of gene expression of this key DNA replication enzyme is being further investigated at the level of transcription rate and message stability. Using the recently isolated promoter region for human DNA polymerase α, we are now able to delineate sequence elements possibly involved in regulation of differentiation.

L 422 **CHARACTERIZATION OF THE COMPONENTS THAT ARE REQUIRED FOR SPECIFIC INITIATION OF LIGHT-STRAND DNA REPLICATION IN HUMAN MITOCHONDRIA**, Robert Genuario, Hilary Gray, and Tai Wai Wong, Dept. of Biochemistry, Robert Wood Johnson Medical School, UMDNJ, Piscataway, N.J. 08854. We have identified and characterized some of the components that are required for initiation at the origin of light-strand mtDNA replication (O_L) in human mitochondria. DNA primase activity that had been purified over 2000-fold consisted of a group of 6 major polypeptides with molecular weight between 58 and 140 kilodaltons. The purified enzyme by itself is not capable of specific initiation at O_L. We have identified a component, distinct from primase, that is required for specific initiation at O_L. This component, mtRF-A, is associated with but distinguishable from DNA polymerase. Disruption of the interaction between mtRF-A and DNA polymerase results in the inactivation of the replication factor. We have also determined that at least one other component, in addition to primase, mtRF-A, and DNA polymerase, is also necessary for specific initiation of replication.

L 423 **A PROCESSIVITY FACTOR FOR A MAMMALIAN DNA POLYMERASE ALPHA/PRIMASE**

M. Goulian, C.J. Heard and S.M. Herrmann, Department of Medicine and Center for Molecular Genetics, University of California San Diego, La Jolla, CA 92093

An accessory factor (AF-1) for DNA polymerase alpha/primase has been identified in mouse cell extracts by its ability to stimulate mouse pol alpha/primase on unprimed single stranded DNA and unprimed poly dT. AF-1 does not stimulate the primase activity of isolated DNA primase nor that of DNA polymerase alpha/primase; under some circumstances it stimulates the polymerase activity of pol alpha/primase functionally uncoupled from the primase, but does not stimulate it with activated DNA.

AF-1 has been purified essentially to homogeneity; it contains two polypeptides of 195 and 45 kDa, respectively, and has a sedimentation coefficient of 6.45S. Experiments testing the effects of dilution, and of competition (by primed templates), on self-initiated reactions (of pol alpha/primase on long single stranded DNA) indicate that AF-1 greatly increases the processivity of pol alpha/primase.

L 424 CIS-ACTING MOUSE DNA SEQUENCES ENABLE ESTABLISHMENT AND PERSISTENCE OF PLASMID DNA POLYMERS IN MOUSE CELLS, Friedrich Grummt, Friedemann Müller, Gerd Zastrow, Andra Klavinius, *U. Köhler and *J. Wienberg, Institute of Biochemistry, University of Würzburg, D-8700 Würzburg, Germany and * Institute of Human Genetics, University of Munich, D-8000 Munich, Germany.
21 distinct cis-acting elements were isolated from mouse genomic DNA confering the ability on plasmid DNA to persist at high copy numbers in mouse L fibroblasts (Holst et al., Cell 52, 355-365, 1988). Inversed field electrophoresis demonstrated that - in contrast to our previous assumption - the vast majority of persisting plasmid DNA does not exist extrachromosomally but as tandem repeats integrated into genomic DNA. Digestion with endonucleases that do not cut within the plasmid DNA results in fragments of 50-300 kb in length indicating reiteration of 10-50 plasmid DNA molecules. Restriction with several enzymes that cut once or twice within the plasmid sequences lead to fragment(s) indicative for head-to-tail tandem repeats. In situ hybridization revealed signals for a long homogeneously stained region (HSR) in one or two chromosomes per cell nucleus. Attempts to elucidate how these cis-acting elements act in the establishment and maintenance of the head-to-tail polymers of plasmid DNA in mouse cells will be discussed.

L 425 **The Role of Auxiliary Sequences Containing Transcriptional Elements in Facilitating Initiation of Simian Virus 40 DNA Replication,** Crisanto Gutierrez, Zong-Sheng Guo, Uwe Heine, James Roberts[1] and Melvin L. DePamphilis, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110, and [1]Department of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The simian virus 40 (SV40) origin of replication (*ori*) consists of a core component flanked by auxiliary sequences that contain promoter elements [large tumor antigen (T-ag) binding region I (*aux-1*) and three of the GC-boxes within the 21 bp repeats (*aux-2*)]. We have found that these auxiliary sequences facilitate initiation of SV40 DNA replication in extracts of SV40-infected monkey cells in the same manner that they do in monkey cell cultures. Replication activity of extracts inactivated by addition of competitor DNA was restored by addition of purified T-ag monomer, revealing that the replication competent form of T-ag was the limiting DNA binding initiation factor. However, ori-auxiliary sequences contributed very little towards binding either the T-ag initiation complex present in cell extracts or purified T-ag. On the other hand, the ability of purified T-ag to unwind specifically the SV40 *ori*-region in purified DNA was as dependent on the presence of *aux-1* and *aux-2* as was SV40 DNA replication. Therefore, we propose that *aux-1* and *aux-2* facilitate SV40 *ori*-core activity by promoting T-ag dependent, *ori*-specific unwinding after the T-ag initiation complex was bound to *ori*-core.

L 426 ANALYSIS OF HUMAN SEQUENCES WHICH REPLICATE AUTONOMOUSLY IN HUMAN CELLS, Steven B. Haase, Scott S. Heinzel, Patrick J. Krysan, and Michele P. Calos, Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305

We previously isolated a heterogeneous collection of human genomic sequences which replicate autonomously when introduced into human cells (see P.J. Krysan, S.B. Haase, and M.P. Calos, *Mol. Cell. Biol. 9*, 1989, in press). The novel strategy for isolation of these sequences involved cloning random human DNA fragments into a defective Epstein-Barr virus vector. The human sequences were shown to have replication activity in the absence of all viral sequences and may represent authentic human origins of replication. We will report on further analysis of these sequences by subcloning and mutagenesis. We will also present data pertaining to the timing of replication of the cloned human sequences and of Epstein-Barr viral vectors.

L 427 DNA POLYMERASES II AND IV FROM THE YEAST *SACCHAROMYCES CEREVISIAE*. Robert K. Hamatake, Alan B. Clark, Hitoshi Hasegawa, and Akio Sugino, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

Multiple DNA polymerase species are present in eukaryotic cells. In yeast, DNA polymerase I (yPol I) is equivalent to DNA polymerase α and DNA polymerase III (yPol III) is similar to DNA polymerase δ. Yeast DNA polymerase II (yPol II), although first described more than a decade ago, has not previously been purified to homogeneity and its relationship to other eukaryotic DNA polymerases is unknown. In order to begin to elucidate the roles of these various yeast DNA polymerases during the yeast cell cycle we have purified DNA polymerases from exponentially growing yeast cells. In addition to yPol I/primase complex and yPol III, we have purified to homogeneity yPol II and a novel polymerase species which we have named yPol IV. yPol II is a single polypeptide of 140 kDa while yPol IV is a complex of polypeptides of 200, 80, 34, 30, and 29 kDa. Polyclonal antibodies raised against yPol I, yPol III, and yPol IV show no cross-reactivity against each other or to yPol II indicating that these four DNA polymerases are distinct species. We will present biochemical and physical data characterizing and comparing these four DNA polymerase species.

Molecular Mechanisms in DNA Replication and Recombination

L 428 EXPRESSION OF THE FUNCTIONAL REPLICATION PROTEIN OF TOMATO GOLDEN MOSAIC VIRUS IN TRANSGENIC TOBACCO PLANTS, Linda K. Hanley-Bowdoin, J. Scott Elmer and Stephen G. Rogers. Plant Molecular Biology, Monsanto Company, St. Louis, MO 63198

We have been studying the molecular genetics of the geminivirus tomato golden mosaic virus (TGMV). Its genome consists of two circular DNA molecules designated as A and B. We have identified a single viral protein (AL1) encoded by the A component that is required for TGMV replication and have constructed transgenic tobacco plants that contain the AL1 coding sequence under the control of the 35S promoter from cauliflower mosaic virus. Several of the transgenic plant lines contain functional AL1 protein, as demonstrated by their capacity to complement defective TGMV A viral components with mutations in the AL1 open reading frame in an infectivity assay. Leaf discs from these transgenic plants also support replication of TGMV B DNA after *Agrobacterium*-mediated inoculation of B DNA. These transgenic plants will be used to locate the cis-acting elements required for TGMV replication and to determine the role of AL1 in replication.

L 429 CO-OPERATIVE INTERACTIONS BETWEEN NFI AND THE ADENOVIRUS DNA BINDING PROTEIN AT THE ADENOVIRUS ORIGIN OF REPLICATION, **Ronald T. Hay and Peter H. Cleat**, Department of Biochemistry and Microbiology, University of St. Andrews, St. Andrews, Scotland KY16 9AL

Nuclear Factor I (NFI) is a cellular protein which binds to the origin of replication and stimulates adenovirus DNA replication in a reaction that is dependent on the concentration of the adenovirus DNA binding protein(DBP). We report here that DBP increases the affinity of NFI for its binding site in the replication origin. DBP did not increase the affinity of other sequence specific DNA binding proteins for their recognition sites and other single strand specific DNA binding proteins could not substitute for DBP in increasing NFI affinity. Kinetic analysis indicated that DBP increased the rate of association and decreased the rate of dissociation of NFI with the DNA template. Cloned NFI is being used to define the regions of the protein that are required for functional interaction with DBP.

Eukaryotic Replication-II

L 430 CHARACTERIZATION OF THE GENE ENCODING THE β SUBUNIT OF YEAST DNA POLYMERASE I, David C. Hinkle, John R. Nelson, Anuradha Chakraborti and Annie Brulfert, Department of Biology, University of Rochester, Rochester, NY 14627

We have used antibodies to isolate the gene encoding the β subunit of yeast DNA Polymerase I. A series of fusion proteins produced in *E. coli* from different regions of the gene react with various polyclonal and monoclonal antibodies raised against the polymerase subunit. The complete nucleotide sequence of the gene has been determined and predicts a protein of 705 amino acids (78.7 kDa). The major form of this subunit present in our preparations of DNA polymerase runs as a 70 kDa protein on SDS-PAGE, but we believe that this is derived from an 85 kDa precursor by proteolysis. We have constructed a plasmid to overproduce the protein in yeast. The overproduced protein runs at 85 kDa by SDS-PAGE and is rapidly converted to a 70 kDa form in extracts. A possible protease sensitive site can be identified at the appropriate location in the amino acid sequence.

A gene disruption experiment indicates that the subunit is an essential protein. The gene is periodically transcribed around the G_1/S phase boundary of the cell cycle and, as with other genes that are regulated in this manner (Pizzagalli et al.(1988) *Proc. Natl. Acad. Sci. USA* **85**, 3772-3776), the sequence 5' ACGCGT 3' is present twice near the start of transcription.

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Molecular Mechanisms in DNA Replication and Recombination

L 431 CHARACTERIZATION OF A DNA PRIMASE ACTIVITY INDUCED IN CELLS INFECTED BY HERPES SIMPLEX VIRUS TYPE 1, Andrew M. Holmes and William T. Ruyechan, Department of Biochemistry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814

A DNA Primase activity identified in Herpes Simplex Virus (HSV) infected HeLa cells(Holmes, et al. (1988) J. Virol. 62: 1038-1045) has been further characterized. Gel filtration chromatography of freshly prepared extracts showed two peaks of HSV induced DNA primase with apparent molecular weights of 130-170kDa and 35-40kDa, indicating the primase may be larger than the previously reported molecular weight of 40kDa and/or that it is associated with other material. Sucrose gradient centrifugation of fresh extracts gave a sedimentation value of 7S, again indicating a molecular weight of 130-140kDa. In antibody binding experiments, antibodies against the HSV DNA polymerase removed the DNA primase from solution when tested against freshly prepared HSV DNA polymerase/DNA primase, but did not remove the DNA primase from solution when tested against stored HSV polymerase/primase, suggesting association between these two activities. The HSV-induced DNA primase, like other eukaryotic DNA primases, can utilize both poly(dT) and poly(dC) as templates, but not poly(dA) or poly(d[I-C]). Finally, time courses of infection in Vero cells indicate the viral DNA polymerase and induced primase appear at similar times, but the DNA primase activity is present over a shorter time span.

L 432 FUNCTIONAL DOMAINS OF CLONED ADENOVIRUS DNA POLYMERASE DEFINED BY INSERTIONAL MUTAGENESIS, Mei Chen and Marshall S. Horwitz, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

Synthesis of enzymatically active adenovirus DNA polymerase (Ad pol) from cloned plasmid templates in CMT4 cells has made it possible to analyse a variety of site-specific mutants of this replicative polymerase. A series of in-frame insertional mutants of 4 amino acids have been constructed at RsaI, HincII, ScaI and AluI restriction endonuclease sites. The effect of mutations on 3 of the Ad pol enzymatic activities, each of which differs in Ad and host-cell gene products required, has been determined. Reaction I, elongation on polydT.oligodA, required Ad pol and DBP. The initiation reaction (II) required the precursor to the Ad terminal protein (pTP), pol, host nuclear factors (NF) and Ad DNA-pro template for covalent addition of dCMP to pTP. Reaction III, the specific elongation on Ad DNA-pro templates, required Ad pTP, DBP, pol as well as NFs. Two of the 16 mutants obtained mapped to 2 of the 5 consensus sequences shared with human DNA pol α, as well as with the herpes simplex and vaccinia virus DNA pols. The other mutants mapped randomly across the Ad pol gene. Four patterns of reactivity were demonstrated. In addition to 3 mutants whose activities in reactions I-III were completely normal, 8 were inactive. Four mutants were active in reactions I and II, but were inactive in reaction III. One mutant that was active in reaction I, but not in II or III, had amino acids inserted between 2 cysteines in a putative Zn finger that might be important in DNA-protein interaction. From the results obtained, it appears that domains that must remain intact to preserve each of the Ad pol activities are distributed across the entire pol gene. The domains that are involved in the binding of Ad pol to other replicative proteins or to template are being determined.

L 433 SEVERAL *ARS* ELEMENTS AT THE LEFT END OF YEAST CHROMOSOME III DO NOT FUNCTION AS REPLICATION ORIGINS, Joel A. Huberman, Dharani D. Dubey, Jiguang Zhu, and Leslie R. Davis, Department of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, NY 14263

ARS elements are *cis*-acting elements, usually derived from yeast chromosomal DNA, which, when placed into plasmids, promote efficient replication of those plasmids in yeast. The *ARS* elements in two different plasmids appear to act as replication origins (Brewer and Fangman, Cell **51**: 463-471, 1987; Huberman, Spotila, Nawotka, El-Assouli, and Davis, Cell **51**: 473-481, 1987). Do *ARS* elements also serve as replication origins in their normal chromosomal environment? Previous work from our lab, in collaboration with Carol Newlon's lab, has identified a chromosomal replication origin about 40 kb from the left telomere of yeast chromosome III which comaps with a previously identified *ARS* element, the A6C *ARS*(Huberman, Zhu, Davis, and Newlon, Nucleic Acids Res. **16**: 6373-6384, 1988). Further studies on the replication of this region suggest that replication forks initiated at the A6C *ARS* proceed all the way to the left telomere and that several additional *ARS* elements located between the A6C *ARS* and the telomere do not function as origins (at least not in the yeast strain tested). These additional *ARS* elements include the two *ARS* elements which flank the silent *HML* locus. Whether derepression of the *HML* locus might activate the flanking *ARS* elements as origins is currently being tested.

L 434 DNA POLYMERASE δ FROM CALF THYMUS. Federico Focher, Max Gassmann und Ulrich Hübscher, Institut für Pharmakologie und Biochemie, Universität Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.
DNA polymerase δ from calf thymus was purified under extreme proteolysis precautions over 6000 fold in a five step isolation procedure including phosphocellulose, hydroxyapatite, Heparin-Sepharose, FPLC mono-S and velocity gradient sedimentation. The apparent homogeneous DNA polymerase δ contains three polypeptides with Mr of 140, 125 and 40 kDa. Tryptic peptide map analysis of the 140 and 125 kDa polypeptides were similar to each other, but different from the catalytic (160-200 kDa) subunit of DNA polymerase α. A 3'→ 5'exonuclease copurified with DNA polymerase δ. The DNA polymerase δ had a processivity of several thousand bases and under optimal conditions the enzyme can fully convert primed single-stranded circular DNA of 7200 bases (M13 mp8) in less than 10 min. For both reactions the proliferating cell nuclear antigen (PCNA) appears to be dispensible. A double stranded replication fork was constructed to test strand displacement synthesis by DNA polymerases δ and/or α. Neither the DNA polymerases alone nor in combination were able to perform strand displacement synthesis. This could be achieved if the two DNA polymerases were supplemented with an additional fraction. Evidence will be presented, which supports the hypothesis (Focher, F., Ferrari, E., Spadari, S. and Hübscher, U. (1988) FEBS Lett. 229, 6-10) that DNA polymerases δ and α act coordinately as leading and lagging strand enzymes, respectively, at the replication fork.

L 435 PROTEOLYTIC CLEAVAGE OF N-TERMINAL PEPTIDE IS ESSENTIAL TO ACTIVE CaMV REVERSE TRANSCRIPTASE, Joh-E Ikeda, Hiroshi Takatsuji, Dep. of Mol. Biol., Natl. Inst. of Agrobiological Resources, Tsukuba Science City, Ibaraki 305, JAPAN
We have previously cloned cauliflower mosaic virus (CaMV) reverse transcriptase gene (ORFV) and it was expressed active in yeast but not in E.coli (Nature 319,240). The active ORFV gene product as well as a virion associated putative reverse transcriptase (RTase) revealed a molecular weight of 60 kDa on activity gel. Although CaMV ORFV gene product in E.coli appears 78 kDa corresponding to the polypeptide fully coded on ORFV gene, no RTase activity was detected. We truncated 5'-terminal end (N-terminus) of ORFV gene ranging over 840 bp. 3'-terminal end (C-terminus) ranging over 540 bp was also deleted. In E.coli cells, those series of truncated ORFV genes were driven by tac promoter in the presence of IPTG. In several clones among them , RTase-like activities were detected. Only the activities of RTase truncated at N-terminus were indistinguishable from that of RTase produced in yeast in enzymatic properties and procession on natural RNA template. These results implicate that CaMV reverse transcriptase is primarily synthesized as an inactiveform (78 kDa polypeptide) and processed to active form by a proteolytic cleavage of its N-terminal region (including protease domain) of the precursor.

L 436 PURIFIED DNA POLYMERASE γ FROM *X. LAEVIS* CONTAINS A 140,000 DALTON CATALYTIC CORE WHICH IS TIGHTLY ASSOCIATED WITH A 3'-> 5' EXONUCLEASE, Neil F. Insdorf and Daniel F. Bogenhagen, Department of Pharmacological Sciences and Graduate Program in Genetics, State University of New York at Stony Brook, Stony Brook, NY 11794. We have purifed DNA polymerase γ approximately 10,000 fold from the mitochondria of *X. laevis* ovaries. Glycerol gradient sedimentation of the final chromatographic fraction revealed that a number of polypeptides cosedimented with DNA polymerase γ activity as determined from SDS-PAGE analysis. We have developed a novel photolabelling procedure which specifically identifies catalytically active subunits from DNA polymerases and have used this technique to identify a 140,000 Da polypeptide which possesses the catalytic site of *X. laevis* DNA polymerase γ. In addition, our photolabelling procedure identified catalytically active polypeptides of 100,000 Da and various smaller molecular weights. These other polypeptides all appear to be degradation products of the 140,000 Da polypeptide and cosediment with DNA polymerase γ activity on glycerol gradients. *X. laevis* DNA polymerase γ copurified with a 3'-> 5' exonuclease activity possessing an identical sedimentation coefficient and Stokes radius.

L 437 CAMPTOTHECIN, A SPECIFIC INHIBITOR OF TOPOISOMERASE I, INHIBITS THE ASSEMBLY OF REPLICATION COMPETENT CHROMATIN, Joany Jackman and Nicholas H. Heintz, Department of Pathology, University of Vermont, Burlington, VT 05405. Camptothecin (CAMP) inhibits topoisomerase 1 by preventing religation of transient protein-linked single stranded breaks in DNA. Several groups have suggested that CAMP directly inhibits elongation by irreversible fragmentation of replication intermediates. The effects of CAMP on initiation and elongation of DNA replication was assessed by examining S-phase specific labeling patterns of amplified chromosomal sequences in nuclear run-on replication assays. Long term effects were monitored by fluorescence-activated cell sorting (FACS) analysis. Nuclei from synchronized cultures exposed to CAMP during traversal of the G1 phase show a dose-dependent reduction in replication capacity; this reduction in synthetic capacity is not restored by cytosolic extracts from replication competent cells. In contrast, nuclei from cells exposed to CAMP just prior to the onset of DNA synthesis demonstrate normal replication capacity and unaltered fragment labeling patterns. Moreover, CAMP has no effect on labeling patterns when added directly to the run-on replication reactions. Interestingly, FACS analysis of drug-exposed cells indicates that exposure to CAMP for 1 hr prior to S does not inhibit entry into the S phase, but rather prevents cells from exiting G2. These results demonstrate that CAMP has minimal effects on elongation in vitro, but rather interferes with events in whole cells required both for assembly of replication competent chromatin in G1 and subsequent exit of cells from G2.

L 438 CLONAL SELECTION OF HUMAN HL-60 DNA SEQUENCES BASED ON INITIATION OF REPLICATION, Shirzad Jenab and Edward M. Johnson, Brookdale Center for Molecular Biology and Department of Pathology, Mount Sinai School of Medicine, New York, NY 10029

We have developed a system for selection of clones of human genomic DNA sequences based on their ability to initiate replication using cell extracts. Bam HI DNA fragments (0.8-2.5kb) from HL-60 promyelocytic leukemia cells were ligated into plasmid PML-2. Following *in vitro* replication using extracts of HL-60 cells, the plasmid library was treated with DpnI, which multiply cleaves unreplicated plasmids. The library thus selected was amplified in HB101 (dam+) and subjected to two more rounds of *in vitro* replication followed by DpnI-treatment and amplification. Several clones obtained showed efficient initiation in the HL-60 cell-free system. With added T-antigen the replication system selects for clones bearing an SV40 origin of replication. No clones were selected with vector pML-2 alone. One selected genomic clone, pREP4 (1.8kb) replicates in transfected cell lines with great preference for HL-60 cells. The insert of pREP4 is repeated 20-100 times in the human and simian genomes. The sequences and distribution of initiating regions are discussed. The method offers advantages for studies of control of initiation at specific human genomic DNA sequences.

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L 439 MITOCHONDRIAL DNA POLYMERASE: STRUCTURE, MECHANISM AND FUNCTION, Laurie S. Kaguni, Catherine M. Wernette, Matthew W. Olson and Richard D. Newcomb, Department of Biochemistry, Michigan State University, East Lansing, MI 48824.

Drosophila DNA polymerase γ has been purified 2500-fold from embryonic mitochondria. The enzyme is a heterodimer comprising subunits of 125,000 and 35,000 daltons. The DNA polymerization function has been assigned to the larger polypeptide.

Unlike DNA polymerization by the replicative α polymerase, that catalyzed by γ polymerase is efficient on single-stranded as compared to double-stranded DNA templates, under conditions of primer-template excess and optimal (high) salt concentration. Under such conditions the processivity of DNA synthesis is only ~ 30 nucleotides. However, because Pol γ preferentially utilizes previously extended primer termini, it generates long products and a pattern of site-specific pausing characteristic of eucaryotic DNA polymerases. Remarkably, in reactions performed in the absence of salt the mitochondrial DNA polymerase is highly processive, and is capable of polymerizing through sites of stable secondary structure to fully replicate singly-primed M13 DNA.

Drosophila DNA polymerase γ replicates DNA with a high degree of accuracy. Although replication fidelity is affected by a nucleotide pool bias, the mitochondrial DNA polymerase shows no preference for dATP or dTTP in unbiased pools. Further, Pol γ does not misincorporate ATP. Our recent results indicate that the enzyme contains a potent 3'-5' exonuclease which edits errors prior to nucleotide polymerization. These catalytic properties will be discussed in regard to the requirements for mitochondrial DNA replication *in vivo*.

Molecular Mechanisms in DNA Replication and Recombination

L 440 INHIBITION OF REPLICON INITIATION FOLLOWING TRANSIENT FREEZING OF THE TOPOISOMERASE-II-DNA COMPLEX. William K. Kaufmann and Jayne C. Boyer, Department of Pathology and Curriculum in Toxicology, University of North Carolina at Chapel Hill, 27599-7295.
DNA topoisomerase II may be required to separate inter-twined daughter chromatids formed during replication of replicon clusters. Certain DNA intercalating agents and epipodophyllotoxins cause DNA topoisomerase II to become frozen in a cleavable complex, with DNA and protein covalently linked at the site of single- and double-strand breaks. A 10 min exposure of diploid human fibroblasts to two such agents, amsacrine or etoposide, produced a concentration-dependent increase in DNA strand breaks as monitored by alkaline elution chromatography. Removal of the drugs resulted in full resealing of these breaks within 30 min. Drug-induced effects on replicon operation were examined by quantification of changes in the size distribution of intermediates of DNA replication. When cells were exposed to 1 uM amsacrine or 10 uM etoposide for 10 min followed by 30 min incubation in drug-free medium, DNA synthesis in newly initiated replicons was inhibited by about 50%. DNA synthesis in replicons that were operating at the time of drug exposure was not affected at these low concentrations, although higher concentrations of the drugs also inhibited chain elongation in operating replicons. The selective inhibition of replicon initiation seen after exposure to a low concentration of drug persisted for up to 90 min without recovery. These results suggest that the topoisomerase II-mediated DNA strand breaks produce the same effect on DNA replication as radiation-induced breaks. This supports the concept that the clastogenesis and cytotoxicity that follows treatment with inhibitors of topoisomerase II may overlap with radiation-induced pathways of cellular injury. Supported by PHS grant CA42765.

L 441 THE INVOLVEMENT OF SV40 IN CARCINOGEN INDUCED OVERREPLICATION AND GENE EXPRESSION. Tamar Kleinberger, Anat Parket, Mirit Aladjem and Sara Lavi, Department of Microbiology, The George S. Wise Faculty of Life Sciences Tel Aviv University, Ramat Aviv 69978, Israel.
Previous work from our laboratory has shown that carcinogens induce transient amplification of SV40 DNA and of the dhfr gene. An in-vitro replication system has been developed which mimicks faithfully SV40 amplification induced in vivo. Extracts from carcinogen-treated, SV40-transformed Chinese hamster cells (C060) facilitate SV40 replication to a higher degree than do untreated cell extracts. The region lying 1.5 kb around the SV40 origin of replication is maximally amplified, unlike the complete replication known in the more permissive HeLa cells. When extracts of carcinogen-treated Chinese hamster embryo (CHE) cells are used in this system, they cannot facilitate SV40 over-replication, even in the presence of excess exogenous T antigen. However, extracts of treated SV40-infected CHE cells can support SV40 over-replication. Extracts of SV40-infected CHE cells which were not treated by carcinogens, do not support viral replication in-vitro. Independently of gene amplification, enhanced gene expression is also observed shortly after exposure to carcinogens. The activity of the chloramphenicol acetyltransferase (CAT) gene linked to promoters of SV40, β-actin and LTR of an intracisternal A particle is increased in both C060 and CHE cells upon carcinogen treatment. The levels of SV40, β-actin and β-actin CAT RNAs are also enhanced in both these cell systems. However, transcription rates are increased only in the SV40-transformed C060 cells and not in CHE cells. Thus both types of carcinogen induced cellular responses, amplification and enhanced transcription, seem to be modulated by SV40. The possible synergistic effect of T antigen, a known transactivating protein, and carcinogen-induced processes is currently being investigated. Possible involvement of cellular T antigen-like transacting factors in carcinogen-induced cellular gene amplification is yet to be approached.

L 442 COMPARISON OF SPONTANEOUS MUTATIONS AT THE APRT LOCUS AND A PLASMID-ENCODED LOCUS IN NORMAL HUMAN CELLS, Donna K. Klinedinst and Norman R. Drinkwater, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706.
The molecular analysis of spontaneous mutations can provide useful insights into the fidelity of DNA synthesis and the nature of errors made by the replication complex. Into a repair-proficient lymphoblastoid cell line (H8S3), we have introduced the HSV-*tk* target gene carried on a plasmid (pND123) that is episomally maintained due to the presence of the Epstein-Barr viral origin. The H8S3 cell line is heterozygous at the APRT locus. We have compared the spontaneous mutation rate of the plasmid-encoded HSV-*tk* gene with that of the endogenous APRT locus. The median mutant frequencies for the APRT locus and the HSV-*tk* gene in independent clonal isolates of H8S3 were $6x10^{-5}$ and $1x10^{-4}$, respectively, after 28-29 generations. The corresponding mutation rates were $1.3x10^{-5}$ and $1.7x10^{-5}$ /cell/generation. However, the two loci differed in the types of spontaneous mutations isolated: 48% of the HSV-*tk* mutations were deletions, while 10% of the APRT mutants showed allele loss by Southern blot. Preliminary sequence data of the HSV-*tk* mutants indicates that 5 of the 6 possible single base changes occurred, the exception being the G->C transversion mutation. Several deletion mutations occurred between very short direct repeats, consistent with the data from *E. coli* and the endogenous APRT locus in CHO cells.

L 443 HELA DNA POLYMERASE α ACTIVITY CHANGES AFTER PROTEIN KINASE C OR ALKALINE PHOSPHATASE EXPOSURE, Sharon W. Krauss and Stuart Linn, Dept. of Biochemistry, Univ. of California-Berkeley, Berkeley, Ca. 94720

Exposure to protein kinase C stimulates DNA polymerase α activity 2-3 fold, increases its fidelity *in vitro* by roughly 2- fold and reduces its K_m for primer-template but not for dNTP's. (Krauss *et al*, JBC,262,3432, 1987) Such effects are not observed with DNA polymerases β, γ, or δ and there is no apparent effect upon the primase activity associated with DNA polymerase α. After protein kinase C exposure, the catalytic subunit of DNA polymerase α is phosphorylated and binding to DNA-cellulose is strengthened. Neither 3'→5' nor 5'→3' exonuclease activities can be detected before or after protein kinase exposure. There is no apparent effect on processivity at 1 mM Mg^{2+}. Exposure of polymerase α to alkaline phosphatase reduces its activity and fidelity 4-5 fold and weakens its binding to DNA-cellulose. Although the lower affinity for DNA-cellulose can be reversed after subsequent exposure to protein kinase C, activity and fidelity can be only partially restored. The alkaline phosphatase treatment does not change the mobility of the α catalytic subunit on SDS gels.

L 444 IDENTIFICATION AND ANALYSIS OF NEW AUTONOMOUSLY REPLICATING PLASMIDS BEARING MONKEY DNA ORIGIN-ENRICHED SEQUENCES (ORS). Suzanne Landry and Maria Zannis-Hadjopoulos, McGill Cancer Centre, McGill University, Montréal, Québec, Canada H3G 1Y6.

A library of one hundred origin-enriched sequences (ors) obtained from monkey (CV-1) DNA replicating at the onset of S phase, was first screened by dot-blot hybridization for sequence homology with the functional ors 3, 8, 9 and 12 that have been previously described (Zannis-Hadjopoulos et al., Mol. Cell. Biol. 5:1621, 1985; Frappier and Zannis-Hadjopoulos, Proc. Natl. Acad. Sci. 84:6668, 1987). Five homologous clones were identified and tested further for transient episomal replication in transfected HeLa cells. Using two assays, Dpn I resistance and bromodeoxyuridine (BrdU) substitution, we showed that four of these five clones, namely ors 13, 14, 15 and 19, contain inserts that can initiate and support their autonomous replication in HeLa cells. Furthermore, additional clones of the ors library chosen at random, were screened by Dpn I resistance and ten out of eighteen were found to be positive. Sequence analysis of some of these clones, as well as of ors 13, 14, 15 and 19, has revealed some common features, such as AT-rich regions and inverted repeats, as well as some high homologies with previously described functional ors.

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L 445 IN VITRO REPLICATION OF SV40 IN EXTRACTS FROM CARCINOGEN TREATED CHINESE HAMSTER CELLS (C060): ONION SKIN MODE OF DNA REPLICATION. S. Lavi, Y. Berko-Flint, D. Hassin, S. Karby. Dept. of Microbiology, Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Israel.

Carcinogens induce exogenous and endogenous SV40 DNA replication in semipermissive Chinese hamster (CH) cells. The overreplication of the viral DNA is transient and requires an origin of replication and T antigen. Sequences flanking the 1.5 kb region around the origin are preferentially replicated in the treated cells. An in vitro replication system was developed to characterize the carcinogen induced factors. In the presence of T antigen the activity of cytosolic extracts prepared from drug treated C060 cells (SV40 transformed CH cells) was markedly increased as compared to the extracts from nontreated C060 cells. Replication products synthesized by the treated C060 extract appeared mainly as replicative intermediates and a heterogenous collection of DNA fragments. Analysis of the newly replicated DNA revealed that only the fragments surrounding the 1.5 kb region around the origin were replicated by the treated C060 extract. The amplification in vitro is bidirectional and symmetrical. The reaction is aphidicolin sensitive and is dependent on cellular factors present in the extract from the treated cells. The newly synthesized DNA from reactions directed by extracts from carcinogen treated cells was digested by DpnI and subjected to two dimensional neutral-alkali agarose gel electrophoresis. The labeled DNA was associated mainly with replicative intermediates which were dissociated after alkali denaturation to non-radioactive template molecules and to newly synthesized labeled short DNA fragments. Reactions were carried out in the presence of 5-bromodeoxy triphosphates as density label. The DNA was digested by BstNI and fractionated on CsCl density gradient. A fraction of the radioactivity migrated as heavy-heavy DNA indicating multiple reinitiation events. Our data are consistent with onion skin mode of DNA replication in extracts from carcinogen treated cells.

Molecular Mechanisms in DNA Replication and Recombination

L 446 THE *REV3* GENE OF *SACCHAROMYCES CEREVISIAE* ENCODES AN INESSENTIAL PRODUCT THAT APPEARS TO BE A DNA POLYMERASE CONCERNED ONLY WITH MUTAGENESIS, A.Morrison, J.F. Lemontt*, A.K.Beck*, E.G.Bernstine*, R.B.Christensen, and C.W.Lawrence, Department of Biophysics, University of Rochester School of Medicine and Dentistry, Rochester NY 14642, and Biology Division*, Oak Ridge National Laboratory, Oak Ridge, TN 37830.
The function of the *REV3* gene is reqired for normal mutagenesis induced by radiations and other DNA damaging agents in bakers'yeast. *Rev3* mutants are substantially deficient in induced mutagenesis but only moderately hypersensitive to killing by mutagens, are slightly deficient in spontaneous mutagenesis (S.K.Quah *et al.*, Genetics 96: 819-839), but are normal with respect to growth and recombination. The *REV3* gene was cloned by complementation of the *rev3-1* defect in induced mutagenesis, using an enrichment procedure based on enhanced survival and mutagenesis in *REV3+* clones. Deletion and sequence analysis of the cloned fragment identified an ORF of 4512 bp, which encodes a predicted protein of 173 kD. Optimal alignment of the predicted protein gives 24% identity with Epstein-Barr DNA polymerase over a region of more than 600 residues. The *REV3* protein also shares the conserved sequences identified by S.W.Wong *et al.*(EMBO J.7:37-47), strongly implying that it is indeed a DNA polymerase. Haploid strains carrying a deletion of the gene are, however, viable, suggesting that the protein is unlikely to be a normal replicase. We tentatively conclude that the protein encoded by *REV3* is concerned only with replication on damaged or distorted templates.

Supported by grant No. GM21858 from NIH, and grant No. DEFG0288ER60626 from DOE.

L 447 THE MITOCHONDRIAL DNA POLYMERASE FROM SACCHROMYCES CEREVESIAE HAS AN ASSOCIATED 3'->5' EXONUCLEASE, Ericka A. Lawson, Robert Vincent and H. Peter Zassenhaus, Department of Microbiology, Saint Louis University, Saint Louis, MO 63104. Crude mitochondrial extracts contain a DNA polymerase which is distinctly different from the nuclear DNA polymerase. The mitochondrial polymerase is not sensitive to aphidicolin, whereas the nuclear polymerases have marked aphidicolin sensitivity. The mitochondrial DNA polymerase also has a magnesium requirement greater than that of other polymerases (25 mM to 50 mM). A 3'->5' exonuclease has been identified in the crude extract. The exonuclease co-purifies through heparin sepharose with the polymerase. The exonuclease has a magnesium requirement similar to that of the polymerase and works in association with it to repair 3' mismatches. The amount of exonuclease activity relative to the polymerization activity is reminiscent of T4 DNA polymerase. Presently no 5'->3' exonuclease has been shown to be associated with the mitochondrial DNA polymerase.

L 448 HUMAN DNA POLYMERASES δ AND α ARE IMMUNOCHEMICALLY RELATED. N. Lan Toomey, Chun-Li Yang, Yunquan Jiang, Fatima De-Freitas and Marietta Y.W.T. Lee, Department of Medicine, University of Miami School of Medicine, Miami, FL 33101.
DNA polymerases δ and α were purified from human placenta by a new procedure which minimizes proteolytic modification of the enzymes. The method uses affinity chromatography with immobilized carboxymethylated β-lactoglobulin and α_2-macroglobulin for the removal of active protease activities from the tissue extracts. DNA polymerases δ and α from the same tissue were found to be discrete enzymes which are different in a number of properties. These include heat stability, sensitivity to N-ethylmaleimide, BuAdATP and DNA polymerase α antibodies. Moreover, factors that stimulate DNA polymerase δ do not stimulate DNA polymerase α. A panel of monoclonal antibodies we have produced against DNA polymerase δ was tested against DNA polymerase α. These antibodies included those which inhibited DNA polymerase δ but not DNA polymerase α. However, several antibodies were found which inhibited and immunoblotted both DNA polymerases. This demonstrates that these two DNA polymerases are immunochemically related and provides the first evidence that DNA polymerase δ and α may have structural similarities.
(Supported by GM 31973; M.Y.W.T.L. is an Established Investigator of the American Heart Association.)

Molecular Mechanisms in DNA Replication and Recombination

L 449 STUDIES ON INITIATION OF REPLICATION IN THE AMPLIFIED DIHYDROFOLATE REDUCTASE DOMAIN OF A METHOTREXATE-RESISTANT CHO CELL LINE. Tzeng-Horng Leu and Joyce L. Hamlin, University of Virginia School of Medicine, Department of Biochemistry, Charlottesville, VA 22908.

We have previously shown in synchronized cells that replication initiates preferentially somewhere within a 28 kb locus in the amplified dihydrofolate reductase domain of the methotrexate-resistant CHO cell line, CHOC 400. However, because of background labelling emanating from other single copy origins firing at the same time in the early S period, we were not able to quantitate the relative labelling of individual amplified bands within this locus, and were therefore not able to locate the origin with great precision. In more recent studies, we have utilized an in-gel renaturation procedure developed by Roninson to eliminate background labelling and have been able to follow the pattern of labelling within the amplicon with great precision. A modification of this technique, termed "hybridization enhancement", has also allowed us to increase the resolution of the labelling method considerably; the results of these studies suggest that there are actually two origins of replication (ori-beta and ori-gamma) separated by ~20 kb within the previously defined initiation locus. Studies on another cell line with a much larger amplicon have identified a third origin of replication that maps ~250 kb upstream from ori-beta. We are currently using the hybridization enhancement method to more precisely localize this third origin so that the sequence of all three early-firing origins can be compared.

L 450 REPLICATION OF YEAST CHROMOSOMAL DNA UNDER VARIOUS GROWTH CONDITIONS, Maarten H. K. Linskens and Joel A. Huberman, Department of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, NY 14263

Our lab has recently demonstrated that some, but not all, ARS elements are used as chromosomal replication origins in the yeast *Saccharomyces cerevisiae* (NAR 16, 6373-6384; MCB 8, 4927-4935). In our study of rDNA replication we found that only 10-30% of the available origins were utilized for initiation in a single S phase. We therefore tested the utilization of replication origins in different yeast strains grown under several temperature and nutrient conditions. The results show that, for the rDNA origin and the origin at A6C in chromosome III, increasingly stringent growth conditions decrease the frequency of initiation. These data, taken together with several reports of non-specific replication initiation under special conditions (i.e. in *Xenopus* egg extracts), suggest that initiation of DNA replication at specific origins may be a stochastic process, possibly dependent on the concentration of *trans*-acting factors and/or chromatin structure.

L 451 A NEW FORM OF MITOCHONDRIAL ENDONUCLEASE APPEARS MORE SITE SPECIFIC, Robert L. Low, William A. Parks and Chris Couper, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262

Mammalian mitochondria contain a potent endonuclease activity that becomes evident once intact organelles are disrupted. In standard DNA replication assays, both double-stranded (ds) and single-stranded (ss) DNA templates are extensively fragmented within minutes at 37°C. The bovine mt endonuclease appears to be a dimer of a 29 kDa polypeptide. It requires Mn^{2+} or Mg^{2+} for activity and degrades DNA templates at acid and neutral pH. This bovine endonuclease as well as the corresponding enzymes from human and rat heart mitochondria show an unusually strong preference for a specific sequence (CSB-II) in the D-loop region. Recently, we have identified a new mitochondrial activity that appears more specific. It also requires Mn^{2+} or Mg^{2+}, nicks (but does not degrade) a recombinant template that contains the D-loop region but not M13 RF DNA or pBR322 DNAs, and fails to degrade M13 ssDNA. The possibility that this activity as a site specific swivelase *in vivo* is being explored.

Molecular Mechanisms in DNA Replication and Recombination

L 452 MECHANISMS OF AMPLIFICATION OF THE DIHYDROFOLATE REDUCTASE GENE IN CHO CELLS. Chi Ma*, Barbara Trask+, and Joyce Hamlin*, University of Virginia School of Medicine, Charlottesville, VA 22908*, and University of California Lawrence Livermore National Laboratory, Livermore, CA 94550+.

We have previously isolated overlapping cosmid clones representing two different amplicon types from the methotrexate-resistant CHO cell line, CHOC 400. These clones have been utilized in combination with pulse-field gel electrophoresis to analyze large SfiI fragments from the amplicons in CHOC 400 cells. These studies indicate that the minor type I amplicons are arranged head-to-tail in the genome, while the major type II amplicons (which represent ~75% of all amplicons) are arranged in head-to-head and tail-to-tail configurations. Because these amplicons are relatively small, and because in earlier studies there appeared to be only one initiation locus per amplicon, we previously suggested that an amplicon might be equivalent to a replicon. However, studies on less resistant cell lines that gave rise to CHOC 400, as well as two other independently isolated highly resistant Chinese hamster cell lines, show that dihydrofolate reductase amplicons are usually at least 650 kb in length. In one of these cell lines, we have identified and cloned an additional upstream initiation locus. Therefore, in this cell line, amplicons are clearly larger than one replicating unit. We will also present in situ hybridization data demonstrating that the two parental dihydrofolate reductase loci in amplified cell lines usually remain intact in their original positions in the chromosomes and are separated from the site of the amplified DNA, which can be found either on the same chromosome or on a different chromosome. The patterns of hybridization observed suggest that sequence amplification is unlikely to result from a simple unequal sister chromatid exchange mechanism.

L 453 PROTEIN-DNA INTERACTIONS AT THE CHROMOSOME III CHORION LOCUS IN DROSOPHILA MELANOGASTER, Angus M. MacNicol and I.R. Lehman, Department of Biochemistry, Stanford University, Stanford, CA 94305.
During Drosophila oogenesis the transcribed sequences within each chorion gene cluster are amplified. It has been proposed that the amplified domains result from multiple initiations within a single region containing a putative origin. The amplified domain extends beyond the area of the chorion genes, where a gradient of amplification extends 40-50 kb in either direction from the centrally located chorion gene cluster. An essential amplification control element (ACE) lies upstream of the s18 chorion gene, and a putative origin resides downstream of the s18 gene, 1.5 kb distant from the ACE. Protein-DNA interactions occurring at the ACE and putative origin sequence have been investigated by gel mobility shift and DNaseI footprinting assays. Nuclear extracts prepared from various developmental stages yield differing protein-DNA complex mobilities when assayed by gel shift. The data suggest that several protein-DNA interactions occur within these regions, and that the interactions may be developmentally regulated. The purification of these proteins will be presented. A detailed biochemical investigation of the proteins interacting with the origin of chorion gene amplification will provide insight into the protein-DNA interactions occurring during the initiation of DNA replication at a eukaryotic chromosomal origin. Supported by NIH grant GM06196.

L 454 AN ORIGIN RECOGNITION PROTEIN ASSOCIATED WITH A MULTIENZYME REPLICATION COMPLEX FROM HELA CELLS THAT SUPPORTS SV40 REPLICATION IN VITRO. L. Malkas, R. Hickey, C. Li, N. Pedersen, and E. Baril, Woro. Fdn. Exp. Bio., Shrewsbury, MA. A protein which specifically recognizes the 17 bp A+T tract in the SV40 replication origin has been purified to apparent homogeneity. This SV40 origin recognition protein and several enzymes for DNA synthesis are recovered in a sedimentable fraction (P-4) obtained by discontinuous gradient centrifugation of a polyethylene glycol (PEG) treated low salt nuclear extract/postmicrosomal supernatant (NE/S-3) (Cancer Cells [1988] 6: 373). The majority of the T-antigen dependent in vitro SV40 DNA replication (Li and Kelly [1984] PNAS 81: 6973) and DNA synthesis enzymatic activities in the NE/ S-3 are found in the sedimentable (P-4) fraction. Several enzymes for DNA synthesis in the P-4 have been identified. These include the 640 kDa multiprotein form of DNA polymerase α (J. Biol. Chem. [1988] 261:6619), topoisomerase I, a DNA-dependent ATPase, RNase H, and DNA ligase. The SV40 replication and enzyme activities co-sediment as an 18-20 S peak during sedimentation analysis. The SV40 replication and DNA polymerase activities associated with the 18-20 S multienzyme complex are extremely sensitive to butylphenylguanine triphosphate (BuPdGTP) and are completely inhibited by the monoclonal antibody (SJK132-20) against human polymerase α. In addition, the resolution of the multienzyme complex by affinity and conventional chromatographic procedures indicates that all of the polymerase activity is attributable to the 640 kDa form of polymerase α. These and other results to be presented suggest that a multienzyme complex functions in SV40 replication in HeLa cells. Supported by NIH grant CA-15187 (EB), NIH fellowship CA-08173 (LM).

L 455 OVEREXPRESSION OF HSV DNA POLYMERASE AND MUTAGENESIS OF CONSERVED REGIONS A. Marcy,[1] J. Gibbs,[1] P. Olivo,[2] M. Challberg[2] and D. Coen[1] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115[1] and Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases Bethesda, MD 20892[2] The DNA polymerase of herpes simplex virus (HSV pol) contains six regions of amino acid similarity with DNA polymerases from a variety of sources including human pol alpha. Regions II, III and V of sequence similarity are thought to include portions of HSV pol involved in substrate interactions since viruses containing mutations in these regions exhibit altered sensitivities to nucleoside and/or pyrophosphate analogs. We have begun efforts to recover viruses containing mutations in two regions of the HSV pol gene in which no mutations have yet been identified (I, IV). We have introduced specific mutations by oligonucleotide mutagenesis of the HSV pol gene contained in M13 vectors and have recombined them back into the HSV genome using a pol mutant which expresses beta-galactosidase. Preliminary results indicate that viruses with mutations in region IV do not grow in Vero cells, but replicate to wild-type levels in cells with a resident HSV pol gene. This suggests that region IV is essential for polymerase function. The baculovirus system permits biochemical comparisons of overexpressed mutant and wild-type enzymes. Insect cells infected with a baculovirus recombinant containing the HSV pol open reading frame express approximately 10-fold more pol than HSV-infected Vero cells. Purification of the HSV pol from infected insect cells has shown that the enzyme has chromatographic and sedimentation properties and specific activity indistinguishable from pol produced in HSV-infected Vero cells.

L 456 The Need for Enhancers to Activate the Polyoma Origin of Replication is acquired in Mammalian Development with the Formation of a Diploid Nucleus, Encarnación Martínez-Salas and Melvin L. DePamphilis, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The activity of the polyoma virus (PyV) origin of DNA replication was used as a sensitive assay for enhancer function in 1-cell and 2-cell mouse embyos by injecting them with plasmid DNA containing different PyV *ori* configurations, allowing them to continue development *in vitro*, and then measuring plasmid DNA replication. Replication always required the PyV origin "core" sequence in *cis* and the PyV large tumor antigen in *trans*. In developing 2-cell embryos, DNA replication and gene expression also required enhancer-elements in the PyV *ori*-region. However, 1-cell embryos did not need an enhancer-element for replication. The core-element replicated 9-fold better in 1-cell embryos than the complete PyV origin did in developing 2-cell embryos. Competition experiments suggested that enhancers bound specific proteins in developing 2-cell embryos, but not in 1-cell embryos. Since 1-cell embryos that replicated the injected DNA were the ones that remained as 1-cell embryos and retained their pronuclei, enhancers are not needed in mammalian development until a diploid nucleus is formed. A particular nuclear composition could be one of the factors involved. Injection into 2-cell embryos causes the plasmid DNA to be relaxed and, later on, it is found as supercoiled DNA (60-70% of the total DNA mass). In constrast, supercoiled form never represents more than 10-20% of the total DNA in 1-cell embryos.

L 457 NUCLEOTIDE MISINSERTION AND EXTENSION EFFICIENCIES OF DNA POLYMERASE α AND AMV REVERSE TRANSCRIPTASE: NEAREST NEIGHBOR EFFECTS ON FIDELITY; Lynn Mendelman, Michael S. Boosalis, John Petruska and Myron F. Goodman, Department of Molecular Biology, University of Southern California, Los Angeles, CA 90089-1340

The inherent fidelity of a polymerase governs an initial step in nucleotide selection during DNA replication. Using a site specific assay to measure the kinetic constants of inserting correct and incorrect nucleotides at specific template sites, we compare two polymerases, Drosophila melanogaster DNA polymerase α and AMV reverse transcriptase, in their ability to replicate single stranded M13 template. Seventeen independent template sites were chosen with twelve representative nearest neighbors. Transitions were made almost equally by both enzymes, with a frequency between 1/1,000 and 1/10,000. Transversions were made more often by DNA polymerase α. CC mispairs were rarely synthesized by both enzymes. Overall, AMV rt made misinsertions less frequently than DNA polymerase α. Conversely, extensions from synthetic mispaired primer ends occurred sometimes ten-fold more frequently with the reverse transcriptase, implying that the reported lower fidelity for AMV rt may stem from its relative ease in extending mispaired primer termini. Finally, we present a model in which stacking interactions at the primer 3' end could explain hot spots for specific nucleotide misinsertions, where ranges in misinsertion efficiencies have been observed to vary five to ten-fold between template sites.

L 458 IMMUNOLOGICAL ANALYSIS OF A CALF THYMUS DNA-DEPENDENT ATPASE
Larry D. Mesner, William M. Sutherland and Joel W. Hockensmith, Dept. of Biochemistry, Univ. of Virginia Sch. of Med., Charlottesville, VA 22908.
We have been intent on establishing a definitive role for eucaryotic DNA-dependent ATPases and have pursued development of monoclonal antibodies (Mab) against a 68 kD DNA-dependent ATPase (Hockensmith et al., Biochemistry 25, 7812-7821). In the course of purifying this ATPase for Mab development, we resolved two chromatographically distinct ATPases, each of which prefers a poly(dA·dT) effector for ATP hydrolysis. We used these two ATPases (ATPase A and ATPase AT) as antigens for murine inoculation and hybridoma cloning. As a result, we have isolated two Mabs (both IgG_1). Mab 2D1 cross-reacts with both antigens, however, Mab 6E12 is specific only for ATPase AT. Both antigens are bound by their reactive antibodies in solution and are removed from solution by binding to a protein G matrix. Both antigens can be recovered by elution of the antigen-antibody complex from the protein G matrix. The antibodies also react with the antigens which have been electroblotted onto nitrocellulose membranes after SDS-PAGE, thereby yielding a definitive identification of the catalytic polypeptides. Probing of the transfers demonstrates that one Mab (2D1) cross-reacts with ~70 kD and ~83 kD bands, while the other Mab reacts only with an ~83 kD band. We conclude that ATPase A (~70 kD) is a proteolyzed fragment of the parent ATPase (~83 kD) (ATPase AT). These observations may account for the prior difficulty in establishing a definitive role for the ATPase A.

L 459 REPLICATION OF SOLUBLE AND NUCLEAR MATRIX-BOUND DNA IN AN IN VITRO SYSTEM,
R. Miskimins and W.K. Miskimins, Department of Biology, University of South Carolina, Columbia, S.C. 29208
We have isolated a fraction from log phase HeLa cells (P15H) that contains DNA polymerase alpha, an ATP-dependent topoisomerase, and several other enzymatic activities known to be necessary for DNA replication. This fraction is capable of initiating replication of DNA in vitro utilizing as templates either supercoiled plasmids or mammalian DNA that is organized in loops that are attached to the nuclear matrix. Using either template there is a lag period of about 10-15 minutes before replication begins. Synthesis is optimum with about 2.3 ug of protein from the active fraction using supercoiled plasmid as the template. When matrix-attached fragments are used optimum synthesis is achieved with about 15 ug of protein. The replication observed is totally inhibited by aphidicolin and is not dependent on preprimed DNA templates. The products of the reaction using plasmid DNA templates are closed circular and supercoiled DNA molecules. Nearly all of the products from the reaction can be positively supercoiled by the intercalator chloroquine, indicating that they are not nicked. Density substitution experiments using BrdUTP and cesium chloride gradients show that replication is semi-conservative on either template. Using plasmids with various inserts we have not seen any site-specific initiation of replication. In the case of replication using loops of DNA bound to the matrix, radioactive label appears first in DNA fragments that are attached to the scaffold and subsequently appears in non-attached sequences. This may indicate that initiation of replication occurs within nuclear matrix attachment sites.

L 460 **THE EFFECT OF ULTRA-VIOLET IRRADIATION ON SV40 DNA REPLICATION *IN VITRO*.**
*Murakami,Y., *,**Miyamoto, N., *,** Kihara, E. and *Inada, T. *Tsukuba Life Science Research Center, RIKEN, Koyadai, Tsukuba, 305, Japan. **Dept.of Radiation biology, Institute for Fundamental Medical Science, University of Tsukuba. Tennodai,Tsukuba, 305,Japan.
The effect of ultra-violet light irradiation on SV40 DNA replication *in vitro* has been analyzed. A plasmid DNA containing SV40 replication origin was irradiated prior to replication reaction. T antigen dependent DNA replication *in vitro* was sharply inhibited by the UV-irradiation. On the other hand, T antigen independent repair synthesis was induced by UV-irradiation. Analysis of replication products suggested the inhibition was occurring not only in the initiation step but also in the step of chain elongation. The effect of UV-irradiation on the initiation reaction will be discussed. *In vitro* system for repair synthesis induced by UV-irradition has been developed using a similar condition to SV40 DNA replication system *in vitro*. The DNA synthsis in this system was highly dependent upon UV-irradiation. The reaction required HeLa cell extract, magnesium, and ATP, however, an omission of ATP-regenerating system had little effect on DNA synthesis. Characterization of this system will be presented.

Molecular Mechanisms in DNA Replication and Recombination

L 461 THE FIDELITY OF REPLICATIVE DNA POLYMERASES FROM CALF THYMUS, Thomas W. Myers*, Robert A. Bambara*, David C. Thomas, and Thomas A. Kunkel, *Dept. of Biochemistry, U. of Rochester, Rochester, NY 14642 and Lab. of Molecular Genetics, NIEHS, Research Triangle Park, NC 27709.
We are examining the fidelity of DNA synthesis catalyzed by two putative replicative DNA polymerases, the four subunit DNA polymerase α-DNA primase complex purified by immunoaffinity chromatography and the PCNA non-stimulable form of DNA polymerase δ (designated Pol δII), having a non-dissociable 3'→5' exonuclease activity. The M13mp2 fidelity assays used include a forward mutation assay that detects a variety of different errors and reversion assays for base-substitutions and one-base frameshifts. Just as for Pol α obtained by conventional chromatography, the Pol α-primase complex commits a variety of base-substitution and frameshift mistakes, at a frequency of 1 error for each 5,000 to 10,000 bases polymerized. Thus, neither the primase subunits nor the higher molecular weight form of the catalytic subunit in the complex substantially enhance base selectivity. Fidelity is greater using reaction conditions known to increase the processivity of polymerization with synthetic polynucleotides, prompting an examination within the M13mp2 target sequence of the site to site variations in error rates versus termination probabilities. Unlike Pol α, DNA polymerase δII is highly accurate, with respect to base-substitution errors, in part due to exonucleolytic proofreading. We are presently examining the error specificity of Pol δII for a wide variety of errors in the forward mutation assay, using reaction conditions that modulate proofreading.

L 462 EVIDENCE FOR A NESTED DISCONTINUITY IN THE SYNTHESIS OF THE LAGGING DNA STRAND OF SIMIAN VIRUS 40, Tamar Nethanel, Tamar Zlotkin and Gabriel Kaufmann, Biochemistry Department, Tel Aviv University, Israel 69978.
Previous work indicated that nascent DNA chains below 40 nucleotides accumulate in SV40 DNA replicating in isolated nuclei in the presence of aphidicolin. These products resemble normal precursors of Okazaki pieces in size-distribution, partial content of RNA primers and lagging replication-fork polarity. The manner in which these precursors (termed DNA primers) configure on the lagging template led us to propose that an Okazaki piece may be synthesized by ligation of shorter precursor chains[1]. Attempts to extend and ligate the lagging nascent DNA chains within replicating SV40 DNA synthesized in vivo provided further evidence in support of this notion. The reactions were catalyzed by T4 DNA ligase combined with E. coli DNA polymerase I large fragment or with MMLV reverse transcriptase which has an associated RNase H activity. The data indicated that a growing Okazaki piece may be devoid of an RNA primer at its 5'-end yet face at its 3'-end a downstream long chain which still contains an RNA primer. Hence, prior to its incorporation onto a long nascent chain, the mature Okazaki piece must regain the RNA primer moiety. Presumably, this entails ligation of a DNA primer unit to the 5'-end of a growing Okazaki piece.
1. Nethanel et al., (1988) J. Virol. 62, 2867.

L 463 DNA POLYMERASE III HOLOENZYME NEEDS ONLY HALF ITS SUBUNITS FOR ATP-ACTIVATED RAPID AND PROCESSIVE DNA REPLICATION
Mike O'Donnell, Maija Skengalis, Todd Stukenberg, Patricia S. Studwell, Rene Onrust
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DNA polymerase III holoenzyme (PolIII H.E.) is the replicase of E. coli and is composed of 10 subunits. PolIII H.E. can be reconstituted from the polIII core subassembly of α(pol),ε(exo), θ , and the accessory proteins β and the γ-complex subassembly of γ,δ,δ',χ,ψ. PolIII H.E. is different from non-replicative polymerases in its high turnover number (500 nucleotides/s) and great processivity (>8kb). With the aim of understanding the individual function of each subunit of polIII H.E. we have determined the minimum number of subunits required to reconstitute the rapid and processive polymerase. The α, ε, β, γ, and τ subunits were purified from their respective overproducing E. coli strains. The αε complex fully substitued for core in reconstituting a processive polymerase with β and the γ-complex, hence θ is not required. The γ-complex was resolved into the δ subunit, the δ' subunit and a complex of γχψ. Gamma and δ were as efficient as the whole γ-complex in reconstituting processive replication with β and αε. Hence full processivity can be achieved with only 5 of the 10 proteins. Neither γ nor γχψ were active with δ'. Surprisingly, τ was fully active with δ' (and δ) which suggests an asymmetry in polymerase structure which could be interpreted as a dimeric polymerase with τδ' on one half, γχψδ on the other and the two halves being connected via a δδ' dimer. Alternatively, polIII H.E. may have only one core molecule with two accessory protein primer binding components: a γχψδβ complex and a τδ'β complex, which may mediate polymerase cycling to multiple lagging strand primers.

L 464 A MISMATCH SPECIFIC 3'-→5' EXONUCLEASE ASSOCIATED WITH THE MITOCHONDRIAL DNA POLYMERASE FROM DROSOPHILA EMBRYOS, Matthew W. Olson and Laurie S. Kaguni, Department of Biochemistry, Michigan State University, East Lansing, MI 48824. Drosophila DNA polymerase γ replicates single-stranded DNA with a high degree of accuracy: the fidelity of in vitro DNA synthesis by the mitochondrial DNA polymerase is comparable to that of E. coli DNA polymerase III holoenzyme. The latter enzyme contains a 3'-5' exonuclease which proofreads errors and increases replication fidelity ~ 100 -fold. Our recent studies demonstrate that although DNA polymerase γ does not catalyze detectable dNTP turnover, it possesses a potent and highly mismatch-specific 3'-5' exonuclease which excises unpaired 3'-termini at ~ 30% of the rate of nucleotide polymerization. Further, under in vitro DNA replication conditions the mitochondrial DNA polymerase removes 3'-terminal mismatched nucleotides prior to primer extension. The high fidelity of DNA synthesis catalyzed by Pol γ suggests that the high rate of evolution of animal mitochondrial DNA may result primarily from lack of post-replicational repair.

Drosophila DNA polymerase γ is a heterodimer comprising two subunits of 125,000 and 35,000 daltons. Our recent results indicate that the 3'-5' exonuclease and DNA polymerase activities are quantitatively associated in the final steps of purification. Data regarding the subunit association of the editing exonuclease will be presented.

L 465 HUMAN CYTOMEGALOVIRUS INDUCES REPLICATION OF THE SV40 DNA ORIGIN ON DNA SYNTHESIS.
Pari G. S. and St Jeor S. Department of Microbiology and the Cell and Molecular Biology Program, University of Nevada, Reno Nevada, 89557.
Human cytomegalovirus, (HCMV) unlike other human herpes viruses, stimulates host DNA synthesis early in the infectious cycle. Recently investigators have identified essential genes that replicate viral origins of replication. These experiments take advantage of the fact that these origins can be replicated by transfection of the cloned origin and subsequent superinfection with virus. The following studies were conducted to determine if HCMV could induce replication of other virus origins. SV40 or Herpes simplex origins were transfected into either Vero or Human fibroblasts, and the cells infected with HCMV. Replication was analyzed using the restriction endonuclease Dpn I, which restricts only methylated (input DNA). HCMV was able to replicate the SV40 origin of replication (in the presence of large T antigen) but did not replicate the cloned HSV-1 origins. Alternatively, HSV-1 did not replicate the SV40 origin of DNA replication. The fact that HCMV can stimulate SV40 DNA synthesis allows us to further define the genes responsible for stimulation of DNA by HCMV.

L 466 REGULATION ELEMENTS CONTROLLING THE EXPRESSION OF HUMAN DNA POLYMERASE α
Barbara.E. Pearson, Heinz-Peter Nasheuer and Teresa S.-F. Wang.
Dept. of Pathology, Stanford Univ. School of Medicine, Stanford, CA 94305

The expression of human DNA polymerase α was found amplified in transformed cells and regulated at the transcriptional level during activation of cell proliferation and in the cell cycle (Wahl et al., 1988). It is of interest to investigate what cis- and trans-acting signals are involved in the expression of this key DNA replication gene in cell proliferation, cell cycle, transformation and terminal differentiation. A genomic clone of 11.5kb containing 1.6kb sequence upstream from the translation start site, the first three exons, two introns and a portion of the third intron was isolated. The transcription start site is mapped at 47 ± 1 nucleotide upstream from the translation start site by S1 nuclease analysis and primer extension. The upstream sequence lacks a TATA, but a CCAAT sequence is found on the opposite strand at -30 relative to the main transcription start site. This upstream region is highly GC rich and contains a 14 base pair palindrome and a stem loop of -39 Kcal/mol. Clustered within the first 240 nucleotides upstream are consensus sequences for Sp1, Ap2 and Ap1 binding sites, and a sequence with similarity to adenovirus E2 early promoter. Further upstream at the -500 bp position, there is a CArG box similar to actin and c-fos SRE. These elements are bound by possible transcription proteins as demonstrated by DNase footprinting and gel shift experiments. A ~1560 bp fragment of this upstream sequence is active during transient transfection of Hela cells and supports the expression of a reporter gene, luciferase, at approximately 1/5 the level obtained from the SV40 early promoter/enhancer. The 5'and 3' boundaries of polymerase α regulatory elements are being defined, and the correlate nuclear factors required for the regulation of this essential DNA replication gene's expression in the above described cellular events are being investigated.(Supported by NIH Grant CA14835 and a gift from Donald and Delia Baxter Fund).

Molecular Mechanisms in DNA Replication and Recombination

L 467 A ROLE FOR 3'→ 5' EXONUCLEOLYTIC PROOFREADING IN HIGH FIDELITY DNA SYNTHESIS BY DNA POLYMERASE-α, Fred W. Perrino and Lawrence A. Loeb, Department of Pathology, University of Washington, Seattle, WA 98195. The primary enzyme responsible for DNA replication in eucaryotic cells, DNA polymerase-α, lacks exonuclease activity when purified as a DNA polymerase-primase complex from calf thymus by immunoaffinity chromatography. We first demonstrated that the frequency of misinsertion of A, C, or G at a template A by the DNA polymerase-α complex is 1/20,000, 1/20,000, and $1/10^6$, respectively. Unexpectedly, the rates of extension from A:A, A:C or A:G mispairs are 600,000, 5000, and $2x10^6$-fold slower, respectively, than from a correctly paired A:T 3'-terminus. This exceptionally slow rate of mispair extension suggests that an independent 3'→ 5' exonuclease might be required to hydrolyze 3'-terminal mispairs that are generated by DNA polymerase-α. Because the contribution of 3'→ 5' exonucleolytic proofreading to highly accurate DNA synthesis is well established in procaryotes, we determined if hydrolysis of 3-teminal mispairs by a separate proofreading exonuclease might contribute to the accuracy of DNA synthesis by DNA polymerase-α. Addition of ε subunit of E. coli DNA polymerase III to DNA polymerase-α complex results in excision of 3-terminal mispairs produced by DNA polymerase-α and permits the resulting correctly base-paired 3'-terminus to be rapidly extended. A sensitive biochemical assay using natural DNA and exploiting the very slow extesion of the A:G mispair has been developed to test mammalian 3'→ 5' exonucleases for their ability to proofread in concert with DNA polymerase-α. Using this assay, we have initiated a search for the putative proofreading exonuclease of DNA polymerase-α.

L 468 MUTATIONS IN THE CLONED ADENOVIRUS PRETERMINAL PROTEIN (pTP) GENE EFFECTS DNA REPLICATION ACTIVITY IN VITRO, Steven C. Pettit[1], Carl Abraham[2], Marshall S. Horwitz[2] and Jeffrey A. Engler[1], [1]Department of Biochemistry, University of Alabama at Birmingham, Birmingham, Alabama 35294 and [2]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York 10461.
The activities of a series of amino terminal (N) pTP mutants cloned in transient expression plasmids transfected into CMT4 cells have been measured, using adenovirus-specific DNA replication assays. Initiation was quantitated as the covalent attachment of [α-^{32}P]dCTP to pTP; elongation was measured by specific incorporation of [α-^{32}P]dTTP into the end fragments of Ad DNA-pro. Specific activities were obtained by quantitating pTP protein by immunoblot analysis. pTP deletion mutations that removed from 2 to 7 amino acids from the N terminus gradually decreased functional activity to 11%; a deletion of 18 amino acids was completely inactive. A cysteine to serine change at the eighth amino acid had no effect on activity. Two amino-terminal in-frame insertion mutants at positions 5 and 11 (described by Freimuth and Ginsberg, Proc. Natl. Acad. Sci. USA 83:7816-7820 (1986)) have also been tested. These mutants have partial activity between 32-60% of wt. Replacement mutations of serine to threonine or alanine at the site of covalent attachment of dCTP at amino acid 580 in pTP have also been tested; either of these changes completely abolished pTP function in both initiation and elongation assays. Further mutagenesis to define other critical sites within pTP are currently in progress.

L 469 THE STRUCTURE OF THE YEAST DNA POLYMERASE-PRIMASE COMPLEX. Paolo Plevani, Marco Foiani, Antonella Pizzagalli, Stefania Francesconi, Marco Muzi Falconi, Paola Valsasnini, Corrado Santocanale, Simonetta Piatti, Alessandra Comedini and Giovanna Lucchini. Dipartimento di Genetica e Biologia dei Microrganismi, Via Celoria 26, 20133 Milano, Italy. The yeast DNA polymerase-primase complex is composed by four polypeptides called p180, p74, p58 and p48. The genes coding for the catalytic core DNA polymerase (p180) and the two subunits of DNA primase (p58 and p48) have been cloned and sequenced in our laboratory. Protein sequence comparison indicate that the structure of the polymerase-primase polypeptides has been highly conserved during evolution. The production of temperature-sensitive mutants in the POL1 gene allowed a tentative definition of the functional domains related to the catalytic activity of the enzyme and its interaction with the other polypeptides of the complex. Finally, an autocatalytic affinity labeling procedure has been applied to map the active center of DNA primase. Both primase subunits partecipate to the formation of the catalytic site, although the NTP binding site is located exclusively on the p48 subunit.

Molecular Mechanisms in DNA Replication and Recombination

L 470 SPECIFICITY OF NUCLEOTIDE MISINCORPORATION BY HIV-1 REVERSE TRANSCRIPTASE AND MAMMALIAN DNA POLYMERASE-α, Bradley D. Preston[1], Fred W. Perrino[2], Bernard J. Poiesz[3] and Lawrence A. Loeb[2], [1]Department of Chemical Biology & Pharmacognosy, College of Pharmacy, Rutgers University, Piscataway, NJ 08855, [2]Department of Pathology, University of Washington, Seattle, WA 98195, and [3]Department of Medicine, SUNY Upstate Medical Center, Syracuse, NY 13210.

Human immunodeficiency virus type 1 (HIV-1) shows extensive genetic variation and evolves at a rate approximately a million-fold greater than eucaryotic DNA genomes. This hypermutability may be central to the pathogenesis of HIV-1 and could thwart efforts to develop effective vaccines. To begin to characterize the biochemical mechanisms of HIV-1 mutagenesis, we have studied the fidelities of purified HIV-1 reverse transcriptase (RT) and mammalian DNA polymerase-α (pol-α) during DNA polymerization in vitro. Analyses of misincorporation rates opposite a single A residue in øX174 am3 DNA showed that HIV-1 RT exhibits a misinsertion frequency (≈ 1/3,000) and mispair specificity (A:C >> A:G > A:A) unique from that of calf thymus DNA pol-α/primase (misinsertion frequency ≈ 1/20,000; A:A ≥ A:C >> A:G). HIV-1 RT and pol-α are also distinct in their abilities to continue polymerization following the insertion of noncomplementary nucleotides. Whereas pol-α extends mispaired primer termini very poorly (Perrino & Loeb, JBC, in press), HIV-1 RT extends nucleotide mispairs with relatively high efficiency. This difference in mispair extension is most prominent opposite template A and C residues. The high error rate and unique specificity of misincorporation by HIV-1 RT point to the importance of this enzyme in HIV-1 evolution and may provide a basis for the systematic design of antiviral nucleosides (e.g. dGTP analogs) that are preferentially incorporated by HIV-1 RT.

L 471 MUTATIONAL SPECIFICITY OF HIV-1 REVERSE TRANSCRIPTASE. John D. Roberts, Katarzyna Bebenek and Thomas A. Kunkel, Lab of Molecular Genetics, NIEHS, Research Triangle Park, NC 27709, and John Abbotts and Samuel H. Wilson, Lab of Biochemistry, NIH, Bethesda, MD 20892

The fidelity of DNA-dependent DNA synthesis catalyzed by HIV-1 reverse transcriptase (RT), isolated both from viral particles and from *E. coli* cells carrying a plasmid expressing the cloned RT gene, has been determined for a wide variety of errors in a forward mutation assay. The results, which are essentially the same with enzyme from either source, demonstrated that HIV-1 RT was exceptionally inaccurate, being on average 10-fold less accurate then either AMV or MMLV RT. This result is consistent with the notion that the exceptional diversity of the HIV genome may result from error-prone reverse transcription.

DNA sequence analysis of mutants generated by the HIV-1 RT indicated that single-base substitution errors and both minus-one-base and plus-one-base frameshift errors were produced. Certain template positions were exceptional mutational hot spots, where the error rate per polymerized nucleotide may be as high as 1 in 40 for base substitutions and 1 in 70 for frameshifts. The frameshift hot spots were at runs of a common base, consistent with a Streisinger slippage model. The base substitution hot spots were at template DNA sequences that would allow these mutations to arise by a dislocation (i.e. transient misalignment) mechanism. The frameshift error frequency was higher at sites at which HIV-1 RT dissociates from the template-primer during synthesis, suggesting that processivity may be important for the fidelity of this polymerase.

L 472 SYNTHESIS AND PROCESSING OF KINETOPLAST DNA MINICIRCLES IN TRYPANOSOMES. Kathleen A. Ryan and Paul T. Englund, Johns Hopkins University, School of Medicine, Baltimore, MD 21205. Kinetoplast DNA, the mitocondrial DNA in trypanosomes, is a giant network containing several thousand topologically interlocked minicircles. Replication occurs on free minicircles which have been detached from the network. Analysis of free minicircles from *Trypanosoma equipedum* by two dimensional agarose gel electrophoresis reveals several species, including the progeny molecules with newly synthesized H or L strands, a knotted species, theta structures and dimeric species. Characterization of newly synthesized strands on these species indicates that L strand synthesis initiates complementary to the 12-mer, GGGGTTGGTGTA, a sequence conserved in minicircles of all trypanosomes. L strand synthesis proceeds continuously and uni-directionally around the circle. The first H strand Okazaki fragment, 73 nucleotides in length, initiates complementary to another conserved sequence, ACGCCC, and terminates within the 12-mer. Joining of some Okazaki fragments appears to occur prior to reattachment of minicircles to the network. Final repair of the interruptions in both newly synthesized strands occurs after reattachment of the progeny molecules to the network. (Supported by grants from NIH (GM-27608) and the MacArthur Foundation).

Molecular Mechanisms in DNA Replication and Recombination

L 473 CELL CYCLE STUDIES IN PHYSARUM: DIFFERENTIAL DNA REPLICATION AND TRANSCRIPTION OF CELL-TYPE SPECIFIC GENES; AND CONSERVATION OF MITOTIC PROTEINS, Helmut W. Sauer, Olga L. Arellano, Jeong W. Cho, John D. Diller, Roland R. Flanagan, Gregory L. Shipley, Department of Biology, Texas A&M University, College Station, TX 77843

A Cdc2 antibody recognized a cytoplasmic 34kDa antigen in yeast, HeLa cells and Physarum and a 60kDa nuclear antigen in Physarum. While the levels of p34 and p60 remained constant during the naturally synchronous cell cycle, MPF activity and phosphoproteins detected by MPM2 (a HeLa mitosis specific Mab) were only present at mitosis. Utilizing the synchronous S-phase of the Physarum plasmodium and a panel of stage-specific cDNA clones, we obtained the following results: 1) the flanking regions of some (2 out of 4) cell-type specific genes are differentially methylated; 2) in the synchronous plasmodium, most (4 out of 5) plasmodial specific genes replicate early while genes expressed in other stages replicate later; 3) expression of these genes is mainly controlled at the transcription level; 4) replication is required for transcription of the plasmodial specific genes; 5) one plasmodial-specific gene does not replicate early, and its expression is mainly post-transcriptionally controlled. We propose that sequential activation of replicons allows early replicating genes to preferentially bind transcription factors. Such temporal compartmentation of S-phase might be involved in establishing cell-type commitment during development.

L 474 REGULATION OF CYCLIN mRNA EXPRESSION IN INTERLEUKIN 2-STIMULATED CLONED MURINE T LYMPHOCYTES. P.M.Shipman, D.E.Sabath, K.Cohen & M.B.Prystowsky. Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104.

Proliferating cell nuclear antigen/cyclin, a nuclear protein synthesized when cells proliferate, is an auxiliary protein for DNA polymerase delta. We identified 6 murine cyclin cDNA clones from a cDNA library representing mRNA from IL2-stimulated cloned T cells (L2), based on hybridization with a rat cDNA. Approximately 90% of one clone was sequenced and matched the rat sequence by >90%. This murine cDNA was used to evaluate cyclin mRNA levels by Northern blot analysis during IL2-driven L2 cell proliferation. In unstimulated cells, cyclin mRNA was minimal but increased 1h after stimulation, and reached maximal levels (30-fold increase) at 24-45hrs. Cyclin mRNA levels dropped to 5-fold above unstimulated levels at 96h, after division. The half-life of cyclin mRNA during G1 to S phase transition was app. 4hrs, and 2hrs in resting cells. We are evaluating the contribution of increased mRNA stability versus transcription to increased cyclin mRNA accumulation. We identified 4 clones from a murine genomic DNA library which hybridize with a 200bp 5'cyclin cDNA fragment on Southern blots. These clones will be used to characterize the organization of the cyclin gene and to identify 5' flanking regions responsible for regulation of cyclin transcription.

L 475 THE *SACCHAROMYCES CDC2*GENE ENCODES DNA POLYMERASE III, A SECOND ESSENTIAL DNA POLYMERASE, Karen C. Sitney, Martin E. Budd and Judith L.Campbell, Division of Chemistry, California Institute of Technology, Pasadena, CA 91125.

Three nuclear DNA polymerases have been described in yeast, DNA polymerases I, II and III. DNA polymerase I is encoded by the *POL1* gene and is essential for DNA replication. Neither DNA polymerase II nor III is affected by *pol1* mutations. The DNA polymerase I gene has been sequenced and contains several regions conserved in human DNA polymerase α, Herpes simplex DNA polymerase, and at least 6 other viral and prokaryotic DNA polymerases. Since the *S. cerevisiae CDC2* gene has recently been shown to have DNA sequence similarity to the active site regions of these other DNA polymerases (A. Boulet and G. Faye, personal communication), but to be different from DNA polymerase I, we examined 3 mutant alleles of *cdc2* for the presence of DNA polymerases II and III. DNA polymerase II was not affected by any *cdc2* mutation. DNA polymerase III activity was significantly reduced in *cdc2-1* extracts. We conclude that *CDC2* encodes DNA polymerase III, and that DNA polymerase III therefore represents a second essential DNA polymerase in yeast. DNA polymerase III is a δ-type polymerase, thus this evidence for two replicative polymerases in yeast supports recent findings that mammalian DNA polymerase δ, in addition to polymerase α, is required for DNA replication. The remaining yeast DNA polymerase, DNA polymerase II, has not yet been cloned. In pursuit of this goal, we have purified this enzyme to near homogeneity. Polymerase II is also a δ-type polymerase, however we think it unlikely that this polymerase is also required for DNA replication.

L 476 PURIFICATION AND CHARACTERIZATION OF A REPLICATION-DEPENDENT CHROMATIN ASSEMBLY FACTOR ISOLATED FROM HUMAN CELLS. Susan Smith and Bruce Stillman. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

An *in vitro* system that supports the assembly of replicating SV40 DNA into chromatin has been developed (Stillman, Cell 45, 555-565, 1986). Authentic SV40 replication occurs in the presence of a cytosol extract from human 293 cells and SV40 T antigen. Addition of a nuclear extract from the same cells assembles chromatin onto the replicating SV40 DNA.

Biochemical fractionation of the nuclear extract has resulted in the identification of a single componant that is required for chromatin assembly. This chromatin assembly factor (CAF) has been purified over several different chromatographic steps. Although gel filtration of CAF suggested a relative molecular mass of >700,000, glycerol gradient sedimentation of the most purified fraction resulted in a single peak of chromatin assembly activity sedimenting at approximately 6.5S (120,000 apparent mol. wt.). The peak of activity corresponds exactly with the migration of several polypeptides which have apparent mol. wts. ranging from 55,000 to 170,000 daltons. This set of polypeptides comigrate with chromatin assembly activity on a wide variety of chromatographic resins and separation techniques (including DEAE, Mono Q and gel filtration). While CAF has no apparent affinity for DNA based upon DNA cellulose chromatography, it does bind tightly to histone agarose.

In the reconstituted system purified CAF assembles chromatin onto replicating SV40 DNA. Surprisingly, it appears that CAF does not supply the histones for this reaction, based upon its polypeptide composition and chromatographic properties. Preliminary experiments show that the chromatin assembled in this cell free system does contain histones and that the histones are supplied by the cytosol extract.

L 477 **Molecular genetic analysis of *SOE1*, an extragenic suppressor of *cdc8* (dTMP kinase) mutations of yeast, Jin-Yuan Su, Lisa Belmont and Robert A. Sclafani, Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, CO 80262.** The *CDC8* gene encodes deoxythymidylate (dTMP) kinase in *Saccharomyces cerevisiae* and is required for nuclear and mitochondrial DNA replication in both the mitotic and meiotic cell cycles. All *cdc8* temperature-sensitive mutations are hypomorphic and are partially defective in meiotic and mitochondrial functions at the permissive temperature. The *SOE1* mutation is an extragenic suppressor which can suppress the temperature-sensitivity of *cdc8* mutations. *SOE1* is a mutation in a single-copy Mendelian gene located <1.5cM from the *CYH2* gene on chromosome VII. Physically, it is contained within a 1.2 kb DNA restriction fragment which is adjacent to the *TSM437-CYH2* region. The order of genes is *SOE1-TSM437-CYH2*. The *SOE1* mutation is dominant and gene-specific but cannot suppress a complete *cdc8* deletion. Therefore, *SOE1* is a gain-of-function mutation which cannot be a bypass of dTMP kinase function. Using chromosome X aneuploids to vary *cdc8* gene dosage, we show that the *SOE1* mutation is unable to suppress the *cdc8* hypomorphic phenotype and that the amount of dTMP kinase which is required for mitotic, meiotic or mitochondrial DNA replication is different. Therefore, we propose the *SOE1* mutation is an interactive suppressor which stabilizes thermo-labile dTMP kinase molecules in the cell (Supported by PHS grant GM35078 to R. Sclafani).

L 478 DNA POLYMERASE δ FROM HELA CELLS THAT IS INSENSITIVE TO PROLIFERATING CELL NUCLEAR ANTIGEN, Juhani Syvaoja, Craig Nishida and Stuart Linn, Department of Biochemistry, University of California, Berkeley, CA 94720.

A large form of DNA polymerase δ from HeLa cells was originally purified as a factor required for conservative DNA synthesis in a reconstituted system by utilizing UV-irradiated permeabilized human diploid fibroblasts. We have now purified this enzyme by assaying its polymerase activity. The enzyme prefers poly(dA)-oligo(dT) template/primer over poly(dA-dT) template/primer and is highly processive in the absence of proliferating cell nuclear antigen (PCNA). Unlike some forms of DNA polymerase δ, neither activity nor processivity of this form is increased by PCNA. Under polymerizing conditions the enzyme removes mismatched but not matched nucleotides from the 3'-terminus of oligo(dT) annealed to poly(dA) suggesting a proofreading function. As determined by SDS-PAGE, two major polypeptides of the preparation have molecular masses of 215 kDa and 55 kDa. Sedimentation and gel filtration data suggest a highly asymmetrical shape for the enzyme with molecular mass estimates of 360 kDa and 170 kDa in low and high salt, respectively. Peptide maps of the 215 kDa peptide of the DNA polymerase δ preparation and the catalytic subunit of the DNA polymerase α purified from a side fraction of this DNA polymerase δ preparation are distinct. The apparent molecular mass of the 55 kDa peptide in the DNA polymerase δ preparation also differs from the apparent molecular masses of any of the subunits of the DNA polymerase α. We have recently found another form of DNA polymerase δ from HeLa cells and are currently in the process of purifying it and studying its properties.

Molecular Mechanisms in DNA Replication and Recombination

L 479 FIDELITY OF DNA REPLICATION *IN VITRO* BY HUMAN HeLa CELL EXTRACTS, David C. Thomas, John D. Roberts, and Thomas A. Kunkel, Lab of Mol. Genetics, NIEHS, Research Triangle Park, NC 27709

The fidelity of DNA replication by extracts of HeLa cells was measured using as a substrate M13mp2 RFI DNA containing the SV40 origin of replication. A forward mutation assay and two reversion assays were used to measure base substitution and frameshift errors by the replication complex. Bidirectional, semiconservative replication by HeLa cell extracts supplemented with purified SV40 T antigen was considerably more accurate than synthesis by the HeLa cell DNA polymerase α-DNA primase complex purified by immunoaffinity chromatography. In the forward assay the cell extract had an estimated fidelity of less than one error per 150,000 nucleotides incorporated, compared to one in 5000 by the pol α-primase complex. The base substitution reversion assay supported this estimate and also demonstrated that replication errors were generated by increasing the concentration of incorrect dNTPs relative to correct dNTPs. Since base substitution errors were so readily detected, it is possible that base substitution fidelity factors are not operative or are missing from the cytosolic extract. The minus-one-base frameshift assay indicated that the replication complex is 100- to 1000-fold more accurate for frameshifts than the pol α-primase. These data suggest that additional factors are present in the extract that contribute to frameshift fidelity. We are searching for such factors and are conducting measurements to determine if exonucleolytic proofreading is occurring during replication and to determine the fidelity of leading-strand versus lagging-strand synthesis.

L 480 HUMAN DNA POLYMERASES δ AND α ARE IMMUNOCHEMICALLY RELATED. N. Lan Toomey, Chun-Li Yang, Yunquan Jiang, Fatima De-Freitas and Marietta Y.W.T. Lee, Department of Medicine, University of Miami School of Medicine, Miami, FL 33101.

DNA polymerases δ and α were purified from human placenta by a new procedure which minimizes proteolytic modification of the enzymes. The method uses affinity chromatography with immobilized carboxymethylated β-lactoglobulin and α_2-macroglobulin for the removal of active protease activities from the tissue extracts. DNA polymerases δ and α from the same tissue were found to be discrete enzymes which are different in a number of properties. These include heat stability, sensitivity to N-ethylmaleimide, BuAdATP and DNA polymerase α antibodies. Moreover, factors that stimulate DNA polymerase δ also stimulate DNA polymerase α. A panel of monoclonal antibodies we have produced against DNA polymerase δ was tested against DNA polymerase α. These antibodies included those which inhibited DNA polymerase δ but not DNA polymerase α. However, several antibodies were found which inhibited and immunoblotted both DNA polymerases. This demonstrates that these two DNA polymerases are immunochemically related and provides the first evidence that DNA polymerase δ and α may have structural similarities.

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L 481 MOLECULAR GENETIC AND BIOCHEMICAL ANALYSES OF VACCINIA-ENCODED PROTEINS WITH ESSENTIAL ROLES IN VIRAL DNA REPLICATION. Paula Traktman, Elizabeth Evans, William McDonald, Rachel Rempel and John Taddie. Depts. of Cell Biology and Microbiology, Cornell University Medical College, New York, N.Y. 10021.

Vaccinia virus, a lytic virus which replicates solely in the cytoplasm of infected cells, is highly autonomous of host cell functions. In addition to its physical separation from host functions involved in DNA replication and recombination, the virus appears to be genetically autonomous and encodes its own replicative machinery. We have used molecular genetic approaches to identify three viral genes which encode essential replicative functions: the viral DNA polymerase, an 82 kDa protein and a 29 kDa protein. Preparation of antisera to these proteins has enabled us to monitor the expression of these proteins following infection, and to determine alterations in the proteins' expression following infection with replication mutants. Moreover, we have overexpressed two of these proteins in vaccinia-infected cells in order to facilitate purification and biochemical analysis. Molecular analysis of alleles of these genes isolated from temperature-sensitive viral mutants has allowed the localization of conditionally lethal mutations to single nucleotide alterations. In addition to temperature-sensitive mutants, we have selected for viral mutants resistant to the polymerase inhibitor aphidicolin, and have mapped these mutations within the DNA polymerase gene. Interestingly, many of these mutants have a significant mutator phenotype, allowing us to analyze polymerase fidelity.

L 482 MOUSE PRIMASE p49 SUBUNIT: CONSERVED AND DIVERGENT PROTEIN REGIONS AND DISTINCTIVE PROMOTER STRUCTURE. Charles E. Prussak, Melissa T. Almazan and Ben Y. Tseng, Eukaryotic Regulatory Biology Program, M-013G, Univ. of Calif., San Diego, La Jolla, CA 92093. The cDNA of mouse primase p49 subunit has been isolated and confirmed by the identity of peptide sequences from p49 with the predicted aa sequence. The cDNA encodes a protein of 49,295 kDa with a net basic charge. A potential metal binding domain is noted with the CxxC motif and suggests the subunit may be involved in DNA binding. Nucleic acid probes for the 3' end of the cDNA (coding) does not detect mRNA from hamster or human cells but a full cDNA probe does detect a similar sized mRNA (1.6 kb) in these cell types indicating a rapid divergence of the C-terminal portion of the subunit. Comparison with the similar sized yeast primase subunit indicated extensive homology over the N-terminal halves of the proteins that remain in phase over 200 aa. The C-term halves do not indicate similarities, except for one short region, consistent with the nucleic acid divergence in the 3' portion of mammalian genes. The potential metal-binding domain of mouse primase is in the homologous N-term region although the motif is conserved except for one of the C residues which is a S in yeast and may serve a similar role in metal ion coordination. We have isolated the 5' promoter region of the gene in order to examine gene expression. The mRNA initiation site was localized by primer extension analysis as a single start site and is designated nucleotide position +1. A portion of the genome extending from -2 kb to + 12 has been linked to a reporter gene to determine the genetically important regions. Unidirectional deletion analysis from -2 kb to -10 indicated that the promoter was active down to -54 with little difference in reporter gene activity whereas a deletion to -10 was inactive when transfected into mouse 3T6 or monkey CV1 cells. The basal level promoter sequence did not indicate similarities to reported cis-acting promoter elements such as TATA, CAAT, or G/C boxes. Additional analysis is underway to fully characterize the promoter. Supported by grants from USPHS GM29091 and ACS NP-594.

L 483 **STUDIES ON DNA REPLICATION ORIGINS OF HUMAN CELLS.** *N. Tuteja, R. Tuteja, E. Csordas-Toth, G. Faulkner, M. Giacca, G. Biamonti, F. Demarchi, S. Riva and A. Falaschi, International Centre for Genetic Engineering and Biotechnology, Trieste - Istituto di Genetica Biochimica ed Evoluzionistica del CNR, Pavia (Italy)*

We have isolated and cloned several sequences replicating immediately after the onset of S-phase in synchronized HL60 cells. Two of the longer fragments were sequenced and studied in more detail; both were shown to contain transcriptional signals associated with promoters and/or enhancers: one of these contained the octamer binding NF-III, with a TATA box at the canonical distance. Another fragment contained putative binding sites for SP1, T-antigen, as well as a sequence recognized by a known cellular transcription factor (USF/MLTF). This nuclear factor is competed away by the similar sequence present in Adenovirus DNA, and in the LTR of HIV-1. The same sequence was shown by CAT assay and Northern blot to contain an active promoter. The purification of the nuclear factor binding this sequence is in progress. None of the putative origin sequences show ARS properties when transfected by different procedures in HeLa or HL60 cells. The exact location of the sequences under study with respect to *in vivo* active origins is under investigation by physical/chemical procedures.

L 484 TEMPERATURE IS A DETERMINANT OF DNA UNWINDING AND INITIATION AT YEAST REPLICATION ORIGINS, Robert M. Umek and David Kowalski, Molecular & Cellular Biology Department, Roswell Park Memorial Institute, Buffalo, NY 14263.

Yeast autonomously replicating sequences (*ARSs*) function as replication origins and consist of 1) an 11 bp consensus sequence, thought to bind the initiator protein, and 2) a broad flanking sequence, the role of which is less clear. We have previously reported that the formation of a single-strand-specific nuclease-hypersensitive site in the flanking sequence correlates with replication competence for a large set of *ARS* sequence mutations (*Cell* 52, 559-567). We have now determined, through the use of 2D gel electrophoresis of plasmid topoisomers, that unwinding of the yeast replication origin is thermodynamically stable in nuclease digestion conditions. The results support our previous interpretation that the *ARS* flanking sequence determines the free energy required for origin unwinding. We have also discovered that temperature is a determinant of unwinding, since the origin is stably unwound at 37°C but not 23°C. Mutant *ARS* derivatives with an increased free enegy requirement for unwinding are cold-sensitive for the initiation of DNA replication in living cells, consistent with a thermal energy contribution to origin unwinding *in vivo*. We call the *ARS* flanking sequence a DNA unwinding element (*DUE*). We have developed a model for the initiation of DNA replication in *S. cerevisiae* which suggests that local unwinding of the *DUE* is critical for entry of the replication machinery.

L 485 TWO-DIMENSIONAL ANALYSIS OF REPLICATION INTERMEDIATES IN THE 240 KB AMPLIFIED DIHYDROFOLATE REDUCTASE DOMAIN OF A METHOTREXATE-RESISTANT CHO CELL LINE. James P. Vaughn and Joyce L. Hamlin, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA 22908.

We have previously demonstrated that ^{3}H-thymidine is preferentially incorporated into a 28 kb initiation locus in the amplified dihydrofolate reductase domain at the beginning of the S period in aphidicolin-synchronized CHOC 400 cells. In recent higher resolution studies, we have shown that there are actually two peaks of labelling within this locus that are separated by ~20 kb, suggesting the presence of two distinct origins. We are presently using a two dimensional gel electrophoretic method developed by Brewer and Fangman to analyze replication intermediates from the dihydrofolate reductase amplicon. Our initial results show that replication forks frequently pass completely through restriction fragments that are centered over each peak of labelling. This result suggests that each origin may sometimes be passively replicated by forks emanating from the adjacent origin, and also that there appears to be no fixed terminus between these two initiation sites. In addition, by using a psoralen crosslinking technique to stabilize replication intermediates, we have observed a bubble arc that is indicative of an active origin in a 5.4 kb BamHI/KpnI restriction fragment that spans one of the peaks of early labelling. Cross-linked DNA from exponentially growing cells has also produced an origin-like pattern in this 5.4 kb BamHI/KpnI fragment. We are presently analyzing the region containing and surrounding the second early labelled peak in order to determine whether this locus also contains replication bubbles in both synchronized and exponential cultures.

L 486 PURIFICATION OF THE YEAST MITOCHONDRIAL DNA POLYMERASE AND IN VITRO CHARACTERIZATION OF ITS ACTIVITY, Robert D. Vincent and H. Peter Zassenhaus, Microbiology Department, St. Louis University School of Medicine, St. Louis, MO 63104.

We have partially purified the yeast mitochondrial DNA polymerase by a combination of ion exchange and affinity chromatography from detergent-salt extracts of mitoplasts derived from a strain of S. cerevisiae deficient in the major mitochondrial nuclease (nuc1-1). The DNA synthetic activity of the partially purified polymerase, as assayed in vitro by monitoring the addition of dNTPs to a singly primed single stranded M13 template, operates over a broad range of [KCl],[$MgCl_2$], pH, and temperature. Its optima are clearly different, however, from those of the yeast nuclear DNA polymerases, and it is insensitive to aphidicolin, unlike those polymerases. Both BSA and spermidine enhance the activity which is extremely stable when stored in buffer at 4°C or in the presence of 50% glycerol at -20°C. We are currently extending the purification of the yeast mitochondrial polymerase to homogeneity and will report on our investigations concerning its kinetic properties and its fidelity of replication.

L 487 PURIFICATION OF REPLICATION PROTEIN C, A HELA PROTEIN REQUIRED FOR IN VITRO SV40 DNA REPLICATION, D.M. Virshup, M.S. Wold, and T.J. Kelly, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Understanding of the mechanism and requirements for in vitro SV40 DNA replication may provide insights into the cellular DNA replication apparatus. A protein required for the efficient replication of SV40 origin-containing plasmids in vitro has been purified from HeLa cell extracts. This protein, Replication Protein C (RP-C), consists of two polypeptides of 32 and 34 kDa. It is purified from a cellular fraction previously shown to stimulate T antigen dependent unwinding of origin-containing duplex DNA. Purified RP-C stimulates in vitro SV40 DNA replication ten-fold. In addition, it stimulates the unwinding of linear duplex DNA in a reaction that requires T antigen, the SV40 origin of replication, and a single stranded DNA binding protein, either the HeLa protein RP-A or the E. coli protein, ssb. Preincubation of DNA and T antigen with the HeLa proteins RP-A and RP-C eliminates the delay normally seen before the onset of DNA synthesis. These observations suggest that these proteins are involved in the earliest steps in SV40 DNA replication.

L 488 BPV-1 DNA INJECTED INTO UNACTIVATED XENOPUS EGGS: EFFICIENT ORIGIN DEPENDENT REPLICATION UPON ACTIVATION, Larry Wangh, Laurie Bankston and J. Aquiles Sanchez, Dept. of Biology, Brandeis Univ. Waltham, MA 02254 Unfertilized-dejellied eggs were injected in 1-2 μM extracellular free-Ca^{2+} without triggering activation. The injection site healed and eggs remained in meiotic metaphase arrest until activated by Ca^{2+} ionophore treatment. Type-1 bovine papilloma virus plasmids were used to analyze DNA replication. These molecules did not replicate in unactivated eggs, but commenced several rounds of efficient semiconservative replication 30 min. after activation. The same plasmids injected into activated eggs initiated replication far less efficiently. Supercoiling analysis revealed that both the kinetics and the extent of chromatin formation were lower in the unactivated egg. Replication efficiencies were not affected by the conformation (Forms I, II or IIr) of the injected molecules. In contrast the absolute amount of injected DNA was critical. Optimal first round replication occurred at 0.5-1.0 ng injected DNA. Efficient replication was dependent on the presence of a BPV-1 origin of replication (either PMS1 or PMS2). Bacterial vectors which lack these sequences did not display efficient first-round replication, nor did constructs containing a mutated PMS1 sequence. We postulate that factors present in the unactivated egg cytoplasm may bind at BPV-1 origins. Supported by NIH DK31695 and BRSG SO7 RR07044.

L 489 Roles for DNA polymerase α and δ in SV40 DNA replication
David H. Weinberg, Marc S. Wold, David M. Virshup, Pamela Simancek, Joachim J. Li, and Thomas Kelly, Dept of Mol. Biol. and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. We have described a cell-free system that is capable of replicating plasmid DNA templates containing the SV40 origin of DNA replication. As an approach to understanding mechanisms, we have systematically fractionated the human cellular extract required for replication activity. DNA polymerase alpha-primase complex is absolutely required in this cell-free system. Additionally, we have obtained direct evidence for a role of a second DNA polymerase activity. This polymerase activity is chromatographically, immunologically and biochemically distinct from alpha. It has biochemical properties similar to those described for calf thymus DNA polymerase delta. Furthermore, polymerase activity on poly dA: oligo dT is dependent upon PCNA, an accessory factor for the calf thymus enzyme . Finally, a highy purified fraction containing this enzyme activity is required for efficient DNA replication . These data, taken with the observation that PCNA is absolutely required for efficient DNA replication in our reconstituted system lead us to conclude a role for DNA polymerase delta in SV40 DNA replication. We have begun to address the role of these 2 polymerases in the replication process using purified proteins to examine initiation and elongation of nascent DNA chains.

L 490 ANALYSIS OF VIRAL GENE PRODUCTS INVOLVED IN HSV-1 DNA REPLICATION. Sandra Weller, Ellen Carmichael, David Goldstein and Reza Seghatoleslami. Department of Microbiology, University of Connecticut Health Center, Farmington, CT 06032
HSV appears to encode many of the proteins involved in viral DNA synthesis. We have isolated and characterized mutants in five genes believed to play a role in HSV DNA synthesis, including the alkaline nuclease gene and four genes of unknown function (UL5, UL8, UL9, and UL52). We have isolated host cell lines capable of supporting the growth of potentially lethal mutants in these five genes. An insertional mutagen (ICP6::lacZ), consisting of the lacZ gene under the control of a strong, inducible HSV promoter has been used to inactivate each of the five genes listed above. Studies with these mutants suggest that the alkaline nuclease is not essential for viral DNA synthesis but that it is required for production of infectious virus particles. We propose that the alkaline nuclease (which exhibits endo and exonuclease activities) may be involved in processing or packaging of replication or recombination intermediates. The function of this gene may be analogous to that of the endonucleases encoded by gene 49 of phage T4 or gene 3 of phage T7 which appear to play a role in the resolution of recombination intermediates and are required for efficient processing of viral genomes. ICP6::lacZ insertion and deletion mutants in the UL5, 8, 9 and 52 genes have been isolated which are defective in the synthesis of viral DNA and late proteins under nonpermissive growth conditions suggesting that these gene products are essential for viral DNA synthesis. The UL5 gene appears to have helicase activity as described in accompanying abstract by Zhu and Weller. UL8 and UL52 genes have recently been found in a complex with the UL5 gene product; this complex exhibits helicase and primase activities (Crute et al, manuscript submitted). The UL9 gene product has recently been identified by Challberg and colleagues as an origin binding protein. Current efforts involve further biochemical and genetic analysis of the products of the UL5, UL8, UL9 and UL 52 genes to more precisely determine function and to begin to identify functional domains of each protein.

L 491 PURIFICATION AND INITIAL CHARACTERIZATION OF HIV POL ORF PROTEINS PRODUCED IN A EUCARYOTIC EXPRESSION SYSTEM,
Anthony B. West, Thomas M. Roberts and Richard D. Kolodner, Department of Cellular and Molecular Biology, Dana-Farber Cancer Institute, Boston, MA 02115. The synthesis of a DNA copy of the human immunodeficiency virus (HIV) genome and its integration into the host genome are obligatory steps in the life cycle of HIV. To better understand these aspects of HIV replication, a recombinant baculovirus (pol1.31) has been made using the *pol* open reading frame (ORF) sequence (minus the *gag* portion) from a biologically active clone of the HIV. This ORF contains the region coding for the viral protease, reverse transcriptase, RNAaseH and integrase. Crude extracts from insect cells infected with pol1.31 show a high level of reverse transcriptase activity and exhibit the template and cation requirements charactersitic of the HIV enzyme. We have demonstrated that the polyprotein expressed by this baculovirus recombinant is made at high levels in insect cells and is correctly processed to yield the 64 kd and 51kd reverse transcriptase proteins and the 34 kd integrase protein that are normally seen in HIV infected human cells. We have purified the reverse transcriptase and are currently characterizing various aspects of its activity. The 64 kd and 51kd forms of the protein copurify and have the amino terminal sequence consistent with correct processing by the HIV protease. Renetauration experiments suggest that both forms of the reverse transcriptase are enzymatically active. Our available data suggest that the baculoviral source for the *pol* ORF proteins will be useful in understanding the role of these enzymes in the biochemsitry of HIV replication.

L 492 POSSIBLE PHYSICAL ASSOCIATION OF THE HERPES SIMPLEX VIRUS ENCODED URACIL-DNA GLYCOSYLASE WITH THE VIRUS ENCODED DNA POLYMERASE,
Marshall Williams and Thomas Winters, Department of Medical Microbiology and Immunology and The Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210
Uracil residues can be formed in DNA either by the incorporation of dUTP by DNA polymerase or by the spontaneous deamination of dCMP residues in the DNA. Uracil-DNA glycosylase, (UNG) an enzyme which is involved with DNA repair, catalyzes the cleavage of the glycosyl bond between the deoxyribose moiety and the uracil base resulting in the release of uracil and the production of an apyrimidinic site in the DNA. Herpes simplex virus (HSV) induces several proteins following the infection of human cells, including a virus encoded DNA polymerase and a UNG. To elucidate the potential role of the UNG in HSV replication the glycosylase was purified from KB cells infected with HSV-2 (HG52). The UNG partially purified from the nuclear extract by Blue Sepharose chromatography, was found to chromatograph as two distinct species on DNA cellulose. One glycosylase species eluted at a NaCl concentration of 100mM and was not associated with other HSV induced enzymatic activities while the second glycosylase species eluted at a higher NaCl concentration and co-eluted with the HSV induced DNA polymerase. These results demonstrate that the HSV induced UNG exists as two chromatographyically distinct but interconvertable species in infected cells and suggest that the distinct UNG species are due in part to the association of UNG with the HSV induced DNA polymerase.

L 493 Identification of Cellular Proteins Required for SV40 DNA Replication,
Marc S. Wold, David H. Weinberg, David M. Virshup, Joachim J. Li, Michael Kauffman and Thomas Kelly, Dept of Mol. Biol. and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205. Using a cell-free system that is capable of replicating plasmid DNA molecules containing the SV40 origin of replication, we conducted a series of fractionation-reconstitution experiments for the purpose of identifying the cellular proteins involved in SV40 DNA replication. In addition to the viral encoded replication protein, T antigen, we have identified and begun to characterize at least eight cellular components from a HeLa cytoplasmic extract that are absolutely required for SV40 DNA replication *in vitro*. These include: i) three partially purified fractions, CF ID, CF IIA and CF IIB, and ii) five highly purified proteins, replication protein-A (RP-A), proliferating cell nuclear antigen (PCNA), replication protein C (RP-C), DNA polymerase α-primase complex, and topoisomerase (I and II). RP-A is a multi-subunit protein that has single-stranded DNA-binding activity and is required for a T antigen-dependent, origin-dependent unwinding reaction which may be an early step in initiation of replication. RP-C can stimulate this unwinding reaction so it also may function during initiation. PCNA, polymerase α-primase, CF IIA and CF IIB all appear to be involved in elongation. We have examined the DNA binding properties of RP-A and have raised monoclonal antibodies to it. Preliminary studies indicate that RP-A level do not change across the cell cycle.

Molecular Mechanisms in DNA Replication and Recombination

L 494 DUAL MECHANISMS OF INHIBITION OF DNA SYNTHESIS BY TRICIRIBINE, Linda L. Wotring, Leroy B. Townsend, Katherine Z. Borysko and Linda M. Jones, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109-1065 The experimental anticancer drug triciribine (TCN) appears to inhibit both DNA replicon initiation and DNA chain elongation. The alkaline elution assay of DNA from L1210 cells labeled (15-min, ^{3}H-dThd) after TCN treatment indicated that the molecular size of actively growing DNA chains was increased by TCN, as expected if replicon initiation were inhibited. DNA from cells labeled and then treated 1 hr with TCN eluted more rapidly than that from control cells, indicating that TCN slowed the increase in size of DNA chains which had already been initated. Further insight into the mechanism of inhibition of DNA chain elongation was gained from studies of the synthesis and ligation of Okazaki fragments. After 1 hr TCN treatment the incorporation of ^{3}H-dThd into Okazaki fragments was not significantly affected, but the incorporation into higher molecular weight DNA was decreased. "Pulse-chase" experiments showed that ligation of ^{3}H-Okazaki fragments into higher molecular weight DNA was not affected by TCN. Therefore, it appears that the inhibition of DNA chain elongation may be due to inhibition of the initiation of Okazaki fragments by DNA primase or to inhibition of DNA polymerase. (Supported in part by Research Grant CH-310 from the American Cancer Society).

L 495 EXPRESSION OF MAMMALIAN ORIGINS OF DNA REPLICATION. Cunle Wu, Maria Zannis-Hadjopoulos and Gerald B. Price, McGill Cancer Centre, McGill University, Montréal, Québec, Canada H3G 1Y6.

Four sequences isolated from CV-1 monkey cells have been shown to function as origins of replication when subcloned and transfected into mammalian cells [Frappier and Zannis-Hadjopoulos, Proc. Natl. Acad. Sci. U.S.A. 84:6668, 1987]. We have assessed their transcriptional activity by RNA dot blot and Northern analyses. Transcripts homologous with these sequences were detected, some in poly-A+ RNA. The level of expression varied from different cell lineages and for different states of differentiation. One of these sequences, ors 3, is highly homologous to the human O-family of middle-repetitive sequences; we have observed that sequences in 2 of 3 human cDNA clones, which contain highly homologous sequences to the O-family, can function as origins of replication. Analysis of these cDNA clones have provided further sequence information about origins of replication and should allow the development of studies in transcriptional activation of mammalian DNA replication. (Research supported by MRC of Canada and C.W. supported by CIDA studentship).

L 496 REPLICATION OF NOVEL RECOMBINANT LINEAR rDNAS ARISING FROM A MUTATED CIRCULAR rDNA PLASMID IN TETRAHYMENA, Guo-Liang Yu and Elizabeth H. Blackburn, Department of Molecular Biology, University of California Berkeley, CA 94720

The macronuclear ribosomal RNA genes (rDNA) in Tetrahymena thermophila are linear 21 kb palindromic molecules. The rDNA from C3 strain has a replication advantage over rDNA from the C3 rDNA mutant *rmm1*. We have constructed a circular plasmid containing a single rRNA gene with a selectable paromomycin resistance marker (*Pmr*) and a polylinker in the 3' nontranscribed spacer, and a tandem repeat of a 1.8 kb segment encompassing the transcription initiation site and the 5' nontranscribed spacer (NTS), including the C3 replication origin. We transformed *rmm1* Tetrahymena macronuclei by microinjection of this plasmid. In 20/21 transformants, homologous recombination between plasmid and endogenous rDNA produced only palindromic rDNA containing both plasmid and endogenous rDNA sequences, as seen previously[1]. However, in one transformant clone, the circular rDNA plasmid initially outreplicated the recipient's palindromic rDNA. Subsequent unequal crossovers between the plasmid and palindromic rDNA resulted in a novel 23 kb linear rDNA which contained three 5' NTSs and outreplicated the circular plasmid, which was then replaced by successively longer linear rDNAs containing up to ~30 tandem copies of the 5' NTSs. We found that in the circular plasmid, the 23 kb and long linear rDNAs in this transformant, the -16 position in the highly conserved promoter sequence was mutated (+G) in the tandem 1.8 kb repeats, while the promoter sequence adjacent to each rRNA gene was wild type. We propose that repeated rRNA promoters are deleterious in this system and conclude that increased origin number increases the replicative advantage of an rDNA replicon.

1. Yu, G.-L., Hasson, M., and Blackburn, E. H. (1988). Circular ribosomal DNA plasmids transform Tetrahymena thermophila by homologous recombination with endogenous macronuclear ribosomal DNA. PNAS USA 85, 5151.

L 497 THE HSV-1 UL5 GENE IS ESSENTIAL FOR DNA REPLICATION AND ENCODES A 99kD PROTEIN WITH HELICASE ACTIVITY. L. Zhu and S. K. Weller. Department of Microbiology, University of Connecticut Health Center, Farmington, CT. 06032

The UL5 gene of HSV-1 is predicted to encode a 99kD polypeptide with a consensus ATP binding site at residues 97-110. Mutants in the UL5 gene have been used to demonstrate that the UL5 gene product is essential and required continuously for DNA synthesis (Zhu and Weller, 1988, Virol 166:366). In order to determine the role of this gene product in replication, it was overexpressed in *E. coli* using a T7 based expression vector system and used to generate polyclonal antibodies (αUL5). αUL5 detected proteins in HSV-1-infected cell extracts from 4 hours post-infection. Although a faint band of the predicted size (99kD) was observed, the majority of the immunoreactive material migrated as smaller bands which either represent proteolytic degradation during extraction or post-translational proteolytic modification of the UL5 gene product. Because of the existence of the ATP binding site in UL5, we have proposed that UL5 may have ATPase and/or helicase activity. We have assayed the UL5 gene product expressed in *E. coli* for DNA-dependent ATPase and helicase activities. Our results suggest that ATPase and helicase activities copurify with the over-expressed UL5 protein through several steps of purification including DEAE-cellulose, phosphcellulose and gel filtration. Thus it appears that UL5 encodes a protein with DNA-dependent ATPase and helicase activities.

Recombination

L 500 CLONING AND CHARACTERIZATION OF THE REC 2 GENE OF USTILAGO MAYDIS. R. Bauchwitz and W. Holloman. Interdivisional Program in Molecular Biology, Cornell Unviersity Medical College, New York, NY 10021. The rec-2 mutant of Ustilago maydis was isolated by Holliday over twenty years ago on the basis of ultraviolet radiation sensitivity. Besides UV, the mutant is also sensitive to ionizing radiation and chemical alkylating agents. There is little induced recombination at heteroallelic loci, although the spontaneous level is normal. Perhaps the most striking effect of rec-2 is on meiosis; it is completely blocked. We now have available libraries of U. maydis DNA in autonomously replicating vectors. We have cloned a genomic fragment of U. maydis DNA that restores the functions interrupted by the rec-2 mutation. Allelic recombination in strains containing multiple copies of the cloned REC2 gene appears to be induced to a far greater extent than the level observed in wild-type cells.

L 502 HIGH EFFICIENT GENE CONVERSION BETWEEN EXOGENOUS AND ENDOGENOUS MOUSE LINES SEQUENCES, Abdellah Belmaaza (1,2), John C. Wallenburg (2), Suzanne Brouillette (2), Serge Montplaisir (1) and Pierre Chartrand (2), (1) Université de Montréal and hôpital Ste-Justine, (2) Research and Development, Canadian Red Cross Society, Montreal, Canada, H1W 1B2.

To explain the concerted evolution of the LINE-1 (L1) family, gene conversion and retroposition have been put forward as alternative models. To see if the L1 elements can undergo gene conversion, we generated a uniquely marked mammalian L1 element, by changing a conserved HindIII site to an XhoI site. Gene conversion between a portion of this element cloned in a shuttle vector plasmid and any of the L1 elements of the genome, should result in a loss of the XhoI site and replacement of the HindIII site at the same position. Transfection of mouse cells with this plasmid was done by DEAE-DEXTRAN procedure and the convertants were rescued in E. coli by cloning a selectable gene into the regenerated HindIII site. Our results suggest that the L1 elements of the mouse undergo a very high frequency of gene conversion despite the less than 100% homology. Furthermore, this non-selective system will allow us to look at the gene conversion process itself. Since these elements are not 100% homologous, we expect that gene conversion event will introduce modifications in about 10% of the bases. This will be used to further determine the extent and borderlines of the conversion event.

L 503 THE DEVELOPMENT OF A MODEL SYSTEM FOR DETERMINING THE CONSEQUENCES OF UNREPAIRED DNA DOUBLE-STRAND BREAKS IN YEAST. C. B. Bennett and M. A. Resnick, Cellular and Genetic Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709. We are interested in the biological consequences of DSBs under conditions where recombinational repair is not possible and DSBs occur in nonessential and nonintegrative extrachromosomal DNA. In essence, do DSBs function as transducing signals for enhanced DNA repair and recombination in yeast cells? To approach this, a selectable (*URA*$^+$) yeast shuttle vector was constructed with a synthetic 24 base pair YZ junction (from *MAT*) situated between nonyeast sequences (pSV2neo and pBR322) at which a unique DSB can be induced. In the presence of galactose, the YZ specific HO endonuclease (fused to the *GAL* promoter) is induced from a second low copy plasmid (pGALHOT) in a strain carrying the YZ target plasmid but incapable of *MAT* switching. In diploid host cells, galactose induction resulted in an increased rate of target plasmid loss *in vivo* over noninduced cells as measured by loss of the *URA*$^+$ marker. Furthermore, HO induced breakage of the target YZ plasmid was visualized both *in vitro* using purified HO endonuclease and *in vivo* using Southern blot analysis. Thus HO induction results in a DSB in a nonintegrating and nongenomic region (chromosomal YZ junctions are mutated). The YZ target plasmid contains *CEN3* and is stably maintained at low copy number; however, higher copy numbers can be obtained and stably maintained due to the presence of the *CUP1* gene on the plasmid. Thus the indirect effects of a known DSB on recombination and repair can now be examined in a dose dependent manner without the lethal effects associated with genomic damage.

L 504 HOMOLOGOUS RECOMBINATION IN HUMAN B-LYMPHOCYTES BY CAPSID MEDIATED TRANSFER OF SHORT DNA FRAGMENTS, Wolf M. Bertling, Petra Harrer, Katharina A. Hunger-Bertling, Max-Planck Research Group at the University of Erlangen, 8520 Erlangen, West Germany. A defect human Hypoxanthine-phosphoribosyltransferase (hprt) gene, a single copy gene on the X-chromosome with a mutation corresponding to a single base pair exchange in exon III was corrected in a male B-lymphocyte cell line. We loaded empty polyoma capsids with double stranded fragments of a functional hprt cDNA. These fragments were 165 bp long and ended at the end of exon III (the transition to the following intron). After transfection cells with a functional hprt gene were selected and analyzed. Hybridization analysis of HPRT positive cells revealed only homologous recombination events. Polymerase chain reaction analysis followed by sequence analysis indicated that most cells had simply replaced the mutated gene segment with one of the introduced fragments. Interestingly, some cells had lost the intron between exon III and exon IV adjacent to the site of mutation. The precise excision of that intron adds a new perspective to the involvement of RNA in DNA repair. The initial pairing of the fragment with the genomic DNA leads to double strand exchange and leaves a complete double stranded break in the genomic DNA that has to be fixed. A proof reading mechanism for this repair might use processed and unprocessed RNA molecules during the double strand re-ligation.

L 505 A SEQUENCE THAT PROMOTES RECOMBINATION IN POLYOMAVIRUS DNA. Daniel Gendron, Danielle Bourgaux-Ramoisy and Pierre Bourgaux, Department of Microbiology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4. RmI is a circular DNA molecule (7,102 bp) consisting of a mouse DNA insert (Ins; 1,628 bp) linked via two direct viral repeats of 182 bp (bp 3092-3273) to an otherwise unrearranged polyomavirus (Py) genome. When transfected into mouse 3T6 cells, RmI not only readily replicates but also recombines to yield unit-length Py DNA. In order to analyze the mechanism underlying the conversion of RmI into Py DNA, we derived from molecularly cloned RmI a series of constructs expected to undergo replication - and possibly resolution - when transfected into mouse 3T6 cells. Because these constructs included additional repeats of bp 3092-3273 (S repeats) as well as other homologies (H repeats), they were predicted to generate different amplifiable products depending on whether recombination occurred between the S or the H repeats. Our experiments demonstrate that S repeats fulfill a unique function in the resolution of RmI. Pairs of S repeats appear to act by inducing crossing-over between themselves or between H repeats in their vicinity. When the respective positions of the S and H repeats within the constructs were such that both sets of repeats could be simultaneously paired, crossing-over occurred more frequently between the S repeats than between the H repeats. When pairing of the H repeats conflicted with pairing of the S repeats, crossing-over took place between the S repeats, even though the S repeats were much shorter than the H repeats. However, crossing-over also appeared more likely to occur between those repeats that were closer to the Py ORI region. These results suggest that the process of recombination initiated by the S repeats is dependent on DNA replication or transcription, or on a protein travelling on the DNA from the viral origin.

L 506 MISMATCH REPAIR IN EXTRACTS OF XENOPUS EGGS: EFFECT OF MISMATCHES ON RECOMBINATION AND ANTI-RECOMBINATION, Peter Brooks, Christiane Dohet, Isabelle Varlet, Jan Filipski and Miroslav Radman, Institut Jacques Monod, CNRS, 2 place Jussieu, 75251 PARIS Cedex 05, France.

Mismatch repair in extracts of *Xenopus* eggs has been characterized. Repair was detected by a physical assay and shown to be specific by mismatch-localized DNA repair synthesis. In addition, mismatch-localized synthesis in non-repaired molecules may represent intermediates in the repair process. The repair synthesis involves kilobase stretches and sensitivity of repair to aphidicolin has been observed. The spectrum of repair efficiency and specificity for the 12 single base-pair mismatches has been determined by using a set of heteroduplex substrates that differ in sequence only at the mismatch position. Nicks placed at a distance of a few hundred base pairs from the mismatch resulted in stimulation of both repair and repair synthesis specifically on the strand bearing the chain breaks. A similar distribution of DNA synthesis was not observed with nicked homoduplex DNA. The sensitivity of recombination and anti-recombination to the presence of single mismatched base-pairs is also being studied. The investigation includes the effect of either single mismatches in one of the parent molecules or mismatches formed during recombination of two sequences that differ at one position.

L 507 A KINETIC STUDY OF λ SITE-SPECIFIC RECOMBINATION, Michael F. Bruist,[#] Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892

The site-specific recombination reaction by which the DNA of bacteriophage λ integrates into the *E. coli* chromosome is believed to proceed as follows (Richet, et al., Cell *52*:9). A complex containing the phage protein Int and the *E. coli* protein integration host factor (IHF) organizes at a specific DNA region, *att*P, on the phage chromosome. This structure, the intasome, then interacts with the bacterial integration site, *att*B. No additional proteins interact with *att*B. This synapsed intermediate is competent to undergo the DNA strand exchanges which produce integration.

The interaction between the intasome and *att*B is rather weak and requires special techniques to be measured. I am using the kinetic properties of the recombination reaction for this purpose. At a fixed concentration of the intasome, increasing the concentration of *att*B should increase the concentration of the synapsed intermediate and, hence, the rate of recombination. When most of the available intasome has synapsed with *att*B, the reaction rate will saturate and no longer increase when more *att*B is added. Although early experiments failed to detect it, improved quantitation of recombination at high *att*B concentrations clearly indicates evidence for saturation above 200 nM *att*B. The observed saturation of recombination is corroborated by other kinetic experiments in which competition between distinguishable *att*B substrates is observed. With the measurement of saturation, the generation of the synapsed intermediate can be monitored separately from the strand exchange reactions, allowing further characterization of each of these individual steps. This is being pursued using modified *att*B DNAs, mutant Int proteins, and other known modulators of recombination (Xis, supercoiling, etc.). [#]Supported by a National Research Council-NIH Research Associateship

L 508 Mechanisms of intramolecular recombination in Escherichia coli. Dominique Brunier, Ben Peeters, Bénédicte Michel, S.Dusko Ehrlich. Lab. Génétique Microbienne, INRA-Domaine de Vilvert, 78350 Jouy en Josas, France. To study intramolecular plasmid recombination in E.coli, we constructed pBR322-derived plasmids which contained direct repeats of 9,18,27 or 180 bp and the replication origin of M13. Recombination between the repeats generated a functional gene (Tc^R or Cm^R). Activation of the M13 origin induced synthesis of single-stranded plasmid DNA and stimulated recombination greatly, so that in a few hours 100% of the cells contained recombinant plasmids. The mechanism of recombination was studied by following the transfer of material from parental to recombinant molecules. No transfer was detected with the 9bp repeats, which shows that they recombine by a copy choice process. In contrast, massive transfer was observed with repeats of 18bp or more which indicates they recombine by a breakage and reunion process. Genetic studies show that RecA is needed for breakage and reunion but not for copy choice process and that helicase II, product of uvrD gene, acts as an anti-recombinase in the copy choice process. Simultaneous inactivation of recA and uvrD changes the recombination mechanism of repeats ≥18 bp from a break-join to a copy choice.

L 509 oriT-ORIENTATION AND THE tra GENE DEPENDENCE OF ENHANCED TRANSDUCTIONAL RECOMBINATION. Jeffrey R. Carter and Ronald D. Porter, Department of Molecular and Cell Biology, The Pennsylvania State University, University Park, PA 16802. Recombination between a cellular lac allele and λplac5 in E. coli is 20- to 50-fold higher when the cellular lac allele is located on F42lac. This enhancement of recombination depends on the RecBCD enzyme of E. coli and the tra regulon of the F factor. Previous work with mini-F-lac plasmid derivatives has shown that this enhancement requires oriT (the F factor origin of conjugational transfer) in-cis to the recombining cellular lac allele, as well as trans-acting tra gene products from both the proximal and distal ends of the main tra operon of the F factor.

Recent work has been concentrated on determining precisely the tra gene products/activities which are essential for this enhancement of transductional recombination. Using an experimental system which involves mini-F-lac-oriT, segments from the proximal and distal ends of the main tra operon have been provided in-trans on separate additional plasmids. We have shown that a plasmid containing traY and traJ is able to provide all of the activity required from the proximal end of the tra operon, while a plasmid containing only traJ cannot. Similarly, only the traI gene product is required from the distal end of the tra operon, and we will present evidence that distinguishes the nicking and helicase activities of the traI gene product in recombination enhancement.

Although the polarized action of the oriT site in conjugal DNA transfer suggests the possibility of an orientation dependence in recombination enhancement, this possibility cannot be examined in a plasmid system. We will present data from chromosomal insertions of oriT near the lac operon that allow orientation dependence to be determined when the necessary tra functions are provided in-trans.

L 510 CONTACTS BETWEEN RecA PROTEIN AND DNA DURING THE FORMATION OF PARANEMIC JOINTS, Samson A. Chow, Department of Biochemistry, University of Hong Kong, Hong Kong.

With certain DNA substrates, RecA protein promotes the pairing of a single strand with its complement in duplex DNA to form a paranemic joint in which the strands are not topologically interwound but are nonetheless base-paired. In this study, we formed paranemic joints by pairing circular, single-stranded G4 DNA with linear G4 duplex molecules that were flanked on both sides with heterologous DNA. The structure of the paranemic joint was studied by probing the complex with pancreatic DNase I or restriction enzymes. The results showed that both the (+) and (-) strands of the duplex in a paranemic joint were protected against nuclease digestion. This is in sharp contrast to the results obtained from plectonemic joints in which the protection is asymmetric and the (-) strand of the heteroduplex is sensitive to nuclease digestion (J. Biol. Chem. 261:6961-6971). The protection of both strands of the duplex molecule in paranemic joints corresponded to the region of shared homology, and the protection of the (-) strand disappeared when the 5' or 3' heterologous flanking sequences were removed. The protection pattern is consistent with the model which proposes that initially during the pairing reaction, RecA protein makes contact with the two strands of a duplex simultaneously. This is then followed by unwinding, base pairing, and under appropriate conditions, intertwining of strands to form plectonemic joints.

L 511 EFFECT OF THE STRUCTURE OF A DOUBLE STRAND BREAK ON RECOMBINATIONAL REPAIR PROMOTED BY HUMAN NUCLEAR EXTRACTS, Bernard Lopez, Eric Corteggiani and Jacques Coppey, Institut Curie, Biologie, 75231 Paris, France

It is well established that double strand break (dsb) stimulates recombination. On the other hand, several pathways can operate for repairing dsb's. We have demonstrated that, in vitro, dsb repair promoted by human nuclear extract preferentially occurs via homologous recombination. The influence of the DNA structure surrounding a dsb on the extent of recombinational repair in human nuclear extract was analysed. The data indicate that the process is initiated at the level of the break and requires an intact homologous DNA. Dephosphorylation of the extremities of the break prevents the ligation pathway but does not affect the recombination pathway. The structure of the extremities strongly influences the effectiveness of this last pathway: blunt or 5' protruding ends can be repaired by recombination whereas 3' protruding ends cannot. These effects could result from polarity of the exonuclease and recombinase activities associated with the process. The effects of insertions and of deletions on recombinational repair were studied. Sequence homology with intact DNA is required on both sides of the break. Heterology can impair the process if the length of homology, between the two substrates, surrounding the dsb is not sufficient. The effect of the length of heterology on the minimal homology requirement surrounding the break was determined. Finally, a double strand gap (30 bp or 935 bp) can be repaired by homologous recombination in our system. The different results throw light on the initiation step of recombination, poorly documented in mammalian cells.

Molecular Mechanisms in DNA Replication and Recombination

L 512 PHYSICAL CHARACTERIZATION OF THE EFFECT OF DNA TOPOISOMERASE I AND II ON THE rDNA CLUSTER OF *S. CEREVISIAE*, Fred S. Dietrich, Michael F. Christman, and Gerald R. Fink, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142. We have found that in *S. cerevisiae* mitotic recombination in the rDNA cluster is suppressed by DNA topoisomerase I and II. We have further investigated the nature of this suppression and the nature of the recombination events in strains carrying *top1* and *top2* mutations using pulsed field gel electrophoresis. Using this technique we can determine the location of plasmids integrated into the rDNA, and detect changes in the size of the repeat. We have observed that under a variety of conditions migration of chromosome XII (containing the rDNA repeat) is different in *top1* vs *TOP1* strains which are otherwise isogenic. The *top2-1* (ts) mutation does not appear to affect migration of chromosome XII under semi-permissive conditions, however, as *TOP2* is an essential gene, the lack of a phenotype is difficult to interpret.

L 513 GENETIC CHARACTERIZATION OF *STP1*, THE GENE ENCODING THE DNA STRAND TRANSFER PROTEIN α. Christine C. Dykstra, Alan Clark and Akio Sugino, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Reseach Triangle Park, N.C. 27709
In order to identifiy the components of the genetic recombination appartus in *S. cerevisiae*, we have begun the biochemical purification of indiviual components expected to be involved. These include DNA strand transfer activities and single-stranded DNA binding proteins that stimulate DNA strand transfer. Now we would like to correlate biochemical activities with a genetic phenotype. We have identified the gene for STPα, a meiosis-specific DNA strand transfer activity and named the gene *STP1* (Clark et al., submitted to Proc. Natl. Acad. Sci. U.S.A.). We have disrupted this gene and found that it is not essential, even during meiosis. Sporulation appears normal in timing as well as viability. We have found that there is a 90% reduction in the meiosis-specific recombination as measured at several alleles. Corresponding to this *in vivo* observation has been the observance that the meiosis-inducible DNA strand transfer activity is also reduced, but not eliminated. We are now testing for the response to DNA damaging agents. We are also beginning the comparison of the *STP1* gene with other recombination and repair genes in order to define potential pathways in which it might be involved.

L 514 GENETIC CONTROL OF RECOMBINATION BETWEEN REPEATED GENES, Rochelle Easton Esposito and Shoshanna Gottlieb, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

In eukaryote genomes there are many repeated gene families and recombination between genes within a family has been amply documented. An important question that has arisen from these studies is why reciprocal recombination between repeated genes does not cause deletions and translocations that are deleterious to the cell. We have addressed this issue by studying the genetic control of recombination between artificially-constructed and naturally-occurring repeated genes in the yeast *S. cerevisiae*. Three genetic systems were used to monitor recombination: 1) a duplication of the *HIS4* locus on chromosome III, 2) a marker inserted in the ribosomal DNA (rDNA) array on chromosome XII and 3) a duplication of *HIS3* sequences located either at the *HIS4* locus or in rDNA. We have used these systems to examine the role of genes (*RAD50* and *SPO11*) known to be required for meiotic recombination between homologs in intrachromosomal exchange. Our results indicate that *RAD50* and *SPO11* are required for meiotic levels of recombination between duplicated *HIS4* and *HIS3* sequences, providing evidence for a common control of exchange between and within chromosomes. In contrast, *RAD50* is not required for meiotic intrachromosomal recombination in rDNA suggesting that exchange in rDNA is, at least in part, under independent genetic control. In addition, we have found that the yeast *SIR2* gene which alters chromatin structure and represses transcription of the silent copies of mating type information on chromosome III, also represses recombination in rDNA while having no effect on recombination elsewhere in the genome. Furthermore, in the absence of *SIR2*, rDNA acquires a partial dependency on recombination functions (*RAD50* and *RAD52*) that are normally dispensable for exchange in the region. These results provide evidence that one mechanism for regulating exchange between repeated genes may be to limit the accessibility of the general Rec system to these regions, perhaps through the control of chromatin structure.

L 516 INTEGRATIVE RECOMBINATION IN WILD-TYPE AND RECOMBINATION DEFICIENT MUTANTS OF USTILAGO MAYDIS. S. Fotheringham and W. Holloman, Department of Microbiology, Cornell University Medical College, New York, NY 10021. Plasmids containing the LEU1 gene of U. maydis, but lacking an autonomously replicating sequence, transform cells at low frequency. One hundred-fold stimuation of the transformation frequency was seen when the plasmid was cut to a linear form, regardless of whether the ends generated lay in stetches homologous to genomic sequences. The majority of integration events examined occurred at apparently random sites throughout the genome when transforming DNA was circular. When the DNA was made linear, integration took place almost exclusively through homologous recombination. Transformation with linear DNA was also observed to take place through a non-integrative mechanism. Concatamers consisting of tandem arrays of unit length plasmid DNA in direct and inverted repeats, and maintained extrachromosomally were found in most cells transformed by linear DNA. Recent studies on transformation have focused on the control of plasmid integration by the REC1 and REC2 genes which control recombination. We have taken advantage of the homologous integration mechanism to disrupt the LEU1 gene by a one-step procedure.

L 517 THE pR uvp1 GENE ENHANCES THE RECOMBINATIONAL ACTIVITY IN E.COLI, Franca Gigliani[1], Elisabetta Sporeno[1], Silvia Perri[2] and Piero A.Battaglia[2]- [1]Dipartimento Biopatologia Umana, Sezione Biologia Cellulare, [2] Laboratorio di Biologia Cellulare, Istituto Superiore di Sanità, 00161 Roma, Italy.
The nucleotide sequence and the expression in minicells of the uvp1 gene of the pR plasmid involved in SOS repair, has been determined.
The uvp1 gene has been found to encode a 20kd protein which appears to be responsible for the enhancement of both UV survival and recombinational activity in E.coli. The uvp1 gene product bears a striking resemblance to Resolvases in comprising two structural domains.One of these (the NH -domain) is apparently responsible for the recombinational activity; the other (the COOH domain) carries the major determinant for the binding to DNA of the 20kd protein. It is surmised that the Uvp1 protein partecipates in UV repair by cooperating with the well known mucAB genes through its recombinational activity.

L 518 DNA DUPLICATIONS IN PLASMID MOLECULES EXCISED FROM A HUMAN CHROMOSOME FOLLOWING FUSION WITH COSI CELLS. Micheline Giphart-Gassler, Andrea Groenewegen and Judith Tasseron-de Jong. Laboratory of Molecular Genetics, Leiden University, Leiden, The Netherlands.
Replication and excision of DNA sequences containing the SV40 origin of replication integrated in the genome of non-permissive cells occurs after fusion of these cells with COS cells. We searched for mutations which occur within excised molecules by fusing with COSI cells a human cell line containing a single copy integrate of a recombinant plasmid, on which a SV40 origin of replication and several marker genes were introduced. The low molecular weight DNA fraction was transformed to E. coli to score for aberrant plasmid molecules. It appeared that 14% of all transforming excised plasmids contained DNA duplications in an either direct or indirect orientation. In all but one plasmid the SV40 origin is part of the duplicated DNA. No DNA duplications of this type were found in plasmids that were transfected to COSI cells and had replicated autonomously, implying that the duplications arise as a consequence of replication within chromosomes. The data indicate that DNA recombination occurs frequently in hyper-replicated DNA. The observed DNA rearrangements may reflect rearrangements involved in or resulting in gene amplification.

Molecular Mechanisms in DNA Replication and Recombination

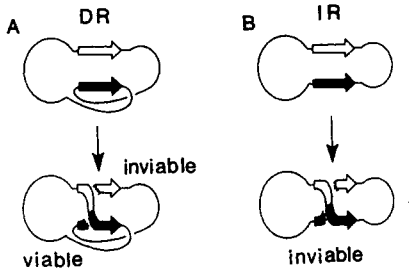
L 519 Characterization of Promoter Region for Human Retinoblastoma Gene, Frank D. Hong, Hoang To, Wen-Hwa Lee, Department of Pathology & Center for Molecular Genetics, University of California, San Diego, La Jolla, CA 92093-0612.
Retinoblastoma, a childhood cancer of the retina, belongs to a class of familiar cancers and it follows both a hereditary and non-hereditary mode of inheritance. The susceptibility to develop the tumor has been associated with inactivation of a genetic locus located within the q14 band of chromosome 13 based on cytogenetic studies. Recently several groups including ours reported cloning of a gene, henceforth designated as Rb gene, found within the 13q14 region, whose altered nature, i.e. lack of expression, truncated transcripts, aberration in the genomic DNA, etc., finds agreement with the gene inactivation concept proposed. We present here a detailed characterization of the promoter region of the Rb gene. Transcription of this gene is initiated at 3 distinct nucleotide positions as reflected from our S1 protection analysis. The sequences surrounding the transcription start sites are extremely rich in G+C nucleotides. Sequences of TATA and CAAT boxes are found 140 bp and 280 bp, respectively, upstream of the transcription initiation sites.These sequences may not participate in transcriptional regulation of Rb gene. Based on deletion analysis, a critical region at -30 through +70 was found to be important in regulating the expression of Rb gene. A fragment containing 8 kb upstream has been tested and seems to lack any enhancer-like elements. In general the mode of expression as assayed via utilization of CAT reporter gene linked to Rb promoter shows little tissue-specificity consistent with the conclusion that the Rb mRNA is expressed essentially in every mammalian cells.

L 520 AMPLIFICATION-ASSOCIATED ILLEGITIMATE DNA REARRANGEMENTS IN CHINESE HAMSTER CELLS, O. Hyrien, M. Debatisse, B. Robert de Saint Vincent and G. Buttin, Unité de Génétique Somatique, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cedex 15.
Chinese hamster fibroblasts cell lines can resist increasing doses of coformycin by increasing amplification of a large stretch of DNA including the gene for AMPD, the target protein of coformycin. We have studied the structure of amplified DNA in several independent lineages of such mutants. The amplified units are chromosomally integrated and have been joined together by illegitimate recombination events which are non randomly distributed along the amplified DNA : the novel joints cluster in a 2kb DNA region which is a mosaïc of Alu-like repeats and palindromic A + T rich DNA segments ; this hotspot contains multiple sequences related to the topoisomerase II cleavage consensus sequence and we are currently testing whether it is a nuclear matrix attachment site. Nucleotide sequences at two independent novel joints show the presence of palindromic sequences and topoisomerase 1 consensus cleavage sites at the breakpoints. The first novel joint formed by joining the hotspot with another distant A + T rich DNA sequence. The second novel joint lies at the center of a large (> 2 x 120kb) inverted duplication. We propose a model by which template switching at a replication fork leads to the formation of a large inverted duplication and to its subsequent amplification. We are presently checking some predictions of this model using pulsed field electrophoretic analysis of amplified DNA.
References : Hyrien et al.(1987) EMBO J. 6, 2401-2408.
Hyrien et al (1988) EMBO J. 7, 407-417.

L 521 THE EFFECT OF TERMINAL NON-HOMOLOGY ON HOMOLOGOUS RECOMBINATION IN *Xenopus laevis* OOCYTES, Sunjoo Jeong-Yu and Dana Carroll, Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah 84132,
DNA molecules recombine efficiently after injection into the nuclei of *Xenopus* oocytes. Recombination in oocytes depends on linear DNA substrates and sequence homology. To investigate the role of molecular ends in this process, we injected variants of a linear DNA substrate with 1250 bp terminal direct repeats, which can undergo intramolecular recombination to yield a circular product. When one or both ends were blocked by nonhomologous sequences, the efficiency of recombination was greatly reduced. This indicates that both ends are involved in determining the yield of products. In addition, the distribution of apparent crossovers within the direct repeats was mapped using restriction site polymorphisms. Without end-blocks, crossovers were concentrated at the ends of homologies; and this distribution was not altered by a nonhomology on one end. These results rule out some simple models for oocyte recombination. Finally, injection of a substrate that should be recombined by double-strand-break-repair as observed in yeast showed that the oocytes perform that type of event very inefficiently, if at all.

Molecular Mechanisms in DNA Replication and Recombination

L 522 HOMOLOGOUS RECOMBINATION IN A MAMMALIAN PLASMID: DIFFERENCE BETWEEN DIRECT REPEATS AND INVERTED REPEATS, Yoshihiro Kitamura, Hiroshi Yoshikura and Ichizo Kobayashi*, Department of Bacteriology, Faculty of Medicine, University of Tokyo, Hongo, Bunkyo-Ku, Tokyo, 113 Japan and *Department of Molecular Biology, Institute of Medical Science, University of Tokyo, Shiroganedai, Minato-Ku, Tokyo, 108 Japan. Bovine papillomavirus shuttle vectors replicate stably as an extrachromosomal plasmid in mouse cell nuclei and can be rescued in E. coli for analysis. They provide unique substrates to analyze homologous recombination in mammalian cells. We found that two homologous sequences placed in direct orientation on this plasmid undergo homologous exchange at a high frequency during multiplication. These repeats did not produce crossing-over plasmids, when placed in inverted orientation. These results are explained by physical non-reciprocality of homologous exchange as only one duplex DNA molecule is produced from two recombining duplexes. When physically non-reciprocal crossing-over (hemi-crossing-over) occurs between direct repeats (Fig. A), a viable molecule can be formed. In the case of inverted repeats (Fig. B), however, the product molecule is linear and inviable.



L 523 EVIDENCE FOR THE DOUBLE-STRAND BREAK REPAIR MODEL OF λ RECOMBINATION, Ichizo Kobayashi* and Noriko Takahashi, National Children's Medical Research Center, Tokyo, Japan. (* Present address: Department of Molecular Biology, Institute of Medical Science, University of Tokyo, 4-6-1 Shiroganedai, Minato-ku, Tokyo 108 Japan (PHONE: 3-443-8111))

Homologous exchanges of bacteriophage λ by its own Red pathway are frequent all over replicated chromosomes but are limited to cos in unreplicated chromosomes. One of us proposed that the double-stranded breaks at cos and at the tail of a rolling circle initiate recombination (Stahl, Kobayashi & Stahl, JMB 181:199).

We now obtained direct evidence for the double-strand break repair model for homologous recombination promoted by λ. A double-stranded gap was made in one of the two regions of homology in an inverted orientation on a plasmid molecule. The gapped plasmid was introduced into E. coli cells expressing λ function, and repair products expected by the model were detected. The gap increased recombination 100 fold. Gene conversion repairing the gap was frequently accompanied by crossing-over of the flanking sequences as in meiosis in eukaryotes.

L 524 BINDING STOICHIOMETRY AND STRUCTURE OF RECA–DNA COMPLEXES – EVIDENCE FOR MULTIPLE HETEROGENEOUS DNA COORDINATION

Mikael Kubista, Bengt Nordén and Masayuki Takahashi, Department of Physical Chemistry, Chalmers Chalmers University of Technology, S-412 96 Gothenburg, SWEDEN.

The interaction between RecA and DNA, in the form of single-stranded DNA, fluorescent ethenoDNA, double-stranded DNA, and poly(dG–dC) in B and Z-conformation is studied with flow linear dichroism, circular dichroism, and fluorescence spectroscopy. RecA is found to form a complex with single-stranded DNA with a binding stoichiometry of four nucleotides per RecA monomer, in which the DNA bases appears to have a random orientation. Addition of ATPγS (a non-hydrolyzable analog of ATP) reduces the stoichiometry to about 3 nucleotides per RECA and causes the DNA bases into an orientation preferentially perpendicular to the fiber axis. This complex is shown to incorporate an additional strand of single-stranded DNA or double–stranded DNA, yielding a total stoichiometry of 3+3 nucleotides, or nucleotides and base-pairs, per RecA. RecA, in presence of ATPγS, is also found to interact with double-stranded DNA in B and Z conformation [the latter studied with poly(dG-dC) and poly(dG-m^{5}dC)], with a stoichiometry of 3 base-pairs per RecA. The association rate of RecA to Z-form DNA is found to be slower than to B-form, and it is shown that RecA does not stabilize the DNA in the Z-conformation but that it can kinetically 'freeze' the polynucleotides in the B-conformation. In all studied complexes the tryptophan residues of RecA are oriented with their aromatic planes preferentially parallel to the fiber axis, whereas the DNA bases, in complexes involving ATPγS, are oriented preferentially perpendicular to the fiber.

Based on these results a model for the search of homology in the RecA-mediated strand-exchange reaction in the genetic recombination process is discussed.

L 525 DNA PAIRING MEDIATED BY recA PROTEIN, Janet E. Lindsley and Michael M. Cox, Department of Biochemistry, University of Wisconsin, Madison, WI 53706

Before strand exchange can begin, recA protein must bring two DNA molecules together in homologous alignment. A key intermediate is formed, in which homology between the two DNAs is detected but no real strand exchange has occurred. This is termed a paranemic joint. We have studied recA-mediated paranemic joint formation between three sets of substrates: (a) single-strands + duplex DNA, (b) gapped duplex DNA + duplex DNA, and (c) duplex DNA + duplex DNA. Pairing has been characterized by DNA underwinding, changes in ATP hydrolysis, and electron microscopy. The results of these experiments will be described.

L 526 **INDUCIBLE RECOMBINATION PROTEINS IN E. coli.** Robert G. Lloyd, Gary J. Sharples, Graham T. Illing, Fiona E. Benson and Stuart J. Morton, Department of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.

The induction of proteins that promote recombination is one of the key features of the LexA-regulated SOS response to DNA damage in E. coli. In addition to recA which provides the "recombinase", four other genes (recN, recQ, ruvA and ruvB) provide recombination activities that are necessary for efficient repair of DNA chain breaks. recN specifies a 63kDal polypeptide that is induced strongly after exposure to radiation and which is needed specifically for the repair of double-strand breaks. This protein has been amplified and shown to have a remarkable affinity for DNA, which is consistent with the properties of the protein predicted by the DNA sequence. ruvA and ruvB form a single operon and specify polypeptides of 22kDal and 37kDal respectively, both of which are necessary for repair. Overproduction of the ruvA product is lethal when induced as part of the SOS response. The ruv genes have been coupled separately to the lambda PL promoter which allows amplification of the gene products to more than 5% of total cell protein. The available evidence suggests that the two proteins function late in recombination to convert intermediates into viable products.

L 527 HOMOLOGOUS RECOMBINATION BETWEEN TWO DUPLEX DNA CATALYSED BY HUMAN NUCLEAR EXTRACT

Bernard Lopez, Eric Corteggiani and Jacques Coppey
Institut Curie, Biologie, 75231 Paris, France.

Homologous recombination is implicated in various genomic rearrangements. We have devised a strategy allowing to study homologous recombination catalysed by human nuclear extract. Our system, which monitors recombination between two duplex DNA molecules, is based on the connection of a phenotypical approach with a molecular one. Recombination intermediates have been isolated and visualized ; their structure have been correlated to the recombined phenotype. Taken together, all results indicate that the recombination process proceeds via a single-strand exchange which can be reciprocal. Restriction analysis on the recombining molecules shows that either DNA strand can be exchanged. Sequence analysis of 20 recombined clones (450 nt per clone) indicates that homologous recombination is error-free in human nuclear extracts. In line with this, DNase I protection assay shows that nuclear extract contains factor(s) which transiently protect the recombining DNA sequences against nuclease(s).

Molecular Mechanisms in DNA Replication and Recombination

L 528 VACCINIA VIRUS CONCATEMER JUNCTIONS: RESOLUTION VIA SITE-SPECIFIC RECOMBINATION, Michael Merchlinsky and B. Moss. Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20892. Bacterial plasmids containing the concatemer junction from vaccinia virus replicative intermediates were replicated and resolved into linear minichromosomes with sealed terminal hairpins after transfection into virally infected cells. A series of symmetrical insertions, deletions, and site-directed oligonucleotide mutations demonstrated that the DNA sequence ATTTAGTGTCTAGAAAAAAA that is found proximal to the hairpin loop and is highly conserved among poxviruses is essential for resolution. In the concatemer junction the orientation of each site, with respect to each other as well as to the axis of symmetry, is critical for resolution. Plasmids containing concatemer junctions composed of two active but different resolution sites are resolved at varying levels, implying the sites are not independently processed. The resolved terminal hairpin is a heteroduplex derived from DNA of both strands of the concatemer junction. A model for resolution involving site-specific recombination and oriented branch migration is consistent with this data. The terminal sequences for conditionally lethal mutants were poorly resolved in mutants defective in late protein synthesis; in contrast to the requirement for early gene products for viral DNA replication.

L 529 A RETROVIRAL-BASED VECTOR SYSTEM FOR STUDYING INTRACHROMOSOMAL RECOMBINATION IN NORMAL AND MUTANT HUMAN CELL LINES, M. Stephen Meyn, Departments of Human Genetics and Pediatrics, Yale University School of Medicine, New Haven, CT 06510 As part of our study of genetic recombination in human cells we have developed a series of retroviral vectors to measure intrachromosomal recombination, sister chromatid exchange and gene conversion events in normal human fibroblast lines and fibroblast lines with altered DNA repair abilities. These retroviral vectors, upon infection, integrate tandem, complementary mutant *neo* genes into chromosomal DNA. The integrated *neo* genes can then undergo recombination, resulting in the reconstitution of a wild-type gene and expression of G418 resistance. A series of vectors were created from the pB2d self-inactivating retroviral vector, the *neo* gene from pCM1polA, and the hygromycin gene from pHyg. These vectors contain two *neo* genes in direct repeat orientation surrounding the hygromycin gene fragment. The two mutant *neo* genes used in these constructions were created by insertion of 8 bp XhoI linkers at their EcoR52 and NaeI sites respectively The retroviral vectors were used to transfect a retroviral-packaging cell line (PA317) and hygromycin-resistant virus-producing subclones isolated. Packaged vectors from these lines were then used to infect a normal human cell line and cell lines established from patients with xeroderma pigmentosa, Bloom syndrome, Fanconi's anemia and ataxia telangiectasia. Hygromycin-resistant clones have been isolated and single-copy integrants identified by Southern blotting. Fluctuation tests to measure the spontaneous rate of recombination in these subclones are underway. Initial results with an excision repair-deficient xeroderma pigmentosa line (complementation group F) indicates a rate of $2x10^{-6}$ $G418^R$ colonies/cell/generation. DNA from $G418^R$ colonies arising during the fluctuation tests is being analyzed to determine the nature of the recombinational events. (Supported by NIH R01 GM38588).

L 530 A MODEL FOR TRANSPOSITION OF *STAPHYLOCOCCUS AUREUS* TRANSPOSON Tn554, Ellen Murphy[1], Leslie Huwyler[1], Ellen Reinheimer[1], Lucy E. Tillotson[2] and Donald T. Dubin[2], [1]The Public Health Research Institute, New York, NY 10016, and [2]UMDNJ–Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Tn554 is a transposable element in *Staphylococcus aureus* that inserts specifically and efficiently into a unique chromosomal site designated *att554*. Transposition does not generate a target duplication, nor do the termini of Tn554 contain repeated elements. Insertion of Tn554 at *att554* occurs mostly in one orientation (+), between nucleotides +3 and +4. Less frequently, insertion occurs in the opposite (–) orientation, in this case between nt –3 and –4. This defines a central "core" of 6 bp, GATGTA. A deletion analysis of *att554* showed that sites retaining nt –28 to +25 possess wild-type *att554* activity, sites retaining only nt –15 to +8 function poorly but retain specificity, and plasmids with deletions extending closer than this to the insertion site, although still retaining the central core, are totally inactive as targets. Studies with mutated attachment sites indicate that some nucleotides within the essential region, most notably that at position +1, can be mutated without affecting *att554* function. Tn554 also transposes at a low frequency ($\leq 10^{-6}$) to secondary insertion sites that have little or no sequence homology with *att554*. The transposon-target junction sequences following serial transposition among secondary sites, mutant *att554* sites and wild-type *att554* were found to vary in a predictable way: the sequence originally present in the target becomes the sequence bounding the left end of the inserted transposon, the 6 or 7 bp originally on the left of Tn554 become the new right end junction, and the original right boundary sequence is lost. A model to account for these observations is presented.

L 531 THE recQ GENE OF Escherichia coli: OVERPRODUCTION, PURIFICATION AND SOME PROPERTIES OF THE GENE PRODUCT, Hiroaki Nakayama, Keiko Umezu and Koji Nakayama, Department of Microbiology, School of Dentistry, Kyushu University, Fukuoka 812, Japan.

The E. coli recQ gene is one of the genes involved in the RecF pathway of conjugal recombination, and its cloning and sequencing have been achieved in our laboratory (MGG 205: 298-304, 1986). We now report a system for its overexpression and purification of its gene product. The multicopy RecQ-overproducing plasmid pKD176 carries a modified recQ gene, with ATG initiation instead of GTG and the promoter/SD region replaced by that of lac. Upon induction with IPTG, the cells harboring pKD176 undergo some growth inhibition, suggesting that excessive production of RecQ is detrimental to the cell. The RecQ protein can be purified to apparent homogeneity through ssDNA-cellulose chromatography, ammonium sulfate fractionation, gel filtration with Sephacryl S200 and hydrophobic chromatography. Preliminary experiments with purified RecQ protein have so far revealed no evidence for nuclease activity, but have given indications that RecQ facilitates the interaction of the RecA protein with DNA.

L 532 A MUTATIONAL ANALYSIS OF THE GENETIC REGULATION OF MAMMALIAN RECOMBINATION WITH Y-CHROMOSOME LINKED SEQUENCES, Ferez S. Nallaseth, Department of Biology, USC, Columbia, SC 29208. We have established that combinations of feral Y-chromosomes and genomes of inbred strains induce murine hybrid dysgenesis (Nallaseth and Whitney, 1988): which is a collection of phenotypic and genotypic traits that includes the loss of genetic control of recombination (Kidwell et al., 1977). Y-chromosome linked repeated sequence probes and combinations of reciprocally backcrossed inbred strain and feral Y-chromosomes have allowed the recovery and analysis of several distinct but reproducible aberrant recombination products. Because of their location in recombinationally suppresed loci and their male limited haploidy these Y-linked probes have allowed the unambiguous detection of traces of Y-chromosomal DNA in females. These sequences are tentatively identified as random interchromosomal gene conversion tracts. Alternative assays for the detection of aberrant recombination products are predicated on the co-ordinated rearrangement of most copies of the above Y-linked repeated sequence families in XY individuals. This class of aberrant recombination products included precise, co-ordinated rearrangement of fragments defining a multi-copy recombination breakpoint and an *Alu* sequence RFLP, on the Y-chromosome. Co-ordinated rearrangements occurred mostly during germline, but also during somatic development. The reproducibility and specificity of substrate-product relationships, the relative precision and high frequencies of these co-ordinated rearrangements in independent germlines, excludes passive genetically non-directed non-homologous recombination as a possible mechanism(s). Distinct autosome or X-chromosome encoded trans activities inducing each of these aberrant recombination events are strongly suggested by the differences in dependence on distinct "genetic backgrounds" for their induction. References: Kidwell, M.G. et al. (1977). Genetics 86, 813-833. Nallaseth, F. S. and J. Barry Whitney III. (1988). XVIth International Congress on Genetics (Abstract No. 32.12.2).

L 533 GENE CONVERSION BETWEEN PLASMIDS IN CHINESE HAMSTER OVARY CELLS EXHIBITS A STRONG POLARITY EFFECT, Nickoloff, J.A. and Reynolds, R.J. Genetics Group, LS-3, Los Alamos National Laboratory, Los Alamos, NM 87545. Derivatives of pSV2neo containing linker insertion mutations at two sites (5' or 3') within the coding region of the neomycin gene were constructed. The mutant coding regions of neomycin were also inserted into pUC19, producing plasmids lacking both the SV40 promoter and the 3'splice/polyadenylation signals required for expression in mammalian cells. Plasmids of each type containing complementary mutations were mixed and electroporated into CHO K1 cells, and recombination was monitored by selecting for G418 resistant colonies. Since cross-overs between the plasmids do not produce a functional expression unit, the system monitors only gene conversions or events involving multiple exchanges. Cleavage near or at the site of the mutation in the pSV2neo derivatives, but not pUC19neo derivatives, stimulated recombination more than 10-fold over levels seen when uncut substrates were used, consistent with gene conversions occurring by a double-strand break repair mechanism. When both plasmids were cleaved at mutated sites, a strong polarity effect was observed. Relative to uncut plasmids, cleavage of both plasmids stimulated recombination 10-fold when the pSV2neo derivative contained the 3' mutation, and more than 50-fold when it contained the 5' mutation. This research was supported by U.S. Department of Energy contract No. W-7405-ENG-36 and National Institutes of Health research grant CA 42390.

L 534 ABNORMAL TOPOISOMERASE I ACTIVITY IN BLOOM SYNDROME FIBROBLASTS. Yves Pommier, Thomas M. RØnger, Donna Kerrigan, and Kenneth Kraemer. Laboratories of Molecular Pharmacology and Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD 20892.

Bloom syndrome cells form numerous sister chromatid exchanges upon exposure to 5-bromodeoxyuridine (BrdU). This effect has been proposed to result from abnormal topoisomerase II (topo II) activity. The possibility that BrdU could induce topo II-mediated DNA breaks was tested by alkaline elution in normal and Bloom syndrome fibroblasts. Five day exposure to BrdU induced similar amounts of DNA single-strand breaks in both cell lines. However, these breaks were not protein-associated and were alkali labile, indicating that they did not result from topo II action. The formation of DNA single-strand breaks induced by UV light (313 nm) was also tested in both cell lines. Bloom syndrome cells formed more single-strand breaks than normal cells. Alkaline elution without proteinase K indicated that the breaks were protein-associated and were associated with approximately one DNA-protein crosslink per DNA break in both cell lines. These characteristics suggested the involvement of topoisomerases in the formation of the breaks. Topo II inhibition by the anticancer drugs, amsacrine and etoposide was tested by alkaline elution in normal and Bloom syndrome cells. Both drugs produced similar DNA single-strand break frequencies in the two cell lines, implying that they had similar topo II activities. By contrast, the topo I inhibitor, camptothecin produced more protein-linked DNA single-strand breaks in Bloom syndrome than in normal fibroblasts, which indicates Bloom syndrome fibroblasts have a greater topo I activity than normal fibroblasts.

L 535 MISMATCH REPAIR AND FIDELITY OF GENETIC RECOMBINATION. C.Rayssiguier and M.Radman Laboratory of Mutagenesis,Institut Jacques Monod,C.N.R.S.,University Paris 7, Paris (France).

M. Radman proposed that the E.coli MutHLSU mismatch repair system may control the fidelity of recombination by destroying the heteroduplexes generated by strand exchanges (Radman,M. 1988,Mismatch repair and genetic recombination. In :Genetic recombination Ed. by R. Kucherlapati and G.R. Smith. American Soc. for Microbiol. p.169-191).This was tested in conjugational crosses involving the two genera E.coli and Salmonella typhimurium.Conjugational crosses involving the two give a frequency of integration of donor DNA of 10^{-8} per donor cell as compared to 10^{-1} in intraspecific crosses(Baron et al.,1968,Intergeneric bacterial matings.Bacteriol. Rev.32 :362-369). When restriction deficient F⁻Salmonella typhimurium recipient of E.coli Hfr DNA is also mismatch repair deficient,the frequency of intergeneric recombinants increases by 10^{3} to 10^{7}fold.The effect is stronger for mutL and S and decreases with mut H and U.It is not observed if the recA mutation is introduced into the recipients.The antirecombinogenic role of the E.coli LPMR(long patch mismatch repair:Mut HLSU system)in the control of the fidelity of recombination and in the molecular mechanism of speciation will be discussed .

L 536 THE LACK OF DNA HOMOLOGY AND RECOMBINATION OPPORTUNITY IN A PAIR OF DIVERGENT CHROMOSOMES GREATLY SENSITIZES THEM TO LOSS BY DNA DAMAGE. [1]M.A. Resnick, [2]M. Skaanild and [2]T.Nilsson-Tillgren. [1]Yeast Genetics Group, National Inst. Environmental Health Sciences, Research Triangle Park, NC, USA. [2]Inst. of Genetics, U. Copenhagen, Denmark.

Chromosomal DNA is considered a priori to be a target for induction of whole chromosome aneuploidy and DNA repair would be expected to play a role. Using the yeast Saccharomyces cerevisiae, we have addressed the importance of recombinational repair (required for double-strand break [DSB] repair) in the maintenance of complete chromosomes. Specifically, we examined aneuploidy induction by ionizing radiation in diploids which had one chromosome III replaced by a DNA divergent chromosome from S. carlsbergensis. While they are functionally equivalent, the lack of precise DNA homology in 1/2 of the chromosome was expected to prevent recombinational repair processes in this region.

The absence of recombinational repair (presumably of DSBs) in the divergent chromosomes results in high aneuploidy levels (5 to 10%) at nonlethal radiation doses. For homologous chromosomes, the aneuploidy frequency is 20 to 50 X lower. Based on genetic and physical analyses, the aneuploidy is due to chromosome loss, rather than deletions or malsegregation. Using other chromosomes that are completely divergent, we are developing a selective system for detecting chromosome loss and have shown that UV is also an effective inducer of chromosome loss. We suggest that nonhomologous regions of otherwise homologous chromosomes may be important targets for the induction of aneuploidy. We are presently examining if relaxation of controls on divergent chromosome interactions enables repair and/or enhances chromosome aberrations.

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L 537 MODIFICATION OF THE MOUSE BETA-2 MICROGLOBULIN GENE IN VIVO BY HOMOLOGOUS RECOMBINATION, Peter Robinson, Donald Palmer and Julian Dyson, MRC Clinical Research Centre, Harrow, Middlesex. U.K.

We have been using the technique of homologous recombination in embryonic stem cells with the aim of producing mice with altered expression of immunologically important cell surface molecules. In addition to the antigen receptor itself, many other cell surface molecules are involved in the process of antigen-driven T lymphocyte activation. One approach to studying the importance of these structures is to replace the normal gene with a modified or defective counterpart in vivo. Our aim is to study the function of T cell accessory molecules such as CD2, CD4 and CD8 using this technique. Initially, we have chosen mouse beta-2 microglobulin, which is essential for cell surface expression of the major histocompatibility class I antigen H-2, as a target for deletion studies. 'Omega' type constructs consisting of a 4.5kb segment of the $B2m^b$ gene modified by addition of a 30bp oligonucleotide designed to cause early termination of translation have been introduced into cultured cells by electroporation or microinjection. Successful integrations are detected without prior selection using the polymerase chain reaction (PCR). Homologous and non-homologous insertion events can be distinguished using appropriate oligonucleotides for the PCR reaction. We are at present attempting to determine the optimal conditions for homologous insertion events in cultured fibroblasts and embryonic stem cells.

L 538 CLONING OF THE LYMPHOID-SPECIFIC RECOMBINATION ACTIVATOR GENE (RAG-1), David G. Schatz, Marjorie A. Oettinger and David Baltimore, Whitehead Institute for Biomedical Research and Massachusettes Institute of Technology, Cambridge, MA 02142.

V(D)J recombination is the central process in the differentiation of B and T cells: it is responsible for the assembly of the genes that encode immunoglobulins and the T-cell receptors. NIH 3T3 cells do not normally contain V(D)J recombinase activity; however, genomic transfection of these cells can stably activate V(D)J recombinase activity. It is likely that expression of a single, lymphoid-specific gene in a fibroblast is sufficient to confer V(D)J recombinase activity on that cell. We have named this putative gene RAG-1 (Recombination Activator Gene). We have used oligonucleotide "tagging" and genomic transfection to identify genomic sequences closely linked to the RAG-1 gene. Identification and cloning of RAG-1 are underway, as are attempts to determine the nature of the activity encoded by RAG-1.

L 539 **INDUCIBLE RECOMBINATION PROTEINS IN E. coli.** Robert G. Lloyd, Gary J. Sharples, Graham T. Illing, Fiona E. Benson and Stuart J. Morton, Department of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.

The induction of proteins that promote recombination is one of the key features of the LexA-regulated SOS response to DNA damage in E. coli. In addition to recA which provides the "recombinase", four other genes (recN, recQ, ruvA and ruvB) provide recombination activities that are necessary for efficient repair of DNA chain breaks. recN specifies a 63kDal polypeptide that is induced strongly after exposure to radiation and which is needed specifically for the repair of double-strand breaks. This protein has been amplified and shown to have a remarkable affinity for DNA, which is consistent with the properties of the protein predicted by the DNA sequence. ruvA and ruvB form a single operon and specify polypeptides of 22kDal and 37kDal respectively, both of which are necessary for repair. Overproduction of the ruvA product is lethal when induced as part of the SOS response. The ruv genes have been coupled separately to the lambda PL promoter which allows amplification of the gene products to more than 5% of total cell protein. The available evidence suggests that the two proteins function late in recombination to convert intermediates into viable products.

L 540 A NEW TOPOLOGICAL MODEL FOR ENZYME MECHANISM, S.J. Spengler*, N.R. Cozzarelli°, C. Ernst+ and D.W. Sumners +, *Program in Math and Molecular Biology and °Dept. of Molecular Biology, U. California, Berkeley, Berkeley CA 94720, and+Dept. of Mathematics Florida State University, Tallahassee, FL 32306.

The topological approach to enzymology posits that facts about enzyme mechanism can be deduced by following the change an enzyme causes in the geometry and topology of the DNA substrate. We show that any 2-strand DNA interaction--site specific recombination or topoisomerase action--that is mediated by a binding enzyme can be modelled using 2-string tangles, i.e., two curves in a bounded 3D space. We compute the topology of the synaptic complex in a single recombination or strand passage event, given the topology of the substrate and product. The synaptic complex is the sum of two tangles. One is associated with the recombination site, either at synapsis, T, or after recombination, R; the second represents the remainder of the DNA, S. The recombination tangle can involve any number of rational crossings of the two strands, not only one. The site specific recombination enzymes, Tn3 resolvase and phage λ Int, produce unique topologically defined products from supercoiled, knotted or relaxed substrates. The rational tangle calculus proves that the supercoils in solution are rational tangles, i.e., plectonemic coils. Similarly, tangle calculus can predict the structure of the product arising from N reiterations of Tn3 resolvase action.

L 541 EXPRESSION OF gene II OF BACTERIOPHAGE f1 IN YEAST LEADS TO ELEVATED RECOMBINATION IN AN INTERVAL CONTAINING A gene II RECOGNITION SITE

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The gene II protein of bacteriophage f1 (M13) makes a site-specific, strand-specific nick at the origin of replication of the phage. We are trying to exploit this activity to determine the degree to which nicks are recombinagenic in yeast and, if so, the nature of the events that are initiated by nicks. We have expressed the gene II protein in yeast and placed its recognition sequence into chromosome III of S. cerevisiae. The site was placed between two genes, trp1 and his3, so that recombination on both sides of the site could be scored. Interchromosomal mitotic recombination in this interval (TRP1 or HIS3 formation) is stimulated about 20 fold in strains with the site on one homologue when gene II is expressed. Using the trp1-his3 locus and closely linked outside markers (cry1 and MAT) we can select recombination in one gene (e.g. TRP1), monitor events in the other gene (e.g. coconversion of his3 alleles), and identify those events that have had an exchange of outside markers. Preliminary evidence indicates that the distribution of recombinants among the various kinds is different in the gene II stimulated events from that of spontaneous events. Experiments are in progress to determine if these events are initiated by a nick in the DNA.

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L 542 THE CONTROL OF SITE-SPECIFIC RECOMBINATION IN PLASMID ColE1.

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In *rec*$^+$ bacteria, the formation of multimers of high copy number plasmids reduces the number of independent segregating units and increases the production of plasmid-free cells. ColE1 and related plasmids containing the 250 bp *cer* site are maintained stably, because monomers are regenerated by site-specific recombination between copies of *cer* within plasmid multimers. This process also requires the products of the chromosomal *xerA*, *xerB* and *xerC* genes.

cer-mediated site-specific recombination is highly directional and intermolecular recombination (which generates multimers) occurs at only 10^{-4} times the frequency of the intramolecular event. An insight into the basis of directionality is provided by the products of *in vivo* recombination between *cer* and *parB* (the multimer resolution site from plasmid CloDF13). Two distinct hybrid sites result, differing by 2 bp. Type I is functionally indistinguishable from wild-type *cer*. It supports only intramolecular recombination and requires the products of *xerA*, *xerB* and *xerC*. Recombination between deletion derivatives of the type II hybrid is independent of the products of *xerA* and *xerB* and of the topological relationship between the participating sites.

We have recently identified another level at which recombination is regulated. A short transcript encoded within *cer* acts *in trans* to inhibit *cer*-mediated recombination. A quantitative analysis of the inhibitory activity of transcripts from deletion derivatives of *cer* has shown that the functionally-important part of the transcript is complementary to the DNA sequences flanking the crossover site.

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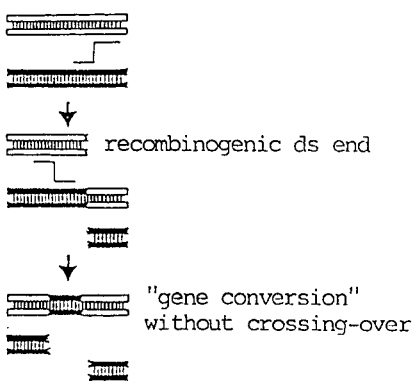
L 543 CHARACTERIZATION OF CLONED REC 1 GENE FROM USTILAGO MAYDIS, T. Tsukuda and W. Holloman, Interdivisional Program in Molecular Biology, Cornell University Medical College, New York, NY 10021. The rec-1 mutant of U. maydis was isolated in 1965 by Holliday on the basis of radiation sensitivity. The mutant was found to be extremely pleiotropic; repair, recombination, viability, growth, and meiosis were found to be affected. Thus, a single mutation alters completely the genetic system of the cell. We cloned the REC 1 gene by complementing the mutant with a genomic DNA library contructed in an autonomously replicating plasmid of U. maydis. The cloned gene restores function to the mutant in every way we have measured, including resistance to radiation and chemical alkylating agents, recovery of viability and growth, and restoration of recombination proficiency. The study of regulation and expression of the REC 1 gene will help reveal the nature of the recombination system operating in this fungus.

L 544 GENE TARGETING OF MOUSE LINES, J. C. Wallenburg, A. Belmaaza and P. Chartrand, Research and Development, Canadian Red Cross Society, Montreal, Canada, H1W 1B2. Gene targeting of single copy endogenous genes in mammalian cells has been shown by several groups to occur at frequencies of about 10^{-3} successfully transfected cells. Although some studies have suggested that target copy number does not play a determining role in the frequency of targeting, no studies have directly addressed this question by targeting high copy numbers of dispersed targets, such as LINES sequences. We have shown that endogenous L1 sequences in mouse cells undergo a very high frequency of gene conversion with exogenously introduced extrachromosomal sequences and have developed a targeting strategy based upon that observation. A plasmid containing a neo gene inserted into a truncated LINES sequence is used to transform mouse L cells to G418 resistance. $G418^r$ clones are pooled and gene targeting events identified by PCR amplification of a diagnostic recombinant fragment. This system (which does not select for or against illegitimate integration or gene targeting) should give a definitive response to the question: is one cell in 10^{-3} competent for gene targeting or is target size a limiting factor? It will also permit us to: 1. "label: endogenous LINES and follow their transcriptional activity and evolution - are they actively transposing, and by what mechanism?; 2. evaluate the role of gene conversion in the maintenance of LINES sequence homogeneity; 3. determine if all copies of endogenous LINES are available targets; 4. to examine factors involved in gene targeting and determine methods with which to improve it efficiency.

L 545 GENE CONVERSION WITHOUT CROSSING-OVER RESULTS FROM SUCCESSIVE "HALF CROSSING-OVERS". Kenji Yamamoto[1], Noriko Takahashi[1,2], Hiroshi Yoshikura[1] and Ichizo Kobayashi[1,2,3]. 1: Department of Bacteriology, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113. 2: National Children's Medical Research Center, Tokyo. 3: Department of Molecular Biology, Institute of Medical Science, University of Tokyo, 4-6-1 Shiroganedai, Tokyo 108 Japan.

Gene conversion is often accompanied by crossing-over of flanking sequences in eukaryotic meiosis. There are, however, some types of gene conversion not accompanied by crossing-over. This mechanism allows information transfer between repeated sequences without altering genome organization.

We found that apparent gene conversion in RecF pathway of E.coli is rarely accompanied by crossing-over. We obtained evidence that this results from two rounds of half crossing-over as illustrated.



L 546 THE CLONING, REGULATION AND CHARACTERIZATION OF THE *recE*$^+$ GENE OF *BACILLUS SUBTILIS*. Ronald E. Yasbin, Rob Marrero, Jeffrey Jackson and Martin Stranathan, Department of Biological Sciences, UMBC, Baltimore, MD 21228.

The major recombination protein of *Bacillus subtilis* (analogous to the RecA protein of *Escherichia coli*) is encoded for by the *recE*$^+$ gene. This gene has been cloned into a phage vector and produces a protein of 42kD Mr. Attempts to subclone this gene onto a multi-copy plasmid in both *E. coli* and *B. subtilis* have resulted in the conclusion that multiple copies of the DNA sequence is lethal in both organisms. Complementation tests indicate that the cloned *recE*$^+$ gene restores recombination to all of the *recE* and *recA* alleles of *B. subtilis* except the *recE45* mutation. Thus the *recE45* mutation represents a different structural gene or a dominant mutation. The *E. coli recA*$^+$ complements the recombination functions of *recE* mutants of *B. subtilis*. However, strains of *B. subtilis* carrying the *recE4* allele are still deficient in certain DNA repair functions even in the presence of a functional copy of the *E. coli recA*$^+$ gene. The *recE*$^+$ gene of *B. subtilis* differs from the *recA*$^+$ gene of *E. coli* in that the former is under two distinct forms of regulation. The *recE*$^+$ gene is autoregulated as well as being under the control of the developmental processes associated with sporulation and competence. The evolutionary significance of the conservation and divergence of Rec genes and their proteins will be discussed.