MECHANISTIC STUDIES OF DNA REPLICATION AND GENETIC RECOMBINATION

Bruce Alberts, Organizer

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Plenary Sessions

Workshops & Poster Sessions

Eucaryotic Model Systems for Replication

802 PROPERTIES OF THE SV40 GENE A PRODUCT, R. Tjian, R. Myers and R. Clark, Department of Biochemistry, University of California, Berkeley, CA 94720

The A gene of simian virus 40 encodes a large molecular weight phosphoprotein which is expressed early after infection of host mammalian cells and is required for the initiation of viral DNA synthesis. This regulatory protein is commonly referred to as large T-antigen because of its apparent involvement in cellular transformation of non-permissive host cells infected with SV40.

Our previous work indicated that T-antigen binds sequentially to 3 tandemly arrayed recognition sites located in a stretch of 120 base pairs of the SV40 genome which contains the origin of viral DNA replication. Dimethyl sulfate methylation experiments showed that T-antigen contacts DNA predominantly in the major groove and that each binding site consists of approximately 20 nucleotide pairs separated by 20-25 intervening base pairs. The recognition sequence contains a penta nucleotide (GCCTC) arranged both in tandem repeats and in mirror symmetrical fashion.

Here we report that T-antigen not only interacts specifically with the origin of SV40 DNA replication but also catalyzes the hydrolysis of ATP. The T-antigen isolated from a variety of sources including monkey cells infected with wild type SV40, human cells transformed by SV40 and cells infected with an Adenovirus SV40 hybrid virus are all efficient ATPases. The ATP hydrolysis activity co-purifies with T-antigen through five chromatographic steps and homogenuously pure T-antigen has a specific activity comparable to that of other eukaryotic ATPases such as muscle myosin. In addition, the T-antigen ATPase is specifically inhibited by both monospecific and monoclonal antibodies directed against large T-antigen. These findings indicate that the ATP hydrolysis activity is an intrinsic property of the SV40 A gene product.

Our preliminary characterization of this enzyme reveals that the rate of ATP hydrolysis is greatly dependent on both the T-antigen concentration as well as the presence of oligo dT. Moreover, the apparent Km also varies with the concentration of enzyme and oligo dT. These findings, together with some recent studies on the subunit structure of T-antigen suggests that there may be some cooperativity involved in the hydrolysis of ATP catalyzed by this viral coded DNA binding protein.

803 METABOLISM OF OKAZAKI FRAGMENTS DURING REPLICATION OF SIMIAN VIRUS 40 CHROMOSOMES, M.L. DePamphilis, S. Anderson, M. Cusick, R. Hay, T. Herman, H. Krokan, E. Shelton, L. Tack, D. Tapper, D. Weaver, and P.M. Wassarman, Department of Biological Chemistry, Harvard Medical School, Boston, MA. 02115.

The metabolism of Okazaki fragments during SV40 chromosome replication has the following characteristics: (i) At least 90% of Okazaki fragments are synthesized on retrograde sides of replication forks by DNA polymerase α. (ii) Specific initiation sites are located an average of once every 10-20 nucleotides with some sites preferred over others (sequencing of these sites is now in progress). (iii) Completed Okazaki fragments are heterogeneous in length; 90% vary from 40-290 nucleotides. (iv) From 30%-50% of Okazaki fragments have an oligoribonucleotide, 8-10 residues, covalently attached to their 5' ends. This RNA primer appears throughout the genome to be randomly synthesized with respect to DNA sequence, but in Py (and presumably SV40) always beginning with a purine rNTP. (v) RNA primers are excised in a two step process at the same rate that Okazaki fragments are joined to longer nascent DNA; only the second step is stimulated by concomitant DNA synthesis. (vi) Completion of Okazaki fragment synthesis (gap-filling) is a unique step that can be uncoupled from RNA primer excision and requires DNA polymerase α plus protein cofactors. (vii) Okazaki fragments are first synthesized and then assembled into nucleosomes. (viii) Nucleosomes are rapidly assembled on both arms of replication forks within 100-150 nucleotides of the sites of DNA synthesis. Overall, nucleosome "maturation" is relatively slow, but occurs faster on the forward sides of replication forks. (ix) Nucleosome assembly is not in phase with DNA replication, which begins at a unique origin sequence. These and other data have been used to construct a model in which Okazaki fragments have a certain probability of being initiated at many sites with in an approximately 290 nucleotide region ("initiation zone") on the exposed retrograde template bounded on one side by the replication fork and on the other by the 5' end of a growing daughter strand. The size of the initiation zone would be determined by the average center-to-center spacing of randomly phased nucleosomes, which is 230 ± 50 bp in SV40 chromosomes. Thus, the regularity of Okazaki fragment initiation could result from chromatin structure rather than DNA sequence.

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M.L. DePamphilis et al., Cold Spring Harbor Symp. Quant. Biol. 43:679 (1979).

804 ADENOVIRUS DNA REPLICATION, Thomas J. Kelly, Jr., Mark D. Challberg and Stephen V. Desiderio, Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

We have characterized a soluble enzyme system from adenovirus-infected HeLa cells that carries out the semiconservative replication of exogenously-added adenovirus DNA in vitro. DNA replication in this in vitro system closely resembles adenovirus DNA replication in vivo. Initiation of replication takes place at or near either terminus of the template. Following each initiation event, a daughter strand is synthesized in the 5' to 3' direction with concomitant displacement of the parental strand of the same polarity. The in vitro rate of chain elongation (approximately 1200 nucleotides/min) is similar to that observed in vivo. The product of the in vitro replication reaction consists principally of long adenovirus DNA strands that are hydrogen-bonded, but not covalently linked, to the input DNA template. A large fraction of the progeny DNA strands approximate full length.

The in vitro replication system exhibits a striking template specificity. DNA replication with the characteristics outlined above has only been observed when a DNA-protein complex isolated from purified adenovirus virions is employed as template. Preparations of DNA-protein complex consist principally of duplex adenovirus DNA molecules whose 5'-termini are covalently linked to a protein with an apparent molecular weight of 55K. Deproteinized adenovirus DNA (or heterologous DNA) is much less effective than adenovirus DNA-protein complex in stimulating DNA synthesis by infected-cell extracts. Furthermore, the limited DNA synthesis that occurs in the presence of deproteinized adenovirus DNA appears to be due to a repair-like reaction. In particular, synthesis begins at many sites within the template, and the synthetic product consists largely of short DNA chains that are covalently linked to template DNA strands. These results suggest that one function of the 55K protein attached to the 5'-ends of the parental DNA strands is to facilitate initiation of daughter strand synthesis at the molecular termini, perhaps via specific interactions with replication proteins in the infected-cell extract.

The termini of the product DNA synthesized in the in vitro system are also covalently attached to a protein. The apparent molecular weight of this protein on SDS-polyacrylamide gels is approximately 80K. Phosphate transfer experiments suggest that the terminal nucleotide of the nascent daughter strand is linked to the 80K polypeptide via a phosphodiester linkage to the β-OH group of a serine residue. (A similar linkage joins the 55K terminal protein to mature adenovirus DNA strands.) The protein-nucleotide linkage is formed early in the course of the in vitro reaction and, to the limit of detection, is present in every nascent DNA chain. The role of the 80K protein in adenovirus DNA replication is not yet clear, although one reasonable possibility is that it serves as the primer for daughter strand synthesis.

805 **THE UNIQUE MECHANISM OF ANIMAL MITOCHONDRIAL DNA REPLICATION,** David A. Clayton, Department of Pathology, Stanford University, Stanford, CA 94305

Mitochondrial DNA (mtDNA) of mouse cells employs a very distinctive replication mechanism, which was proposed several years ago on the basis of electron microscopic analysis of replicating molecules. Subsequent pulse-chase labeling experiments in our laboratory have corroborated and refined this model. These studies have revealed a marked asynchrony in the synthesis of the two complementary strands of mtDNA, which are separable into a heavy (H) and a light (L) strand by equilibrium centrifugation in alkaline CsCl gradients. The map positions and sequence properties of the origin regions of different mtDNAs provide a basis for comparison of the replication mechanism employed in each individual case. In mouse cells, up to 70% of the mtDNA molecules contain a partially replicated region at the H-strand replication origin, termed a displacement loop (D-loop). This triple-stranded structure is created by synthesis of ~500-600 nucleotides of the H-strand, which remains hydrogen bonded to the L-strand template and causes displacement of the parental H-strand. The newly synthesized H-strand segment is termed 7S mtDNA based on its sedimentation coefficient and in vivo labeling data indicate that the 7S mtDNA strand is unstable, with a mean lifetime of 90 minutes. Synthesis and turnover occur without nicking of the parental DNA strands and hence without changing the topological winding number of the mtDNA molecule. Thus, synthesis and turnover of 7S mtDNA entail interconversion of mtDNA between the supercoiled form and the D-loop form, which has a low apparent superhelix density.

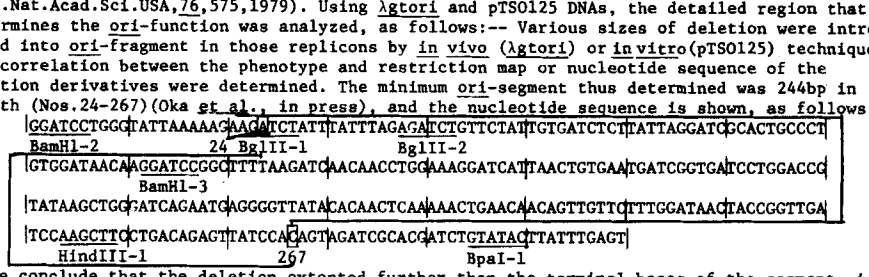
Synthesis of the full length H-strand precedes that of the L-strand and presumably begins by extending a 7S mtDNA molecule. Although most 7S mtDNA strands cannot serve as primers because they are turned over at a relatively rapid rate, electron microscopic observations of replicating forms of mtDNA suggest that a 7S mtDNA segment is a primer for H-strand replication. L-strand synthesis begins only after the H-strand is approximately 60% replicated. The detailed mechanics of H- and L-strand synthesis are expected to differ significantly. H-strand synthesis requires unwinding of the DNA helix and repeated cycles of nicking and closing of the parental strands. In contrast, L-strand synthesis occurs on a displaced single-stranded template. Pulse-chase labeling experiments have shown that a complete round of mtDNA replication requires 60 minutes which corresponds to fewer than 5 nucleotides polymerized per second per strand if synthesis were occurring at a constant rate. It is conceivable that topological constraints may limit the overall rate of mtDNA replication.

Following a round of replication, both mtDNA daughter molecules are isolated as closed circular molecules lacking superhelical turns, referred to as E-mtDNA. After approximately 40 minutes, E-mtDNA molecules are converted to the supercoiled form containing approximately 100 superhelical turns, termed C-mtDNA. A low frequency of molecules of intermediate superhelix density has been demonstrated. The behavior of these molecules in pulse-labeling expeirments is consistent with their role as intermediates in the supercoiling of newly replicated mtDNA molecules.

Chromosome Origins

806 DISSECTION OF ESCHERICHIA COLI ORIGIN OF DNA REPLICATION : STRUCTURE AND FUNCTION OF ORI DNA, Y. Hirota*, M. Yamada*, A. Oka+, K. Sugimoto+ and M. Takanami+, * National Institute of Genetics, Mishima, Shizuoka-ken, Japan 411. + Institute for Chemical Research, Kyoto University, Uji, Kyoto-fu, Japan 611.

The replication origin of Escherichia coli has been cloned on a non-replicating DNA fragment coding for ampicillin resistance. This recombinant DNA, named pSY211, replicates autonomously depending on a 6 Mdal EcoRl chromosomal fragment which contains the origin of DNA replication (Yasuda and Hirota, Proc.Nat.Acad.Sci.,74,5458,1977). The 6 Mdal EcoRl fragment was recloned on the bacteriophage λgtλC. λgtori existed in the recA⁻ host cells in an extrachromosomal state using the E. coli replication origin (Hirota et al., Cold Spring Harb. Symp.,43,1979). From a sub-fragment of the EcoRl fragment, an E. coli ori-pBR322 named pTSO 125 was constructed. It replicated in polA⁻ cells and the minimal size of DNA fragment determining the replication function was shortened within 422bp (Nos.1-422)(Sugimoto et al., Proc.Nat.Acad.Sci.USA,76,575,1979). Using λgtori and pTSO125 DNAs, the detailed region that determines the ori-function was analyzed, as follows:-- Various sizes of deletion were introduced into ori-fragment in those replicons by in vivo (λgtori) or in vitro(pTSO125) techniques, and correlation between the phenotype and restriction map or nucleotide sequence of the deletion derivatives were determined. The minimum ori-segment thus determined was 244bp in length (Nos.24-267)(Oka et al., in press), and the nucleotide sequence is shown, as follows:--

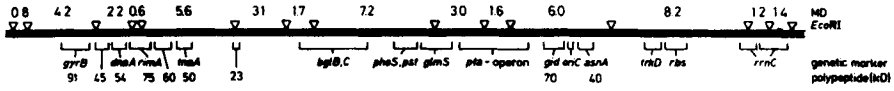


We conclude that the deletion extented further than the terminal bases of the segment, i.e. No.24A or No.267C, resulted defective DNA replication origin. Furthermore, deletion or insertion mutation was introduced into the ori-segment at the restriction sites either by repair-ligation or nuclease S1-ligation, the mutant DNAs cloned, base sequence of the DNAs determined, and the ori-function of the mutants examined. The function of the mutants deleted (Δ) or inserted (Ω) several bases at the restriction sites are as follows: mutants of Ω4bp or Δ16bp at BglII-2 site were ori⁺ and ori⁻, respectively. Mutants of Ω4bp and Δ6bp at BamHl-3 site were ori⁻. Mutants of Δ4bp, Δ5bp, Δ7bp, Δ8bp at the HindIII-1 site were ori⁻, however Δ12 at the HindIII-1 site was ori⁺.

Based on our results we shall present a model for the DNA-replication origin of E. coli.

807 THE ORIGIN OF REPLICATION OF THE ESCHERICHIA COLI CHROMOSOME, Kaspar von Meyenburg and Flemming G.Hansen, Department of Microbiology, Technical University of Denmark, 2800 Lyngby-Copenhagen, Denmark.

The origin of replication, oriC, of the E.coli chromosome has been located at 82 min counterclockwise of the asnA gene on the genetic map through genetic and physical analysis of deletion derivatives of an F'ilv (1) and of specialized transducing phages λasn (2) and through in vitro and in vivo construction of minichromosomes (3,4,5,6,7). The genetic organisation of the chromosome in the vicinity of oriC has been determined through genetic,physical and functional analysis of the chromosomal DNA carried on various specialized transducing phages λasn, λglmS, λbglB and λtna (2,5,8):



The map location of oriC is in accordance with physiological data (9,10). In order to approach the question whether the E.coli chromosome carries additional, alternative replication origins, we have constructed ΔoriC derivatives of the oriC carrying λasn phages (2) making use of the deletogenic properties of the tetracycline resistance transposon Tn10 (11), inserted into the asnA gene. oriC deletions as large as 5 MD extending from within the asnA gene into the pta operon (=unc, proton translocating ATPase)(see Fig.) flanked by chromosomal DNA and genetically marked by the tetᴿ gene of Tn10, could be transduced into the E.coli chromosome if this contained an integrated F or R-plasmid (or a therefrom derived replicon). Minichromosomes like pCM959 (5) or pOC1-pOC34 (6) can replicate in these ΔoriC strains and appear to be more stably inherited and to be present at an even higher copy number than in the wild-type oriC⁺ strains (5). We conclude that oriC (2) is the only functional replication origin on the E.coli K-12 F⁻ chromosome and that there are no gene products essential for the initiation of replication at oriC encoded between pta and asnA. Also the F_0 part of the membrane bound proton translocating ATPase and the 70kD protein mapping just counterclockwise of oriC (see Fig.) are dispensible. The ΔoriC mutant strains appear to be ideally suited for the functional analysis of oriC mutations in vivo and of the interrelationship between minichromosome and chromosome replication and partitioning.

(1)von Meyenburg,K., et.al.(1977),Mol.Gen.Genet.158:101; (2)von Meyenburg,K., et.al.(1978), Mol.Gen.Genet.160:287; (3)Yasuda,S. and Y.Hirota (1977),Proc.Natl.Acad.Sci.74:5458; (4)Messer,W., et.al.(1978) Mol.Gen.Genet.162:269; (5)von Meyenburg,K., et.al.(1978) Cold Spring Harb.Symp.Quant.Biol.43:121; (6) Messer,W., et.al.(1978) ibid.43:139; (7)Meijer,M.,et.al. (1979),Proc.Natl.Acad.Sci.76:580; (8)Hansen, F.G. and K.von Meyenburg(1979) Mol.Gen.Genet.175:135; (9)Fayet,O. & J.Louarn(1978)Mol.Gen.Genet.162:109; (10) Marsh,R.C. & A.Worcel(1977)Proc.Natl.Acad.Sci. 74:2720; (11)Kleckner,N. et.al.(1977) J.Mol.Biol. 116:125.

808 ORIGIN AND INCEPTOR OF DNA REPLICATION IN BACTERIOPHAGE LAMBDA, Gerd Hobom and Monika Lusky, Institut für Biologie III, Universität Freiburg, Schänzlestr.1, 7800 Freiburg i.Br., W.-Germany

Hybrid plasmid dissection and reconstruction procedures have shown that two different signal structures for initiation of DNA replication can be separated within the replicator region of various lambdoid phages. From our hybrid plasmid experiments we conclude that the origin of replication (ori) serves as the initiation site for leftward primer RNA synthesis while the replicational inceptor (ice) signal controls termination of that primer RNA and inception of daughter strand DNA synthesis. Hybrid plasmids containing only ori are unable to initiate lambdoid replication, whereas plasmids containing ice in connection with a leftward promoter do show replicational activity (minimal replication system, 1).

Initiation in the ori-ice maximal replication system (2) is for its full activity dependent upon rightward transcriptional activation of ori, which has been interpreted to result in local DNA strand separation thereby exposing a single-stranded initiation site for primase catalysed RNA synthesis. Origin-specific strand separation appears to be facilitated by highly symmetrical DNA sequence organizations and stabilized by interaction with phage-specific O-proteins.

Initiation in the p_O-ice mini system will be in cis repressed in ori-p_O-ice (maxi + mini) hybrid plasmids. This replicational repression effect is dependent only on the left section of ori(3), on transcriptional ori activation, and on the N-terminal domain of the O-protein, as revealed by employing C-terminal variants or amber mutants of O which also retain full repressive control over the cryptic minimal replication system.

Further studies with lambdoid ice$^-$-mutants obtained by site-directed mutagenesis in vitro support our interpretation of the ice signal structure to consist of a single stranded hairpin loop and an attached hexa-G tail sequence. This structure resembles transcriptional terminator sequences, and a more generalized hypothesis on how RNA polymerase recognizes these various signal structures will be presented.

(1) Lusky,M. and Hobom,G. Gene 6, 137-172 (1979)
(2) Lusky,M. and Hobom,G. Gene 6, 173-197 (1979)
(3) Grosschedl,R. and Hobom,G. Nature 277, 621-627 (1979)

809 SEQUENCE ORGANIZATION OF LAMBDOID ORIGINS OF REPLICATION(λ/φ80/82/iteron/A·T rich zone/ice/ori); Frederick R. Blattner, Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706; Katherine Denniston-Thompson, National Institute of Health, Bethesda, Maryland 20205; David D. Moore, Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143. We have determined the wild type DNA sequence of the origin of replication region of bacteriophage lambda and of several mutants. These include the substitution hybrids λrep82:λ and λrep80:λ, a pseudorevertant of the r93 λori− mutant, λr93hot5, and the insertion mutant λpk35. The 428 base pair substitution of 82 DNA in λrep82:λ lies entirely within the amino terminal part of the O gene and codes for both an O gene and origin of replication with 82 specificity. λrep80:λ is a more extensive substitution which confers the replication specificity of φ80. The origin sequences of all three phages feature iterons and A·T rich zones. λ and φ80 have four iterons, and 82 has five. We note a similar arrangement of DNA is also found in all sequenced bidirectional replication origins. The sequence of the origin of λr93hot5 is unusual in that it contains only three iterons, yet the phage grows normally. Analysis of this mutant shows that the spacing of iterons is crucial to origin function, whereas their number is not. This argues against the cloverleaf model for origin structure.

In λpk35 the drug resistance element Tn903 is inserted into the "inceptor" (ice) site, proposed by Hobom et.al. to be critical for λ replication initiation.

810 REPLICATION ORIGIN, STRUCTURE, AND THE REGULATION OF PLASMID REPLICATION, Donald R. Helinski, Roberto Kolter, Manabu Inuzuka, Noriko Inuzuka, Christopher Thomas, and David Stalker, University of California, San Diego, La Jolla, CA 92093

Two antibiotic resistance plasmids RK2 (56 Kb) and R6K (38 Kb) have been analyzed in detail for regions that are essential for replication and maintenance in the extrachromosomal state in bacteria. The broad host range plasmid RK2, differs from most other plasmids examined in that the essential replication regions are not clustered. Two regions essential for replication (trfA, 2.7 Kb and trfB, 2.0 Kb) are separated from each other by 16 Kb and specify products that act in *trans* in unidirectional replication from the RK2 origin. The RK2 replication origin, located at a site distinct from the trfA and trfB regions, has been localized on a 393 base pair HpaII restriction fragment containing five direct repeats. Plasmid R6K exhibits three origins of replication α, β and γ. Replication from the α and β origins has been shown to proceed by a sequential, bi-directional mode. The α, β and γ origins and a structural gene specifying the initiation protein π (mol. wt. of 35,000) are located within a contiguous segment of DNA that is 4 Kb in length. The nucleotide sequence has been determined for a functional replicon consisting of the γ origin and the π gene. The γ origin is contained within a 380 bp region of this replicon. The π protein has been shown both *in vitro* and *in vivo* to be required for replication from the γ origin. One striking feature of the γ origin region is the presence of seven 22 bp tandem direct repeats. In addition, both the R6K γ origin and the RK2 origin contain specific regions unusually rich in A-T and/or G-C. The possible role of the direct repeats and other unusual features of the origin regions of these plasmids in the regulation of initiation of plasmid DNA replication will be discussed.

Multienzyme in Vitro Systems

811 BACTERIOPHAGE T7 DNA REPLICATION IN VITRO, Fuyuhiko Tamanoi, Michael J. Engler, Robert L. Lechner, Terry L. Orr-Weaver, Louis J. Romano, Haruo Saito, Stanley Tabor, and Charles C. Richardson, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

The DNA of bacteriophage T7 is a linear duplex molecule containing an origin of replication located approximately 17% from the genetic left end. In an attempt to further define the origin of T7 DNA replication we have used mutants of T7 carrying deletions around this region. One such mutant, LG37, although able to replicate its DNA normally, carries a deletion between 14.55 and 19.35% on the physical map. In LG37-infected cells initiation near 17% is abolished, but secondary initiation sites, the predominant one at 4%, are observed by electron microscopy. Similar experiments with four other deletion mutants show that the primary origin is located between 14.55 and 16.0% on the physical map.

We have studied the initiation of T7 DNA replication *in vitro* using a plasmid into which we have cloned a DNA segment (HpaI G fragment) derived from the region between 12 and 18.2% on the physical map of T7. When this hybrid plasmid DNA is used as template in an *in vitro* T7 DNA replication system prepared by gentle lysis of $T7_{3,6}$ - infected *E. coli*, 5 to 10% of the DNA molecules contain replication bubbles (theta structures). Replication initiates within the HpaI G fragment, and the newly synthesized DNA is not covalently attached to the template.

The reactions occurring at the replication fork have been reconstituted using homogeneous T7 DNA replication proteins: DNA polymerase, gene 4 protein, and DNA-binding protein. T7 DNA polymerase and gene 4 protein alone can carry out extensive leading-strand synthesis. Unwinding of the duplex DNA is facilitated by the hydrolysis of NTPs, a reaction catalyzed by the gene 4 protein. In the presence of rNTPs and DNA-binding protein, the gene 4 protein catalyzes the synthesis of RNA primers having the sequence pppApCpCpC/A. The recognition sequence for RNA primer synthesis catalyzed by the gene 4 protein has been determined by using DNA templates of known sequence. On ϕX174 DNA 13 unique sites are used, and sequence determination of the 5'-terminal DNA regions of the RNA primed fragments show that all are initiated at the common sequence, 3'-CTGGG/T-5'. Relative efficiency of the utilization of the sites suggests that the gene 4 protein travels along single-stranded DNA in a 5' → 3' direction. Extension of the RNA primers by DNA polymerase leads to the accumulation of fragments with chain lengths of 5000-6000 nucleotides. Removal of the RNA primers and joining of the fragments can be accomplished by T7 gene 6 exonuclease / *E. coli* DNA polymerase I and T7 DNA ligase / *E. coli* DNA ligase, respectively.

812 STUDIES OF REPLICATION MECHANISMS WITH THE T4 BACTERIOPHAGE IN VITRO SYSTEM, Bruce Alberts, Jack Barry, Pat Bedinger, Rae Lyn Burke, Urszula Hibner, Chung Cheng Liu, and Richard Sheridan, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

The T4 bacteriophage in vitro replication system, as presently constituted, consists of 7 highly purified T4-induced proteins. The protein components can be grouped into functional classes as follows: DNA polymerase (gene 43 protein), helix-destabilizing protein (gene 32 protein), polymerase accessory proteins (gene 44/62 & 45 proteins), and RNA priming proteins (gene 41 & 61 proteins). Mutants in each of these genes have been shown by others to have a severe effect on the in vivo replication process. DNA synthesis in this 7 protein in vitro system starts by covalent addition onto the 3'OH end at a nick on a double-stranded DNA template, and proceeds to generate a replication fork which moves at about the in vivo rate, and with approximately the in vivo base-pairing fidelity. DNA is synthesized at the fork in a continuous fashion on the leading strand, and in a discontinuous fashion on the lagging strand (generating Okazaki fragments with 5'-linked pppApCpXpYpZ penta-ribonucleotide primers).

Both the 44/62 protein complex and the 41 protein catalyze a single-stranded DNA-dependent hydrolysis of nucleoside triphosphates to nucleoside diphosphates and inorganic phosphate; these hydrolyses can be differentially blocked by ATPγS and GTPγS respectively, revealing that each hydrolysis has an essential role in a functioning replication fork. The DNA synthesis on the leading and lagging side of the fork are linked via the dual role of the gene 41 protein: this protein appears to use nucleotide hydrolysis energy to run along the lagging-strand template at the fork, simultaneously helping to drive open the parental DNA helix (to permit rapid leading-strand synthesis), while acting as a mobile site at which new RNA primers are formed (to prime Okazaki piece synthesis on the lagging strand). In a second reaction requiring nucleotide hydrolysis, the gene 44/62 and 45 proteins act in a synergistic manner to tie down the DNA polymerase, causing it to move through these double-helical templates in an enormously processive fashion.

813 ENZYME STUDIES OF φX174 DNA REPLICATION, Joseph Shlomai, Ken-ichi Arai, Joan Kobori, Naoko Arai, Laurien Polder, Robert Low, LeRoy Bertsch and Arthur Kornberg, Department of Biochemistry, Stanford University, Stanford, CA 94305.

Enzymology of phage φX174 replication, which employs E. coli replication proteins, is a guide to their action in replicating the host chromosome. Recent findings include: (1) identification and purification of the 6 proteins (n, n', n", i, dnaB, dnaC) needed to form the prepriming intermediate in the conversion of the viral single-stranded circle (SS), coated with single strand binding protein, to the duplex replicative form (RF), the SS→RF system; (2) recognition by protein n', a φX DNA-dependent ATPase, of a unique sequence within a hairpin region in φX SS, a likely origin for complementary strand synthesis; (3) analysis of the order and manner in which the prepriming proteins perform; (4) multiple binding sites and functions of dnaB protein as a mobile replication "promoter;" (5) priming by primase coupled to chain elongation by DNA polymerase III holoenzyme (see Hübscher and Kornberg, these Abstracts) to form an incomplete (gapped) RFII; (6) conversion of the latter to supercoiled RFI by DNA polymerase I, ligase and gyrase; (7) complex of RF with φX-encoded gene A protein, rep protein and holoenzyme in the looped, rolling circle intermediate which generates viral circles (SS), the RF→SS system; and (8) coupling of the RF→SS and the SS→RF systems in a rapid and efficient multiplication of RF.

DNA Topoisomerases

814 DNA TOPOISOMERASES, James C. Wang, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

DNA topoisomerases are enzymes that catalyze the concerted breaking and rejoining of DNA backbone bonds. The action of such enzymes is usually manifested by the interconversion between topological isomers (topoisomers) of DNA: examples are the relaxation of a supercoiled DNA, the supercoiling of a relaxed DNA, the linking of single-stranded DNA rings of complementary nucleotide sequences, and the interconversion between DNA rings with and without knots. It has been postulated that catalysis of DNA topoisomerization involves the formation of an intermediate in which at least one DNA phosphodiester bond is broken and that this bond breakage is accompanied by the simultaneous formation of a covalent protein-DNA linkage. Experimentally, a number of topoisomerase-DNA complexes have been observed under certain conditions. For E. coli or M. luteus DNA topoisomerase I, the covalent linkage has been identified as a phosphodiester bond between a DNA 5' phosphoryl group and a protein tyrosyl hydroxyl group. For M. luteus DNA gyrase, the same covalent linkage is again formed between DNA and the subunit A of the enzyme. E. coli DNA gyrase presumably form the same type of bond with DNA. For enzymes of the E. coli DNA topoisomerase I type, catalysis of DNA topoisomerization appears to require the formation of a complex in which a short segment of the DNA helix is unwound. For E. coli and M. luteus DNA gyrase, there appears to be no unwinding of the DNA double helix; topoisomerization of the DNA appears to be related to the wrapping of the bihelical DNA around a tetrameric protein core containing two protomers of each of the two subunits. "Gyrasomes" resulting from staphylococcal nuclease digestion of gyrase-DNA complex have been purified and characterized by physicochemical measurements and by probing with chemical and enzymatic treatments. Similar measurements have also been applied to gyrase bound to unique sequences of DNA.

815 THE MECHANISM OF THE REACTION CATALYZED BY THE EUCARYOTIC DNA NICKING-CLOSING ENZYME, James J. Champoux, Department of Microbiology and Immunology, University of Washington, Seattle, WA 98195

We have previously shown that if a nicking-closing reaction catalyzed by the rat liver enzyme is terminated by the addition of NaOH (to pH > 12.5), broken DNA strands can be shown to contain the enzyme covalently attached at their 3'-phosphoryl termini (1). Because this structure may represent the nicked intermediate in the unperturbed nicking-closing reaction,we have been interested in determining the nature of the linkage between the protein and the DNA. The linkage has been shown to be resistant to either 1 N NaOH or 1 N HCl for 5 hr at 37°C. The bond is also resistant to neutral or acidic hydroxylamine. These properties rule out serine and threonine phosphoesters as well as most phosphamide type bonds. The results do not rule out a tyrosine phosphoester linkage.

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816 MECHANISTIC STUDIES ON DNA GYRASE, L. Mark Fisher, Kiyoshi Mizuuchi, Mary O'Dea and Martin Gellert, National Institutes of Health, Bethesda, Maryland 20205

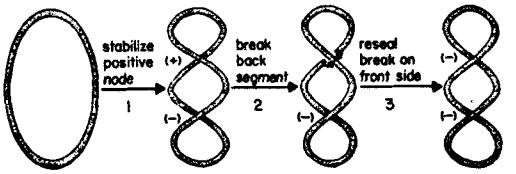
A fundamental question raised by Liu, Liu, and Alberts about DNA topoisomerases is whether they function by breaking one or both DNA strands in each cycle of action. We have shown that in the supercoiling reaction catalyzed by DNA gyrase, the linking number of closed circular duplex DNA changes in multiples of two. This implies that DNA gyrase acts by introducing breaks into both strands of DNA and passing a double-helical segment through the break in each supercoiling step.

DNA gyrase is known to cleave DNA at specific sites in the presence of oxolinic acid. We have studied in some detail the interaction of gyrase with a specific DNA fragment containing a strong cleavage site. In footprinting experiments, DNA gyrase protects a region about 60 base pairs long on each side of a cleavage site against DNase digestion. The enzyme can be shown to bind in a directional manner to the DNA. The same region of DNA is protected, although more weakly, in the absence of oxolinic acid, thus indicating that cleavage sites are also preferred sites of normal binding of the enzyme.

We have recently purified from E. coli a protein that complements the gyrase A protein to generate a DNA relaxing activity. The protein appears to be a fragment of the gyrase B subunit. The B fragment-gyrase A protein complex exhibits some but not all of the multiple activities of gyrase itself. Thus, the B fragment complex will induce double-strand DNA breaks in the presence of oxolinic acid at the same sites as are found with DNA gyrase. However, the complex does not catalyze DNA supercoiling or DNA-dependent hydrolysis of ATP.

817 DNA GYRASE CATALYZES THE SUPERCOILING, RELAXATION, AND CATENATION OF DNA BY A SIGN INVERSION MECHANISM, Nicholas R. Cozzarelli, Patrick O. Brown, Kenneth N. Kreuzer, Alan Morrison, Richard J. Otter, Stephen P. Gerrard, Akio Sugino, Craig L. Peebles, and N. Patrick Higgins, Department of Biochemistry, University of Chicago, Chicago IL 60637

DNA gyrase from E. coli is an ATP-requiring, 400,000 dalton enzyme composed of two reversibly associated subunits termed A and B. DNA breakage-and-reunion and energy transduction, the two basic components of supercoiling, are particularly associated with subunits A and B, respectively, but a complex of both subunits is needed for all gyrase activities. Gyrase not only supertwists and relaxes DNA but also catenates, knots, and decatenates duplex DNA rings. Catenation is an efficient reaction requiring ATP, Mg^{2+}, and spermidine but not DNA sequence homology, and two to thousands of rings are interlocked depending on the tertiary structure of the substrate. In the presence of ATP, these huge networks can be resolved by gyrase almost entirely into supertwisted monomers. the generation and dissolution of complex topological forms shows that gyrase makes a transient double-strand break, a property shared with several newly discovered prokaryotic and eukaryotic topoisomerases. All the reactions of gyrase are explained by the process called sign inversion which is illustrated for supercoiling. 1) Binding of gyrase to specific DNA sequences juxtaposes two DNA segments so that the intervening DNA forms a positive supercoiled loop. 2) & 3) upon addition of ATP, one segment of DNA is passed through a transient double-strand break in the other, thereby inverting the sign of the loop. The net result is a decrease of two in the linking number. The broken DNA ends in the intermediate are held by gyrase so that no loss of supercoiling occurs during sign inversion. Indeed, subunit A protomers are covalently attached to the protruding, transient, 5'-phosphate ends. Gyrase remains bound stationarily to the DNA, supertwisting the DNA processively by successive cycles of sign inversion until its binding is sufficiently weakened by increased negative supertwist density that it is released. An E. coli topoisomerase has been discovered that is structurally and mechanistically related to DNA gyrase but has distinct properties.



818 TYPE II DNA TOPOISOMERASES: ENZYMES THAT CATALYZE THE DNA STRAND PASSING REACTION VIA REVERSIBLE DOUBLE STRAND BREAK, Leroy F. Liu, Chung C. Liu and Bruce M. Alberts Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

The T4 DNA topoisomerase is a recently discovered multisubunit protein which appears to have an essential role in the initiation of T4 bacteriophage DNA replication(1,2). Treatment of double-stranded circular DNA with large amount of this topoisomerase in the absence of ATP yields new DNA species which are knotted topological isomers of the double-stranded DNA circles. These knotted DNA circles, whether covalently-closed or nicked, are converted to unknotted circles by treatment with trace amounts of the topoisomerase in the presence of ATP. Bacterial DNA gyrases and an ATP-dependent activity in extracts of early embryos of Drosophila melanogaster are also ahown to be capable of unknotting DNA. We propose that these unknotting enzymes constitute a new general class of DNA topoisomerases (type II DNA topoisomerases). These enzymes must act via mechanisms that involve the concerted cleavage and rejoining of two opposite DNA strands, such that the DNA double-helix is transiently broken. As predicted by this type of mechanism, type II DNA topoisomerases change the DNA linking number only by multiples of two. Other related DNA topoisomerization reactions catalyzed by the type II DNA topoisomerases such as relaxation, supercoiling and segregation of interlocked DNA rings are also believed to follow the same mechanism. The possible roles of such enzymes in a variety of biological functions are discussed, along with their likely molecular mechanisms.

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Genetic Recombination: Purified Enzymes and DNA Sites

819 ORIENTATION-DEPENDENCE OF CHI'S ABILITY TO STIMULATE GENETIC EXCHANGE IN PHAGE λ, Franklin W. Stahl, Nancy Dower, Ezra Yagil, and Mary Stahl Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Chi is a genetic element that stimulates exchange near itself via the E. coli RecBC pathway (1). The elements can arise by single base change (2) at four or more widely separated sites in phage λ (3); they can also be substituted into λ on fragments of wild-type E. coli (4) and S. cerevisiae (5) DNA. Inversion of E. coli fragments leads to reversal of Chi phenotype (6); fragments originally classified as able to stimulate exchange (Chi^+) become unable to do so (Chi^-) when inverted and vice versa. Chi has been introduced by mutation into the previously Chi-free transposon Tn5. At any given site, the mutant transposon manifests Chi^+ phenotype in one orientation and is Chi^- when flipped. The active orientation is the same throughout λ (from J to R on the vegetative map) independent of the direction of transcription in the neighborhood or the location of the transposon relative to λ's origin of replication. This behavior of Chi is likely to be related to the preferential leftward activity of Chi wherever located throughout that same region of λ (7). Observations will be presented that bear on two very different models for the action of Chi, its orientation dependence, and its directionality.

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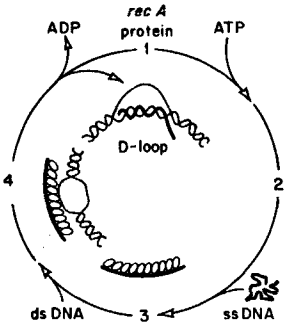
820 PROMOTION OF HOMOLOGOUS PAIRING BY PURIFIED E. COLI REC A PROTEIN, C. M. Radding, T. Shibata, R. P. Cunningham, C. DasGupta, Department of Human Genetics, Yale Univ. School of Medicine, New Haven, CT 06510.

The recA protein, which is essential for genetic recombination in E. coli, catalyzes homologous pairing of DNA molecules (1,2). Observations on the formation of D-loops (See Figure) from single-stranded fragments and circular duplex fd DNA show that single strands play a key role in initiating the pairing reaction (3,4). The amount of single-stranded DNA determines the stoichiometric requirement for recA protein, which reflects the need to unfold single strands (Stage 3, Figure). More significantly, single-stranded DNA, whether homologous or not, as well as oligodeoxynucleotides, stimulate recA protein to bind and partially unwind duplex DNA (Stage 4, Figure) in the presence of ATPγS, a non-hydrolyzable analog of ATP (3,4). These observations led to the hypothesis that recA protein first brings single-stranded and double-stranded DNA into proximity and then partially unwinds the duplex DNA in order to search for homology (See Figure). Unlike annealing reactions, which are second order, the kinetics of synthesis of D-loops by recA protein resemble classical Michaelis-Menten kinetics, which confirms that a ternary complex consisting of recA protein and two DNA molecules (Stage 4, Figure) is a precursor whose conversion to a D-loop is rate-limiting. Experiments with various substrates reveal that recA protein will make stable joint molecules when one molecule is single-stranded or partially single-stranded and either of the two molecules is not a closed circle. This specificity is explained by the apparent lack of topoisomerase activity of recA protein, and more importantly by the ability of recA protein to unwind duplex DNA when stimulated by single strands.



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821 FUNCTION OF recA PROTEIN IN GENERAL RECOMBINATION IN E. COLI, K. McEntee, G. M. Weinstock and I. R. Lehman, Department of Biochemistry, Stanford University Medical School, Stanford, CA 94305

RecA protein function is required in vivo for homology-dependent recombination. In vitro, purified recA protein catalyzes two reactions that are likely to be important for its role in recombination: (i) the renaturation of complementary single-stranded DNA chains and (ii) the assimilation of single strands into homologous duplex molecules. In both reactions, hydrolysis of a nucleoside triphosphate is required. Although recA protein acts catalytically in the renaturation reaction, it appears to act stoichiometrically in strand assimilation.

We have investigated the mechanism of these reactions, in particular, the role of nucleoside triphosphate hydrolysis. In the presence of the nucleoside triphosphate analogs ATP[γ-S] or UTP[γ-S] recA protein binds and unwinds duplex DNA at low pH (6.2-6.7). At higher pH values (7.5-8.5), the rate of binding is slow but is significantly stimulated by single-stranded (ss) polynucleotides. RecA protein binds rapidly and preferentially to ss DNA in the presence of duplex DNA and this binding is enhanced by either ATP[γ-S] or UTP[γ-S]. These results suggest that the first step in strand assimilation is the binding of recA protein to ss DNA, followed by the binding of the recA protein-ss DNA complex to duplex DNA. In the renaturation reaction, an analogous complex has been detected in which recA protein is bound to several ss DNA molecules. The preference of recA protein for ss DNA explains the requirement for high levels of the protein in strand assimilation; the ss DNA sequesters recA protein thereby preventing it from binding duplex DNA. By complexing ss DNA with single-stranded DNA binding protein (SSB) we have observed a significant stimulation of strand assimilation. SSB not only stimulates the rate and extent of strand assimilation but also reduces (i) the rate of ATP hydrolysis during assimilation and (ii) the requirement for high concentrations of recA protein. SSB purified from an SSB mutant strain (lexC) is considerably less effective than the wild type protein in stimulating strand assimilation by recA protein. Thus, SSB is likely to participate with recA protein in general recombination in vivo. These results suggest a mechanism for strand assimilation and provide insight into the role of recA protein in strand recognition.

822 THE PROTEINS OF LAMBDA INTEGRATIVE RECOMBINATION: STRUCTURE AND FUNCTION, Howard A. Nash, NIMH, Bethesda, MD 20205

In E coli cells infected with bacteriophage lambda, a recombination between specific sites on the phage and host genomes results in the integration of viral DNA into the host chromosome. This recombination can be achieved in cell free extracts and has been under study as a biochemical reaction for several years. The following facts about the substrates and products of this reaction have been established: a) The natural substrate involves supercoiled DNA - efficient recombination requires that at least the phage specific site, attP, must be located as part of a supertwisted circle of DNA. b) DNA gyrase can be used to produce suitably supertwisted substrate DNA but appears to play no further role in integrative recombination. c) The products of integrative recombination in vitro are those expected from a break and rejoin mechanism with two special features. First, between the breakage and rejoining few if any superhelical turns are lost from the recombining DNA. Second, the rejoining of the broken attachment sites occurs in the absence of ATP and other cofactors normally required to form phosphodiester bonds. These two features imply a mechanism for recombination in which the two att sites are first drawn together and then broken, realigned, and rejoined in a concerted reaction that involves transient bond formation between DNA phosphate and a recombination protein.

We are in the process of studying how recombination proteins carry out the steps of synapsis and strand exchange. The only viral protein required for integrative recombination , the product of the int gene , has been purified to near homogeneity (1) . The purified protein displays two activities that seem relevant to its role in recombination - specific binding to the phage att site (1,2) and a topoisomerase function (3). Although the topoisomerase activity of Int displays no striking sequence specificity, we have proposed a model for how such an activity could be used to exchange strands of DNA during integrative recombination (3) . The purification of the bacterial component of integrative recombination has been simplified by the existence of mutants of E coli defective in this reaction. Complementation in vitro has been shown between extracts made from cells carrying mutants in two distantly located cistrons, himA and hip (4). Although this suggests the existence of two separate bacterial proteins required for recombination we find that himA and hip complementing activities of sonic extracts from wild type cells copurify (1000 fold with a ten percent yield). SDS gel electrophoresis of the most purified material displays two separate but closely spaced bands of equal intensity, each having a mobility consistent with Mr∿ 7,500 - 10,000. The hydrodynamic behavior of this material indicates that the two activities are present in a 1:1 complex.

823 GENETIC RECOMBINATION IN VITRO PROMOTED BY BACTERIOPHAGE T7-INFECTED CELL EXTRACTS, Paul D. Sadowski, William Bradley, Donald Lee, Dan Vetter and Linda Beatty, Department of Medical Genetics, University of Toronto, Toronto, Canada, M5S 1A8

We have previously reported the development of two assays which measure the recombination between exogenously added, mature T7 DNA and the DNA present in a crude cell-free extract of T7-infected cells (1,2). In the first case recombinants were detected after packaging the DNA in vitro whereas in the second they were detected by observing a shift in buoyant density of the exogenously added heavy DNA.

Since the above assays had limited usefulness for genetic and biochemical studies, Roeder and Sadowski (3) developed a two-stage assay in which two exogenous DNA's could undergo recombination but not packaging in the first stage. In the second stage the recombinant DNA molecules were packaged in vitro to yield viable phage progeny. We have used this assay to define three genetically distinct pathways of in vitro recombination: a) the exonuclease pathway; b) the endonuclease pathway, and c) the wild-type pathway.

This assay was designed to eliminate recombination events in the second stage (packaging) of the reaction; however, two lines of evidence have caused us to reconsider the validity of this assumption. The first is that purified T7 exonuclease or T7 DNA polymerase/exonuclease alone promote recombination in this 2-stage system. Secondly, the two exogenous DNA's, when denatured, can be packaged in the second stage of the reaction to yield recombinant phage. Thus it seems that production of single stranded DNA is an important step in the formation of genetic recombinants in vitro. While single stranded DNA is recombinogenic in the second stage of the assay, buoyant density shift experiments of the reaction products of the first stage suggest that recombinant intermediates are formed during the first stage.

We have subsequently found that the second stage infected-cell extract contains an activity which promotes the renaturation of complementary single strands. This activity copurifies with the T7-induced DNA binding protein. This protein also stimulates the recombination promoted by the gene 6 exonuclease. This effect can be explained because the binding protein specifically stimulates the activity of the exonuclease.

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Selected Details of the Replication Process

824 MAPPING OF INITIATION SITES OF DNA REPLICATION ON PROKARYOTIC GENOMES, Tuneko Okazaki, Susumu Hirose, Tohru Ogawa, Asao Fujiyama, Yuji Kohara, Institute of Molecular Biology, Faculty of Science, Nagoya University, Nagoya, Japan 464

Initiation Sites of Bacteriophage T7 Discontinuous DNA Replication

Bacteriophage T7 DNA is replicated discontinuously using the tetra- or pentaribonucleotide primer (1). Structure of tetranucleotide primer is pppApCp$\begin{matrix}C\\A\end{matrix}$pN, where N is rich in A and C (1). Short chains with covalently linked primer RNA at 5' ends accumulate in T7 phage infected E. coli cells when activities of T7 gene 6 exonuclease and E. coli DNA polymerase I are suppressed (1). The accumulated short chains were treated with alkali and uncovered 5' hydroxyl ends of the DNA were labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase at 0°C (2). H-short chains which have ^{32}P-labeled termini on a restricted genome segment (Hpa II-H3 fragment: chain length 339 nucleotides) were purified and the exact location of the ^{32}P-labeled 5' terminal nucleotides (the transition sites of RNA to DNA synthesis) were mapped on the fragment. Five sites have been found and all of which shared a common sequence, 5' GAC$\begin{matrix}C\\A\end{matrix}$NN'N" 3', in which transition from RNA to DNA synthesis occurs at N' or N". These results along with in vitro studies (3, 4) indicate that T7 phage primer RNA is synthesized in situ. The sequence on the complementary strand, 3' CTG$\begin{matrix}G\\A\end{matrix}$ 5', may be a signal for the primer synthesis. The probability of the appearance of the observed common base sequence is roughly once every 130 nucleotides assuming a random base sequence. Only one out of several such potential initiation sites may be used every 1000 to 2000 nucleotide fork migration.

Initiation Site at the Replication Origin of E. coli

DNA fragments synthesized with RNA primer in the E. coli oriC region may represent the initiation fragments of genome replication. The nascent fragments which have a homology to oriC region (5) were accumulated in E. coli dnaC2 cells during the synchronized initiation of DNA replication by temperature shift down performed in the presence of arabinosyl-cytosine. The 5' hydroxyl ends of DNA uncovered by an alkaline treatment of these fragments were labeled with ^{32}P (2). 5'-^{32}P labeled short chains having a homology to oriC region were purified by hybridization with the 538 bp oriC containing DNA segment inserted in pBR322 plasmid DNA and the ^{32}P labeled 5' terminal nucleotides were mapped on the oriC region. Two sites were detected on the one of the two complementary strands but none on the other strand. Exact position of the transition sites will be presented.

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825 PRIMING OF OKAZAKI FRAGMENTS DURING POLYOMA DNA SYNTHESIS, Peter Reichard, Rolf Eliasson and Giorgio Mastromei, Department of Biochemistry, Medical Nobel Institute, Karolinska Institute, S-104 01 Stockholm, Sweden.

Short DNA strands (average chain length 100-200 nucleotides = Okazaki fragments) are formed transiently during polyoma DNA synthesis. The strand elongation process can be studied in vitro in isolated virus infected nuclei or in nucleoprotein complexes, extracted and purified from such nuclei. In both systems a limited initiation of new Okazaki fragments takes place and part of them contain at their 5'-ends covalently attached decaribonucleotides (= initiator RNA), starting with a triphosphate group from ATP or GTP, probably involved in the priming of Okazaki fragments. Initiator RNA consists of a large family of molecules without defined nucleotide sequence but of identical length.

Initiator RNA is present at the 5'-ends of only 1/4 to 1/3 of in vitro labeled Okazaki fragments. In vivo inhibition of polyoma DNA synthesis by hydroxyurea results in accumulation of short strands. It has been suggested that labeled Okazaki fragments both in vitro and in vivo could arise from the transient incorporation of dUTP followed by a repair process involving uracil excision and strand scission. With the aid of a new enzymatic method we have analyzed dUTP pools in polyoma infected cells, including the effects of hydroxyurea. The size of the dUTP pool in no case exceeded 0.4% that of the dTTP pool suggesting that incorporation of uracil did not contribute significantly to the formation of Okazaki fragments.

826 MOLECULAR ASPECTS OF THE INTERACTIONS OF T4-CODED GENE 32-PROTEIN AND DNA POLYMERASE (GENE 43-PROTEIN) WITH NUCLEIC ACIDS, John W. Newport, Stephen C. Kowalcyzkowski, Nils Lonberg and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The structure and overall function of the T4 DNA replication complex has been outlined by Alberts et al. (1). In order to further refine this picture we have been studying the molecular details of the interactions of some of the proteins of this replication system with relevant nucleic acid lattices, and with one another. We have shown that gene 32-protein binds to short (ℓ = 2→8 residues) oligonucleotides essentially independent of base composition or sugar type; this binding is also relatively independent of salt concentration. In contrast, the cooperative binding of gene 32-protein to polynucleotides shows an appreciable dependence on base composition and sugar-type, and a large dependence on salt concentration. This salt concentration dependence resides in the binding constant to the nucleic acid lattice (K), and not in the cooperativity parameter (ω); it has been shown that this salt concentration dependence involves a significant anion, as well as a cation, displacement reaction on binding. These results are interpreted in terms of an explicit two-conformation model of the interaction of this protein with nucleic acid lattices. In addition, the results provide a quantitative molecular interpretation of the autogeneous regulation by this protein of its own synthesis (2), and lead to general principles for the development of binding specificity via cooperative (cluster) protein binding.

T4 DNA replication in vivo involves at least seven proteins (1). We have characterized the binding properties of the T4 DNA polymerase to various DNA substrates, and will present a model which describes how T4 DNA polymerase might bind to the primer-template substrate during replication based on these results. At in vivo salt concentrations ($\simeq$ 170 mM NaCl), we have shown that T4 DNA polymerase, by itself, synthesizes DNA "dispersively"; synthesis in the "processive" mode (3) requires T4 DNA polymerase, gene 32-protein, the proteins encoded by T4 genes 44, 62, 45, and ATP. ATP hydrolysis is required only for the initial assembly of these proteins into a multiprotein complex. This complex has a lifetime of less than 90 seconds. A model in which the slow relaxation of this complex could serve as a timing mechanism to control the length of DNA synthesized during lagging strand synthesis will be presented. (Supported in part by USPHS Research Grant GM-15792 and Training Grant GM-07759.)

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827 ON THE MOLECULAR BASIS OF MUTAGENESIS: ENZYMOLOGICAL AND GENETIC STUDIES WITH THE BACTERIOPHAGE T4 SYSTEM, Myron F. Goodman, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90007.

Spontaneous and base analogue induced mutagenesis is dependent upon deoxyribonucleoside triphosphate pool sizes, properties of the replication complex, in particular DNA polymerase, hydrogen bond stabilities of nascent base pairs, and DNA sequence-specific effects. The bacteriophage T4 provides an ideal system to study the individual properties of these components, their coupled interactions and effects on mutagenesis. We have utilized the base analogue 2-aminopurine (AP) as an indicator of aberrant DNA synthesis. The pathway for AP-induced mutagenesis is A · T ⇶ AP · T ⇶ AP · C ⇶ G · C. AP · T base mispairing frequencies have been measured in vitro and in vivo. We will show that these measurements when taken in conjunction with genetic determinations of AP-induced A · T ⇶ G · C transition frequencies allow us to predict dAPTP pool sizes and AP · C heteroduplex heterozygote (het) mispair frequencies in vivo in DNA polymerase (gene 43) mutator, wild type, and antimutator backgrounds. The versatile T4 system is used to measure AP · C hets and dAPTP pool size; these measurements are in excellent agreement with simple theoretical predictions based on a study of the properties of purified mutator, wild type, and antimutator T4 DNA polymerases. The measured frequencies for "slightly" mispaired AP · T and "badly" mispaired AP · C are consistent with a model in which hydrogen bonding free energy differences between competing correct and incorrect nucleotides plays an active role in determining the fidelity of DNA replication; the T4 polymerase is regarded as a more passive component which either inserts or excises a base non-selectively, depending primarily on hydrogen bonding stability of the previously inserted nucleotide and the concentrations and relative base pairing stabilities of succeeding "competing" nucleotides.

828 METHYL-DIRECTED MISMATCH REPAIR--A MECHANISM FOR CORRECTING DNA REPLICATION ERRORS, Matthew Meselson, Patricia Pukkila, Mary Rykowski, Janet Peterson, Miroslav Radman and Robert Wagner, The Biological Laboratories, Harvard University, Cambridge, MA 02138 and Gail Herman and Paul Modrich, Duke University Medical Center, Durham, NC 27710

A role for the considerable amount of DNA methylation not implicated in restriction-modification may lie in the possibility that errors of DNA replication are corrected by mismatch repair selective for the unmethylated DNA chains transiently present in newly replicated DNA duplexes (1). We have tested this possibility by investigating the effect of DNA chain methylation on the repair of mismatches in bacteriophage lambda heteroduplexes transfecting E. coli. We find that the overall frequency of repair at a given mismatch is essentially the same whether only one chain or neither are methylated. The presence of a methylated chain, however, strongly affects the specificity of repair, favoring repair of the unmethylated chain. If both chains are methylated, little if any repair occurs. The methylation responsible for these effects results from DNA adenine methylase, which transfers methyl groups to the adenine residues in the symmetric sequence GATC, giving 6-methylaminopurine (2,3). Our findings demonstrate the occurance of methyl-directed mismatch repair and, together with the observed hypermutability of bacteria deficient in adenine methylation (4-6), support the hypothesis that such repair serves to increase the fidelity of DNA replication.

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DNA Replication and Chromosome Structure

829 STUDIES ON THE ORIGIN OF ØX RF DNA REPLICATION, P.D.Baas[1,2], F.Heidekamp[1,2], H.S.Jansz[1,2], S.A.Langeveld[1,3], G.A.van der Marel[4], G.H.Veeneman[4] and J.H.Boom[4], [1]Institute of Molecular Biology, [2]Laboratory for Physiological Chemistry, [3]Department of Molecular Cell Biology, State University of Utrecht, [4]Department of Organic Chemistry, State University of Leiden, The Netherlands

Bacteriophage ØX174 RF DNA replication starts after introduction of a nick in the viral strand of supertwisted RFI DNA by the phage coded gene A protein. Previous work has shown, that ØX gene A protein also nicks the viral strand of RFI DNA of the related bacteriophage G4. Comparison of the ØX and G4 DNA sequence around the gene A cleavage site has revealed a conserved region of 30 nucleotides. This region is thought to be the maximum origin signal for ØX DNA replication. In order to study which nucleotides within the conserved region are essential for origin function two different approaches are followed.

1. ØX gene A protein also nicks RFI DNA of the phages St1, α3, G14 and U3. The DNA sequence around the nick site of St1 has been determined and shows two remarkable differences within the conserved region.
2. We have synthesized by pure chemical means a set of deoxyribonucleotides complementary to the origin region of ØX, except for one nucleotide change. After annealing to wild type ØX DNA these oligodeoxyribonucleotides were used as primers for Klenow DNA polymerase in the presence of DNA ligase. From the resulting heteroduplex RFI DNA ØX complementary strands with the preselected mutation were isolated and tested for biological activity in the spheroplast system. Progress on both approaches will be reported.

830 CLONING OF EUCARYOTIC DNA REPLICATION ORIGINS, R.F. Baker and M.T. Harrigan, Molecular Biology Division, University of Southern California, Los Angeles, CA 90007.

We have selected for and have cloned sequences of monkey DNA containing replication origins. TC7 African green monkey kidney cell DNA pieces were selected in a eucaryotic replication system and inserted into the tetracycline resistance gene region on the bacterial plasmid PBR322. Ten different cloned monkey DNA sequences have been obtained. After DEAE dextran-induced transfection into TC7 tissue culture cells, all ten of these hybrid DNAs have been shown to replicate in vivo. After multiple passage of the transfected cells in tissue culture, the hybrid DNAs retain their ability to confer ampicillin resistance on E. coli.

We have compared the different cloned replication sequences and also the sequences flanking the origin sequences. We are also using the PBR322-eucaryotic replication hybrids as cloning vectors for the insertion of additional DNA sequences into tissue culture cells. In these studies we are selecting for sequences which allow integration of the cloning vectors into the host genome of the transfected tissue culture cells. We are using a calibrated E. coli transfection system to measure the number of copies (per haploid genome) of the ampicillin gene portion of PBR322 that have been integrated into the eucaryotic DNA.

831 ISOLATION OF REPLICATION ORIGINS FROM YEAST CHROMOSOMES, David Beach, Melanie Piper and Sydney Shall, University of Sussex, Brighton, England.

We have developed a method allowing isolation of any piece of yeast chromosomal DNA containing an origin of replication. The yeast Leu 2 gene was cloned in the bacterial plasmid pBR325. The resulting 6 kb plasmid, pDAM1, can transform an appropriate strain of E.Coli (JA221) to Leu^+ and chloramphenicol resistance. This molecule can also transform an appropriate leucine requiring strain of Saccharomyces cerevisiae (M16) to Leu^+ (10^4 transformants per µg DNA) but transformants grow extremely poorly on a leucine free medium due to low copy number of the plasmid. However, after insertion of certain restriction fragments of yeast chromosomal DNA into pDAM1 the growth of transformants is improved by approximately two orders of magnitude, and copies of the transforming plasmid are detectable in the yeast by the Southern hybridization technique. These restriction fragments are presumed to contain origins of replication, thereby allowing more efficient replication of the plasmid and hence better growth of transformants. Origins of replication, identified in this way, occur with a frequency of one per 40 kb of chromosomal DNA. There are therefore about 500 origins of replication in a haploid cell. Several origins identified by our assay are now being sequenced.

832 Tertiary structure is involved in the intiiation of DNA synthesis by the dnaG protein. Edmund W. Benz, John Sims*, Jerard Hurwitz and David Dressler*. Albert Einstein College of Medicine, Bronx, NY 10461 and the *Biological Laboratories, Harvard University, Cambridge, MA 02138. The dnaG protein of E. coli initiates DNA replication by synthesizing primer oligonucleotides for elongation by DNA polymerase. The experiments described here probe the nature of the nucleic acid element recognized by the dnaG protein. Three well-separated groups of nucleotides within the negative strand origin of the single-stranded phage ØK are protected by the dnaG protein against nuclease digestion. DNA as far as 115 bases from the start site of primer synthesis is involved in binding of the dnaG protein to the replication origin. One molecule of dnaG protein could protect all of these nucleotides if the DNA were folded into a higher-order, tertiary structure.

Protection of the ØK origin by dnaG protein requires DNA binding protein, and does not occur if the group of protected nucleotides most distant from the start site is removed from the template. There is no binding of dnaG protein to the complementary strand of the ØK origin-region DNA. The observed protection of the positive strand is due to a functional nucleic acid-protein complex.

833 CHROMOSOMAL REPLICATION ORIGINS IN YEAST, Clarence S.M. Chan and Bik-Kwoon Tye, Cornell University, Ithaca, NY 14853

Eukaryote chromosomes, unlike prokaryote chromosomes, contain multiple replicons. There are eighteen chromosomes in yeast, and each chromosome is estimated to contain 25-35 replicons. Experimental evidence from D. melanogaster and Triturus suggests that the rate of DNA replication in rapidly dividing embryonic cells versus slow growing somatic cells is regulated not by the rate of DNA chain growth but rather by the efficiency of the utilization of initiation sites. The nature of the mechanism which controls when and which origins are to be activated is unknown. A first step towards an understanding of this control mechanism in eukaryotes is to study the structure, organization and complexity of these initiation sites. We have individually cloned approximately 200 yeast chromosomal replication origins using as vector, the chimeric pBR322 plasmid carrying the yeast leu2 gene. These plasmid clones are characterized by their ability to transform leu^- spheroplasts to LEU^+ at a high frequency and their ability to replicate autonomously. Analysis of some of these self-replicating plasmids divide them into two categories; those that are represented as unique sequences in the yeast genome and those that are represented repeatedly. Further analyses of these replication origin clones will yield information on the structure, organization and complexity of the replication origins in eukaryotic chromosomes.

834 TRANSFORMATION OF *STREPTOCOCCUS PNEUMONIAE* WITH *S. PNEUMONIAE* - LAMBDA PHAGE HYBRID DNA : INDUCTION OF DELETIONS, J.P. Claverys, J.C. Lefevre and A.M. Sicard, CRBGC - CNRS, 118, route de Narbonne, 31077 Toulouse Cedex - France -

The genetic fate of a fragment of *Streptococcus pneumoniae* DNA cloned into a derivative of the *Escherichia coli* bacteriophage λ has been studied in pneumococcal transformation. Transforming activity of this hybrid DNA is eight times higher than standard *S. pneumoniae* DNA. Hybrid DNA is mutagenic for the recipient bacteria. Mutations are induced at a rate of two per thousand transformation events. These mutations are deletions adjacent to the cloned pneumococcal fragment, starting at or near its extremities and extending outside. The length of these deletions estimated by genetic analysis or by gel electrophoresis of restriction endonuclease generated DNA fragments is quite variable from 150 base pairs to more than 3800 base pairs. Insertion of λ DNA has been detected in two large deletions using DNA-DNA hybridization as a probe. This suggests that non homologous regions adjacent to the cloned fragment may be illegitimatly integrated by the transformation process. During the genetic analysis of these induced mutations we have observed that not only these deletions but also spontaneous deletions drastically increase recombination rates when present on donor DNA in transformation of neighboring markers. Such an effect is interpreted as partial exclusion of deletions from synapsis between donor and recipient DNA.

835 REPLICATION OF A PLASMID UNDER CONTROL OF THE CLONED REPLICATION ORIGIN OF PHAGE M13
Joseph M. Cleary and Dan S. Ray, Department of Biology and Molecular Biology Institute University of California, Los Angeles, California 90024

The viral and complementary strand replication origins of bacteriophage M13 are located near the center of a 507 base pair intergenic region of the viral genome. In order to characterize those DNA sequences in the intergenic region essential for replication of the duplex replicative form (RF) of M13, we have cloned specific restriction fragments from this region into the plasmid pBR322. Replication of these co-integrated origin plasmids, under conditions non-permissive for the plasmid replicon, depends on specific segments of the M13 origin region and the presence of M13 helper virus. More than 45% of the region, including three segments of potential hairpin structures, is non-essential for replication of one of these chimeric DNAs in a polA host. A central 142 base pair fragment, containing the nucleotide sequence for the RNA primer for the complementary strand and the nicking site for the M13 gene II protein, is not sufficient for replication under the non-permissive conditions. Efficient replication of the chimeric plasmids requires additional sequence(s) located within an AT rich 128 base pair segment of DNA on the gene II side of the intergenic region.

836 HUMAN INTERSPERSED REPEATED DNA SEQUENCES SHOW HOMOLOGY TO VIRAL ORIGINS OF REPLICATION AND HUMAN HnRNA, Prescott L. Deininger, Doug J. Jolly, Theodore Friedmann, Dept. of Pediatrics, University of California, San Diego, La Jolla, Calif. 92093 and Carol M. Rubin, Catherine M. Houck and Carl W. Schmid, Dept. of Chemistry, University of California, Davis, Calif. 95616.

Three percent or more of the human genome is made up of one major family of repeated DNA sequences, with an approximate length of 300 nucleotides, which are interspersed throughout the genome. We have cloned members of this repeated DNA family and have determined the nucleotide sequence of several of these clones. This repeated DNA family contains nucleotide stretches which are homologous to a number of the major fingerprint spots demonstrated for the repeated sequences found in human heterogeneous nuclear RNA molecules (1). Furthermore, these DNA sequences show long A, T runs adjacent to G, C rich regions, features often found in the vicinity of origins of DNA replication. There is also a region of strong base sequence homology to approximately 14 nucleotide-long region of the inverted-repeated DNA sequences found in the vicinity of the papovavirus origins of DNA replication.

1) H.D. Robertson, E. Dickson & W. Jelinek (1977) J. Mol. Biol. 115,571-589.

837 PSORALEN PHOTOADDITION AS A PROBE FOR THE IN VIVO NUCLEOPROTEIN STRUCTURE OF SV40,
Lesley M. Hallick and Gordon W. Robinson, Dept. of Microbiology, Univ. of Oregon Health Sciences Center, Portland, OR 97201

Psoralen derivatives enter live cells and in the presence of long wavelength UV light form covalent photoadducts to DNA. Previous work has shown that photoaddition occurs preferentially in the "interbead" region between nucleosomes on eucaryotic cellular DNA (Wiesehahn et al. (1977), Bioch. 16, 925) and intracellular SV40 "minichromosomes" (Hallick et al. (1978), J. Virol. 27, 127). We have developed a technique for the quantitative analysis of the extent of photoadditions to specific restriction enzyme fragments. Radioactive psoralen derivatives (kindly provided by J. Hearst) are utilized and quantitation of the specific activities of individual restriction fragments is achieved by coupling a modification of the parabolic approximation of ethidium bromide stained polyacrylamide gels suggested by Pulleyblank et al. (NAR (1977), 4, 1409) with fluoragraphy or with digestion and counting of gel slices. In both the DNA and the fluorographic radioisotope determinations, step wedges have been constructed to provide an internal calibration for each analysis. This system should have wide applicability to replication studies. We are currently employing this approach to analyze the in vivo structure of the replication origin of SV40 DNA.

838 SEQUENCE VARIATION AT THE ORIGIN OF BOVINE MITOCHONDRIAL DNA REPLICATION, Philip J. Laipis and William W. Hauswirth, University of Florida, Gainesville, FL 32610

Mitochondrial DNA constitutes the only example of a functional, non-viral eukaryotic genetic element which can be easily isolated in its entirety from animal tissue. Because of this, the mitochondrial genome has been recognized as a model system for understanding gene organization, control, and evolution in eukaryotic organisms. We have begun a detailed characterization of the bovine mitochondrial genome. Of particular interest has been the origin of mitochondrial DNA replication (D-loop), which we have located and oriented on the physical map. In order to deduce which nucleotide sequences within this region are of functional importance, we have begun to compare D-loop sequences between cattle breeds, between apparently unrelated animals of the same breed, between maternally related animals, and between cloned sequences derived from an individual animal. Previous results from other mammalian species suggest that the D-loop contains unconserved sequences, even between individuals of the same species (Upholt and Dawid, Cell 11:571 [1977]). Certain segments of the D-loop appeared highly conserved by heteroduplex mapping. We will present sequencing and restriction enzyme analyses that confirm and extend our knowledge of conserved and unconserved domains within the bovine D-loop.

839 EXPRESSION OF THE E.COLI REPLICATION ORIGIN IN A SINGLE STRANDED DNA PHAGE, Laurie Simon La Verne, Jon Kaguni, and Dan S. Ray, UCLA, Los Angeles, CA 90024

Studies of the replication systems of the single-stranded DNA phages of E. coli have revealed three distinct mechanisms of RNA primed initiation. The mechanism of the M13 type filamentous phages involves priming by the rifampicin-sensitive RNA polymerase, while the phage øX and G4 initiate complementary strand synthesis by rifampicin-resistant mechanisms involving the dnaG primase. Because the RNA primed synthesis of the complementary strand in each case involves only host encoded proteins, the reactions potentially mimic systems operating in E. coli replication.

In order to examine the possibility of functionally separating a cloned bidirectional origin into the separate unidirectional initiation components, we have cloned the duplex origin of E. coli into an M13 single-stranded DNA phage vector, in both of the two possible orientations. The first stage of M13 replication, the conversion of the single-stranded viral DNA to the duplex form, is strikingly inhibited by rifampicin, and consequently provides a convenient means for assessing the functionality of cloned rifampicin-resistant origins. In an in vivo experiment in which the M13 complementary strand origin is inactivated by rifampicin, we have found that both of the separated strands of the cloned E. coli origin are active in the conversion of the chimeric single-stranded DNA to duplex form. The site specificity of the reactions, and the requirements for E. coli gene products are currently under investigation.

840 REPLICATION AND RECOMBINATION OF T7 DNA, Richard Smith and R.C. Miller, Jr., University of British Columbia, Vancouver, B.C. V6T 1W5

Marker rescue of a mutant bacteriophage by a recombinant plasmid carrying a wild-type allele is dependent on phage and plasmid replication. Recombinant plasmids of pBR322 and T7 DNA were constructed, and plasmids carrying alleles of T7 gene 5 were isolated. Recombination between T7-am5-28 or T7 ts5 and the recombinant plasmid was monitored under conditions where either the phage or the plasmid or neither could replicate. Plasmid replication was controlled by use of a dna B mutation. The results show a decrease in normal rescue frequencies if either the phage or the plasmid cannot replicate, and they show no rescue above the background level, if neither can replicate. Hybridization experiments by the procedure of Southern show that the plasmid DNA is not degraded after infection, and copy number determinations show that a large amount of plasmid is produced in a dna B mutant at 30°C. Copy number does not decrease for at least 45 minutes at 42°C. Therefore, the dependence of marker rescue on replication is not likely to be due simply to the production of a critical DNA concentration. This dependence probably reflects the use of regions of replication for recombination as postulated in the unisex circle and Meselson-Radding models.

841 REPLICATION OF SMALL CHROMOSOMES IN YEAST, Carol S. Newlon and Wylie Burke*
Department of Zoology, University of Iowa, Iowa City, IA 52242 and *Dept. of Medicine, University of Washington School of Medicine, Seattle, WA 98195.
Chromosomal DNA's of the yeast, Saccharomyces cerevisiae are very small, averaging only 6.2×10^8d (940 kb) in size (Petes and Fangman, PNAS 69:1188, 1972). Despite their small size, the chromosomal DNA's replicate like those of higher eukaryotes, with bidirectional replication from multiple initiation sites (Newlon, Petes, Hereford and Fangman, Nature 247: 32, 1974). In order to study the position of initiation sites and the temporal control of replication initiation, replicating molecules have been prepared under conditions expected to yield unbroken DNA's, and small chromosomal DNA's have been analyzed topologically by electron microscopy. Replication maps have been prepared for limited size classes of molecules. The results suggest that initiation sites are at specific places on molecules, but that the temporal sequence of initiation events on a particular molecule is not constant (Newlon and Burke, in prep'n.). In order to confirm these findings, this analysis is being extended to a physically unique molecule, a mutant, circular derivative of chromosome III which has recently been characterized (Strathern, Newlon, Herskowitz, and Hicks, Cell 18:309, 1979).

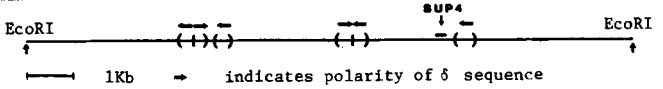
842 EARLY REPLICATION OF SECOND GENERATION CHROMOSOMES IN MOUSE MYELOMA CELLS, Tatsuo Matsushita and Geraldine Matsushita, Division of Biological and Medical Research, Argonne National Laboratory, Argonne, IL 60439
Mouse myeloma cells grown in suspension culture can be utilized to measure sister chromatid exchanges when chromosomes are differentially stained after growth in BrdUrd during two replication (S) periods. In addition, differentially stained chromosomes can be utilized for the determination of DNA replication rate in the second S period of BrdUrd exposure. While correlating this DNA replication rate with simultaneous growth studies, we observed the complete (100%) replication of second generation chromosomes by 1.5 generations of cell growth. Further studies revealed an inverse relationship between cell doubling time and the early appearance of second generation chromosomes. A cell cycle model will be proposed to explain these results. (Work supported by the U. S. Department of Energy under contract No. W-31-109-ENG-38.)

843 SV40 AND AMPLIFIED XENOPUS RIBOSOMAL DNAs DO NOT REPLICATE AUTONOMOUSLY IN YEAST. Virginia A. Zakian and Ronald H. Reeder, Hutchinson Cancer Res. Cntr,Seattle, WA 98104.
Yeast cells can be transformed at characteristic low frequencies with recombinant plasmids containing a selectable, structural yeast gene. A small and specific fraction of yeast DNA is capable of transforming cells at elevated frequencies. Recombinant vectors containing such fragments are found as extrachromosomal plasmids in transformed cells. It has been hypothesized that those fragments capable of transformation without integration into chromosomal DNA contain sequences normally used for the initiation of chromosomal DNA replication. Moreover, Stinchcomb and Davis have reported that a small percentage of DNA fragments from other eukaryotes (eg. Dictyostelium, corn, Drosophila) but not E. coli is also capable of automomous replication in yeast. These results suggest that yeast can use specific sequences from a foreign DNA for the initiation of DNA synthesis and raise the possibility that such sites might be conserved throughout the eukaryotic world.

We have transformed yeast with two DNAs, SV40 and amplified Xenopus laevis ribosomal DNA (rDNA), both of which contain sites used for initiation of DNA synthesis in situ. Neither DNA was capable of autonomous, extra-chromosomal replication in yeast. In contrast, the trp-1 region of yeast inserted into the same vector transformed cells at a high frequency. The failure of yeast to use either SV40 or rDNA initiation sites for DNA replication may reflect the atypical nature of both sites; ie., the initiation of SV40 replication is T-antigen mediated and that of amplified rDNA occurs via a rolling circle mechanism. It is still possible that those sites used for bi-directional replication of chromosomal DNA in other eukaryotes would function as initiation sites in yeast.

844 DELETION AND INVERSIONS AT THE SUP4 LOCUS IN YEAST. Rodney Rothstein, New Jersey Medical School, Newark, N.J. 07103

The SUP4 locus in yeast codes for a tyrosine tRNA. An ochre allele at this locus has an altered anticodon such that it recognizes the UAA termination signal. Mutations which cause loss of suppressor activity genetically map at the locus. Among these second site mutations, complete deletions of the gene occur at high frequency- 3×10^{-7}. This high frequency is unusual since deletions are rare events in yeast- 10^{-10} to 10^{-11}. DNA from the deletion strains was examined by probing genomic blots with the appropriate restriction fragment. The deletions fall into two classes: 2.1 Kb and 2.8 Kb in size. For each deletion size class, there is one case with an inversion in an adjacent DNA segment. A number of small 250 base pair elements (δ sequences) have been found on both sides of the SUP4 gene (Cameron, Loh and Davis, Cell 16 739, 1979):



The arrangement of these δ sequences and the size of the deletions and inversions is compatible with the notion that the repeated sequences are intimately involved in the observed events. These complex events are analogous to those associated with transposition elements in prokaryotes.

845 CHROMOSOMAL SITE OF NUCLEOSOME ASSEMBLY, Ronald L. Seale. Dept. Cellular Biology, Scripps Clinic and Research Foundation, 10666 North Torrey Pines Road, La Jolla, CA 92037.

The chromosomal sites of nucleosome assembly were investigated with respect to newly replicated versus non-replicating DNA. The problem was investigated from two experimental approaches. In one set of experiments, new chromatin DNA was marked with ^{14}C-BUdR to effect its resolution from non-replicated chromatin by buoyant density difference. The second set of experiments utilized intrinsic properties of newly replicated chromatin to differentiate new from old; these properties are the light buoyant density, increased nuclease susceptibility and shorter multimer size of pulse-labelled chromatin DNA relative to the respective properties of whole chromatin.

The buoyant density and electrophoretic patterns of ^{14}C-thymidine uniformly labelled chromatin and 2.5 min ^{3}H-thymidine pulse-labelled chromatin were compared to the patterns obtained by pulse-labeling chromatin for 2.5 min with ^{3}H-arginine. Both total chromatin, and salt-washed chromatin containing only the nucleosomal histones, were investigated. In all experiments, the new nucleosomal histones followed the properties of whole chromatin, and gave no indication of assembly on the newly replicated DNA. This data supports the evidence in support of nucleosome assembly on non-replicating DNA, and re-cycling of parental histones to the replication fork such that both daughter chromatids become complexed primarily with old, parental nucleosomal protein.

846 CHROMATIN SUBUNITS OF THE DEVELOPING SEA URCHIN EMBRYO. Barbara Ramsay Shaw, Randall G. Richards, and Goffredo Cognetti, Duke University, Durham, NC.

Previous studies from our laboratory have demonstrated a protamine-release method that allows for ready analysis of covalently modified and variant histones in nucleosomes. The protamine in combination with 8M urea effectively releases histones as well as other proteins from the chromatin subunits. We have employed the protamine-release method to monitor the types of nucleosomes that are produced upon digestion of chromatin in Strongylocentrotus purpuratus sea urchin embryos. We find that core particles isolated from different stages of development contain different histone variants that reflect accompanying switches in histone synthesis. The complexity of monomer and dimer nucleosome products from micrococcal nuclease digestion increases with increasing time of development. At 6 hr development there is one each major type core particle, monomer, and dimer nucleosome species. At blastula a new nucleosome class emerges. By gastrula many nucleosome species reflect heterogeneity of the packaging of the DNA by proteins.

Newly replicated DNA from nuclei at the gastrula stage that were pulse labeled with ^{3}H-thymidine and treated with micrococcal nuclease is digested considerably faster than bulk DNA. When 16% of the ^{3}H-thymidine DNA is released, less than 2% of the bulk DNA has been digested. With longer labeling times, the newly replicated DNA forms core particles and becomes more resistant to nuclease. Pulse-labeled nucleosomes isolated from a nuclease digest exhibit lower sedimentation coefficients than ^{14}C-bulk labeled nucleosomes on sucrose gradients. Using a specific salt-fractionation procedure, we have identified a 0.1 M NaCl-soluble nucleosome subfraction which is enriched in newly replicated DNA and certain proteins.

847 TENSION IN THE DNA DOUBLE HELIX MEASURED IN VIVO WITH 4,5',8-TRIMETHYLPSORALEN, R.R. Sinden and D.E. Pettijohn, University of Colorado, Denver, CO 80262

The association constant of the drug psoralen in binding to double helical DNA is greater when the DNA contains negative superhelical tension than when the DNA is relaxed. Therefore when DNA is irradiated with long-wavelength UV light in the presence of psoralen the rate of covalent photobinding of psoralen to DNA is dependent on the helical tension in the DNA; in vitro this rate is directly proportional to the negative superhelical density of the DNA. Thus, measurement of the rate of covalent binding provides a sensitive and reproducible method for determining helical tension. The rate of photobinding of ^3H-psoralen to DNA in Escherichia coli cells has been determined and compared to the rates obtained when: a) DNA inside the cells contain 300 single-strand breaks per genome equivalent (introduced by gamma irradiation); or b) cells were incubated with coumermycin to relax DNA supercoiling. The rate of photobinding is 2.0 fold greater in the untreated cells than after the treatments of (a) or (b), above. In addition, the rate of psoralen photobinding to ribosomal RNA in all three populations of cells is equal, suggesting no significant differences in the intracellular psoralen concentrations. The differences in the rates of psoralen binding to DNA are the same as those found in vivo between relaxed DNA and DNA containing a superhelical density of -0.07. This suggests that E. coli DNA in vivo is under tension which is not completely restrained by nucleosome like structures. Preliminary studies show that multiple single strand breaks (at least 200 per genome equivalent) are required to relax this tension in vivo, suggesting that the chromosome in vivo is segregated into domains of supercoiling.

848 ISOLATION AND CHARACTERIZATION OF A EUKARYOTIC ORIGIN OF REPLICATION
Dan T. Stinchcomb, Eric Selker, Morjorie Thomas, Jeffrey L. Kelly, and Ronald W. Davis, Stanford University, Stanford, CA 94305

A segment of yeast chromosomal DNA has been isolated, ars1 (autonomously replicating sequence), that behaves as an origin of replication. When reintroduced into yeast by transformation, ars1 allows autonomous replication of all colinear DNA. ars1 can function as a dimer and can integrate into other chromosomal sites without seriously impairing sequence stability. Genetic analysis defines ars1 as a cis-acting element contained within 850 base pairs. This region shows sequence similarities to known prokaryotic and viral eukaryotic origins of replication.

The behavior of ars1 upon transformation suggests a selection for other putative eukaryotic origins. Segments of DNA from yeast, N. crassa, D. melanogaster, D. discoideum, and Z. mays have been isolated that allow autonomous replication in yeast. These will prove useful in both determining the sequence(s) responsible for autonomous replication and in developing other eukaryotic DNA transformation systems.

849 CLONED FRAGMENTS OF ØX174 RF CONFER RIFAMPICIN RESISTANT INITIATION ON M13 SS TO RF REPLICATION, Michael D. Strathearn and Dan S. Ray, University of California, Los Angeles, California 90024

Conversion of the single-stranded DNA (SS) of bacteriophages G4 and ØX174 to a duplex replicative form (RF) occurs by a mechanism involving rifampicin-resistant priming of the complementary strand. In the case of G4, the *dnaG* protein interacts with a specific sequence on the viral DNA to initiate primer synthesis. The ØX174 initiation mechanism is more complicated, possibly involving a mobile replication promoter (the *dnaB* protein) and initiating at a large number of sites on the single-stranded template. To identify the ØX174 sequences responsible for determining this specific initiation mechanism, we have cloned restriction fragments of ØX174 RF into the filamentous single-stranded DNA phage M13 and screened for inserts capable of directing rifampicin-resistant initiation of the complementary strand. Since M13 is normally converted to a duplex RF by a mechanism involving priming by the rifampicin-sensitive RNA polymerase, only those chimeric phages capable of initiating by the ØX174 mechanism are converted to RF in the presence of rifampicin. Several phage carrying inserts that include the intergenic space between the ØX174 genes F and G have been found to carry out the SS to RF reaction in the presence of rifampicin. Further sub-cloning of the ØX174 genome is in progress in order to more precisely localize the ØX174 sequences responsible for the appropriation of the specific enzymes required for this initiation mechanism.

850 SV40 DNA REPLICATION: THE TERMINATION REGION, Douglas P. Tapper and Melvin L. DePamphilis, Department of Biological Chemistry, Harvard Medical School, Boston, Ma. 02115

Replicating SV40 DNA molecules on average 90±2% completed are two to three times more prevalent than molecules at any other stage of replication. Positions of replication forks within the termination region were mapped by labeling nascent DNA chains at their 3' and 5' ends. The longest end-labeled chains were annealed to SV40 linear DNA opened at the origin of replication and then treated with restriction endonucleases that cut near the termination region. Labeled DNA fragments were analyzed by gel electrophoresis which revealed DNA fragments of defined lengths rather than a continuous distribution of lengths. The most intense fragments from 3' labeled DNA defined a gap of 0.5 Kb centered around the expected termination site. Less intense fragments could be aligned to produce a series of gaps each approximately 0.5 Kb and centered 0.45 Kb to the left or right of the normal termination point. 5' end-labeled nascent DNA chains yielded a family of discrete DNA fragments whose positions could be correlated with those observed for the 3' ends. The fact that 5' ends of nascent DNA chains are found at specific loci about 40 nucleotides apart suggest that Okazaki fragments are initiated at preferred, rather than random, DNA sequences. The accumulation of replicating SV40 DNA occurs when the two converging replication forks are about 0.5 Kb apart. The variability in the location of the termination region results from the degree of asynchrony in the arrival of replication forks and the possibility of preferred termination sequences. Electron microscopic analysis of replicating molecules revealed that in 78% of the molecules at any stage in replication, one fork traveled up to 20% further than the other fork. This predicts that the termination regions should vary over 19% of the genome, as observed.

851 NUCLEOTIDE SEQUENCE OF THE *SALMONELLA TYPHIMURIUM* ORIGIN OF REPLICATION, Judith W. Zyskind and Douglas W. Smith; Department of Biology, C-016; University of California, San Diego; La Jolla, CA 92093

Construction of deletion derivative plasmids, and cloning of restriction fragments, from plasmids containing the *Salmonella typhimurium* origin of replication (*ori*) were used to locate the functional origin to within a DNA fragment of 296 basepairs between the genes *uncB* and *asn*. The nucleotide sequence of the *S. typhimurium ori* region was determined and compared with the *Escherichia coli ori* sequence. In the 296 basepair fragment, 85.8% of the bases are conserved between the two species. A nearly equal number of transition and transversion type differences, with no insertions or deletions, occurs between the two bacterial origins, such that the relatively high %(adenine plus thymine) of 59.5% is conserved. The 296 basepair fragment contains fourteen GATC sequences, all of which are conserved. The high frequency of the sequence, GATC, which is the site of methylation under control of the *dam* gene, may explain, in part, why the bacterial *ori* region appears to be so highly conserved. A large number of secondary structures are possible. One such structure, with a "cloverleaf", is favored by *ori* nucleotide sequence comparisons, and leads to potential novel macromolecular interactions.

852 DNA SYNTHESIS IN E. coli CELLS MADE PERMEABLE WITH HYPOTONIC BUFFER*, Erik Boye and Stein Alver, Dept of Medical Physics, The Norwegian Radium Hosp. & Dept of Biophysics, Norsk Hydro's Institute for Cancer Research, Montebello, Oslo 3, Norway.

Hypotonic buffers render cells of E. coli permeable to small-molecular weight compounds while maintaining cell viability. In such buffers the cells are unable to perform DNA replication and DNA repair without the addition of precursors and cofactors. Deoxyribonucleotide triphosphates (dNTP's) are normally not able to penetrate into E. coli cells. However, after treatment with 40 mM Tris buffer, pH 8.0, exogenous dNTP's are incorporated into TCA-insoluble DNA in a process requiring Mg^{++} and ATP. This ATP-dependent synthesis is sensitive to novobiocin, is independent of the PolA gene product, and ceases immediately in dnaB mutants after transfer to the restrictive temperature. The rate of synthesis is slightly lower than in toluene-treated cells. It is concluded that the synthesis observed represents semiconservative replication. Repair of radiation-induced single-strand breaks in cells treated with 40 mM Tris can only occur in the presence of Mg^{++} and all four dNTP's. It is proposed that hypotonic buffers may be used to study the lethal and mutagenic effects of perturbations in semiconservative replication and repair replication.

* Work supported by The Norwegian Cancer Society.

Replicating Linear DNA Molecules

853 *IN VITRO* SYNTHESIS OF PARVOVIRAL DNA, Robert C. Bates, Cynthia Pritchard, and Ernest R. Stout, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

We have analyzed several different types of subcellular systems for the ability to synthesize double- and single-stranded unit length parvoviral DNA. Lysates of synchronized cells infected with bovine parvovirus or Kilham rat virus are capable of continuing viral DNA synthesis *in vitro*, as are nuclear lysates from infected cells, when these are supplemented with polyamines. Polyamines are required for the protection of both template and product viral DNA from degradation. The template DNA in the lysates, labeled *in vivo*, and the product DNA synthesized *in vitro* were analyzed for size, strandedness, and bouyant density, and found to be identical. The DNA products were shown to be more than 98% viral by hybridization analysis. In addition, the template and product DNA are being compared by restriction enzyme analysis. Optimal conditions for the synthesis of viral DNA in nuclear lysates were determined, and the incorporation of labeled substrate into this DNA was linear for at least one hour. The results of studies with inhibitors of and antibodies against cellular DNA polymerases indicated that DNA polymerase α was responsible for viral DNA synthesis. Viral DNA synthesis was not affected by the addition of RNase, RNA polymerase inhibitors, or ribonucleoside triphosphates. The viral DNA synthesized *in vitro* in the presence of these or inhibitors of cellular DNA polymerases β and γ was found to be identical to viral DNA synthesized in their absence.

854 INITIATION OF ADENOVIRUS DNA REPLICATION, John W. Bodnar and George D. Pearson, Dept. of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

The time required to synthesize an adenovirus DNA molecule is roughly 20 minutes. This would suggest that the amount of viral DNA per cell should double every 20 minutes, but the accumulation of viral DNA is considerably slower. We propose that this is due to a low probability per molecule to initiate a round of DNA replication. We have directly measured the rate of initiation in density-shift experiments. The measured rates at 14, 18, and 22 hr after infection were, respectively, 0.0035, 0.0012, and 0.0005 initiations/min/end. However, the pattern of density-labeled molecules indicated that recently replicated molecules were preferentially initiated. This preference is slight early in infection, but increases markedly later in infection. We interpret these results to mean that adenovirus DNA is partitioned into several pools, and at least one pool represents molecules destined for replication. The average rate of initiation in this pool is 0.0042 ± 0.0007 initiations/min/end. We have measured the size of the replication pool to be 50,000 ± 7,000 molecules/cell.

855 STRUCTURE OF THE LINKAGE BETWEEN THE 5'-ENDS OF ADENOVIRUS DNA AND THE 55 K TERMINAL PROTEIN, Stephen V. Desiderio, Mark D. Challberg, and Thomas J. Kelly, Jr., Department of Microbiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The 5'-termini of mature adenovirus DNA molecules isolated from purified virions are covalently linked to a protein of apparent molecular weight of 55K. Since this protein may play a role in the initiation of adenovirus DNA replication (see abstract by Kelly, Challberg, and Desiderio, this volume) we have studied the nature of the DNA-protein linkage. Purified adenovirus 5 DNA-55K protein complex labeled uniformly with ^{32}P was digested with DNase and exonuclease I to yield 55K protein linked to [^{32}P] oligonucleotides. After purification by gel electrophoresis the [^{32}P] 55K protein was digested with trypsin and the resulting [^{32}P] peptides were analyzed with the following results: 1) treatment of the [^{32}P] peptide preparation with snake venom phosphodiesterase yielded exclusively pC, pA, and pT as expected (no guanylate residues are present in the first 25 nucleotides at the 5'-termini of adenovirus DNA); 2) treatment with alkaline phosphatase did not release inorganic phosphate; 3) acid hydrolysis yielded a single phosphorylated amino acid which was identified in four independent chromatographic or electrophoretic systems as O-phosphoserine; 4) prior treatment of the [^{32}P] peptide preparation with snake venom phosphodiesterase resulted in a substantial reduction in the yield of O-phosphoserine upon subsequent acid hydrolysis, while prior treatment with alkaline phosphatase did not affect the yield of O-phosphoserine. We conclude from these data that the 5'-end of adenovirus DNA is bound to the 55K terminal protein by a phosphodiester linkage to the β-OH of a serine residue.

856 Mechanism of Poliovirus RNA Replication in vitro. James B. Flanegan, Terry A. Van Dyke and Richard J. Rickles, University of Florida, Gainesville, Florida 32610.
Poliovirus has a single-stranded RNA genome ($2.5x10^6$ daltons) that has a poly(A) sequence at its 3'-terminal end and a small protein (VPg) covalently linked through a phosphodiester linkage at its 5'-terminal end. We and others have suggested that VPg may be required to initiate RNA synthesis by a mechanism analogous to that proposed for the protein covalently linked to the 5'-ends of adenovirus DNA. To study the specific mechanisms involved in the initiation and elongation of poliovirus RNA in vitro, we have isolated the poliovirus RNA dependent RNA polymerase from the cytoplasm of infected cells. One virus-coded protein that has a molecular weight of 62,500 (p63) was found to copurify with polymerase activity. This appears to be the only protein, either host or virus-coded, that is required for elongation activity in vitro. The polymerase will copy poliovirion RNA in the presence of added oligo(U) into a full genome-length molecule of complementary RNA. The oligo(U) apparently functions as a primer for the reaction and is incorporated into the full-sized product RNA. The possibility that VPg-pU might function in place of oligo(U) is being investigated.

857 EVIDENCE FOR REPAIR OF HERPES SIMPLEX VIRUS TYPE 1 BY GENETIC RECOMBINATION, Jennifer D. Hall, University of Arizona, Tucson, Arizona 85721.
Experiments have been conducted in order to access the role of genetic recombination in the repair of viral DNA damage in herpes virus-infected cells. Human fibroblasts infected with two or more UV-irradiated viral genomes were found to repair virus more efficiently than singly-infected cells. The increased survival of multiply-infected cells could not be attributed solely to the increased chance of infection of these cells by undamaged virus. These results suggest that recombination between damaged viral genomes in multiply-infected cells may be an important repair mechanism in these cells. Multiplicity-dependent repair of herpes virus damaged by chemical agents will also be discussed.

The effects of UV-irradiation on the yield of temperature resistant viral recombinants in cells infected with pairs of temperature sensitive mutants was also investigated. Increased recombination frequencies were observed after UV-irradiation of either one or both viral parents, suggesting that damaged sites on the herpes genome stimulate genetic exchanges. These data provide further support for the idea that recombination is involved in repair of herpes virus DNA.

Multiplicity-dependent repair and UV-induced recombination in repair deficient host cells (xeroderma pigmentosum) will be described. In addition, the effect on multiplicity-dependent repair of herpes virus temperature sensitive mutations which alter viral DNA replication will be discussed.

858 VACCINIA VIRUS DNA REPLICATION, J. A. Holowczak, CMDNJ, Rutgers Medical School, Piscataway, N. J. 08854 and M. Esteban, SUNY, Downstate Medical Center, Brooklyn, N. Y. 11203
Poxviruses contain linear, double-stranded DNA genomes whose complementary strands are naturally cross-linked. Following infection of an appropriate host-cell, vaccinia virus DNA replication occurs in association with characteristic cytoplasmic inclusion bodies called "factories". Single-stranded DNA regions per se or proteins having affinity for ssDNA bind the replicating DNA molecules together in these complexes. Viral DNA replication can be shown to proceed in a semiconservative, discontinuous manner, at about twice the rate that DNA is replicated in uninfected mammalian cells. Permeabilized, infected Hela cells actively incorporate deoxytriphosphates into viral DNA but the synthesis is limited to one round of DNA replication. This "limited" viral DNA synthesis reflects an inhibition of protein synthesis in the permeabilized cells. Analysis of the events during viral DNA replication show that active protein synthesis is required at three stages: (a) to complete uncoating of the infecting virions and allow synthesis of the viral coded enzymes necessary for DNA replication; (b) to permit synthesis of proteins which function in a non-catalytic manner and are required to initiate each new round of DNA replication; and (c) synthesis of proteins which release newly replicated viral DNA molecules from the cytoplasmic "factories" (probably a ssDNase).

859 STUDIES ON THE EFFECT OF INHIBITORS OF DNA POLYMERASES ON SYNTHESIS OF PARVOVIRUS H-1 DNA, Regine Kollek, Ben Y. Tseng and Mehran Goulian, University of California, San Diego, La Jolla, CA 92093

A nuclear in vitro system of parvovirus H-1 infected human NB cells has been used to characterize the DNA polymerase requirements for replication of viral DNA, by comparison of the effects of inhibitors of DNA polymerases. Each of the three inhibitors used influenced both the infected and uninfected nuclei in a very similar way. ddTTP, which inhibits primarily DNA polymerases γ and α,inhibited the replication in both systems only slightly (~20%;ddTTP/dTTP=1). Aphidicolin, considered to be a specific inhibitor of DNA polymerase α, caused significant inhibition (~70%;1 µg/ml) of infected and uninfected nuclei. araCTP, another inhibitor of DNA polymerase α, also inhibited both infected and uninfected nuclei (~60%; araCTP/dCTP=1). It is concluded that DNA polymerase γ is probably not involved in the replication of parvovirus H-1, and that DNA polymerase α, thought to be responsible for cellular DNA synthesis on the basis of similar evidence, is probably required for viral DNA synthesis, as well. However, these studies also indicated problems with specificity of the inhibitors. Although results as described by others (inhibition by ddTTP:γ>β>α; inhibition by aphidicolin: α>>>β,γ) can be reproduced under optimal assay conditions, with the conditions used for viral or cellular DNA synthesis the specificity towards γ polymerase was much reduced. Using "activated" DNA as template-primer and the conditions for DNA synthesis in nuclei (i.e. salt, ATP, etc.), γ polymerase was sensitive to aphidicolin and relatively insensitive to ddTTP. In view of these results, the usefulness of aphidicolin and ddTTP for distinction between the activities of α and γ polymerases in DNA replication has to be reconsidered.

860 STUDIES ON CONCATEMERIC T7 DNA, Robert L. Lechner and Charles C. Richardson, Harvard Medical School, Boston, MA 02115

Several studies have led to the conclusion that, in vivo, linear T7 DNA molecules are derived from linear concatemeric DNA. Analysis of restriction endonuclease cleavage patterns indicate that T7 concatemers consist of unit-length T7 DNA covalently linked head to tail, presumably through the terminal redundancy. It is likely that the conversion of T7 concatemers to mature T7 DNA molecules requires staggered nicks generated by a sequence - specific endonuclease. Generation of a full length duplex T7 DNA molecule and its release from the concatemer would then require DNA synthesis with concomitant strand displacement.

We have developed an assay to monitor the processing of concatemers that is based on the identification of a unique restriction enzyme fragment present in a concatemer as a result of the joint between two T7 DNA molecules. Such an assay is being used to identify a site - specific endonuclease. We have found that T7 DNA polymerase alone is sufficient to catalyze the DNA synthesis and strand displacement which must occur as a final step in the processing of concatemers. Artificial concatemers can be formed by exposing the terminal redundancy with E. coli exonuclease III followed by annealing. Treatment of such molecules with T7 DNA polymerase produces full length T7 molecules.

We are currently proceeding to describe defects in concatemer processing conferred by amber mutations in T7 genes 8, 9, 10, 18 and 19, and to purify those T7 proteins required for the processing of concatemers.

861 MECHANISM FOR REPLICATION OF PARVOVIRUS H-1 DNA, Dennis Revie and Mehran Goulian, University of California, San Diego, La Jolla, CA 92093

H-1 is an autonomous parvovirus with single-strand DNA genome of 1.6×10^6 daltons, and produces intracellular double-strand replicative form (RF) during infection. Studies in this laboratory thus far on the mechanism of replication have shown: 1) a protein is covalently associated with the 5' terminal restriction fragments of both viral (V) and complementary (C) strands of RF; 2) both in intact infected cells, and nuclei from infected cells, 4-5S nascent pieces characteristic of discontinuous DNA synthesis are absent even with labeling times as short as 20 sec; 3) in nuclei from infected cells, there is no transfer of ^{32}P from incorporated α-^{32}P-dNMP to rNMP, after treatment with alkali; 4) in completed strands of RF uniformly labeled with ^{32}P and pulse-labeled for varying intervals with ^{3}H-dThd, there is progressive increase in ratio of $^{3}H/^{32}P$ in restriction fragments from 5' to 3' termini, in both V and C strands. The results are consistent with continuous synthesis of both strands of RF. This may take place by strand displacement in RF followed by re-conversion of the displaced single strand to RF. The function of the 5' terminal protein is unknown.

862 PROTEIN p3, COVALENTLY LINKED TO THE 5' TERMINI OF Ø29 DNA, IS INVOLVED IN THE INITIATION OF REPLICATION, Margarita Salas, Juan A. García, Miguel A. Peñalva, Rafael P. Mellado, José M. Hermoso, Cristina Escarmís, Marta R. Inciarte and José M. Sogo, Centro de Biología Molecular, Madrid, Spain

Bacteriophage Ø29 contains a double-stranded DNA ($Mr=11.8x10^6$) with a protein, product of cistron 3(p3;Mr = 27 000), covalently linked to the two 5' termini. Protein p3 is linked to the DNA by a phosphodiester bond to serine. Sequence analysis of the 80 first nucleotides at the 5' termini of Ø29 DNA has shown the existence of an inverted terminal repetition 7 nucleotides long.

The analysis of Ø29 replicative intermediates with the electron microscope has shown that replication starts non simultaneously at either end of the DNA and proceeds by a mechanism of strand displacement.

With the use of ts 3 mutants we have shown that protein p3 is required for the initiation of DNA replication. Moreover, the parental protein itself is able to initiate one round of replication, as indicated by the fact that viral DNA of hybrid density is mainly synthesized after restrictive infection of bacteria grown in heavy medium with light sus 3 mutants. The mechanism by which protein p3 initiates replication will be discussed.

863 AGAROSE GEL ELECTROPHORESIS OF CONCATEMERIC BACTERIOPHAGE T7 DNA: FRAGMENTS OF FAST SEDIMENTING, REPLICATING DNA, Philip Serwer, Department of Biochemistry, The University of Texas Health Science Center, San Antonio, Texas 78284

To develop a technique for fractionating concatemers of bacteriophage T7 DNA, agarose gel electrophoresis of duplex DNA molecules with molecular weights between 2×10^6 and 110×10^6 daltons has been performed in multiple-concentration agarose gels (a procedure described in Serwer, P. [1980]. Anal. Biochem., in press). Using DNA concentrations low enough so that the distance migrated was not a function of DNA concentration, the distance migrated by DNA at .4 volts/cm was measured as a function of the molecular weight of the DNA and the concentration of agarose in the gel (Agarose concentrations were between .075% and .70%). A semilogarithmic plot of the molecular weight of duplex DNA as a function of the distance migrated was linear for .1% agarose and for molecular weights between 14×10^6 daltons and 110×10^6 daltons. This plot became progressively nonlinear at higher and lower concentrations of agarose.

Most fragments released from fast sedimenting, replicating T7 DNA ($100S^+$DNA, Serwer, P. [1974]. Virology 59, 70-88) by S1 nuclease were electrophoretically heterogeneous in .15% gels. However, a weak, but sharp, band at the position expected of a T7 dimer was found. The following observations indicate that a linear dimer of mature T7 DNA is the most abundant form of concatemer within $100S^+$DNA: (a) Completion digests of $100S^+$ DNA with the restriction enzyme BgIII (cuts mature T7 DNA in one place) contain roughly equal amounts of the mature T7-migrating DNA and the fragments of mature DNA; (b) Subcompletion digests of $100S^+$ DNA with Bgl II have no detectable concatemer-migrating fragments.

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864 CELL FREE SYSTEMS FOR DNA REPLICATION AND RECOMBINATION IN ADENOVIRUS INFECTED HUMAN CELLS, Ernst-L. Winnacker, Johannes Fütterer, Christian Meinschad and Theresia Reiter, Institut für Biochemie, Universität München, Karlstrasse 23, 8000 München 2, Germany.

This communication adresses itself to the mechanism of initiation of the adenovirus replicon as well as to a biochemical approach to the study of general genetic recombination in adenovirus infected cells.

Properties of a soluble, nuclear extract from adenovirus type 2 infected HeLa cells will be described which is able to efficiently initiate viral DNA replication with a fidelity comparable to the situation in the infected cell. Evidence will be presented for the role and function of the 55 K terminal protein in the initiation process as well as on the nature and size of the DNA replicon at the molecular termini of adenovirus DNA. Finally, it will be demonstrated that these nuclear extracts are able to catalyze a genetic exchange between the closely related adenovirus type 2 and type 5 genomes. These activities which could be envisaged to represent enzymatic steps in general genetic recombination, will be discussed in terms of their relation to DNA replication as well as their viral and/or cellular origin. (Supported by the Deutsche Forschungsgemeinschaft, grant Wi 319/5/6).

865 DEOXYPYRIMIDINE TRIPHOSPHATASE FROM HERPES SIMPLEX VIRUS INFECTED CELLS, Franz Wohlrab and Bertold Francke, Yale University, New Haven, CT 06510
Nuclei from BHK-cells infected with herpes simplex virus type 1 contain a virus-specific deoxyribonucleoside triphosphate degrading activity. The reaction proceeds at 4°C and can thus be distinguished from host enzymes. Under these conditions the enzyme is specific for deoxyribopyrimidine triphosphates and catalyzes pyrophosphate-cleavage to produce the monophosphates, dUTP being the best substrate followed by dCTP and dTTP. The appearance of the activity after infection parallels that of viral DNA-synthesis related functions. Of a series of eight temperature sensitive mutants tested, two (tsD and tsK) exhibit significantly reduced triphosphatase levels after infection at non-permissive temperature, while a viral deoxypyrimidine kinase deficient mutant induced wild type levels.

Initiation of Replication Forks

866 REPLICATION OF THE RIBOSOMAL RNA GENES OF YEAST, Bonita J. Brewer, Virginia A. Zakian, and Walton L. Fangman, Dept. of Genetics, Univ. of Washington, Seattle, WA 98195
The yeast genome contains about 100 copies of the ribosomal RNA genes tandemly repeated on chromosome XII. Replication of these genes was examined in two ways. 1) The pattern of replication of rDNA was determined by analyzing the density classes (HH,HL,LL) of rDNA after a culture transfer from dense to light medium. By hybridizing ^{32}P-labeled, cloned rDNA to CsCl gradient fractions containing total yeast DNA, the behavior of rDNA was found to be identical to the behavior of chromosomal DNA at each sampling time. Quantitative analysis of these data indicates that the replication of any given rDNA unit occurs during the same short interval ($\leq$1/6) of each cell cycle. 2) The time of rDNA replication in the cell cycle was determined in synchronous cultures. Cells pregrown with ^{14}C-uracil were arrested at the G1/S phase boundary, then released from the block in the presence of ^{3}H-uracil. The increasing ^{3}H/^{14}C ratio for the rDNA paralled exactly the ratio for total chromosomal DNA during the synchronous S phase (1/2 the cell cycle). The difference in replication intervals ($\leq$1/6 vs. 1/2 the cell cycle) obtained with the two experiments indicates that replication of rDNA copies is temporally programmed; i.e., early, mid, and late S replicating copies maintain their time of replication in subsequent S phases. The program could be accounted for if the single large rDNA cluster is replicated from two replication origins, in flanking non-ribosomal DNA sequences, which are activated at the beginning of the S phase. The data are inconsistent with the existence of an origin in each rDNA unit. However, it is possibile that a few of the rDNA units contain an origin and that activations are temporally programmed.

867 DNA REPLICATION IN DNA TEMPERATURE SENSITIVE INITIATION MUTANTS OF ESCHERICHIA COLI. H. Eberle, N. Forest, J. Hrynyszyn and J. Van Knapp, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642.
We have examined the replication pattern of several temperature sensitive mutants with apparent defects in the initiation process. We have found that in temperature reversible dna A mutants it is possible to demonstrate prolonged DNA synthesis for up to 5 hrs. at nonpermissive temperatures after a certain growth regimen. The conditions which are prerequiste for the prolonged DNA synthesis at nonpermissive temperature (NPT) include: a prior period at nonpermissive temperature of 40-60 min, followed by return to the permissive temperature in the presence of chloramphemicol for at least 10 min. The rec A gene product also appears to be essential for the prolonged DNA synthesis at NPT in these dna A mutants. A characterization of the DNA synthesized for prolonged period at NPT suggests that the synthesis is semiconservative and is probably chain elongation which commences at 4-5 forks which had previously stopped at random sites on the chromosome. We have also examined DNA binding proteins which were synthesized at NPT and during periods after return to permissive temperature. A DNA binding protein of 60-65 kd was found to be synthesized during periods when active dna A product was present. The implication of these results to the regulation of DNA replication will be discussed.
This paper is based on work performed under contract with The U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics and has been assigned Report No. UR-3490-1747.

868 REPLICATION PATTERN OF A LARGE, TANDEMLY-REPEATED NUCLEOTIDE SEQUENCE IN CHINESE HAMSTER CELLS. Joyce L. Hamlin , Jeffrey D. Milbrandt, Nicholas H. Heintz, and Stephen Rothman, University of Virginia School of Medicine, Department of Biochemistry, Charlottesville, Va. 22908. We are studying a series of methotrexate-resistant Chinese hamster cell lines each of which carries on one chromosome arm a large, tandem repetition of genetic material (ca. 300-500 kb amplified approximately 300 times). The repeated DNA sequence includes the gene coding for dihydrofolate reductase. This gene is expressed, producing high levels of the enzyme, which accounts for resistance to methotrexate. Evidence is presented that this highly repeated nucleotide sequence behaves as a single replicon type in each cell line. We show that the unit repeated sequences initiate and terminate replication synchronously with respect to one another in the early S period. Since the nucleotide sequence in each unit is presumably identical, this result argues that the origin of DNA synthesis in an animal cell replicon is determined by the sequence itself, as it is in microorganisms. We also present studies in which the movement of the replication fork through the dihydrofolate reductase gene is utilized in order to estimate the location of the origin of DNA synthesis in the replicon.

869 T4 DNA REPLICATION AND ITS REGULATION BY THE DNA-DELAY PROTEINS, Wai Mun Huang, Gretchen J. King and Gary L. Stetler, University of Utah Medical Center, Salt Lake City, Utah 84132

Mutants of bacteriophage T4 defective in genes 39, 52 or 60 are characterized by a delay in the on-set of DNA replication and the proteins coded by these genes are called DNA-delay proteins. The requirement of these proteins for T4 DNA replication can also be demonstrated *in vitro* using concentrated cell extracts prepared on cellophane discs. These proteins appear to be involved in the initiation of the T4 chromosomal replication because of their mutant phenotypes and the role of gene 39 protein in positively regulating the specific DNA replication. By using the *in vitro* complementation assay which measures the stimulation of DNA synthesis in extracts of the corresponding DNA-delay mutant infected cells, the DNA delay proteins of T4 and T6 have been purified. In both cases, the purified fractions consist mainly of the proteins of genes 39 and 52 and possibly another minor protein subunit. The DNA delay proteins so purified form a complex that gives, in addition to the DNA synthesis stimulating activity, a new DNA topoisomerase activity which untwists both positive or negative superhelical DNA in an ATP, Mg^{++} dependent reaction. In this process, ATP is hydrolysed to ADP and Pi.

870 TERMINATION OF CHROMOSOME REPLICATION IN *ESCHERICHIA COLI*, R. Bitner, D. Damm, G. Romansky, R. Binding and P. Kuempel, MCDB, Univ. of Colorado, Boulder, CO 80309.

Studies of the terminus region of *E. coli* have demonstrated that replication forks traveling in either a clockwise or counterclockwise direction are inhibited in the region between the *rac* and *man* loci. These loci are at min 31.3 and min 35.6, respectively, on the current genetic map (Bachman *et al.*, Bacteriol. Rev. *40*, 116-167, 1976).

In order to define the terminus region more precisely, we are constructing a genetic map of this region. We have used P1 transduction to confirm that the *nirA* locus is at min 29.2, and we have determined that the *ksgB* locus is at min 34.5, adjacent to the *relB* locus. We have also determined that the *rac* locus is adjacent to the *nirA* locus. The *rac* locus contains a defective prophage, and it extends from min 29.3 through min 29.8. Because of the absence of known loci in the region between min 29.8 and min 34.6, we have used Tn1 and Tn10 insertions to define this region genetically. These insertion transposons are also being used as hybridization probes to determine the location of the replication terminus more precisely.

In our studies of the *rac* locus, we have determined that the defective prophage at this locus is excised after the rac^+ prophage is transferred to a rac^- recipient by means of Hfr or F' mediated conjugation. This excision has been studied by DNA-DNA hybridization using cloned *rac* locus DNA, and also by the excision of Tn10 transposons inserted in different parts of the *rac* locus.

871 CHARACTERIZATION OF THE ORIGIN OF CHROMOSOME REPLICATION FOR B. SUBTILIS, Kenneth F. Bott, Dept. Bacteriology, Univ. of North Carolina Medical School, Chapel Hill, N.C.
We have isolated and cloned in the λgt WES·λB vector a 5Kb EcoRl fragment from a nalidixic acid resistant mutant of strain 168 which transforms Nal^r to competent recipients. We believe this fragment also contains a functional origin of replication since it will replicate autonomously in recA⁻ recipients when circularized with DNA ligase. Since expression of the Nal^r phenotype is normally inefficient, this marker functions only poorly as the primary selection for the self-replicating fragment. However, when subcloned into the chimeric plasmid pCS540 of Chang and Cohen where chloramphenicol could be used as the selective agent, replication in B. subtilis occurred normally. (The parent pCS540 lacks a functional origin of replication for B. subtilis.) Since pCS540 carries a portion of the E. coli plasmid pSC101 it can replicate in E. coli. Derivatives of the plasmid which contain the 5Kb B. subtilis fragment convert both E. coli and B. subtilis to Nal^r (and replicate in either host). When the 5Kb fragment was labeled by nick translation and used as a probe for DNA-DNA hybridizations against EcoRl digests of different laboratory strains of B. subtilis or digests using other restriction enzymes, we noted that the homologous fragment of some strains including 168 thy⁻, W23 and 168 Marburg did not have similar restriction enzyme cleavage sites and were not the same size as fragments from strain 168. Our results suggest that the B. subtilis cultures used routinely in many laboratories may be derived from distinctly different backgrounds whose origins of replication are situated in substantially different molecular environments.

872 INTERACTION OF BACTERIOPHAGE LAMBDA REPLICATION PROTEINS WITH HOST CELL COMPONENTS
Albrecht Klein, Walter Reiser and Alfons Anderl, Microbiology, University of Heidelberg, W. Germany.
The replication genes O and P of phage λ were cloned on multicopy plasmids and rendered inducible under the control of the lac or λP_L promoters. Expression in minicells and subsequent fractionation revealed that the O protein is membrane associated.
Biochemical properties of a λP/dnaB protein complex isolated from cells harboring a λP gene containing plasmid have been studied. It was found that λP protein masks dnaB complementing activity in a ØX single strand to double strand DNA replication reaction in vitro and DNA dependent dnaB ATPase in an assay employing ØX ss DNA. Results of further studies using DNA from fd and fd containing either the L-strand or the R-strand of λdv in ATPase tests with free dnaB protein or λP/dnaB complex will be reported.
Nucleotide sequences of the P genes of λ π missense mutants will be discussed with respect to P protein areas involved in the interaction with the dnaB protein.

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873 INITIATION OF E. COLI CHROMOSOME REPLICATION IN VITRO, Steven J. Projan and James A. Wechsler, University of Utah, Salt Lake City, Ut 84112
Initiation of rounds of chromosome replication begin at a specific region, the origin, on the E. coli genome. An in vitro assay of initiation has been developed. The initiation reaction is dependent on an exogenous source of $dnaA^+$ gene product. The DNA synthesized in vitro hybridizes to chromosomal DNA from the region near the origin and not to DNA from a region far removed from the origin. Data on the role of RNA polymerase in the initiation reaction will be presented.

874 ISOLATION OF A REPLICATION ORIGIN COMPLEX FROM ESCHERICHIA COLI, M. Schaechter, K. Nagai, W. Hendrickson, R. Balakrishnan, H. Yamaki and D. Boyd, Tufts University School of Medicine, Boston, Mass. 02111.
A complex consisting of replicative origin DNA and several proteins was isolated from Escherichia coli. Cells of temperature sensitive mutants were labeled at the origin and fractionated by sucrose gradient centrifugation. A complex highly purified in origin DNA sedimented as a unique band. This complex dissociated at high concentration, above 0.2 M KCl. Upon dialysis, the complex reformed, allowing further purification of its constituents. Three major protein bands were found, corresponding to proteins of the outer membrane.
The complex did not sediment with membrane fractions, but adhered to the outer membrane in the presence of magnesium.

875 PREFERENTIAL UTILIZATION OF A YEAST CHROMOSOMAL REPLICATION ORIGIN AS TEMPLATE FOR ENZYMATIC DNA SYNTHESIS, John F. Scott, MBI, UCLA, Los Angeles, Ca., 90024

Restriction fragments of cloned Saccharomyces cerevisiae chromosomal DNA were used as templates for DNA synthesis by yeast enzymes. Fragments used included two adjacent EcoRl-HindIII fragments derived from a region shown previously to contain the functional trp1+ gene and an autonomously replicating sequence (ARS) (1). The ARS presumably represents a chromosomal origin of DNA replication. The sources of enzymes were yeast cell-free extracts. The (^{32}P)-labelled products of the DNA synthesis reactions were used to probe patterns of agarose gel bands transferred to nitrocellulose strips. The bands represented the same fragments which were used as templates in the reactions. Autoradiography of the resulting patterns of the hybridized products revealed that the yeast enzymes had used as template the fragment which had been shown by functional cloning experiments to contain an ARS, in preference to the adjacent yeast fragment shown not to contain an ARS by the same test. This analysis provides a means by which cell-free extracts or reconstituted replication enzyme mixtures can be tested for their ability to recognize DNA replication origins and to utilize them in a specific fashion.

1. Stinchcomb, D.T., Struhl, K. and Davis, R.W., Nature, 282, (1979) pp39-43.

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876 PSEUDOVIRULENT MUTANTS OF λporiC asnA RESULTING FROM ALTERATIONS WITHIN THE oriC REGION, Larry Soll and Stephen P. Eisenberg, Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309

Mutants of the specialized transducing phage λb221cI⁻poriCasnA which form plaques on lambda lysogens have been isolated. Genetic and physical evidence places the mutations responsible for the pseudovirulent phenotype in or near the oriC locus. The most probable explanation of the effect of these oriC^{c} alleles is an increase in the frequency of replication initiation at the oriC locus.

877 STRUCTURE AND FUNCTION OF "A+T"-RICH REGION IN MITOCHONDRIAL DNA FROM DROSOPHILA, Akio Sugino*, Koji Nakayama, and Hitoshi Kojo, NIEHS/NIH, Research Triangle Park, NC 27709

Drosophila mitochondrial DNAs (9.9-12.4x10^{6} MW) contain high "A+T"-rich region whose size is 0.31-3.4x10^{6}. Orgin and direction of replication of mitochondrial DNA from some species of Drosophila have been determined by electron microscopic studies. DNA replication starts in the "A+T"-rich region and proceeds unidirectionally around the molecule. However, the precise location of the origin is still unkown. In order to determine more precisely the location of the origin and elucidate unique feature of its nucleotide sequence, we have cloned mitochondrial DNA fragments containing the "A+T"-rich region from Drosophila virilis embryos and such "A+T"-rich region nucleotide sequence has been determined. We will present the nucleotide sequence and discuss its unique structure.
Using both native mitochondrial DNA and the chimeric plasmid DNA containing "A+T"-rich region of mitochondrial DNA for a prove, we have been developing in vitro mitochondrial DNA replication system which mimic in vivo. Our current progress of this attempt will be presented.

878 ISOLATION OF B. SUBTILIS DNA FRAGMENTS CONTAINING REPLICATING FORKS, Manuel S. Valenzuela and María del Pilar Aguinaga, University of Wisconsin, Madison, WI 53706

When gently lysed B. subtilis cells are treated with a restriction endonuclease, about 2% of the total DNA can be recovered as part of a fast sedimenting complex. This fraction contains putative replicating forks and also appears to be enriched for markers near the origin and terminus of B. subtilis DNA replication.

879 DNA-MEMBRANE INTERACTIONS DURING THE INITIATION OF DNA REPLICATION IN BACILLUS SUBTILIS. Scott Winston, Ron Korn, and Noboru Sueoka. MCDB University of Colorado, Boulder, Co. 80309

The plasmid pSL103, whose replication function is provided by the Staphylococcus aureus plasmid pUB110, replicates in B. subtilis at 40 copies per cell and expresses Nm^r. This plasmid was found predominantly in the DNA-membrane fraction from B. subtilis. Inhibition of plasmid initiation by shifting the temperature-sensitive initiation mutant, dna-1, to non-permissive temperature resulted in a decreased membrane enrichment of pSL103. Host chromosomal initiation was also inhibited, and this was concomitant with a decreased membrane enrichment of the host origin marker, purAl6. The membrane association of both pSL103 and purAl6 was restored by returning the dna-1 cells to permissive temperature. In another B. subtilis initiation mutant, dnaB19, only host chromosomal initiation and membrane association were affected when the cells were shifted to non-permissive temperature. During these studies it was observed that the mode of plasmid replication was random and plasmid molecules were initiated asynchronously throughout the replication cycle. In vitro studies demonstrated the selective release of purAl6 DNA and pSL103 from DNA-membrane fractions prepared from dna-1 cells, while only purAl6 DNA was released from DNA-membrane fractions of dnaB19 cells. The plasmid, pSL103, and purAl6 DNA selectively reassociated with the membrane fraction when the dna-1 gene product was reactivated in vitro by lowering the temperature. These and other studies have demonstrated a direct relationship between the initiation of DNA replication and DNA-membrane attachment.

880 REPLICATION OF PLASMIDS CONTAINING oriC, Alan C. Leonard and Charles E. Helmstetter, Roswell Park Memorial Institute, Buffalo, NY 14263

Control aspects of chromosome replication in E. coli are being analyzed by examining the properties of cells harboring plasmids which replicate from a copy of the chromosomal origin (oriC). Some of these plasmids exhibited a degree of instability in recA mutants. At each cell division, a percentage (up to 50%) of the newborn cells were plasmidless. In order to examine plasmid and chromosome replication during the division cycle in this experimental system, three different approaches are being used. First, some oriC-containing plasmids which encode resistance to kanamycin can be stably maintained (at approx. 3 copies per cell) in recA mutants grown in media supplemented with high concentrations of kanamycin. Second, a composite plasmid has been constructed which contains both oriC and the origin of replication from the cloning vector pACYC 184. Replication of this composite (pAL 3) was studied in a temperature-sensitive polA mutant. At 25°C, plasmid replication ensued from the replication origin of pACYC 184. At this temperature plasmid copy number was high, the plasmid was stably inherited, and amplification with chloramphenicol was possible. Upon shift to 42°C, plasmid copy number decreased, plasmid instability became apparent, and chloramphenicol amplification was no longer possible. Third, pAL3 has been introduced into dnaA and dnaC mutants of E. coli B/r in order to determine the kinetics of pAL3 replication in cells induced to initiate chromosome replication synchronously by temperature shifts. The relationship between plasmid and chromosome replication will be presented as determined by each of the experimental techniques.

881 BINDING SITES FOR A MEMBRANE - ORIGIN - BINDING PROTEIN IN THE E. COLI REPLICATION ORIGIN, H. Lother, A. Jacq, M. Kohiyama and W. Messer, Max-Planck-Institut für molekulare Genetik, Berlin, Germany and I.R.B.M., Université de Paris VII, Paris

A protein has been isolated from the membrane of E. coli, which binds specifically to single-stranded DNA pulse-labelled early in the replication cycle. The binding sites for this membrane DNA binding protein have been localized within oriC by preparing restriction fragments from the minichromosome pCM959.

These have been labelled at their 5' ends with $^{32}PO_4$, were denatured and allowed to react with the protein. Their binding to nitrocellulose filters was monitored in the presence of different amounts of competing nonradioactive E. coli chromosomal DNA.

Fragments which contain the whole 422 bp oriC segment plus a segment of 75 bp between the XhoI site at position 417 and the PstI site at position 489 bind to the protein efficiently and specifically. Both single strands of this segment are bound. Fragments which contain either the BamHI segment (position o-92) from oriC or the XhoI - PstI segment bind to the protein with kinetics which are compatible with the specific binding of one of the two single strands only. The portion of oriC between these segments ($HindIII_{244}$ to $XhoI_{417}$) does not bind to the protein specifically.

Fragments labelled at one of their 5' ends only were used to define the strand specificity in the individual binding sites. In the "left" binding site (position o-92 bp) the strand reading 3'→5' in the direction of the E. coli map is bound, the "right" binding site (position 417 - 489 bp) is specific for the complementary strand.

882 PLASMID REPLICATION FROM BACTERIOPHAGE T4 ORIGINS, T. Mattson and G.Van Houwe Univ. of Geneva, Geneva, Switzerland

Since essential T4 replication genes are distributed over the genome, no single cloned restriction fragment can be expected to code for all of the T4 replication functions. This means that it is unlikely that any meaningful replication could be initiated from a cloned T4 origin unless the cell was infected by a phage which could provide all of the trans acting replication functions. Therefore, we have sought to develop an assay that could detect cloned T4 origins after infection. The assay is based on our observation that T4 infection shuts-off plasmid PBR322 as well as chromosomal replication. The idea then, is simply to screen different clones for those that will allow PBR322 vector DNA to replicate after phage infection. This can be done by hybridization of pulse labelled DNA to filters charged with only PBR322 DNA. One simple problem that we have overcome by using phage mutant in the denA and denB genes is the phage induced breakdown of host DNA. A more difficult problem is to distinguish between automonous plasmid replication and replication following recombination between the cloned sequences and the infecting phage DNA. Although we cannot, at the time of writing completely exclude the recombinational possibility, we will present evidence supporting the validity of this approach. Using this assay, we have been able to identify restriction fragments that may contain T4 origin sequences. The availability of small restriction fragments containing T4 origins would be of obvious use for a number of different kinds of experiments.

883 STRUCTURE OF THE E. COLI REPLICATION ORIGIN. JOINT REPLICONS CONTAINING THE E. COLI AND THE colE$_1$ REPLICATION ORIGINS, Walter Messer, Barbara Heimann and Stephan Hall, Max-Planck-Institut für molekulare Genetik, Berlin, Germany

A 422 bp DNA fragment from the E. coli origin region can serve as an origin for replication of minichromosomes.

This fragment was cloned into the BamHI site of pBR322 (pOC44). Deletions were obtained resulting in a loss of varying parts of the fragment. A 95 bp fragment (pOC41) and a 1.98 kb fragment (pOC42) of oriC was also cloned into pBR322.

Replication of these plasmids was tested under conditions where either one of the two origins could not function (growth at 42° C in ts mutants of polA and in dnaA204 integratively suppressed by F).

In all cases either the colE$_1$ origin (dnaA) or the fragment from the E. coli origin (polA) allowed replication of the plasmids and thus supported growth of the host cells at 42° C in the presence of antibiotic.

In addition replication at 42° in polA^{ts} was measured by pulse-labelling with ^{3}H-thymidine followed by CsCl-propidium diiodide equilibrium centrifugation in order to be certain that growth behaviour was not due to artefacts.

These experiments showed that a fragment containing only about 100 bp of the E. coli origin region was able to serve as an origin. Experiments on the control of this "mini-origin" will be discussed.

884 A FIXED SITE OF DNA REPLICATION IN EUCARYOTIC CELLS, Drew Pardoll, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Recently, a number of laboratories have reported the existance of a skeletal framework in the nucleus. This framework, often termed the nuclear matrix, is revealed by extraction of nuclei with neutral detergents, low Mg and high salt buffers. The role of the nuclear matrix in DNA replication was studied both in vivo and in a cell culture system. When regenerating rat liver or exponentially growing 3T3 fibroblasts are pulse labeled with ^{3}H thymidine and nuclear matrix is subsequently isolated, the fraction of DNA remaining tightly attached to the matrix is highly enriched in newly synthesized DNA. After a 30 second labeling period and limited DNase I digestion, the matrix DNA of 3T3 fibroblasts, which constitutes 15% of the total DNA, contains virtually all of the labeled newly synthesized DNA. Over 80% of this label can be chased out of the matrix DNA if the pulse is followed by a 45 minute incubation with excess unlabeled thymidine. These and other kinetic studies suggest that the growing point of DNA replication is attached to the nuclear matrix. Studies measuring the size distribution of the matrix DNA also support this conclusion. Electron microscopic autoradiography shows that, as with intact nuclei, sites of DNA replication are distributed throughout the nuclear matrix. A fixed site of DNA synthesis is proposed in which DNA replication complexes are anchored to the nuclear matrix and the DNA is reeled through these complexes as it is replicated.

885 ROLE OF THE NUCLEAR MATRIX IN POLYOMA DNA REPLICATION, Vincent Pigiet, Alicia Buckler, and Glenn Humphrey, The Johns Hopkins University, Dept. of Biology, Baltimore, MD 21218

The Nuclear matrix is a proteinaceous framework that plays a structural role in providing shape and rigidity to the nucleus and a functional role in DNA replication by providing (a) physical site(s) for replication. Polyoma DNA replication, like replication of the host genome, also takes place in association with the matrix as evidenced by the retention of viral genomes after extensive extraction using high salt buffers and shearing the bulk of the cellular DNA and their associated proteins. Replicative intermediates (RIs), as well as superhelical form I DNAs are matrix-bound. Removal of these matrix-bound genomes requires conditions severe enough to denatture the protein matrix (1% SDS, 4 M urea). As much as 20%-30% of viral genomes are matrix-bound at the onset of replication (16 hr post-infection), and the relative amount (NM/N) decreases as mature forms accumulate as the lytic cycle progresses. Kinetic studies on the fate of in vivo and in vitro labelled DNA also suggest the critical role for the matrix in replication. Protection of viral DNA to nuclease digestion offers evidence for a non-random attachment site on the DNA for the matrix.

The polyoma tumor antigen (T-Ag) is also bound, in part, to the matrix with a constant amount of T-Ag/cell through the replication period (16-30 hr). The ratio of T-Ag to Py DNA on the matrix decreased from 10 (16 hr) to 1 (30 hr) while in bulk nucleoplasm this ratio changed more dramatically, from 13 (16 hr) to 1 (30 hr).

886 ON THE ROLE OF THE DNAB PROTEIN OF ESCHERICHIA COLI IN THE REPLICATION OF λ BACTERIOPHAGE DNA, Robert C. Shuster, Department of Biochemistry, Emory University, Atlanta, GA 30322

The interaction of the E.coli dna β gene product and λ P gene product was investigated by measuring the stability of superhelical phage DNA after λ infection of two temperature sensitive dna β mutants, JG28 and TO534 groP⁻B.

Results with λcI60, which does not form plaques on the groP⁻B host, showed that superhelical phage DNA persisted after infection of TO534 but was lost in cultures of JG28 during incubation at both permissive and non-permissive termperatures. When infected cells were incubated in the presence of BUdR, λcI60 DNA did not undergo a shift in density in cultures of TO534 at 30° or 42° or in JG28 at 42°. Bacterial DNA synthesis in uninfected cells abruptly ceased in JG28 upon transfer to 42° but persisted at a decreasing rate for 1 hr after the temperature shift in cultures of TO534.

In contrast to the results with λcI60, closed circular DNA of λcI60πB was unstable at either 30° or 42° after infection of both dnaB strains. When a culture of TO534 was pre-incubated at 42° for 2 hrs prior to infection, a similar loss in closed circular λπB DNA was observed. Phage DNA synthesis could be detected in cultures of TO534 pre-incubated at 42° for 1 hr prior to infection with λπB, but was not observed in cells pre-incubated for 2 hrs. From these results, it is concluded that in conditions where the dnaB protein has lost the capacity to function in nucleotide polymerization, it is capable of interacting with the λ replication gene products to introduce a break in the phage DNA.

887 INTERACTIONS BETWEEN ORI-C & A MEMBRANE PROTEIN OF E.COLI.
A. Jacq, H. Lother*, M.Kohiyama, & W.Messer*. Institut de Recherche en Biologie Moléculaire - Université Paris 7 & Max Plank Institüt für Molekulare Genetik - Berlin*.

Three proteins (A, B & B') were purified from E.coli membranes. One of them called B' (MW. 65 000) has been shown to attach specifically to early replication region of E.coli (Eur. J. Biochem. in press). Using ori c containning minichromosome pCM 959 (see V.Mayenburg) we have demonstrated that B' protein attaches to this clone with twelve times higher affinity than to coli DNA. This specificity is only seen with heat-denaturated DNA. Detailed analyses on the mechanism of specific attachement of B' protein, using various fragments made by restriction enzymes, have shown that i) two regions, Bam HI - Bam H92 (A) and Xho 417 - Pst 489 (B) are required ii) (A) region is recognized as DNA having 3' end at Bam HI and finally iii) (B) region needs to have 5' end at Xho 417 for specific attachement. (A) region is in ori-C but not (B) region.

Eucaryotic DNA Polymerases and Other Eucaryotic Replication Proteins

888 KINETIC CHARACTERISTICS WHICH DISTINGUISH FORMS OF CALF THYMUS DNA POLYMERASE ALPHA
Robert A. Bambara and Joel W. Hockensmith, Dept. of Biochemistry, Univ. of Rochester, Rochester, N.Y. 14642

DNA Polymerase alpha was isolated according to the technique of Holmes et al., Eur. J. Biochem. 43, 487-499 (1974). This procedure yields five nuclease-free forms of alpha polymerase; A1, A2, B, C, D. Two of these, A1 and C have been studied in detail. Holmes et al., Biochem. Soc. Symp. 42, 17-36 (1977) demonstrated the conversion of form A1 to an enzyme with the physical properties of form C, by removal of a protein subunit. Apparent reconstitution of form A1 could be accomplished by readdition of this subunit to form C. The function of the subunit was not investigated because the A1 and C forms could not be distinguished using biochemical kinetics. We have been able to demonstrate three kinetic differences between the A1 and C forms: 1. If the endonuclease activated DNA is treated with E. coli exonuclease III, a gapped DNA template is produced. The synthetic activity of the A1 form is greater on this gapped template than on the original activated DNA, whereas the activity of the C form does not increase in a similar comparison. 2. The A1 form adds more nucleotides per binding event to activated DNA (is more processive) than does the C form. 3. The synthetic activity of the C form is inhibited by high concentrations of DNA (>400μM), whereas the A1 form is not susceptible to such substrate inhibition. These kinetic differences provide us with a tool for purification of the protein subunit that converts C to A1, and provide a basis for isolation and characterization of other DNA replication associated proteins. Supported by NIH grants GM24441, T32GM07102 and Cancer Center Core Grant 5-P30-CA11198-11.

889 PROPERTIES OF MONOCLONAL ANTIBODIES TO A EUKARYOTIC DNA POLYMERASE,
Alan B. Blumenthal and Ching-Hung Kuo, University of California, San Francisco, San Francisco, CA 94143

Embryos of Drosophila melanogaster contain high concentrations of DNA polymerase. This enzyme activity has been purified to homogeneity and appears to be a single species of DNA polymerase, similar to vertebrate alpha polymerases.

We have used a partially purified (1000 fold) Drosophila DNA polymerase to produce mouse monoclonal antibodies. 60% of the initial cell cultures produced antibodies that reacted with the immunizing antigen. Of these 20% made antibodies that inhibit the in vitro activity of DNA polymerase. Cells producing these inhibitory antibodies are being cloned. Initial experiments will be described which use these antibodies to (1) investigate the roles of the several polypeptides in DNA polymerase; (2) study regulation of DNA synthesis in polytene chromosomes, by in situ localization of DNA polymerase; and (3) detect other proteins that function with the DNA polymerase to replicate DNA.

890 6-(p-n-BUTYLANILINO)URACIL: A SELECTIVE INHIBITOR OF HELA CELL DNA SYNTHESIS AND DNA POLYMERASE ALPHA,
Neal C. Brown, Earl F. Baril and George E. Wright, UMass. Medical School, Worcester, MA 01605 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

6-Anilinouracils are dGTP analogues which selectively inhibit specific bacterial DNA polymerases. The inhibitor mechanism, determined with B.subtilis DNA polymerase (pol) III, involves: (1) base-pairing of the pyrimidine moiety with template cytosine, and (2) binding of the aryl group to a specific aryl site on the enzyme, sequestering the latter in a DNA:drug:protein complex. When a series of analogues was screened against purified DNA polymerases from several sources, the p-n-butyl derivative (BuAU) was found to inhibit HeLa cell pol α with a K_i of approximately 60μM. The inhibitory effect of BuAU was reversed specifically by dGTP, and was selective for pol α. HeLa-specific pol β and γ were not inhibited by BuAU at 1mM. BuAU also was inhibitory in vivo in HeLa cell culture; at 120μM it reversibly inhibited cell division and selectively depressed DNA synthesis by approximately 80%. These studies indicate that BuAU may be a useful probe for studying the structure of mammalian pol α and its putative role in cellular DNA synthesis. (Supported by U.S.P.H.S. Grants CA15915, CA15187, and GM21747).

891 PURIFICATION, SUBUNIT STRUCTURE AND SEROLOGICAL ANALYSIS OF CALF THYMUS RNASE H I, Werner Büsen, M.P.I. für Virusforschung, Tübingen

Calf thymus RNase H I (Büsen and Hausen, E.J.B. 52, 179-190 (1975)) has been 1,000 fold purified to near homogeneity. The purified enzyme sample contains 2 forms of RNase H I, possibly isozymes, named RNase H I 1 and RNase H I 2 with MW s of ~ 80,000, S values of ~ 5 and IEP s of ~ 4.9 under non-denaturing conditions. Each enzyme is composed of three subunits. RNase H I 1 and RNase H I 2 have 2 subunits in common and differ from each other in the third. Subunit models are presented. RNase H I is an endonuclease. The final preparation is free of RNase and endo-DNase activity.

Rabbit antibodies produced against purified RNase H I precipitate the subunits and neutralize the enzyme activity to more than 90 %. The enzyme is predominantly located in the cell nucleus.

RNase H I is a candidate for the excision activity required for the removal of the RNA primers during DNA replication, since its activity rises in parallel with DNA synthesis after stimulation of resting bovine lymph node cells with concanavalin A (Büsen et al., E.J.B. 74, 203-208 (1977)).

892 THE ROLE OF DNA POLYMERASES α AND γ IN THE SYNTHESIS OF ADENOVIRUS DNA, Shih Min Chen and Arthur Weissbach, Roche Institute of Molecular Biology, Nutley, New Jersey 07110

The replication of human adenovirus by a single strand displacement mechanism has been suggested by electron microscope observations and biochemical analysis. In the initial step, only one of the parental strands is copied while the other strand is displaced and replicated in a subsequent step. We are studying the role of DNA polymerase α and γ in this mechanism by taking advantage of relatively specific inhibitors, aphidicolin (for DNA polymerase α) and dideoxynucleoside triphosphates (for DNA polymerase β and γ). At a concentration of 25 μg/ml in the media, aphidicolin inhibits the formation of adenovirus type 5 in HeLa cells by 98%. The incorporation of [^{3}H]-thymidine into the viral DNA is also inhibited up to 70%. Analysis of the physical structure of the viral DNA synthesized in the presence of this drug showed that both replicating forms containing single stranded region and pure single strand viral DNA was accumulated. This data suggest that DNA polymerase α may be involved in the copying of the displaced single stranded adeno DNA.

893 EPSTEIN-BARR VIRUS-INDUCED SEQUENCE SPECIFIC ENDONUCLEASE ISOLATED FROM HUMAN LYMPHOBLASTOID CELLS, Wendy Clough, Molecular Biology, University of Southern California, Los Angeles, California 90007

Epstein-Barr virus (EBV) induces an endonucleolytic activity in virus-producer human B lymphoblastoid cell lines. This virally induced enzymatic activity has been purified from crude cell extracts through DEAE cellulose, phosphocellulose and native DNA cellulose. It has been shown to partially reduce phage lambda DNA to a series of large fragments which are sensitive to digestion by S_1 nuclease. Furthermore this EBV-induced endonuclease shows a high degree of specificity in its digestion of lambda DNA. For example, it preferentially attacks those regions of the lambda genome which are contained in the 2.25 and 2.0 kilobase Hind III fragments. Similar studies are currently being taken with BAM HI, EcoRI and Hpa II treated lambda DNA. We have already shown that the EBV-induced endonuclease will digest lambda DNA (pretreated with a variety of procaryotic restriction enzymes) to produce families of new small fragments of DNA in the 0.1 to 0.9 kilobase size range. In summary the S_1 sensitivity of DNA treated with the EBV-induced endonuclease, the generation of large fragments from lambda DNA and the alteration caused by EBV-induced endonuclease treatment of the banding pattern of lambda DNA previously treated with procaryotic restriction enzymes all indicate that this enzyme has specificity for particular sequences in the lambda genome. Furthermore cuts at these specific sites must lead to digestion of one strand to generate large amounts of single stranded S_1 sensitive DNA. We propose that such enzymatic activities play important roles in the generation of large single stranded regions recently found in replicating DNA isolated from many eucaryotes.

894 VIRAL DNA REPLICATION IN THE PRESENCE OF CONCENTRATIONS OF n-BUTYRATE WHICH INHIBIT CELL DNA SYNTHESIS, Ellen Daniell, Department of Molecular Biology, University of California, Berkeley, CA 94720

Recent experiments have shown that n-butyrate in low concentrations inhibits cell DNA synthesis and cell division in cultured cells, and causes an increase in the level of acetylation of histones (Ref. 1 for example). Both of these effects are readily reversed upon removal of butyrate.

I am studying the replication of human and simian adenoviruses and of SV40 in cells treated with n-butyrate. SV40 normally stimulates cellular DNA and histone synthesis, and uses cellular histones to form its own chromatin. Adenoviruses, in contrast, have histone-like virus coded proteins which complex viral DNA. I have found that both adenovirus and SV40 stimulate DNA synthesis in cells in which DNA synthesis (and histone synthesis) were inhibited by more than 95% at the time of infection. Infection proceeds in the presence of n-butyrate. The production of infectious adenovirus is the same in butyrate treated cells as in untreated cells, but SV40 virus production is reduced by several orders of magnitude.

Our results suggest that n-butyrate acts indirectly to inhibit DNA synthesis, as both adenoviruses and SV40 utilize host DNA polymerase in replication. Work in progress includes characterization of the DNA synthesized in butyrate-treated infected cells as to its viral or cellular origins, and measurement of histone synthesis during this virus-stimulated replication.

1. Hagopian et al. (1977) Cell 12: 855.

895 GAP-FILLING: A UNIQUE STEP IN THE METABOLISM OF OKAZAKI FRAGMENTS THAT REQUIRES BOTH DNA POLYMERASE α AND PROTEIN COFACTORS, David T. Weaver, Hans Krokan and Melvin L. DePamphilis, Department of Biological Chemistry, Harvard Medical School, Boston, MA.02115

DNA polymerase α is required for both continuous and discontinuous DNA synthesis on native replicating chromosomes [Krokan et al., Biochemistry 18:4431(1979)], including incorporation of the final nucleotides on Okazaki fragments (gap-filling). Gap-filling is blocked by aphidicolin which inhibits α-polymerase with no effect on DNA ligase, but not by d_2TTP which inhibits β and γ-polymerases [DePamphilis et al., Cold Spring Harbor Symp. Quant. Biol. 43:679(1979)]. Only those Okazaki fragments awaiting ligation are rapidly joined in the presence of aphidicolin. However, the host (CV-1) α-polymerase alone cannot complete Okazaki fragment synthesis despite the absence of RNA primers in washed nuclei [Anderson and DePamphilis J. Biol. Chem., in press]. This deficiency is not corrected by addition of host α-polymerase and DNA ligase, but is corrected by a cell fraction devoid of DNA polymerase activity. Furthermore, host α-polymerase is unable to fill in randomly placed gaps of 40 ± 5 nucleotides in model compounds or complete Okazaki fragments on purified replicating SV40 DNA as measured by their conversion to DNA ligase substrates. However, gaps of 4 ± 2 nucleotides are completed In contrast, α-polymerases from calf thymus and HeLa cells, as well as CV-1 β and γ-polymerases, HSV-1 DNA polymerase and T4 DNA polymerase, readily complete gaps of all sizes. These data suggest that CV-1 DNA polymerase α looses a cofactor during purification that permits extensive DNA synthesis.

896 RAT LIVER DNA BINDING PROTEINS - PROPERTIES AND PHYSIOLOGICAL VARIATIONS, A.M. de Recondo, C. Bonne and M. Duguet. Institut de Recherches scientifiques sur le Cancer - 94800 Villejuif - FRANCE

A DNA binding protein (25 000 dalton subunit) has been purified to apparent homogeneity from normal or regenerating rat liver by a procedure including a differential DNA cellulose affinity chromatography. The level of this protein seems to be independent of the regeneration process. The protein is present in about 10^6 copies per cell, indicating that its function is rather structural or regulating than catalytic.

The protein purified from regenerating rat liver (HD25) exhibits the main characteristics of a helix destabilizing protein, indeed it depresses the melting point of poly d(A-T) by more than 40°C and stimulates the homologous DNA polymerases α and β. In contrast, the protein from normal rat liver (S25) is inactive in respect to these two properties. Modification that confers functional properties to HD25 seems to be correlated with the occurence of subspecies bearing different electric charges.

897 RNA- AND DNA- POLYMERASES ASSOCIATED WITH SV40 CHROMOSOMES, Howard J. Edenberg, Department of Biochemistry, Indiana University School of Medicine, Indianapolis, Indiana 46223.

We are analyzing the enzymatic composition of SV40 chromosomes which have been extracted in a way that allows continuation of replication in vitro. We have obtained strong evidence that DNA polymerase α is responsible for SV40 DNA synthesis. DNA polymerase α is associated specifically with replicating SV40 chromosomes, and is the only cellular polymerase resistant to the nucleotide analog 2',3'dideoxythymidine-5'triphosphate. Since all measureable aspects of DNA synthesis in vitro are resistant to ddTTP, these data strongly argue that DNA polymerase α is responsible for all SV40 DNA synthesis detected.

Since RNA primers are utilized in SV40 DNA replication, we have looked for the presence of an RNA polymerase activity associated with SV40 chromosomes. Our very low salt extraction yields a transcription complex containing SV40 chromosomes with strongly bound RNA polymerase II. These complexes retain proteins that are lost upon exposure to the high salt previously used to extract transcription complexes, and thus might prove useful in studies on the control of transcription. The bulk of this RNA polymerase activity is not, however, involved in SV40 DNA replication, since DNA synthesis in vitro is not sensitive to α-amanitin. Attempts to detect an RNA primase will be complicated by the presence of these transcription complexes.

898 RAT LIVER MITOCHONDRIAL NICKING-CLOSING ENZYME: PROPERTIES AND PRELIMINARY STUDIES ON ITS ROLE. F. R. Fairfield, M.V. Simpson, and W.R. Bauer, SUNY, Stony Brook, NY 11794.

Recently, we have isolated a DNA nicking-closing (N-C) enzyme from mitochondria (J. Biol. Chem. 254, 9352 (1979)). It is distinguished from the rat nuclear enzyme and other N-C enzymes that we have tested by its sensitivity to ethidium bromide and the trypanocidal drug Berenil and by its severe instability to handling and storage. We now report further properties. The enzyme is membrane bound as suggested by the requirement for detergent extraction and the amount of enzyme remaining in the membrane fraction after extraction. Substitution of variant chromatographic purification procedures, when effective, always lead to a Berenil and ethidium bromide sensitive enzyme. The purified enzyme is sensitive to NEM, to the specific chymotrypsin inhibitor, TPCK, and, reversibly,to DMSO which converts it to a nuclease. Five analogs of Berenil show a range of inhibition from 10 times to 0.01 times that of Berenil. The enzyme relaxes positively superhelical pSM1 DNA, whose positive supercoils were generated by salt effects (Anderson and Bauer, Biochemistry 17, 594 (1978)) rather than by ethidium bromide, which inhibits the enzyme. Berenil could prove to be an important tool in the elucidation of the locus of action of the N-C enzyme in mitochondria. Preliminary experiments using isolated intact mitochondria, previously shown to carry out replicative DNA synthesis, demonstrate that this drug, in the same concentration range used to inhibit the purified enzyme, inhibits the replication of all forms of mitochondrial DNA. These results suggest that the enzyme acts at more than one point on the replication pathway. (Supported by NIH grant 22333 (to MVS) and 21176 (to WRB) and Amer. Can. Soc. grant NP87K (to MVS)).

899 ENZYMOLOGIC MECHANISM OF HOMOGENEOUS KB CELL DNA POLYMERASE α, Paul A. Fisher, Jane T. Chen and David Korn, Stanford University School of Medicine, Stanford, CA 94305

The catalytic properties of a homogeneous preparation of human KB cell DNA polymerase α have been studied in detail. With respect to the enzyme's interaction with DNA, we have identified two kinetically distinguishable classes of sites on the enzyme for the binding of nucleic acid substrates. One of these is specific for single-stranded DNA (template site) while the second recognizes 3'-hydroxyl termini (primer site). Template binding is not dependent on the participation of 3'-hydroxyl termini. In contrast, the primer site appears to be able to bind a 3'-hydroxyl terminus only when the template site is occupied, and such binding is restricted to 3'-terminal residues that can base-pair with the template. Our data indicate that only minimal terminal complementarity (2 to 5 base pairs) is required. Spermidine is a specific effector of the primer-binding event and acts to destabilize the polymerase-DNA complex in such a way as to increase dramatically both the apparent K_m for primer-template and the maximal velocity (V_{max}) of the polymerization reaction. Kinetic data suggest that there are two template binding sites on each molecule of DNA polymerase α and that these sites interact cooperatively (positively) by a simple allosteric mechanism. Evidence has also been obtained for two strongly cooperative primer-binding sites per molecule of enzyme. Investigations are currently underway regarding possible sequence specificity of the template binding site.

(These studies were supported by Grant CA14835 and Training Grants GM01922, CA09151 and CA09302).

900 THE ADENOVIRUS-SPECIFIC DNA BINDING PROTEIN INHIBITS THE HYDROLYSIS OF DNA BY DNase IN VITRO. Gerald D. Frenkel and Kathryn Nass, N.Y. State Dept. of Health, Div. of Laboratories and Research, Albany, N.Y. 12201.

Adenovirus DNA replicates by a displacement mechanism which results in the presence of extensive single-stranded regions in the replicating DNA molecules. An adenovirus-coded early protein has been shown to be a single-strand specific DNA binding protein (DBP). Analysis of a temperature sensitive mutant of Ad5 (ts125) has shown that this protein is essential for viral DNA replication, but has not, as yet, demonstrated the actual role of the DBP in this process. We have previously shown that after infection by Ad5, there is a progressive decrease in detectable DNase activity in the cell, and that this decrease does not occur after infection with ts125 at the non-permissive temperature (J.Virol.26:540,1978). Based upon these observations, we suggested that at least one role of the DNA binding protein in viral DNA replication may be in protecting the single-stranded regions of the replicating molecules from degradation by DNase. We have now isolated the DBP and have shown that it is in fact able to inhibit the hydrolysis of single-stranded DNA by DNase in vitro. This inhibition represents a decrease in the rate of DNA hydrolysis, proportional to the amount of DBP added. The most likely mechanism is a decrease in the effective substrate concentration for the DNase as a result of the binding of the DBP to the DNA. The inhibitor activity of the viral DBP can be distinguished from that of the cellular DBP which we described previously (J.Biol.Chem. 254:3407,1979) by the differential effect of temperature on the two activities.

901 DNA POLYMERASE OF EPSTEIN-BARR VIRUS, Dario Grossberger and Wendy G. Clough, University of Southern California, Los Angeles, CA 90007

The DNA polymerase induced by Epstein-Barr Virus (EBV) in P3-HRI cells has been further purified on denatured DNA-cellulose. This purification separates an apparently singular phosphocellulose peak into three peaks, one of which is found to possess a 3'→5' exonuclease activity like that of procaryotic DNA polymerases. ^{3}HdATP or ^{3}HdTTP are turned over to monophosphates in a poly d[A-T]-dependent manner, and roughly equal amounts of triphosphate are incorporated and turned over, suggesting a very active exonuclease.
Phosphonoacetic acid (PAA), a specific inhibitor of herpesvirus DNA polymerases, is found to differentially inhibit the DEAE, phosphocellulose and denatured DNA-cellulose purified DNA polymerase. The least pure DEAE fractions are found to be more sensitive to PAA than the denatured DNA-cellulose fractions, which are rather insensitive. Since EBV is very sensitive to PAA in vivo it is suggested that some other components of EBV replication confer PAA sensitivity and are being removed through purification. The differential sensitivity is observed only with a low DNA concentration.

902 DIADENOSINE TETRAPHOSPHATE - A LIGAND OF DNA POLYMERASE α AND TRIGGER OF DNA REPLICATION, Friedrich Grummt, Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, Germany

Diadenosine tetraphosphate (Ap_4A) induces replicative DNA synthesis in quiescent mammalian cells. By equilibrium dialysis an Ap_4A binding activity is shown to be present in mammalian cells. The Ap_4A binding activity co-purifies with DNA polymerase α during the isolation procedure comprising chromatography on phospho-, DEAE- and DNA-cellulose, gel filtration, sucrose gradient centrifugation and electrophoresis in non-denaturing polyacrylamide gels. After these purification steps DNA polymerase α appears as a homogeneous protein complex in non-denaturing polyacrylamide gels with an apparent M_r of 413 000, consisting of seven different subunits with apparent M_r of 64 000, 63 000, 62 000, 60 000, 57 000, 55 000 and 52 000. By affinity labeling the protein with M_r of 57 000 has been shown to be the Ap_4A binding constituent of DNA polymerase α. The Ap_4A binding site is lost in neuronal cells during maturation of rat brains concomitantly with the loss of DNA polymerase α and the mitotic activity in those cells. From these results DNA polymerase α seems to be the intracellular target of Ap_4A. For the elucidation of the mechanism of Ap_4A action during DNA synthesis a structural analog has been synthesized in this laboratory. This analog, methylene-bis-ADP, has been shown to act as an antagonist to Ap_4A during binding to DNA polymerase α as well as in DNA replication in vivo and in vitro, and inhibits cell proliferation.

903 PURIFICATION AND CHARACTERIZATION OF, AND FLUORESCENCE SPOT TEST FOR, DNA NICKING-CLOSING ENZYME FROM BOVINE LIVER, David Kowalski, James V. Tricoli and Janet P. Sanford, Roswell Park Mem. Inst., Buffalo, NY14263

DNA nicking-closing enzyme has been purified ∿500 fold from nuclei of calf liver. Purification was facilitated by a spot test for screening many samples for activity. The relaxed DNA formed is not cleaved by single-strand specific endonuclease while the superhelical DNA is. Only the relaxed DNA renatures after heating at pH ∿12 and cooling and so enhances the fluorescence of ethidium bromide. Microdroplets containing 20 ng DNA are spotted on Saran Wrap atop a shortwave UV lamp. Spots which fluoresce indicate the presence of activity in the sample assayed. The purification procedure employs sonication of 1M KCl-treated nuclei, high speed centrifugation, polyethylene glycol treatment (Fr. III), and elution from columns of hydroxylapatite and Matrix Gel Blue (Fr. V). Passage of Fr. V through a column of Sephadex G-150 yields one major peak of protein (M_r range 70K-180K) with no change in specific activity. SDS-PAGE of the reduced protein shows three major bands (M_r range 68K-80K). Experiments to determine which band or bands correspond to the activity are in progress. The high M_r of the native vs. the denatured proteins suggest either a subunit structure or an asymmetric conformation for the native enzyme. Fr. III activity is inhibited by N-ethyl maleimide, potassium phosphate, novobiocin, and ATP. Other ribo- and deoxyribonucleoside triphosphates and nalidixic acid do not inhibit.

904 EUCARYOTIC DNA POLYMERASE-α : EVIDENCE FOR TWO STRUCTURAL FORMS.

Marcel Méchali, and Anne-Marie de Recondo - Institut de Recherches Scientifiques sur le Cancer, B.P. N°8, 94800 - Villejuif, France.

The DNA polymerase-α from regenerating rat liver has been purified to homogeneity (J. Biol. Chem., in press). The purified enzyme gave a single stained band correlating with DNA polymerase-α activity on native polyacrylamide gel electrophoresis. On pore gradient gel electrophoresis, a molecular radius of 72 Å was obtained for the enzyme. Under denaturing conditions, five polypeptides with molecular weights 156,000, 64,000, 61,000, 58,000, and 54,000 daltons, were resolved.

The 156,000-dalton polypeptide correlated with the catalytic unit which can be purified to homogeneity. The 54,000 - to 64,000 polypeptides interacted among themselves to constitute a hetero-oligomer of high molecular weight, without DNA polymerase activity. Electron microscopy studies confirmed the biochemical result.

The specific activity of the catalytic unit was to six-fold enhanced when it was associated with the 54,000- to 64,000- dalton structure, to form a putative DNA polymerase-α holoenzyme. Thermal denaturation studies, binding to DNA, and competition experiments, suggest a role of the (54,000 - 64,000) unit in the stabilisation of the catalytic unit to the template-primer.

905 INTERACTION OF A BIDIRECTIONAL, DOUBLE-STRAND SPECIFIC EXONUCLEASE (DNASE V) WITH DNA POLYMERASE-β FROM THE NOVIKOFF HEPATOMA, Ralph R. Meyer and Dale W. Mosbaugh, University of Cincinnati, Cincinnati, OH 45221.

Exonucleolytic excision is an essential step in DNA replication and repair. While prokaryotic DNA polymerases commonly contain exonuclease as an integral part of the polymerase, mammalian enzymes generally do not, suggesting these functions are carried out by separate interacting polypeptides. We have identified such an exonuclease from the Novikoff hepatoma. The enzyme physically interacts with the β-polymerase in a 1:1 stoichiometry. This nuclease, purified to homogeneity, has a molecular weight of 12,500, is specific for double-stranded DNA and yields 5'-dNMP products. It cleaves hydroxyl- or phosphoryl-terminated polynucleotides in both 3'→5' and 5'→3' directions at equal rates and can remove a single terminally mismatched base from the 3' end; it cannot excise pyrimidine dimers from uv-irradiated DNA and lacks RNase-H activity. Since these properties distinguish it from all bidirectional prokaryotic exonucleases and from all other mammalian exonucleases, we have designated this enzyme as mammalian DNase-V. (This work was supported by NIH grants CA-17723 and American Cancer Society grant NP-277.)

906 STUDIES ON DNA REPLICATION OF SV40 CHROMATIN IN VITRO, Bernd Otto and Arndt Richter, University of Konstanz, D-7750 Konstanz FRG

As a model system for mammalian DNA replication we use the minichromosome of simian virus 40 (SV40). SV40 chromatin isolated from nuclei of SV40 infected monkey cells was fractionated by sucrose gradient centrifugation into replicating and nonreplicating chromatin. DNA synthesis in replicating chromatin was stimulated by highly concentrated protein extracts regardless of whether these extracts were prepared from SV40 infected cells or noninfected growing cells. However DNA synthesis in nonreplicating chromatin was 3-10 fold higher in the presence of protein extracts from infected than from noninfected cells suggesting that this typ of DNA synthesis depends on a viral protein. We will discuss the dependence of this DNA synthesis on low molecular weight factors and the effect of antiserum against highly purified T-Antigen on DNA synthesis in non-replicating chromatin.

907 DNA BINDING AND STRUCTURE OF MOUSE HELIX-DESTABILIZING PROTEIN-1, Stephen R. Planck and Samuel H. Wilson, National Cancer Institute, NIH, Bethesda, MD 20205

A helix-destabilizing protein (HD protein-1) that shares many properties with calf thymus HD protein-1 has been purified from mouse myeloma. The HD protein had higher affinity for ssDNA-cellulose than dsDNA-cellulose and was resistant to elution from ssDNA cellulose by dextran sulfate. This protein was heterogeneous with regard to apparent molecular weight; individual species were obtained in near homogeneous form by isoelectric focusing. The protein is a helix-destabilizing protein, since it lowered the Tm of poly[d(A-T)] by more than 20°C. Binding to ssDNA is non-cooperative and the binding site size on ssDNA is about 6 nucleotide residues as indicated by sedimentation boundary analysis. Subcellular localization studies suggest that mouse HD protein-1 is nuclear. HD protein undergoes a conformational change upon binding to ssDNA. This was revealed by a change in intrinsic tryptophan fluorescence and also by a change in sensitivity to trypsin. Mild trypsinization of native HD protein-1 (Mr=27,000) produces discrete fragments with Mr ranging from 19,000 to 24,000 and whose relative abundance is changed by the presence of DNA during the digestion. Thus, a 22,000-Mr fragment (22 HDP*) predominated in the absence of ssDNA and a 19,000-Mr fragment (19 HDP*) predominated in the presence of ssDNA. Poly d(T) and denatured calf thymus DNA were more effective than other polynucleotides tested in promoting accumulation of 19 HDP*; $d(T)_8$ was as effective as longer molecules of $d(T)_n$, but $d(T)_4$ and $d(T)_6$ were much less effective. Both 19 HDP* and 22 HDP* have the same N-terminal end and the same affinity for ssDNA-cellulose as the native HD protein-1, indicating that a 8,000-Mr sequence at the C-terminal end is not required for binding to ssDNA. (NIH grant F32 CA05569)

908 STRUCTURE OF A HIGH MOLECULAR WEIGHT DNA POLYMERASE FROM DROSOPHILA EMBRYOS, Brian Sauer, Giuseppe Villani and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

The DNA polymerase of early embryos of Drosophila melanogaster has been purified to near homogeneity [Banks, G. R., Boezi, J. A. and Lehman, I. R. (1979) J. Biol. Chem. 254, 9886]. The purified enzyme gives a single, catalytically active protein band after polyacrylamide gel electrophoresis under nondenaturing conditions. Four polypeptides with molecular weights of 43,000, 46,000, 58,000 and 148,000 are resolved when this single band is electrophoresed on a SDS polyacrylamide gel. At high ionic strengths the DNA polymerase has a sedimentation coefficient of 8.7S, a Stokes radius of 78Å and a frictional ratio of 1.81, parameters that yield a molecular weight of 280,000. Because it is inhibited by low concentrations of N-ethylmaleimide and Aphidicolin, this Drosophila DNA polymerase most closely resembles the α DNA polymerases purified from mammalian cells.

The four bands displayed by this DNA polymerase upon electrophoresis in the presence of SDS appear to be structurally distinct from one another, as demonstrated by limited proteolytic digestion of each of these bands. Thus, the complexity of this enzyme does not appear to derive from a generalized proteolysis during purification. Further analysis of the structure of the enzyme using rabbit antiserum and monoclonal antibodies is under way.

909 SIMIAN VIRUS 40 DNA-DEPENDENT SIMIAN VIRUS 40 DNA SYNTHESIS IN VITRO, Chikako Sumida-Yasumoto, Laboratory of Molecular Virology, National Cancer Institute, N.I.H. Bethesda, Maryland 20205

A cell-free system which catalyzes simian virus 40 (SV40) DNA replciation has been developed from African green monkey kidney cells infected with SV40. DNA synthesis depends on exogenously added SV40 DNA, ATP and the four deoxyribonucleoside triphosphates. Sedimentation analysis of DNA products labeled in vitro showed the products to be SV40 form I and form II DNA. This SV40 DNA-dependent SV40 DNA replication also seems to depend on the product of A gene of SV40.

910 PURIFIED DNA POLYMERASE ALPHA MAKES SHORT DNA PIECES, Samuel H. Wilson and Sevilla D. Detera

We have determined the size of DNA products formed by purified mouse α-polymerase after the enzyme initiates on a model template-primer with a long stretch of single-stranded template. The model replication systems studied were: oligo r(A) initiated poly d(T) replication, RNA initiated denatured calf thymus DNA replication, and restriction fragment initiated ϕX174 DNA replication. The results of RNA:DNA joint analysis and polyacrylamide gel electrophoresis indicate that essentially all of the DNA molecules formed were oligonucleotides 10 to 20 residues in length. These results are explained by a model in which α-polymerase is highly processive during formation of each short DNA molecule.

911 ACTIVATION OF THE DROSOPHILA EMBRYO DNA POLYMERASE BY PROTEOLYTIC CLEAVAGE, Christine L. Brakel, New York University, New York, N.Y.10003

The Drosophila melanogaster embryo DNA polymerase can be isolated in distinct forms that differ in chromatographic and electrophoretic behaviors, isoelectric points, molecular weights, and subunit compositions. Studies suggest that the multiple forms arise from a common precursor as a result of proteolytic events and because of subunit loss or dissociation, which might also result from proteolysis. In vitro trypsinization of the largest (9.0S) enzyme form results in the production of smaller (7.3S and 5.5S) enzyme forms which are similar in structure and activity to those produced by endogenous proteases. When the trypsin treated preparations are fractionated by sedimentation, chromatography or electrophoresis, 2 to 5 times more enzyme activity is recovered than in untreated preparations whereas when activity is tested after treatment but prior to fractionation, 50-100% of the activity is lost. If trypsinization is carried out in the presence of appropriate template-primers, the initial rate as well as the extent of polymerization are increased. The site of proteolytic cleavage that results in the altered kinetics may reside in the largest (160,000 or 140,000 dalton) subunit of the 9.0S enzyme form. These results suggest that portions of the enzyme modulate the polymerizing activity of the enzyme and the proteolytic conversion may have significance in the regulation of DNA replication in vivo.

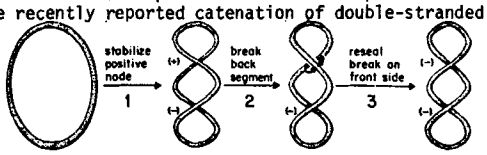
DNA-Dependent ATPases

912 DNA TRANSLOCASE REQUIRED FOR DNA PACKAGING INTO THE T4 CAPSID
Lindsay W. Black & Veeraswamy Manne, Dept. Biochemistry, U. of Maryland Med. Sch., Baltimore, Md., 21201

Two phage T4 late gene products(gp16 and gp17) are known to be directly required for DNA packaging, apparently in association with a third(gp20), a structural protein situated at the neck of the capsid. We have isolated phage T4 mutants which overproduce gp16 and gp17, and have identified these nonstructural proteins. A DNA dependent ATPase, absent from gene 16^-17^-, 16^-, and maturation gene defective mutant infections, has been isolated. Activity of the highly purified ATPase(also dATP, CTP, dCTP&UTPase) is completely dependent upon addition of DNA, and is inhibited by Ethidium Bromide and 9-aminoacridine, the latter compound a known specific inhibitor of T4 DNA packaging in vivo. Activity is observed on various DNAs(e.g. CT, T4, Lambda, Adeno, and ColE1), with dsDNAs about 10-20% as active as denatured DNAs. Although limited double stranded breaks are induced by the enzyme preparation under some assay conditions, covalent changes in the DNA under ATPase conditions appear minimal. This packaging gene-associated activity supports the model we proposed some time ago on the basis of genetic results, that T4 DNA condensation into the empty head occurs by an enzymatic translocation of DNA.

913 THE SIGN INVERSION MECHANISM FOR DNA SUPERCOILING, Patrick O. Brown and Nicholas R. Cozzarelli, The University of Chicago, Chicago, IL 60637

Both the introduction and removal of supercoils by DNA gyrase change the linking number of DNA in steps of two. This observation provides strong evidence that gyrase acts by a mechanism we call sign inversion which is illustrated below and consists of the following steps. Step 1: Gyrase binds to a DNA molecule at two points such that the two bound segments cross to form a right-handed node (the upper of the two nodes in the figure). This is sufficient to stabilize a positive supercoil and induce a counterposing negative supercoil (represented by the lower node). Step 2: Gyrase introduces a double-strand break in the DNA at the back of the right-handed node and passes the front segment through the break, inverting the handedness and thus the sign of the node. Step 3: The break is resealed on the front side of the now left-handed node. The net result of this procedure is to reduce the linking number by two. The reverse process, inversion of an initially left-handed node, would be the path by which gyrase relaxes negatively supercoiled DNA. Our model provides a natural explanation for the double-strand cleavage of DNA and the recently reported catenation of double-stranded DNA circles by gyrase. It may apply generally to topoisomerases that make a transient double-strand break. We present the evidence for sign inversion and discuss some implications of the model and our recent work on the details of its operation.



914 RecA-DEPENDENT INTERACTIONS BETWEEN DUPLEX DNA MOLECULES, Era Cassuto, Steve West and Paul Howard-Flanders, Yale University, New Haven, Ct. 06520

Purified recA protein has been shown to promote pairing of duplex DNA with homologous single-stranded fragments. However, single-stranded fragments are not normally found in cells and are not a likely substrate for homologous pairing in vivo. We found that recA protein can also promote interactions between duplex DNA molecules, producing a highly complex structure which is stable in the presence of detergents. The reaction requires ATP, the presence of small single-stranded regions (gaps) in some of the molecules, and genetic homology. The formation of the DNA complex is not associated with nicking of the intact DNA molecules, indicating that recA protein alone is not sufficient for the resolution of the intermediate structure into recombined DNA molecules.

915 DNA-DEPENDENT ATPase FROM REGENERATING RAT LIVER, Michel Duguet and Anne-Marie De Recondo, Institut de Recherches sur le Cancer 94800 Villejuif, FRANCE.

A DNA-dependent ATPase has been isolated from regenerating rat liver using phosphocellulose and DNA cellulose chromatographies. The enzyme degradates ATP in the presence of single-stranded DNA and to a smaller extent in the presence of poly(dT) or poly(dA). The km value for ATP is about 0.1 mM. The activity of the enzyme requires magnesium ions (2 mM).

916 A POLY(dT) STIMULATED ATPase ACTIVITY ASSOCIATED WITH SV40 LARGE TUMOR (T) ANTIGEN, Don Giacherio and Lowell P. Hager, University of Illinois, Urbana, Ill. 61801

Genetic evidence indicates that large T antigen, the A gene product of SV40, is required for the initiation of viral replication and the initiation and maintenance of cellular transformation. Highly purified preparations of large T antigen exhibit an ATPase activity which can be stimulated approximately seven to ten fold by the DNA homopolymer poly(dT). We have characterized this activity with respect to substrate specificity, ion requirements, stimulation by other polynucleotides, and the effect of chain length of poly(dT) on the level of stimulation of the ATPase activity.

Large T antigen has previously been shown to bind to both single and double stranded DNA and to specific regions on the SV40 genome. Studies using sedimentation analysis on glycerol gradients and filter binding competition experiments reveal that T antigen does bind to poly(dT). However, the ATPase stimulating ability of poly(dT) can not be explained simply on the basis of binding affinity. While both single and double stranded calf thymus DNA yield only low level stimulation of the T antigen ATPase activity, T antigen binds much more efficiently to single strand DNA than to poly(dT) or double strand calf thymus DNA.

A possible model for the role of T antigen in the initiation of SV40 DNA replication might involve specific binding at the origin of replication and then utilization of ATP hydrolysis to create and maintain strand separation in this localized region. This would create a new site for cellular polymerases and replication enzymes to bind and initiate replication.

917 STRUCTURAL AND FUNCTIONAL STUDIES OF T4 GENE 32 HELIX-DESTABILIZING PROTEIN BY LIMITED PROTEOLYSIS, Junko Hosoda*, Rae L. Burke*, Herbert Moise*, Akira Tsugita†, and Bruce Alberts†, *Lawrence Berkeley Lab., University of Calif., Berkeley, CA 94720, Medical School, University of Calif., San Francisco CA 94143 and †EMBO, Heidelberg, West Germany

Two domains of the T4 gene 32 helix-destabilizing protein are readily removed by limited proteolysis: 1) the acidic A region composed of 55 to 60 amino acids at the carboxyl terminus and 2) the basic B region composed of 21 amino acids at the amino terminus. The amino acid sequences of these two regions were determined. Loss of the A region is accompanied by 1) a greatly increased helix-destabilizing potential, 2) an apparent increase in affinity for ds-DNA and for 3) ss-DNA, 4) loss of affinity for T4 DNA polymerase (43 protein) and 5) for RNA-priming protein (61 protein). Loss of the B region is accompanied by a loss in 1) self-association and 2) cooperative tight binding to ss-DNA.

We have used these discrete fragments of 32 protein to probe its essential role in the in vitro T4 replication system. 32*I protein (minus A region) stimulates DNA replication catalized by DNA polymerase (43 protein) plus polymerase accessary protein complex (44/62 and 45 protein) on both nicked ds-DNA and primed ss-DNA templates, even more efficiently than intact 32 protein. 32*I, however, strongly inhibits 1) primer pentaribonucleotide synthesis by 41 and 61 proteins, 2) primer utilization by the replication complex and 3) DNA synthesis by polymerase alone.

Based upon these results models of 32 protein interactions with DNA and proteins in the replication complex are presented.

918 INVOLVEMENT OF BASIC AMINO ACID RESIDUES IN THE BINDING OF A HELIX-DESTABILIZING PROTEIN TO SINGLE-STRANDED DNA, David J. Merkler, Richard L. Karpel and Brian K. Flowers, Dept. of Chemistry, University of Maryland Baltimore Co., Catonsville, MD 21228. At low ionic strength, ribonuclease A lowers the Tm of DNA helices (Jensen, et al, JBC 251, 7215 (1976)). Since the 3D structure of RNase in known to high resolution, this protein could serve as a model for understanding the molecular basis of the selective recognition of single-strands by helix-destabilizing proteins. The Tm depressing activity of RNase is strongly inhibited by Na^+, suggesting that ionic interactions, hence basic amino acids, play a role in the interaction. Thus, whereas unmodified RNase lowers Tm of polyd(A-T) by 20°C or more, modification of arginines by phenylglyoxalation leads to a Tm depression of only $\sim$ 5°C. Modification of lysines indicates that several of these residues are also sites of interaction with DNA. Maleylation of lysines, leading to negatively charged derivatives, destroys the Tm depressing activity. Although reductive alkylation of these residues generally has only a slight effect, isopropylation halves the Tm depression. In the presence of excess single-stranded DNA, methylation with labeled formaldehyde followed by $NaBH_4$ reduction yields a significantly lower level of labeling relative to that obtained in the absence of DNA. When RNase is methylated with $^3HC^3HO$ in the presence of protecting single-stranded DNA, and then subjected to methylation with $H^{14}CHO$ under conditions (0.1MNa^+) where the protein is no longer bound to the DNA, the resulting $^{14}C/^3H$ activity is as much as four times higher than the same experiment performed in the absence of DNA. We are presently attempting to identify the particular lysine residues protected against methylation (Supported by NIH Grant CA 21374).

919 DETAILED MAPPING OF THE DNA-DNA GYRASE INTERACTION, Karla Kirkegaard and James C. Wang, Harvard University, Cambridge, MA 02138.
In order to investigate the binding of DNA gyrase to DNA at the sequence level, unique complexes have been formed between Micrococcus luteus DNA gyrase and DNA fragments of known sequence. Fragments of pBR322 DNA have been isolated which are preferentially retained on glass fiber filters by the binding of gyrase. Staphylococcal nuclease digestion of complexes formed between gyrase and these fragments yields characteristic 143 base pair subfragments; protected DNA of this length has been previously observed with random double-stranded DNA gyrase complexes. The 143 base pair nuclease protected regions map to specific sites on the original restriction fragments. This localization of unique gyrase binding sites permits the application of sequencing techniques to investigate the topography of the gyrase-DNA complex and the effects of ATP and inhibitors on this interaction.

920 "Gyrasomes", Physical Characterization of a Unique Gyrase-DNA Complex, Leonard Klevan and James C. Wang, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138
The enzyme, DNA gyrase, forms a stable complex with DNA which, when digested with micrococcal nuclease, protects a 140 base pair fragment of DNA. If nicked circular DNA is ligated in the presence of gyrase, a supercoiled DNA substrate results upon removal of the protein (Liu and Wang, Cell 15, 979(1978)). These results suggest that the mechanism of gyrase action may involve a wrapping or coiling of DNA around the protein core as in the case of histone-DNA complexes. Recent advances in the purification procedure of M. luteus gyrase have resulted in the isolation of a stable Gyrase-140 base pair DNA particle containing equimolar ratios of the two gyrase subunits. The protein-protein and protein-DNA interactions in the "gyrasome" particle are being investigated by several techniques including sedimentation, electrophoresis, circular dichroism and nuclease digestion. Furthermore, the subunit structure of DNA gyrase is being probed by chemical crosslinking reagents. A tetrameric complex of the form A_2B_2 has been demonstrated for the enzyme.

921 KINETICS OF THE INTERACTION OF T4 CODED GENE 32-PROTEIN WITH SINGLE-STRANDED NUCLEIC ACIDS, Stephen C. Kowalczykowski and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The kinetics of association and dissociation of gene 32-protein (G32P) have been studied in order to determine whether the protein can move along the DNA by some facilitated translocation mechanism (e.g., "sliding") in addition to undergoing macroscopic dissociation and reassociation. The kinetic measurements obtained, together with general considerations, suggest that some form of protein sliding is necessary in order to maintain G32P saturation of the single-stranded DNA sequences produced during DNA synthesis. Evidence will be presented which suggests that G32P is capable of at least limited mobility while bound to single-stranded DNA, and the implications of this finding for possible models of the kinetics of DNA replication fork structure and movement will be considered. [Supported in part by USPHS grant GM-15792 to PHvH, and American Cancer Society Postdoctoral Fellowship (PF-1301) to SCK.]

922 FORMATION AND RESOLUTION OF CATENANES BY DNA GYRASE, Kenneth N. Kreuzer, Mark A. Krasnow, and Nicholas R. Cozzarelli, University of Chicago, Chicago, IL 60637

We have discovered that DNA gyrase can efficiently catenate and decatenate duplex DNA rings. Both reactions require gyrase subunits A and B, ATP, Mg^{++}, and spermidine, and are inhibited by the gyrase antagonists novobiocin and oxolinic acid. The sharp ionic strength optimum for catenation explains the failure to observe the reaction previously. Since hybrid catenanes were produced between ColE1 and ØX174 DNA, sequence homology is not required; the homologous reaction however is apparently preferred. A native DNA substrate is converted primarily into dimer and trimer catenanes. Nicked DNA was a poor substrate even in the presence of native DNA, suggesting that a superhelical tertiary structure is strongly preferred for both participating DNA molecules. The most surprising result was that relaxed substrate was converted into vast interlocked networks of DNA with a maximum molecular weight of about 10^{11}. These networks may be formed by processive catenation. After treatment of relaxed DNA with gyrase, we have also observed knotted DNA molecules, as had been found with T4 topoisomerase products by Liu, Liu, and Alberts (personal communication). All the reactions catalyzed by DNA gyrase will be explained by a unitary mechanism.

These newly-discovered reactions of DNA gyrase provide direct evidence that gyrase makes a double-stranded break, passes duplex DNA through the break, and then reseals it in an efficient, concerted reaction. These results also suggest possible new physiological roles for DNA gyrase, e.g., the resolution of catenanes arising from replication and/or recombination. Finally, almost unlimited amounts of catenanes can now be prepared for physical and biological studies, and catenanes can be formed for the first time from unrelated monomers.

923

CONSTRUCTION, PROPERTIES AND SEQUENCE OF A HYBRID PLASMID CONTAINING BACTERIOPHAGE T4D DNA BINDING PROTEIN (GENE 32)

Krisch, H.M., B. Allet and R. Duvoisin, Dept of Molecular Biology, University of Geneva, CH - 1211-Genève-4 (Switzerland).

The gene 32 product has a central role in viral DNA replication, recombination, and repair. Control of its expression is unusual, being self-regulatory at the level of translation. Difficulties encountered in isolating a hybrid plasmid containing gene 32 were probably due to expression of this gene which was lethal or deleterious to the host cells. Utilizing a non-sense mutation in gene 32 (32amA453) and a restriction enzyme which cuts T4 DNA frequently, we have obtained a chimeric plasmid which contains gene 32 (pKSK12). The T4 insert in this hybrid has been defined genetically as extending from the middle of gene 33, covering gene 59 completely and going beyond gene 32. The plasmid containing cells complement phage mutant in gene 59. As expected, the complentation of gene 32 mutants is poor because the non-sense mutation is weakly suppressed by the host cell. Using antibody against gene 32 protein, we have demonstrated gene 32 expression in plasmid containing cells. This expression is increased 5 to 10 fold when the cells are shifted to 42°C. We have sequenced plasmid DNA covering the first 40% of the gene 32 as well as 65 base pairs immediately preceding the initiation codon.

924 THE ASSOCIATION AND DISSOCIATION KINETICS OF T4 HELIX DESTABILIZING PROTEIN-SINGLE STRANDED NUCLEIC ACID INTERACTIONS, Timothy M. Lohman*, Department of Chemistry, University of California, San Diego, La Jolla, CA 92037

Both the association and dissociation kinetics of the T4 helix destabilizing protein (T4HDP)-single stranded nucleic acid interaction have been investigated using fluorescence stopped-flow methods. The association kinetics of binding to isolated sites indicate that the reaction is not a single bimolecular process, but involves an intermediate. Furthermore, an extremely slow process has been observed which is likely to reflect the formation of contiguously bound T4HDP clusters. This very slow redistribution poses severe restrictions as to how T4HDP is able to move with the replication fork. The dissociation kinetics have shown a dramatic dependence of dissociation rate on both sugar and base type as follows in order of increasing complex lifetime: poly rA < poly rU < poly dA < poly dC << S.S.M13DNA < poly dT. This "specificity" is the same as that observed by Newport, Lonberg, Kowalczykowski and von Hippel (in press) in equilibrium experiments. The dissociation kinetics also indicate that there is only limited "sliding" of the T4HDP along a single-stranded lattice and hence redistribution should be slow. [This research has been supported by grants to Dr. Bruno Zimm from NIH (GM-11916) and the American Cancer Society (NP-150) and an NIH postdoctoral fellowship (GM-07272) to TML.]

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925 INTERACTIONS OF DNA GYRASE WITH DNA, Alan Morrison and Nicholas R. Cozzarelli, University of Chicago, Chicago, IL 60637

Escherichia coli DNA gyrase makes transient, double-strand breaks in DNA during the normal supertwisting cycle. Treatment of gyrase with the inhibitor oxolinic acid and a protein denaturant fixes the broken DNA intermediate, thus providing a means of studying gyrase mechanism. Cleavage creates protruding 5'-DNA termini that remain covalently attached to gyrase. The covalent attachment preserves the energy released from DNA breakage so that resealing does not require an exogenous energy source. Unexpectedly for a topoisomerase, cleavage occurs at discrete locations. However, no fixed DNA sequence dictates the sites of cleavage. Different cleavages have variable but similar, asymmetric DNA sequences with a generally constant TG core sequence. Gyrase binds tightly to DNA and protects from nuclease attack an extensive region of DNA that asymmetrically surrounds the cleavage site. ATP is the necessary cofactor for supercoiling. ATP-binding induces a conformational change in gyrase that drives the mechanical movement required for each supercoiling cycle. This conformational change is mirrored by a change in the proportion of enzyme in the cleaving mode. However, the gross region of DNA bound by gyrase is not changed by ATP or by oxolinic acid. Gyrase binds stably to relaxed DNA but not to negatively supercoiled DNA. This stability differential allows gyrase to turn over. Thus, addition of ATP to the complex between gyrase and relaxed, circular DNA triggers processive rounds of supercoiling which in turn lead to rapid release of the enzyme.

926 CHARACTERIZATION OF PROCESSIVE DNA SYNTHESIS: A MODEL SYSTEM FOR "DNA-WALKING" PROTEINS, John W. Newport and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

Many DNA polymerases synthesize DNA "processively"; i.e., each time a polymerase binds to a primer it incorporates more than one nucleotide residue prior to dissociation. We have developed a simple homopolymeric assay for measuring polymerase processivity under different conditions. Using this procedure we have shown that the number of nucleotide residues incorporated, per association, decreases exponentially with the length of the extended primer for both E. coli DNA polymerase I, and for T4 DNA polymerase. This behavior suggests that polymerases incorporate nucleotides at a constant forward rate, and dissociate from the DNA substrate in a first-order fashion. The overall processivity will depend on the ratio of these rates. In support of this model we have shown experimentally that either reducing the forward rate, or increasing the dissociation rate, reduces the processivity of T4 DNA polymerase. A model will be presented which describes processive synthesis as the product of three steps: dXTP binding, catalytic incorporation, and polymerase translocation. A change in rate of any of these steps will effect the processivity of the polymerization process. We have shown that the 3'→5' exonuclease associated with T4 DNA polymerase also digests both single- and double-stranded DNA processively. Some of the implications of these results for other "DNA-walking" proteins will be discussed. (Supported in part by USPHS Research Grant GM-15792 and Training Grant GM-07759.)

927 Function of nucleotide in E. coli recA protein-dependent repressor cleavage.

Jeffrey W. Roberts, Nancy L. Craig, Eric M. Phizicky, & Christine W. Roberts, Cornell University, Ithaca, N.Y. 14853.

The E. coli recA protein inactivates temperate bacteriophage repressors by directing their cleavage into two fragments. The recA protein is activated for this reaction by binding to polynucleotide and to ATP, or to an analogue of ATP. We have studied the regulation of this reaction and its relation to the strand pairing activity of recA protein.

928 INITIATION OF GENETIC RECOMBINATION IN E. coli: RECOGNITION OF DUPLEX DNA CONTAINING SINGLE-STRANDED REGIONS BY PURIFIED recA PROTEIN, Stephen C. West, Era Cassuto and Paul Howard-Flanders, Yale University, New Haven, Connecticut, 06520

Genetic recombination in E. coli is dependent upon the recA gene. We have investigated the interaction of purified recA protein with duplex DNA molecules and have shown that recA protein is capable of recognizing and acting upon duplex DNA that contains short single-stranded regions. The association of this activity with recA protein is shown by (a) stimulation of the single-strand DNA-dependent ATP-ase activity of recA protein by double-stranded plasmid DNA that contains short single-stranded gaps and by λ DNA that contains single-stranded cohesive ends; (b) formation of stable complexes between recA protein and substrate DNA in the presence of the γ-thio analog of ATP and Mg^{++}. The protein-DNA complexes formed sediment rapidly through neutral sucrose gradients and have been visualized by electron microscopy. Neither undamaged plasmid DNA, nor λ DNA treated with SI nuclease to digest the single-stranded cohesive ends, are capable of stimulating the recA protein-dependent ATP-ase activity, nor are they able to form the heavy protein-DNA complexes. Recognition of substrate DNA and the formation of this protein-DNA intermediate may be an integral function of recA protein prior to the subsequent steps - homologous pairing, cutting and joining - of genetic recombination.

Plasmid Replication

929 PROTEINS PRODUCED BY A MINI-R PLASMID, Karen A. Armstrong and William Bauer, SUNY, Stony Brook, NY 11794.

pSM1 is a mini-plasmid (5.68 kb) that was derived in vivo from the large drug resistance plasmid R100 by spontaneous deletion of 93% of the R100 genome. (Mickel, Ohtsubo, and Bauer, Gene 2:193). We are investigating the proteins produced by pSM1 in vivo with the goal of assigning the plasmid-encoded functions of replication and incompatibility to specific polypeptides. pSM1 is useful because it appears to code for functions involving plasmid replication and incompatibility and not for other R100 functions. Intact pSM1 produces four polypeptides of apparent molecular weights 11,700, 13,000, 22,000 and 23,000. When a pSM1 fragment which is 60% the size of pSM1 is cloned into a vector plasmid that carries a promoter for the expression of proteins from inserted DNA fragments, a new (fifth) polypeptide is produced. Three of these five pSM1-encoded polypeptides correspond closely in size to three of the four polypeptides predicted from the nucleotide sequence of the replication region of pSM1 (Rosen et al., in preparation). We are currently isolating mutants of pSM1 in order to assign specific functions to the pSM1-encoded polypeptides we have identified.

930 DNA REARRANGEMENTS IN A HYBRID PLASMID CARRYING THE λ IMMUNITY REGION.
Alberto BERNARDI and Françoise BERNARDI, Centre National de la Recherche Scientifique, Laboratoire d'Enzymologie, 91190 Gif-sur-Yvette, France.

The hybrid plasmid consisting of pSC101 and the immunity region of phage λcI857 persists in cells grown at 30°C but not in cells grown at 37°C. In the latter case the plasmid was found to undergo several modifications.

We have determined the restriction maps of these new plasmids and detected the following modifications : (1) the insertion of an IS1 element into gene N carried by the λ fragment, (2) a mutation in the P_L O_L site of the same fragment, and (3) four large deletions (30 to 50 % of the hybrid plasmid size) which remove almost entirely the λ fragment. The four deletions have one of their endpoint in the same restriction fragment of pSC101 ; the other endpoint is located in four different positions on the λ fragment, suggesting the site specific recombination events.

931 REPLICATION AND RECOMBINATION FUNCTIONS ASSOCIATED WITH THE YEAST PLASMID, 2μ CIRCLE.
James R. Broach, SUNY, Stony Brook. The yeast plasmid, 2μ circle, is a 6000 base pair double stranded DNA specie which is present in S. cerevisiae at 60 to 100 copies per cell and which replicates extrachromasonally under control of the cell cycle. The plasmid consists of two unique regions separated by two regions of 600 base pairs in length which are inverted repeats of each other and which in yeast serve as sites for intramolecular recombination. By examining both the transformation efficiency of yeast of various plasmids containing defined regions of the 2μ circle genome and the characteristics of the resultant transformants, we have identified several regions of the 2μ circle genome which are involved in 2μ circle replication and recombination. First, by identifying those DNA fragments from the molecule which promote high frequency transformation of yeast, we have localized the origin of replication to a sequence within the large unique region, which, as determined by subsequent deletion analysis, lies adjacent to and perhaps extending into one of the inverted repeat regions. Second, by examining the relative efficiency of replication in yeast of hybrid plasmids containing either the entire 2μ circle genome or a fragment of 2μ circle encompassing the origin of replication, we have determined that efficient use of the 2μ circle origin requires some function or functions encoded in the molecule at a site away from the origin. In addition this function apparently can be provided in trans, thus suggesting that it is a diffusible product. Third, by examining the ability of a mutant 2μ circle specie to undergo intramolecular recombination in yeast, we have identified a 2μ circle gene which codes for a product required for this process.

932 ROLE OF PLASMID-CODED RNA IN PLASMID DNA REPLICATION CONTROL, Judith L. Campbell, California Institute of Technology, Pasadena, CA 91125

An in vitro replication system has been used to study the control of DNA replication of the relaxed plasmids ColE1 and RSF1030. An RNA transcript approximately 100 nucleotides long is synthesized approximately 450 base pairs away from the origin of replication. Although plasmids ColE1 and RSF1030 have no extensive sequence homology, a 100 nucleotide RNA is synthesized during RSF1030 replication that hybridizes to the ColE1 origin region, suggesting that the RNA may be an important determinant of the relaxed mode of replication. A small insertion in the coding sequence for the RNA made from ColE1 DNA leads to a larger RNA species and simultaneously to a high copy number phenotype. Revertants missing the specific insertion show shorter RNA transcripts and wild-type copy number. The high copy number mutation is both cis- and trans-recessive. We propose that the 100 nucleotide RNA is involved in regulating the DNA replication of these plasmids. Derivatives of ColE1 that are missing part or all of the RNA coding sequence have been constructed and the replication of these mutants in vivo and in vitro is being investigated to define the mechanism by which the RNA exerts its effect.

Finally, extracts prepared from mutants of Escherichia coli deficient in ribonuclease III do not replicate RSF1030 or ColE1 plasmids in vitro. When supplemented with homogeneous RNase III, such extracts do support DNA replication on these templates, indicating that RNase III is required for DNA replication. Thus, RNA processing may be involved in DNA replication.

933 R-DETERMINANT PRODUCTION FROM R100.1 IN ESCHERICHIA COLI, M. Chandler, A. Bruschi, E. Boy de la Tour and L. Caro, Dépt de Biologie Moléculaire, 30, quai Ernest-Ansermet CH - 1211 - Genève 4 (switzerland)

Integration of the multiple antibiotic resistance plasmid R100.1 into the E. coli chromosome can result in the appearance of small (22 kb) closed circular molecules. These molecules are circular forms of the r-determinant (r-det) of R100.1. They are formed by recombination between two flanking directly repeated IS1 elements and their formation is dependent on the recA gene product. They carry most of the R100.1 resistance genes and are unable to replicate autonomously in E. coli. Since autonomously replicating R100.1 does not produce such molecules in this host, integration must be responsible for this behaviour.

We have isolated an R100.1 derivative from an r-det producing Hfr which retains the ability to produce r-det. This demonstrates that integration has resulted in a permanent inheritable change in R100.1. Some properties of this plasmid and its derivatives will be described.

934 CLONING OF THE E. coli K12 ORIGIN oriC IN THE cI GENE OF A 434 PHAGE VECTOR GENERATES PHAGEMIDS. Raymond DEVORET, Alvaro LEITAO*and Alberto BERNARDI, Centre National de la Recherche Scientifique, 91190 Gif-sur-Yvette, France.

Linear molecules of the pOC15 plasmid (Messer et al., 1978) carrying the E. coli K12 replication origin oriC and an ampicillin resistance determinant were inserted at the single Hind III site located in the 434 cI repressor gene of phage vector 590 (Murray et al., 1977) and gave rise to two different replicons AT1 and AT2. We call phagemids the two replicons AT1 and AT2 since they display the following dual phenotype : they develop as mature phages upon infection of non-immune hosts whereas they are stable plasmids upon infection of host cells with elevated levels of 434 repressor. In host cells with discrete decreasing 434 repressor concentrations plasmidization efficiency decreases whereas phage virulence increases correspondingly. Phagemid AT2 has a more pronounced virulent phenotype than AT1 and differs also from AT1 by the orientation of the oriC fragment. Insertion of oriC in phage DNA leads to a relatively high number of plasmid copies (about 20) upon infection of a 434 lysogen. It is likely that oriC-dependent plasmidic replication switches to phage replication and maturation when the number of plasmid copies overtitrates the cellular 434 repressor concentration.

*CNPq (Brazil) Post-doctoral fellow.

935 CHARACTERIZATION OF PROPERTIES AND INDUCTION OF COLE1 RELAXATION COMPLEX, Samuel V. Kelly and Paul H. Johnson, Wayne State University School of Medicine, Detroit, MI 48201.

The nonconjugative plasmid ColE1 can be isolated as a specific protein-supercoiled DNA complex under physiological conditions which minimize catabolite repression. Treatment of this complex with protein denaturing agents results in phosphodiester bond cleavage at a unique nucleotide sequence on the heavy strand of the DNA. Conjugative plasmids such as sex factor F promote efficient mobilization of ColE1 which occurs autonomously and depends on the integrity of its relaxation site and gene(s) specifying at least one and perhaps all the proteins of the relaxation complex. We have demonstrated that ColE1 relaxation complex is stable during agarose gel electrophoresis in low ionic strength buffer and migrates as a distinct species having a 3% reduction in electrophoretic mobility relative to uncomplexed native supercoiled DNA. Treatment of complex with sodium dodecyl sulfate (SDS) results in the apparent activation of a repressed endonucleolytic activity which converts it to a species having a mobility coincident with nicked circular DNA. If SDS is applied to an agarose gel at times subsequent to the application of relaxation complex, SDS will overtake the DNA, cause induction and result in complete separation of induced complex from all other DNA forms. This technique constitutes a rapid, sensitive and quantitative assay for induction as well as a single step purification procedure. Induced complex can be readily isolated from preparative gels by electroelution and demonstrated to be a substrate for DNA polymerase I and radioactive nucleoside triphosphates in a nick translation reaction. Incorporation of radioactive label occurs specifically in a 460 base pair restriction fragment known to contain the relaxation cleavage site, the likely origin of transfer of heavy strand during F-factor mediated mobilization of ColE1.

936 REGULATION OF PLASMID R6K REPLICATION, Roberto Kolter and Donald R. Helinski, Univ. of California, San Diego, La Jolla, CA 92093

The replication region of plasmid R6K was separated into two components: (a) a gene coding for a protein essential for the initiation of replication (π) and (b) the origin of replication. The π protein acts *in trans* to initiate replication from the origin. Nucleotide sequence analysis of this replicon revealed the presence of seven tandem twenty-two base pair repeats in the origin region. A similar twenty-two base pair sequence is present in the region preceeding the start of the structural gene for π. Using restriction enzymes a 380 base pair fragment containing the origin of replication was isolated. The size of the origin was further reduced to about 280 base pairs by analysis of insertions in the region using Tn5. A single *Hin*d III site in this region, 100 base pairs away from the tandem repeats, cannot be interrupted without loss of origin function. A spontaneous origin mutant having a deletion of one repeat was obtained by M. Inuzuka. This mutant origin can now be interrupted at the *Hin*d III site. To study the control of π expression we have analyzed transcripts made *in vitro* using purified DNA fragments. The promoter for π has been localized in the region of the eighth repeat. In addition, the fragment containing the π promoter was fused to the lactose operon such that lactose expression is under π control. The β-galactosidase levels from such fusions were measured in cells with or without an R6K replicon. The enzyme levels are reduced when there is a functional π in the cell, suggesting that the synthesis of π is autogenously regulated. Additional operon fusion experiments are in progress to test the regulatory role of π in the initiation of R6K replication.

937 THE STABILITY LOCUS OF THE PLASMID NR1. Jean McKell and Robert H. Rownd, Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706.

Although the EcoRI fragment B of the R plasmid NR1 is capable of autonomous replication it is not stably inherited in the absence of selection for the recombinant plasmid. The EcoRI fragment A confers stability in cis but not in trans, suggesting firstly a stability (stb) locus in that region, and secondly, a structural role in plasmid inheritance for that locus. We have mapped the stb locus to a region 4-7 kb from $IS1_a$ by subcloning stable derivatives of NR1 using PstI and BglII. BglII fragment B contains the replicator region of NR1 (i.e., part of EcoRI B), while BglII D contains part of EcoRI A. Together these fragments encompass the r-determinants and IS1's of NR1. Plasmids consisting only of BglII fragments B and D were unstable. Stable plasmids containing BglII fragment G, which is adjacent to BglII D within EcoRI A, were isolated. Plasmids with the parental copy number and incompatibility properties always contained one or more BglII fragments between fragments G and B, which in some instances were novel BglII fragments not present in NR1. Stable plasmids consisting of only BglII fragments B + D + G were copy mutants with mutant incompatibility properties. These and other experiments suggest that regions adjacent to the replicator of NR1 may affect copy number and incompatibility, perhaps due to transcriptional read-through from the adjacent fragment. BglII fragment G is apparently necessary for plasmid stability and cannot be joined directly with BglII fragment B to form a recombinant plasmid with wild-type NR1 properties.

938

CONTROL OF REPLICATION AND STABLE INHERITANCE OF PLASMID R1, Søren Molin and Kurt Nordström, Dept. of Molecular Biology, Odense University, Odense, Denmark.

Plasmid R1 is present in a few copies per cell in E. coli. All functions required for replication and control of replication are located within a 2000 bp region of the plasmid molecule. We have located within this region a gene responsible for maintaining the low plasmid copy number. The function of this gene is to inhibit initiation of plasmid replication via a cytoplasmic effector molecule (protein). Mutants existwhich have a much reduced activity of this inhibitor and which phenotypically are copy mutants. Plasmid incompatibility is primarily excerted by this control function. Copy mutants exist which seem to have normal inhibitor function. One mutant that replicates without control at temperatures above 37°C also has an unchanged activity of the inhibitor. Small plasmid derivatives of R1, though replicating as the full-size plasmid, are not inherited stably. The segregation kinetics indicate that a partitioning function responsible for the stable inheritance of plasmid R1 is lacking in the small plasmids. The gene(s) for this function thus probably maps outside the replication region.

939 CONTROL OF COLE1 PLASMID DNA REPLICATION. Barry Polisky and Michael Shepard, Department of Biology, Indiana University, Bloomington, IN 47405

The control of ColE1 DNA replication has been investigated using copy number mutants isolated from the ColE1 derivative pBGP120. Deletion derivatives of the Cop^- plasmid pOP1 exist at 200-300 copies per chromosome in E. coli compared to 15-20 for the Cop^+ parent plasmid. In E. coli containing both a Cop^- deletion derivative (pOP1Δ6) and the Cop^+ parent pBGP120, the copy number of the Cop^- plasmid is lowered to 4-6 copies per chromosome. Thus the copy control mutation in pOP1Δ6 is recessive. In addition, co-residence of the two plasmids is a stable phenomenon in E. coli indicating that incompatibility and replication control characteristics have been altered. Co-residence of an unrelated plasmid (pSC101) with pOP1Δ6 has no effect on pOP1Δ6 copy number. These results suggest that a plasmid specific, diffusible repressor appears to act negatively to control plasmid copy number. In this scheme, pOP1Δ6 produces a defective repressor or is altered in repressor production.

To map the mutation in pOP1Δ6 we have constructed small derivatives in vitro containing specific cognate regions of either Cop^+ or Cop^- plasmids. These experiments have resulted in the localization of the mutation in pOP1Δ6 to a 1.2 kb region containing the ColE1 origin of replication. We are currently determining the nucleotide sequence of the Cop^- replication origin.

940 INCOMPATIBILITY AND COPY NUMBER CONTROL GENES OF THE R PLASMID NR1. Robert H. Rownd, Alan M. Easton, Carolyn R. Barton, Jean McKell, Padmini Sampathkumar and Verne A. Luckow, University of Wisconsin, Madison, Wisconsin 53706.

The incompatibility, copy number control, and origin of replication of the R plasmid NR1 (90 kb) and its copy mutant pRR12 have been located on two PstI fragments of size 1.1 kb (inc, cop) and 1.6 kb (ori). A plasmid function required for stable plasmid inheritance (stb) is located in another region. stb confers stability in cis, suggesting that it plays a structural role in plasmid inheritance, rather than providing a diffusible gene product. The Cop- mutant pRR12 is compatible (Inc^-) with NR1, but is incompatible with itself. Hybrid plasmids have been constructed which contain the PstI 1.1 fragment of NR1 ligated to the PstI 1.6 fragment of pRR12 (and vice-versa). Incompatibility studies with these plasmids suggest that, if incompatibility is due to the interaction of a repressor with a receptor site on a plasmid, both the structural gene for the repressor and its receptor site are located on the PstI 1.1 fragment. Plasmids have been constructed which contain two copies of the PstI 1.1 (inc, cop) fragment. It has not been possible to construct plasmids containing two PstI 1.6 (ori) fragments in cis, even when the ori fragments are derived from the cop- pRR12. This suggests that some DNA structural feature prevents the stable inheritance of plasmids containing two ori fragments. Some cloned plasmids have been isolated which are Inc^- Cop^- owing to a deletion or a Tn element insertion outside of the replication functions. This suggests that plasmid regions adjacent to the replication functions may affect copy number and incompatibility, perhaps owing to transcriptional read-through. Several RNA polymerase binding sites have been located in the replication region of NR1.

941 COPY NUMBER CONTROL OF THE PLASMID PROPHAGE P1, June Rothman Scott, Martha M. Kropf, Jack A. Cowan and Sharon D. Langley, Microbiology Department, Emory University, Atlanta, Georgia 30322

The heteroimmune P1 and P7 plasmid prophages are stringently controlled to maintain about one copy per chromosome. We have isolated mutants of prophage P1 that are maintained at 10-20 copies per chromosome. One of these mutants (copE38ts) expresses the high copy number only at high temperature, so it is a conditional mutant. Lysogens for copy mutants grow at the same rate as wild type lysogens in L broth. All seven of the cop mutations studied are closely linked on the P1 genetic map and are located near c7 and am115. Copy mutant plasmids express little or no incompatibility in relation to wild type P1, but retain incompatibility to marked derivatives of themselves. To test dominance of each mutation, a recA(P7Cm) lysogen was infected with each P1Apcop mutant in turn and, at different times after infection, the copy number of each plasmid was determined. Following superinfection with cop N3, N20, N22, and N24, the number of P7Cm's per cell increases above the wild type level; after superinfection with copN26 and E38ts the number of copy mutant plasmids per cell drops to the wild type level. Following segregation of cells containing both wild type and copy mutant plasmids, copN20, N22 and N24 are retained in preference to wild type, while wild type is retained in preference to copN26 and E38ts. This suggests that N3, N20, N22, and N24 are dominant and that N26 and E38ts are recessive to wild type and implies that these mutations define at least two separate genes which control plasmid copy number.

942 MULTIPLE LOCI OF REPLICATION CONTROL IN COL E1, H. Michael Shepard and Barry Polisky, Indiana University, Biology Department, Bloomington, IN. 47401.
Spontaneous high level copy number mutants of Col E1-derived plasmids have recently been described and characterized by Gelfand et al. (PNAS 75: 5869,1978) and Shepard et al. (Cell 18: 267, 1979). These plasmids are present at a 5-50 fold increased copy number per chromosome equivalent over that of the wild type replicons. The degree of amplification depends upon replicon size, the stage of cell growth and the growth medium (Shepard et al., op.cit., and Muesing et al., in preparation). Another in vitro generated copy number mutation has been isolated by Heffron et al. (PNAS 75: 6012, 1978). This mutation is derived from the insertion of an Eco R1 linker within the Col E1 Hae III-F fragment and leads to DNA overproduction in vivo and to alteration of an in vitro synthesized 110 base RNA (Conrad and Campbell,Cell 18:61, 1979). Sequence data from our laboratory will be described which show that the site of the mutation we have defines a second locus of replication control in Col E1.

943 BROAD HOST RANGE PLASMID RK2: NUCLEOTIDE SEQUENCE OF THE REGIONS CONTAINING THE ORIGIN OF REPLICATION AND INCOMPATIBILITY FUNCTION, David M. Stalker, Christopher Thomas and Donald R. Helinski, University of California, San Diego, La Jolla, CA 92093
The nucleotide sequence of a 617 base pair region containing the origin of replication of the broad-host range plasmid RK2 has been determined. When linked to an antibiotic resistance marker, a 393 base pair HpaII restriction fragment within this sequence provides a functional origin of replication when two other regions of the RK2 genome are provided in trans in the same cell. Included in the 617 bp sequence is a region adjacent to this HpaII origin fragment which is involved in determining RK2 specified plasmid incompatibility. Major features of the sequence within the 393 origin fragment include a 74% A-T rich region followed by a 79% G-C rich region and the presence of five 17 base pair direct repeats. Deletion analysis indicates that the regions containing these features are required for functional origin activity. Three 17 base pair repeated sequences are present adjacent to this region and coincide with the region involved in incompatibility expression. This region alone is not, however, sufficient for the expression of incompatibility since deletions which remove part of the group of five direct repeats also reduce incompatibility expression from cloned fragments. Thus all eight direct repeat units are required for the incompatibility function specified by the 617 base pair region of DNA. Working models include the involvement of the high A-T and G-C containing regions and five direct repeats in the initiation and control of RK2 DNA replication and an interaction between regions of DNA containing the direct repeat units in the specification of the RK2 incompatibility function.

944 AUTONOMOUS REPLICATION OF R-DETERMINANTS DNA IN PROTEUS MIRABILIS. David D. Womble and Robert H. Rownd, University of Wisconsin-Madison, Madison, WI 53706.
Composite R plasmids are composed of two distinguishable elements, the resistance transfer factor (RTF), which contains genes which mediate sexual transfer, replication and incompatibility, and the r-determinants segment, which contains genes which confer resistance to antibiotics. When harbored in P. mirabilis, amplification of the r-determinants region relative to the RTF DNA takes place when selection is made for very high levels of drug resistance. Dissociation, mediated by recombination between the directly repeated IS1 elements which are found between the RTF and r-determinants regions, followed by autonomous replication of the r-determinants DNA during the amplification had been postulated. However, no direct demonstration of autonomous r-determinants DNA replication had been demonstrated. We have selected for high ampicillin resistance from a strain of P. mirabilis harboring the composite R plasmid NR84, and found it to contain, in addition to the composite plasmid, autonomous monomeric r-determinants DNA molecules with a copy number of about 100. Experiments using buoyant density analysis and electron microscopy have shown that the autonomous r-determinants can initiate DNA replication and form classical theta-type structures. In limiting concentrations of thymine, more radioactive thymine is incorporated by r-determinants replication forks than into the chromosomal or composite plasmid DNA, suggesting that a large number of r-determinants molecules can replicate. Experiments are currently in progress to determine the origin and direction of replication on these autonomous r-determinants molecules.

945 THE CLONING OF THE ORIGIN REGION OF COLICIN E1 DNA REPLICATION INTO BACTERIOPHAGE M13, Nobuo Nomura and Dan S. Ray, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, California 90024

ColE1 DNA replication initiates at a fixed origin and proceeds unidirectionally. In vitro experiments have shown that the 6S L-strand fragment, which hybridizes to the H-strand DNA of ColE1, is synthesized first. RNA polymerase participates in the synthesis of the 6S L-strand fragment by making an RNA primer. Once the primer is formed, RNA polymerase is not required for further replication. In order to study the initiation of replication of each strand, the origin region (HaeII E fragment) of ColE1 was cloned into the single-stranded DNA phage M13 genome in both orientations. Because the first step of M13 replication, conversion of the phage SS DNA to parental RF DNA requires RNA polymerase for priming, RNA polymerase independent initiation of replication of a cloned origin can be detected under the condition where RNA polymerase activity is inhibited. Although M13 phage and M13 phage in which the viral strand was ligated with H-strand of HaeII E were not converted to parental RF DNA in vivo in the presence of rifampicin, an inhibitor of RNA polymerase, M13 phage in which the viral strand was ligated with L-strand was converted to parental RF DNA. These results suggest that an origin for H-strand synthesis of ColE1 DNA is located within the L-strand of HaeII E and that initiation at the H-strand origin occurs on the single-stranded template by a rifampicin-resistant mechanism.

DNA Transposition Mechanisms

946 GENETIC AND BIOCHEMICAL ANALYSIS OF THE TRANSPOSON Tn10, Christoph F. Beck, Fakultät für Biologie, Universität Konstanz, Germany

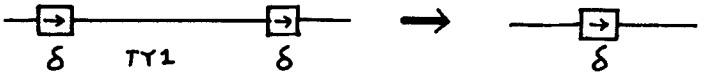
A mini ColE1 plasmid was constructed which contained Tn10 in the lacI portion of a lacI-Z gene fusion. In this genetic set up, deletion mutants were isolated in which DNA was excised from one end of Tn10 resulting in the fusion of Tn10 genes with lacZ in such a manner that fusion proteins with ß-galactosidase activity are produced (1). The extent of the deletions entering Tn10 was determined by analysis with restriction endonucleases and agarose gel electrophoresis. The mutants obtained were used for the analysis of functions encoded by the transposon. Results obtained provide evidence for: 1) The presence on Tn10 of three genes inducible by tetracycline and of several genes not regulated by the antibiotic. The data allow for the construction of a plausible genetic map of the transposon. 2) Synthesis of several proteins encoded by a cloned 3500 base pair piece of Tn10 DNA which contains the tetracycline resistance genes. In an in vitro protein synthesizing system, four proteins encoded by the cloned Tn10 fragment with molecular weights of 41 000, 30 800, 22 500 and 10 800 could be demonstrated. 3) A control mechanism for translocation of Tn10. A region of the Tn10 genome was found to exert a powerful inhibition on the translocation of Tn10 at 37°C but not at 30°C.

(1) Beck, C.F. (1979) Proc.Natl.Acad.Sci.USA 76, 2376-2380

947 INSERTION AND EXCISION OF A YEAST TRANSPOSABLE ELEMENT ANALYSED BY DNA SEQUENCING Philip J. Farabaugh and Gerald R. Fink, Cornell University, Ithaca, NY 14853

The his4-912 mutation of S. cerevisiae is the result of the insertion of a 6200 base pair transposable element into the promoter of the his4 gene. The inserted element is similar to the TY1 element described by Cameron, Loh and Davis (Cell, 1979); it consists of a 5600 base pair sequence flanked by a direct repeat of a 333 base pair element (delta). The mutation has a his⁻ phenotype.

I have determined the sequence of his4⁺ and his4-912. The insertion of TY1 occurred 162 base pairs from the initiator ATG of his4. Insertion of the element creates a twelve-base palindrome at the junction between his4 and delta. One class of his⁺ revertants involves the nearly-precise excision of the TY1 element, leaving a single delta sequence (see figure). Most revertants of this kind are his⁺ at 37° and his⁻ at 23°; a class of revertants existswhich are his⁺ at both temperatures. I am sequencing a number of revertants of both types to determine the molecular basis for this difference. The existence of two classes of revertants implies a variability in the product of nearly-precise excision; analysis of these revertants should provide clues to the mechanism of this process.



948 INTEGRATION OF BACTERIOPHAGE MU AT HOST CHROMOSOMAL REPLICATION FORKS DURING LYTIC DEVELOPMENT, Renee A. Fitts and Austin L. Taylor, University of Colorado Health Sciences Center, Denver, Col. 80262

The target site for bacteriophage Mu integration in a lytic cycle of infection was investigated. DNA synthesis in five Hfr strains of E. coli K-12 was synchronized by amino acid starvation and was allowed to proceed for zero, 8, or 15 min before infection. The Hfr cells were then infected with Mu and were subsequently mated with nonimmune F^- recipient cells. Mating was interrupted mechanically at 5 min intervals and samples were assayed for infective centers. Conjugal transfer of Mu was delayed in Hfr strains that have transfer origins 18 map units or more from the E. coli replication origin, and the delays increased as the distance between an Hfr point of origin and the replication origin increased. When a gene A mutant of Mu was used for the infection, no infective centers were generated. Infection with a gene B mutant resulted in infective center formation only after long periods of mating. These data are most consistent with a model in which infecting Mu DNA or its progeny integrate at host chromosomal replication forks.

949 STRUCTURE AND FUNCTION OF THE TRANSPOSON, Tn903, Nigel D.F. Grindley and Catherine M. Clarke, University of Pittsburgh, Pittsburgh, PA 15260

The kanamycin resistance transposon, Tn903, consists of a unique region of about 1,000 b.p. bounded by a pair of 1,050 b.p. inverted repeat sequences (IRs). Each IR contains two PvuII cleavage sites separated by 520 b.p. We have constructed derivatives of Tn903 in which this 520 b.p. fragment is deleted from one or both IRs. Those derivatives that lack both 520 b.p. fragments cannot transpose, while those that lack just one remain transposition proficient. One such transposable derivative, Tn903 del1, has been selected for further study. We have determined the sequence of the non-deleted IR. The 18 b.p. at each end of an IR are identical, and inverted relative to one another: a structure characteristic of insertion sequences. Additional experiments indicate that a single IR from Tn903 can, in fact, transpose. To correlate the DNA sequence with genetic activities, we have created mutations by inserting a 10 b.p. DNA fragment at several sites within the IR of Tn903 del1, and have examined the effect of such insertions on transposability. The results of these studies will be discussed.

950 SITES, FUNCTIONS, AND INTERMEDIATES IN Tn10-PROMOTED TRANSPOSITION AND RELATED EVENTS, Nancy Kleckner, Timothy J. Foster and Shirley M. Halling, Harvard University, Cambridge, MA. 02138

Genetic and physical analysis of Tn10 has demonstrated that all sites and functions essential for Tn10-promoted transposition, inversions and deletions lie within Tn10's 1400 basepair inverted repetitions (hereafter referred to as IS10). Isolation of point mutants in IS10 (in progress) is giving more detailed information as to the functional organization of this element.

The left and right IS10 sequences of Tn10 are not identical, either physically or functionally. Physical analysis of certain Tn10 insertions has revealed that frequently information is exchanged between the left and right IS10's during transposition. These observations have suggested to us the occurrence during transposition of a particular molecular intermediate. The structure of this intermediate makes it possible to see how the two ends of the transposon are readily juxtaposed in space prior to joining to a target molecule, and to understand transposition and co-integration as alternative fates of this intermediate structure.

Analysis of Tn10 and host mutants which affect various Tn10-related recombination events strongly suggests that precise excision and nearly-precise excision of the element can often occur by pathway(s) which are unrelated to the pathway(s) for transposition, deletions and inversions.

951 STUDIES ON TN 3 SPECIFIC RECOMBINATION USING λ. Carol J. Muster, Lorne A. MacHattie and James A. Shapiro, University of Chicago, Chicago, Ill. 60637

To look at Tn 3-specific insertion events, a bla$^+$ transducing phage, λCb 1, was made by insertion of Tn 3Δ596 (TpnA$^-$TpnS$^-$TpnR$^-$bla$^+$) into λb2red3cI857 at ral. In the presence of exogenous TpnA gene product (RSF103 = RSF1010::Tn 1ΔAp. TpnA$^+$TpnS$^+$TpnR$^-$bla$^-$) the Tn 3Δ596 transposes at high frequency forming only co-integrates in recA hosts. λCb 1 can transduce recA, recA(R388), recA(RSF103) or recA(RSF103, R388) cells to carbenicillin (cb) resistance. In recA or recA (R388) transduction frequencies are low (6/10^6 pfu) due to the defective phage attachment site Most transductants are unstable with respect to cb resistance and λ immunity. In recA(RSF103) and recA(RSF103, R388) transduction frequencies are 10-50 fold higher, and transductants are stable. For cb resistant transductants in recA(RSF103, R388), replica mating shows co-transfer of cb resistance with R388 drug resistance markers to a λ-resistant λ-immune recipient. Plasmid DNA isolated from transductants capable of this transfer showed two DNA species; one co-migrates with RSF103, one migrates slower than R388 DNA on gels. Restriction analysis of plasmid DNAs shows λCb 1-specific DNA bands in transductants but not in controls. These data suggest the structure is that of a λCb 1-R388 co-integrate. Transduction frequencies and stability data are unaltered in the presence of λ prophage indicating λ functions are not critical for insertion of λCb 1 in the presence of RSF103. Tn 3 specific recombination has also been studied using a packagable plasmid form of λ::Tn 9, pλCm 1, as transposition recipient. The packaged form of this recipient, synchronously introduced into and recovered from cells, was used to follow the kinetics of recombination events with small plasmids carrying forms of Tn 3 derepressed for transposition.

952 HITCHHIKING TRANSPOSONS: A NEW CLASS OF MOBILE GENETIC ELEMENTS, Richard Novick and Ellen Murphy, Public Health Research Institute, 455 First Ave., New York, NY, 10016

Several chromosomally located determinants have been described in Staphylococcus aureus that are frequently cotransduced with unlinked replicons such as plasmids. Following co-transfer, the passively transferred element returns to its original chromosomal site and there is no change in the structure of the carrier replicon. Two examples of this phenomenon involve the transfer of methicillin resistance and enterotoxin B production in conjunction with a Tc^r plasmid (Shafer and Iandolo, Inf. Imm., 25:902, 1979) and transfer of erythromycin and spectinomycin resistances in conjunction with a Pc^r plasmid (Phillips and Novick, Nature, 278:476, 1979). We have found that the latter element, Tn554, is capable of occupying sites other than its primary chromosomal locus and can be transferred to a rec$^-$ recipient as efficiently as to a rec$^+$. The presence of one copy of Tn554 in the recipient inhibits the transfer of a second by 100-1000 fold even if the resident copy is not at its primary site. The carrier-transposon complex can be trapped by transduction to a rec$^-$, transposon-containing recipient; it consists of a 6 kb insert into the carrier plasmid, lacking a visible stem. This complex is stable upon transfer to a Tn554$^+$ recipient; however, it dissociates upon transfer to a Tn$^-$. In this case, the transposon returns to its primary site, regenerating the original carrier plasmid. On the basis of these observations, it is suggested that: i) transposons of this type operate by a transposon-encoded, site-specific excision-insertion system; ii) this excision-insertion system is controlled by transposon-encoded repressor; and iii) the transposition intermediate is a free circular molecule generated by the Campbell mechanism.

953 UNUSUAL GENETIC EVENTS ASSOCIATED WITH A TRANSPOSABLE ELEMENT IN YEAST, G. Shirleen Roeder, Deborah T. Chaleff and Gerald R. Fink, Cornell University, Ithaca, New York, 14853.

The his4-912 mutation is the result of insertion of 6.2 kilobase pairs of DNA into the regulatory region of the his4 locus of yeast. His$^+$ revertants derived from the his4-912 mutant carry a number of chromosomal rearrangements. The transposable element is a hotspot for these rearrangements since they do not occur in the absence of the element. The majority of His$^+$ are cold-sensitive for their His$^+$ phenotype and are the result of nearly precise excision of the insertion element. Other revertants are the results of deletions which have one endpoint in the his4-912 insertion element and one endpoint in the adjacent his4 DNA. Some revertants carry reciprocal translocations where one of the translocation breakpoints occurswithin the his4-912 insertion element.

A number of His$^+$ revertants carry mutations which are unlinked to the his4 gene and which influence the behavior of the his4-912 mutant. The phenotypes of these unlinked mutations include suppression of the his$^-$ phenotype of the his4-912 mutant, suppresssion of the cold-sensitivity of the cold-sensitive revertants and enhancement of the reversion frequency of the his4-912 mutation. These controlling elements are being investigated at the biochemical and genetic level.

954 PROPERTIES OF PHAGES CONTAINING BOTH ENDS OF MU, James W. Schumm and Martha M. Howe, Department of Bacteriology, University of Wisconsin, Madison, WI 53706

We have constructed λ phages containing both ends of Mu by in vitro recombination of EcoRI fragments from λpMu transducing phage derivatives of λCharon 4. Analysis of the DNA structure of these phages by agarose gel electrophoresis of restriction fragments and electron microscopic observation of heteroduplexes has revealed that the phages differ from one another with respect to the orientation of the Mu fragments relative to each other and to the λ DNA. The specific orientations of the Mu fragments with respect to each other determine the properties of the phages. Only those phages with Mu ends in their normal relative orientation, "type 1" phages, express Mu-specific phenomena, and this expression does not occur in the presence of Mu immunity. In "type 1" phages expression of Mu functions appears to inhibit the growth of the λ;these phages grow much more efficiently in a Mu lysogen than in a non-lysogen. In addition, infection of λ-immune Mu-sensitive cells with "type 1" phages causes an increase in the frequency of mutations in host genes. Many of the mutant cells release plaque-forming λ particles and contain Mu immunity and Mu and λ markers present on the infecting "type 1" phage. Removal of the host mutation by generalized transduction results in the loss of both Mu and λ markers. These Rec^+ cells containing host mutations segregate derivatives which have lost the λ markers but which retain Mu immunity and Mu markers. This segregation suggests that the integrated 'type 1" phage is in a cointegrate structure, Mu-λ-Mu, in which the Mu DNA copies are in the same orientation.

955 DNA REARRANGEMENTS IN THE R-DNA OF YEAST, Jack W. Szostak, Sidney Farber Cancer Institute, 44 Binney St. , Boston MA 02115

DNA rearrangements may be conveniently studied in yeast by using hybrid plasmids for the insertion of useful genetic markers at desired points in the genome.

Mitotic unequal crossovers between sister chromatids of haploid yeast in the rDNA locus were demonstrated as follows. The genetic marker LEU2 was inserted in the rDNA locus by transformation of yeast with a hybrid plasmid constructed in vitro . Leu^+/leu^- sectored colonies in which the insertion had been spontaneously lost were picked for analysis.The leu^- side was shown to have a complete deletion for the inserted plasmid; the Leu^+ side often had a duplication of the inserted plasmid. The duplication events involved 6 to 8 rDNA repeat units. The frequency of unequal crossing over was calculated to be sufficient for the maintenance of sequence homogeneity among the rDNA repeat units.

Extrachromosomal copies of the rDNA repeats were detected by a gel hybridization analysis of total and supercoiled circular yeast DNA. Different strains vary in the amount of free rDNA circles they contain from roughly 0.5 to 5 copies per cell. Genetic evidence that these circles were generated by intrachromosomal reciprocal recombination was obtained by inserting a hybrid plasmid in the rDNA locus and observing transposition events in which the plasmid was excised from the rDNA locus and reintegrated at a different locus on another chromosome. These events were detected by selection for recombination between two defective his3 alleles, one on the plasmid, and one at the his3 locus. A generalization of this procedure for the construction of specific chromosome rearrangements, including deletions, inversions, transpositions, translocations, and ring and dicentric chromosomes, will be discussed.

956 NUCLEOTIDE SEQUENCE ANALYSIS OF THE CHLORAMPHENICOL RESISTANCE TRANSPOSON Tn9. Daniel Vapnek and N. Kirby Alton, Dept. of Microbiology, Univ. of Georgia, Athens, Georgia, 30602

The chloramphenicol resistance transposon Tn9 is a 2800 bp segment of DNA composed of two directly repeated sequences of IS1 flanking a region of 1102 bp which carries the chloramphenicol transacetylase (CAT) gene. We have utilized the Sanger chain termination method of DNA sequencing to determine the nucleotide sequence of this region. Two methods were used to prepare the single-stranded templates needed for the primed-synthesis reaction. One of these involved the use of Exonuclease III digestion of double-stranded DNA and the other cloning in the single-stranded DNA bacteriophage M13.

By utilizing these templates the nucleotide sequence of the 1102 bp region bounded by the IS1 sequence was determined. Analysis of the sequence reveals an uninterrupted coding sequence for 219 amino acids beginning 224 base pairs from the left end of IS1. Amino acid sequencing data of CAT by W.V. Shaw confirms this as the coding sequence for the CAT gene. No other coding sequence for a protein of greater than 8,000 daltons is found within this region. Since IS1 itself is transposable, this result suggests that the 1102 bp region of Tn9 is not directly involved in the transposition. Transposition of Tn9 results in the duplication of a 9 bp sequence at the site of integration. The internal 9 bp sequences at the IS1 junction of Tn9 have no homology with each other nor with any of the 9 bp sequences found to be duplicated at the sites of Tn9 integration.

Supported by USPHS grant GM20160

Replication Fidelity

957 DECREASED FIDELITY IN ONE SUBSPECIES OF CYTOPLASMIC DNA POLYMERASE DURING N-2 ACETYLAMINOFLUORENE HEPATOCARCINOGENESIS, John Y.H. Chan and Frederick F. Becker, The University of Texas System Cancer Center M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

During the exposure of rat livers to the hepatocarcinogen N-2 Acetylaminofluorene it was demonstrated that the cytoplasmic α-DNA polymerase (E.C.2.7.7.7.) was strikingly error-prone, when compared with that of the normal regenerating rat liver (Proc. Natl. Acad. Sci. 76,814-818, 1979). The fidelity of polymerization of these enzymes was assayed by determining the incorporation of non-complementary deoxribonucleotide triphosphates (misincorporation) on a poly (dA-dT) template. In an attempt to identify the mechanism of infidelity we modified and extended our purification scheme. As a result, cytoplasmic α-DNA polymerase activity was separated into two subspecies which were designated Cα1 and Cα2. Cα1 activity eluted from a phosphocellulese column at 0.07-0, 17M NaCl while Cα2 eluted at 0.2-0.25M. Cα2 demonstrated a normal fidelity throughout the various steps of purification while, despite being purified some 10,250 fold Cα1 continued to demonstrate a severe degree of infidelity.

958 ISOLATION OF UNEXPECTED MUTANTS DURING THE CONSTRUCTION OF A SITE-SPECIFIC NONSENSE MUTATION IN AN ESSENTIAL GENE OF ØX174, Robert W. Chambers and Opinder S. Bhanot, New York University School of Medicine, New York, New York 10016.

Using a combination of chemical and enzymatic techniques, we have synthesized øX174 RF DNA containing a single preselected G → A substitution at position 2401 of the minus strand. This corresponds to a nonsense mutation in the third codon of gene G, an essential gene coding for one of the viral spike proteins. Infectious virus was produced by transfection of spheroplasts derived from E. coli C600 Su+2 that inserts the w.t. amino acid, Gln, at amber codons. Mutants were detected with a recA host bearing the plasmid, pøXG, that carries a functional copy of øX gene G. Of 1355 plaques examined, 15 carried gene G mutations. Of these, 11 gave identical results with different suppressors: Su+1 (Ser) = thermosensitive; Su+2 (Gln) = w.t.; Su+3 (Tyr) = lethal. One isolate has been sequenced and shown to contain the expected change. From their biological properties, the other 10 isolates of this group seem to have the expected change. The 4 remaining isolates were not suppressed by any of the available strains. One has been partially sequenced, and it does not contain any change at or near residue 2401. The nature and origin of these mutations are unknown but controls seem to rule out mutations in the template DNA used for the enzymatic synthesis or copy errors by DNA polymerase I (Klenow). Gene G from each of these unexpected mutants is being sequenced to locate and characterize the mutations. This is the first report of unexpected mutants in site-specific mutagenesis studies. They were detected only because our plasmid system, pøXG is permissive for all kinds of mutations in øX gene G. It is important to establish whether these mutations arise during the in vitro replication of template DNA or in the spheroplasts after transfection.

959 SOME ALTERNATIVE POSSIBILITIES FOR SOS AND MISMATCH CORRECTION. H. Echols, University of California, Berkeley, CA 94720.

There are instances in which information in a replication-blocked parental DNA strand is likely to be transferred to a daughter strand with more fidelity than can be explained on the basis of random incorporation of precursors (e.g. "SOS repair" of a pyrimidine dimer). Popular models for SOS repair involve either recombination (Rupp/Howard-Flanders) or an error-required random polymerization across the lesion (Radman). An alternative idea is the following (presented initially at the Nethybridge Meeting in 1975). One or more DNA-binding proteins recognize the distortion in the helix of a non-replicatable lesion (or perhaps any lesion). The DNA-binding protein(s) provide a replicative switch in which the daughter strand of the non-blocked parent is used as a template. Past the block the newly replicated DNA strand switches back to the original template to produce a replicative bypass to the lesion. This switch might be "error-prone" because under-methylated DNA or RNA serves as template. Such a distortion-directed switch mechanism also introduces an alternative possibility for "mismatch correction" of closely spaced mutations without excision.

960 MODE OF ACTION OF ARABINONUCLEOSIDES ON DNA SYNTHESIS IN HUMAN LYMPHOBLASTS, Arnold Fridland and Douglas E. Bell, St. Jude Children's Research Hospital, Memphis, TN 38101
The effects of 9-β-D-arabinosyladenine (ara-A), 1-β-D-arabinosylcytosine (ara-C) and 2'-deoxyadenosine (dAdo) on DNA replication in cultured human lymphoblasts (CCRF-CEM line) were studied by pulse labeling cells with [^{3}H]thymidine and analyzing the nascent DNA by velocity sedimentation in alkaline sucrose gradients. At doses that reduced the overall rate of DNA synthesis to 50-70% of control values, both ara-A and ara-C profoundly inhibited the formation of new replicons, with secondary effects on chain elongation contributing to the total inhibition of DNA synthesis. In contrast, the suppression of DNA synthesis by dAdo stemmed mainly from an inhibition of chain elongation. These studies also disclosed that about 100 times more ara-A than ara-C was required to produce a similar inhibition of DNA replication in CCRF-CEM cells. Determination of intracellular concentrations of the nucleoside triphosphates (ara-CTP and ara-ATP) indicated that 90% inhibition of DNA synthesis was achieved at 1.6 and 25 pmol per $1x10^6$ cells, respectively. Studies with cell lysates revealed that the replicative machinery in CCRF-CEM cells is more sensitive to ara-CTP than to ara-ATP. This finding contrasts with earlier research, in which the inhibition of purified DNA polymerases by either ara-ATP or ara-CTP yielded essentially the same K_i value. The difference in sensitivity of the cell lysate to these arabinonucleotides may reflect either a target enzyme other than DNA polymerase or, more plausibly, some subtle interaction of the polymerase with other components of the replicative process.

961 "MISINCORPORATION" OF dUMP INTO ANIMAL CELL DNA RESULTING FROM INHIBITION OF THYMIDYLATE SYNTHETASE. M. Goulian, H. A. Ingraham, B. Bleile and B. Y. Tseng, University of California, San Diego, La Jolla CA 92093
Intracellular dUrd and dThd nucleotide pools were measured by a combination of labeling with ^{3}H-dUrd plus measurements of specific activity, direct measurements by high pressure liquid chromatography, and an enzymatic assay for thymidine using thymidine kinase. Inhibition of thymidylate synthetase in cultured human lymphoblasts by methotrexate or FdUrd results in a rise in intracellular dUMP of ~10^3-fold (~1 pmol/10^6 cells → ~1 nmol/10^6 cells). Associated with this is a similar increase in dUTP, from undetectable levels (<0.2 fmol/10^6 cells) to ~0.2 pmol/10^6 cells. Combined with the fall in dTTP to 1/10-1/100 of normal, this results in an increase in ratio dUTP/dTTP from 10^{-5} to 1/10-1/1. The high ratio of dUTP/dTTP implies that in residual DNA synthesis in the drug-inhibited cell dUMP is incorporated at rates close to the rate for dTMP; this was supported by the presence of dUMP in DNA (15-25 pmol labeled dUMP/10^9 cell, for 2-h labeling period). This net amount results from concurrent removal (Ura-DNA glycosylase/AP endonuclease incision/excision) and re-incorporation of dUMP during the gap filling step of repair (because of the high ratio dUTP/dTTP). This is reflected by 2-4-fold increase of dUMP in DNA by addition of Ura (which inhibits Ura-DNA glycosylase) during cell treatment, and by rapid removal of dUMP from DNA by a chase with unlabeled dThd. The cyclic removal and re-incorporation of dUMP indicated by these results may play a role in the cytotoxicity of these drugs and thymine-less states, in general.

962 THE FIDELITY OF DNA SYNTHESIS *IN VITRO* WITH *E. COLI* REPLICATION PROTEINS, Thomas A. Kunkel and Lawrence A. Loeb, The Joseph Gottstein Memorial Cancer Research Laboratory, University of Washington, Seattle, WA 98195
In vitro mutagenesis of natural DNA is observed during copying with purified DNA polymerases. When ΦX174 viral DNA is primed with a single restriction endonuclease fragment 83 nucleotides away from an amber mutation (*am3*) and copied *in vitro* with *E. coli* DNA polymerase I (Pol I), the reversion frequency of this DNA is greater than that of uncopied DNA. This reversion frequency is increased in proportion to the concentration of dATP or dCTP relative to the other three dNTPs in the reaction misture. DNA sequence analysis of revertants from substrate pool bias experiments demonstrates that the revertants contain the selectively biased nucleotide as an incorrect substitution at the *am3* locus. Two of the three predicted nucleotide substitutions are observed, an A for T transversion and a C for T transition, both at position 587. Analyses of the product of the copying reaction indicate that *in vitro* DNA synthesis proceeds past the *am3* locus. Using a heteroduplex molecule (wt(-) fragment hybridized to *am3*(+) circle) similar in structure to the product of the copying reaction, the expression of the minus strand is 40%. From these data we estimate the error rate of Pol I with 5 mM $MgCl_2$ is 1/50,000.
Using the ΦX174 assay we have determined the accuracy of several DNA polymerases, including *E. coli* DNA polymerase III holoenzyme (containing eight subunits), in copying natural DNA. In addition we have shown that single-strand DNA binding protein (SSB) enhances this accuracy as much as 20-fold. Thus, in the presence of SSB, Pol III holoenzyme copies natural DNA *in vitro* with an error rate of approximately 10^{-6}.

963 UNDAMAGED DNA IS REPLICATED WITH LOW FIDELITY IN UV-IRRADIATED YEAST, Chris Lawrence and Roshan Christensen,University of Rochester Medical School,Rochester N.Y. 14642. Witkin and Wermundsen (Cold Spring Harb.Symp.43:881-886) have argued that at least 99% of all mutations induced by UV in E.coli are targeted, that is result from misincorporation of bases at the site of the causal lesion. The remaining untargeted mutations are thought to reflect the decreased fidelity of normal DNA replication in SOS-repair induced cells,in which the 3'-5' proofreading activity of DNA polymerases is inhibited (Caillet-Fauquet et al.,J.Mol.Biol. 117:95-112). We have measured the proportion of targeted mutations induced by UV and gamma rays in the lower eukaryote Saccharomyces cerevisiae, by mating an irradiated excision deficient haploid carrying a non-revertible deletion of the whole CYC1 locus to an unirradiated excision deficient haploid carrying a revertible cyc1 allele. The reciprocally irradiated mating and control crosses were also made. Using two different deletions and two point mutations,we find that irradiating the deletion strains induces about as many revertants as irradiating the point mutants themselves,indicating that targeted mutations are much rarer than untargeted events in yeast. This result is consistent with the observation that no more than about 1% of UV-induced mutations at the CYC1 locus are tandem double base-pair changes (Lawrence et al., J.Mol.Biol.85:137-162). Only targeted mutations are likely to involve such double events. Since loss of proofreading is unlikely to decrease fidelity by more than a few fold (Loeb et al.,Cold Spring Harb.Symp.43:921-927),we suggest that UV-irradiation may actively promote infidelity,perhaps by promoting non-polymerase directed base selection or by stimulating the insertion of incorrect bases into intact DNA. Supported by PHS grant GM21858 & DOE. UR report no. 3490-1772.

964 ANALOGUE NUCLEOTIDE TRIPHOSPHATES AS PROBES OF T4 DNA POLYMERASE FIDELITY MECHANISMS David C. Mace, National Institute of Environmental Health Sciences, RTP, NC 27709

Direct Watson-Crick base pairing of substrate and template base moeities has long been assumed to be the basis of DNA polymerase insertion fidelity. Yet the results of Engel and von Hippel (JBC, 253, 935-939 (1978)) argue that either such direct base pairing does not occur, or if occuring, does not play a role in the formation of the enzyme-template-triphosphate ternary complex. Using the same modified nucleotide, N_6methyl dATP, the involvement of base-pairing has been approached with the replicative polymerase of phage T4. For this enzyme, kinetic analysis shows that dATP is used in preference to N_6me dATP, and that the preference can be accounted for by the difference in the apparent K_M,s for the two substrates. Additionally, an incorporated N_6methyl dAMP residue is more frequently excised than dAMP (as might be expected from the reduced equilibrium stability of the N_6medA-dT pair compared to the dA-dT pair). Unexpectedly however, this differential "proofreading" by the 3'-5' exonuclease does not affect the overall polymerase fidelity. Such a result seems in direct conflict with currently acccepted views of the relationship between triphosphate turnover and proofreading by the exonuclease of T4 DNA polymerase.

965 SITES OF TERMINATION OF IN VITRO DNA SYNTHESIS IN AAF REACTED DNA. Peter D. Moore, Kallol Bose, Samuel Rabkin and Bernard S. Strauss, University of Chicago.

AAF adducts in DNA act as blocks to synthesis by E. coli DNA polymerases I and III, T4 DNA polymerase, AMV reverse transcriptase and a mammalian DNA polymerase α. Under normal reaction conditions there is 100% termination of synthesis catalysed by pol I. Variation in the site of termination was observed with the different enzymes and for one, the α polymerase, there was variation apparently due to differences in DNA sequence at the site of the adduct. Such variations in the site of termination were not observed at UV induced pyrimidine dimers. Substitution of Mn^{2+} for Mg^{2+} permitted the incorporation of an extra nucleotide opposite the AAF adduct with pol I. These changes were not due to inhibition of the 3'$\longrightarrow$5' exonuclease activity of this enzyme. It is concluded that termination of DNA synthesis on damaged DNA templates may be determined by the nature of the lesion, the properties of the DNA polymerase catalyzing the reaction, the DNA sequence at the site of the lesion and the choice of divalent cation used in the reaction.

966 A Novel Mechanism for DNA Replication, A. Richard Morgan, Biochemistry Department, University of Alberta, Edmonton, Alberta, T6G 2H7.

In vitro replication of nicked circular DNA with *E. coli* DNA polymerase I gives extensive net-fold synthesis in which the daughter strands are covalently linked. Kornberg originally postulated nucleotide addition at the 3' end with displacement of single-stranded DNA followed by strand switching of the polymerase to give covalently-linked complementary (clc) DNA. Several lines of evidence are inconsistent with this interpretation. A simple fluorescence assay for clc DNA showed all the replication product to ±2% was clc DNA. This was confirmed by electronmicroscopy in which single-stranded DNA was not observed and yet the double-stranded tails growing from the circles increased in length with time and were clc as shown by the additional criterion of supercoiling of the circles on adding ethidium (circles topologically closed). The two analogues of dATP, 6 Me dATP and dATPαS (the phosphate containing sulfur in the S configuration) both repaired exonuclease III treated DNA at the same rate as dATP, but 6 Me dATP did not give any clc DNA and dATPαS gave clc DNA at 1/40 the rate of dATP. This cannot be explained by a strand displacement mechanism for clc DNA synthesis. A model is proposed for clc DNA synthesis involving base tetrads at the replication fork with incorporation of nucleotides synchronously 5' → 3' and 3' → 5' while preserving a phosphodiester link between the daughter chains. Several predictions are born out by this model. Pulse-labelling experiments are consistent with incorporation into the interior of a polynucleotide chain. In repair processes the configuration of dATPαS is inverted but during clc DNA synthesis evidence from SV phosphodiesterase digestion is that both configurations are present in the product as predicted by the new mechanism.

967 THE FIDELITY OF DNA POLYMERASES FROM AGEING HUMAN FIBROBLASTS, Vincent Murray and Robin Holliday, National Institute for Medical Research, London, NW7 1AA, U.K. Orgel's error catastrophe theory of ageing predicts that the *in vitro* error frequency of DNA polymerase will increase in ageing cells. Experimental observations confirming this prediction have been obtained with MRC-5 human diploid fibroblasts. With the poly[d(A-T]/dGTP/Mg^{2+} system, "cytoplasmic" DNA polymerase α from ageing cells had an average of 3.4 fold higher error frequency than from young cells. With poly[d(A-T)]/dGTP/Mn^{2+} and poly[d(I-C)]/dTTP/Mg^{2+} the average increase with α-polymerase was 1.9 and 2.0 fold respectively. The error frequency of DNA polymerase γ with poly(A).oligo (dT)/dGTP/Mn^{2+} was increased 3.8 fold in ageing cells. In addition the error frequency was measured at intervals during the lifespan of MRC-5 and the kinetics of error accumulation were consistent with an exponential or slightly greater than an exponential relationship. This indicates that an error feedback mechanism is operating. Nearest neighbour analysis, degradation to 5'dNMPs, mixing and other control experiments confirm that DNA polymerase-directed errors are responsible for the error frequencies observed in young and old cells.

968 EXAMINATION OF NEWLY SYNTHESIZED DNA IN THYMINE AUXOTROPHS OF *E. COLI*, Michael H. Patrick, Karen E. Rice, and T. Jesse Kwoh, University of Texas at Dallas, Richardson, TX 75080.

The observation that the concentration of thymine in the growth medium has a specific effect on the DNA replication velocity in thy^- strains of *E. coli* was exploited to probe the coupling between DNA chain elongation and replication fork movement. If the two events can be made to proceed at different rates by altering the dTTP pools, there may be more frequent initiation of fragments on the leading strand.

When newly synthesized DNA was examined in the thy^- strain, W3110 (ung^+), and the isogenic strain, BD10 (ung^-), the fraction of pulse-label radioactivity in < 20S fragments was found not to vary for growth in media with thymine concentrations ranging from 0.2 μg/ml to 10 μg/ml. These results suggest that the extent of discontinuous synthesis on the leading strand is not influenced by variation in the dTTP pools. We interpret the findings to mean that DNA chain elongation and replication fork movement keep apace of each other in spite of an apparent non-constant rate of elongation.

969 THE ROLE OF GENE 43 POLYMERASE IN FRAMESHIFT MUTATION, Lynn Ripley, and Nadja Shoemaker, NIEHS, Research Triangle Park, NC 27709

ts alleles of gene 43 polymerase of T4 influence the fidelity of replication with respect to base pair substitutions. The role of the polymerase in maintaining proper alignment of DNA strands and thus, the influence of the polymerase on frameshift mutations has not been systematically defined.

We are characterizing the influence of ts gene 43 alleles on spontaneous and induced frameshift mutation frequencies.

970 INDUCTION OF AN ERROR-PRONE MODE OF DNA REPLICATION IN MONKEY CELLS TREATED BY UV-IRRADIATION OR CHEMICAL CARCINOGEN, Alain Sarasin, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France.

UV-irradiation or chemical carcinogen treatment of monkey kidney cells induces a new recovery pathway which enhances the survival of UV-irradiated simian virus 40 (SV40). We carried out experiments to determine whether this phenomenon called induced virus reactivation is a mutagenic process. Using phenotypic reversion towards wild type behaviour of temperature-sensitive SV40 mutants, we found that replication of UV-irradiated SV40 in UV-irradiated or carcinogen-treated monkey cells is indeed a very mutagenic process. The revertants produced during the induced virus reactivation grow at 41° as well as at 33°, while the mutant we started with cannot grow at 41°. The induced reversions occurred both with an early mutant (tsA 58) and a late mutant (tsB 201) of SV40. The molecular mechanism of UV-induced mutagenesis is not known. However, the analysis of revertant genomes using restriction enzymes indicates that no detectable deletions or additions are responsible for the reversion pattern.

These results suggest that enzymes from UV-irradiated or chemical-carcinogen treated cells are able to replicate UV-irradiated DNA using an error-prone mode of DNA replication. Induced virus reactivation and error-prone replication are probably among the expressions of SOS functions in mammalian cells.

971 INDUCTION OF TRANSITION AND TRANVERSION MUTATIONS DURING DNA REPLICATION IN VITRO BY PURIFIED REPLICATION PROTEINS OF BACTERIOPHAGE T4. Navin Sinha and Melanie Haimes, Rutgers University, Piscataway, N.J. 08854

Purified proteins coded by phage T4 replication genes 32, 41, 43, 44, 45, 61 and 62 replicate DNA in vitro with great fidelity so that no increase in the frequency of revertants is observed when ØX174 DNA containing an amber codon is used as template. By using appropriate imbalances in the concentrations of the deoxynucleotide substrates, however, it is possible to increase significantly the reversion frequency of an amber codon. The error rates are linearly related to the ratio of the concentration of the correct and the incorrect nucleotide and sequencing studies show that the revertants do have the predicted DNA sequence. We have measured in vitro the frequency of A-C and G-T mispairs leading to transition mutants and A-G and C-T mispairs leading to transversion mutants with 4 different amber mutants of ØX. The exact mispairing frequency varies from 9×10^{-6} to $< 10^{-8}$ depending upon the mispair involved. Even for the same mispair, the error rates are dependent upon the neighboring DNA sequence. High error rates during DNA synthesis in vitro are seen with an amber mutant (am3, gene E) that has a high reversion rate in vivo, while lower error rates are seen with other amber codons (am16, gene B and am9, gene G) that have lower in vivo reversion rates. The data are consistent with the Watson-Crick and Topal-Fresco models of base mispairings.

972 EFFECTS OF CHEMICAL MUTAGENS ON REPLICATION FIDELITY, M. D. Topal, C. A. Hutchison, III M. Baker and C. Harris, University of North Carolina Medical School, Chapel Hill, N.C.

Alkylating agents induce high levels of mutations in both prokaryotes and eukaryotes. To begin to discern the mechanisms by which these mutagens change the genetic content of DNA we reacted dATP with N-methyl-nitrosourea (MNU) - a methylating agent and potent mutagen and carcinogen. The products of this reaction were separated by ion exchange chromatography to yield five pure compounds. These are being identified by chromatographic and spectroscopic techniques and results suggest that they are all methylation products of intact dATP. The DNA biosynthetic properties of these modified dATPS were analyzed by a novel application of the plus-and-minus DNA sequencing procedure (C. A. Hutchison, III, M. D. Topal, M. H. Edgell, and M. Smith, in preparation). Several of the modified dATPs were shown to incorporate into DNA but with a loss of specificity - covalent incorporation into DNA occurring opposite G and C as well as T template residues. Moreover, incorporation was dependent on the sequence of surrounding DNA, occurring opposite a template residue other than T only when that residue was part of a particular sequence. The sequence dependence varied with the modified dATP and may account for mutational hot spots such as those observed by Seymour Benzer while mapping the rII locus of phage T-4. These results suggest that: a) mutations can be induced in vivo by alkylation of nucleic acid residues in the DNA precursor pools; b) methylation of adenine in dATP and possibly in DNA will induce both transition and transversion mutations; and c) the DNA sequence at the daughter strand growing end exerts a very strong influence on the occurrence of errors during DNA replication. Supported in part by DHEW grants (GM24798 and P30 CA16086) to MDT and (GM21313 and A108998) to CAH III).

973 A ROLE FOR ESCHERICHIA COLI ENDONUCLEASES III, IV AND VI IN THE REPAIR OF APURINIC, APYRIMIDINIC OR PYRIMIDINE DIMER LESIONS IN DNA, H.R. Warner, B.F. Demple, W.A. Deutsch, C.M. Kane and S. Linn, Univ. of Minnesota, St. Paul, MN and Univ. of California, Berkeley, CA. The characteristics of the nicks introduced into damaged DNA by Escherichia coli endonucleases III, IV and VI and by phage T4 ultraviolet (UV) endonuclease have been investigated using E. coli DNA polymerase I. Nicks introduced into depurinated DNA by endonucleases IV or VI are translated easily by the polymerase, whereas the translation of nicks introduced into depurinated DNA by endonuclease III or into irradiated DNA by T4 UV endonuclease is much slower. T4 UV endonuclease also possesses apurinic (AP) endonuclease activity which generates nicks in depurinated DNA, which are poor substrates for nick translation. Nick translation with DNA treated with endonuclease III or the T4 UV endonuclease can be enhanced, however, by an additional incubation with endonuclease VI, and to a lesser extent by incubation with endonuclease IV. These results are taken to indicate that endonuclease III or T4 UV endonuclease (acting either upon depurinated or irradiated DNA) generate nicks containing AP sites at their 3'-termini, and that such sites are not rapidly excised by the 3'→5' activity of DNA polymerase I. However, such terminal sites apparently can be removed by either endonuclease IV or VI, which were previously shown to be able to cleave on the 5'-side of un-nicked AP sites of the AP lesion. Taken together, these results suggest roles for endonucleases III, IV and VI in the repair of apurinic/apyrimidinic sites as well as pyrimidine dimer sites in DNA. They also imply that the T4 UV endonuclease cleaves irradiated DNA by a two-step process, first cleaving a glycosylic bond, and then the phosphodiester bond.

974 MECHANISMS FOR MAINTENANCE OF FIDELITY IN DNA REPLICATION, Susan M. Watanabe and Myron F. Goodman, University of Southern California, Los Angeles, CA 90007.

Homopolymers containing adenine or the analogue 2-aminopurine and copolymers with both of the bases have been utilized to investigate the mechanism involved in distinguishing between mismatches during DNA synthesis involving relatively large differences in free energy. Previous studies of adenine:thymine and 2-aminopurine:thymine base pairs in vitro have substantiated a kinetic model in which discrimination between right and wrong base pairs is based on the differences in free energy. Described here are studies which challenge enzymes with varying editing abilities (wild type and mutant bacteriophage T4-induced DNA polymerases) or with no detectable editing ability (calf thymus α DNA polymerase) to distinguish between dCTP or TTP as candidates for polymerization opposite adenine or 2-aminopurine in the templates. Determinations of the frequency of each type of pairing event and the K_m and V_{max} values for the deoxynucleotides with each type of template will be discussed in terms of the kinetic model.

RNA Priming

975 INTERACTION OF DNA POLYMERASES α AND β WITH TEMPLATE-PRIMER DNA'S OF DEFINED STRUCTURE AND SEQUENCE, Joel A. Huberman, Julian F. Burke, Joan Plummer, and Mary Jo Evans, Roswell Park Memorial Institute, Buffalo, New York 14263.

We have constructed several defined template-primer DNAs and tested their interaction with DNA polymerases α and β from calf thymus. The template-primers, and the conclusions drawn from them, are: (1) Double-stranded, covalently-closed, circular PM2 DNA was nicked at a specific location. The nicks were then enlarged to gaps of 2-150 nucleotides by treatment with exonuclease III for precise times. The initial rate of DNA synthesis by DNA polymerase α was significantly higher at a gap size of about 40 nucleotides than at smaller or larger sizes. In contrast, the rate for polymerase β was constant over this range. (2) Separated single strands were prepared from restriction fragments of ϕX174 RF DNA. DNA polymerase α used different restriction fragment strands with a wide range of efficiencies, suggesting that utilization of single-stranded DNA as template-primer by polymerase α depends on the secondary structure of the DNA. (3) Complementary single strands from ϕX174 restriction fragments were annealed to ϕX174 single-stranded viral DNA. The resulting template-primers were used by DNA polymerase α but not by DNA polymerase β. A protein factor from calf thymus capable of stimulating DNA polymerase α up to 10-fold on this template-primer, or on single-stranded DNA (but not on activated DNA) has been identified, partially purified, and partially characterized.

976 PROCESSING OF OKAZAKI FRAGMENTS OF BACTERIOPHAGE T7, Michael J. Engler and Charles C. Richardson, Harvard Medical School, Boston, MA 02115

Lagging strand DNA synthesis in bacteriophage T7 is primed by an oligoribonucleotide having the sequence pppApCpCpA/C. Conversion of these RNA-primed DNA pieces into long continuous DNA strands requires removal of the oligoribonucleotide, synthesis of DNA to fill all gaps, and joining of the DNA pieces.

Two likely candidates for the primer removal are the 5'→3' exonuclease function of E. coli DNA polymerase I and the T7 gene 6 exonuclease which also initiates hydrolysis at the 5'-termini of duplex DNA. The gene 6 exonuclease has been purified and compared with DNA polymerase I for the ability to hydrolyze the oligoribonucleotide primers attached to in vitro synthesized T7 DNA. Either enzyme is capable of nearly complete removal of RNA primers. The products of hydrolysis by DNA polymerase I include pppApC, ATP, CMP, and AMP, while the action of gene 6 exonuclease generates ATP, CMP and AMP.

Joining of DNA pieces can be catalyzed by either the E. coli DNA ligase or the T7-coded DNA ligase. The T7 DNA ligase has been purified to homogeneity and characterized. In combination the T7 DNA polymerase and T7 DNA ligase can accomplish the gap filling and joining reaction. Reconstitution of the entire process of primer removal, DNA synthesis to form a nick, and ligation is presently under way.

977 INITIATOR RNA OF OKAZAKI PIECES IN REPLICATING SV40 DNA, Gabriel Kaufmann and Gail Dinter-Gottlieb, Weizmann Institute of Science, Rehovot, Israel

Replicating SV40 (RI)DNA was pulse-labeled in isolated nuclei with α-^{32}P rCTP and ^{3}H-TTP in presence of α-amanitin. The labeled viral (RI)DNA was purified by SDS-NaCl extraction and Sepharose 4B gel filtration. Upon DNase I digestion greater than 80% of the (RI)DNA ^{32}P ended up in ribooligonucleotides (iRNA) of ca. 8-10 residues. Treatment of the native (RI)DNA with RNase A had no effect on iRNA, indicating that it was entirely duplexed. Denaturation followed by gel-electrophoresis resolved the nascent DNA into long chains and Okazaki pieces, iRNA being mostly associated with the latter. The retrograde fork-polarity of the iRNA-tagged Okazaki pieces was determined by its exclusive hybridization to unidirectional probes (separated strands of Hpa_1+Bgl_1, fragments) of forward polarity, as previously seen with α-^{32}dNTP labeled pieces. The in vivo priming of DNA chains was studied on SV40 (RI)DNA isolated from infected cells. Cells were pulse-labeled with ^{3}H-TdR, SV40 (RI)DNA extracted and purified by gel-filtration, BND-cellulose chromatography and banding in a CsCl-EtBr gradient. The purified (RI)DNA was denatured, Okazaki pieces isolated and labeled with ^{32}P 5'-P by polynucleotide kinase. The extent of labeling did not depend on prior dephosphorylation. Subsequent DNase digestion converted the labeled pieces into a mixture of oligonucleotides up to 6-7 residues. These were alkali and RNase T_2 sensitive, yielding a mixture of all four ^{32}P-pNp's. Thus, the in vivo synthesized Okazaki pieces contained a 5'-terminal ribooligonucleotide, presumably partially degraded iRNA. Less than 2% of the ^{32}P in the DNase digest was in RNA chains of 15-20 residues. Their possible role as primers of the continuously synthesized strands is being investigated.

978 RIBONUCLEOTIDES IN DNA NEWLY SYNTHESIZED IN 3T6 CELLS *IN VIVO*. Jacek Kowalski, Monica Meuth, and David T. Denhardt, Dept. Biochem., McGill University, Montreal, Que, Canada

We have examined DNA newly synthesized in intact mammalian cells for the presence of ribonucleotides empolying sensitivity to digestion by spleen exonuclease after alkali or ribonuclease treatment. Cellular DNA pulse or pulse-chase labeled with [^{3}H]dThd was purified, mixed with uniformly ^{32}P-labeled DNA, fractionated according to size, and treated with polynucleotide kinase and ATP under conditions that rendered micrococcal nuclease-treated DNA 95% insensitive to digestion by spleen exonuclease. Treatment of the DNA with alkali or ribonuclease resulted in a significantly increased sensitivity to spleen exonuclease digestion in pulse labeled DNA that was absent in pulse-chase labeled DNA and in the ^{32}P-labeled internal control DNA. This sensitivity varied inversely with the size of the molecules. About 40% of all nascent molecules were rendered sensitive to spleen exonuclease digestion by alkali or ribonuclease. We interpret these data to indicate that about half of the earliest intermediates of synthesis of DNA in intact cells possess ribonucleotides. Qualitatively similar results have been obtained with short nascent DNA molecules derived from polyoma replicative intermediates. The results of further characterization of these *in vivo* nascent polyoma molecules possessing ribonucleotides will be presented.

979 RNA PRIMER SYNTHESIS IN THE T4 BACTERIOPHAGE DNA REPLICATION SYSTEM, Chung-Cheng Liu and Bruce Alberts, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

In the presence of single-stranded DNA, bacteriophage T4 gene 41 and gene 61 proteins catalyze the synthesis of a group of penta-ribonucleotides. These ribonucleotides are homogeneous in chain length, but heterogeneous in nucleotide sequence. When most single-stranded DNAs are used as template, half of the penta-ribonucleotides start with pppA and the other half with pppG. However, only penta-ribonucleotides starting with pppA are synthesized when 5- hydroxymethyl C containing T4 DNA is used as template. On either T4 or fd DNA, the sequence of the penta-ribonucleotides which start with pppA is pppApCpXpYpZ, while the sequence of the primers which start with pppG on fd DNA is pppGpWpXpYpZ (the W,X,Y and Z here denote any of the 4 ribonucleotides). In the presence of the other T4 replication proteins, the penta-ribonucleotides can serve as RNA primers to initiate denovo DNA synthesis catalyzed by T4 DNA polymerase on single-stranded DNA. The structure of the RNA primers reisolated from the 5' end of the in vitro synthesized DNA is similar to that described above. Moreover, identical RNA primers have been detected by the Okazaki laboratory at the end of the short T4 DNA fragments made in vivo. Using the complete in vitro DNA synthesis system developed in this laboratory, we have found that there are multiple, but specific initiation sites for initiating Okazaki fragment synthesis on a natural single-stranded DNA. However, since the synthetic random copolymer poly d(I,T) serves as a good template for pppA-start penta-ribonucleotide synthesis, the DNA sequence requirements for making a primer are probably not extensive.

980 EVIDENCE THAT THE φX174 VIRAL STRAND IS SYNTHESIZED DISCONTINUOUSLY, Margaret Matthes and David T. Denhardt, Dept. Biochem., McGill University, 3655 Drummond Street, Montreal, Quebec, Canada, H3G 1Y6

Using a new method of stopping DNA synthesis we have studied the nascent intermediates present during the final stage of φX174 DNA replication when progeny single-stranded circular DNA molecules are synthesized. We find that 40-50% of the [^{3}H]thymidine incorporated in a brief pulse stopped by bringing the infected culture rapidly to 100°C is present in DNA molecules shorter than unit-length. The molecules, which range from very short to unit length, are not generated by the stopping and isolating procedure since ^{32}P-labelled infecting parental viral strands remain relatively intact. The proportion of pulse-label found in short intermediates varies with pulse length, stopping procedure, aeration level of the infected culture, and host strain. There is no significant difference in the abundance of short nascent intermediates in *ung* and *ung*$^{+}$ strains, suggesting that the short molecules are not the result of uracil excision by uracil-DNA glycosylase. The ^{3}H-labelled short molecules hybridize to all regions of the φX genome, but preferentially to the region around the origin/terminus of replication. Sensitivity to spleen exonuclease after alkali or RNase treatment suggests that some of the short molecules isolated both during viral strand synthesis and during RF replication have at least one ribonucleotide at the 5' end. Resistance to spleen exonuclease after phosphatase treatment indicates that some of the short molecules have blocked 5' termini. The block is alkali labile. We conclude that the major mode of φX viral strand DNA replication is a discontinuous process, not continuous as proposed by the rolling circle model.

981 ORIGIN AND INITIATION OF SHORT DNA CHAINS IN E. COLI, Deborah W. Siegmann and Rudolf Werner, University of Miami, Miami, Florida 33101

We had previously shown that short nascent DNA chains can be eluted from hydroxylapatite at unusually low phosphate concentrations because of their non-covalent association with protein. Further analysis revealed that these DNA preparations contain only nascent chains and are essentially free of short, unlabeled DNA chains that often contaminate pulse-labeled DNA when other purification techniques are used. If nascent DNA is isolated under denaturing conditions, where the protein is removed prior to hydroxylapatite chromatography, then substantial amounts of unlabeled DNA are contained in the preparation. These results indicate that hydroxylapatite chromatography under non-denaturing conditions is an effective method for the purification of nascent DNA chains. Analysis of the 5'-terminal nucleotide composition of the nascent DNA chains purified by this method showed that all four deoxyribonucleotides are present at the 5'-end, although the relative amounts of the nucleotides differ depending upon the pulse-labeling conditions. These results suggest that DNA chain initiation is random with respect to the nucleotide used in the initiation process. Earlier results which we had interpreted as evidence for the presence of an oligonucleotide carrier molecule at the 5'-end of nascent DNA chains were complicated by the non-covalent binding of small amounts of RNA to the pulse-labeled DNA. We now find that it is difficult to completely remove this RNA since its total digestion is often not accomplished by the usual alkali treatments. The interaction of the RNA and DNA interfered with our analysis of the 5'-termini, and our conclusion as to the existence of the carrier molecule was wrong.

982 RNA PRIMING OF DNA REPLICATION BY BACTERIOPHAGE T4 PROTEINS, Lynn L. Silver and Nancy G. Nossal, National Institutes of Health, Bethesda, Maryland 20205

The nature of the RNA primers synthesized by bacteriophage T4 DNA replication proteins has been determined using single-stranded ϕX174 and f1 DNA as model templates. The T4 gene 41 protein and a protein controlled by T4 gene 61 together synthesize pentanucleotides with the sequence pppA-C-N_3, where N can be G, U, C, or A. The same group of sequences is found in the RNA at the 5' terminus of the ϕX174 DNA product made by the T4 gene 41, "61", 43 (polymerase), 44-62, 45, and 32 proteins. The DNA product chains begin at multiple discrete positions on the ϕX174 DNA template. The size and sequence of the primers formed *in vitro* by the T4 41 and "61" proteins correspond well with those observed *in vivo* after bacteriophage T4 infection (Okazaki *et al.*, Cold Spring Harbor, 1978).

The "61" protein is identified as an activity, absent in extracts of *E. coli* infected with T4 61⁻ mutants, which is required for the synthesis of ribooligonucleotides and for DNA synthesis with unprimed single-stranded templates. This activity has been extensively purified by chromatography on single-stranded DNA cellulose, phosphocellulose, hydroxylapatite, and valine Sepharose. During purification, synthetic activity correlates best with a protein with a denatured molecular weight of approximately 40,000. The apparent molecular weight of "61" protein measured on Sephadex G-150 is about 45,000, indicating that it is active as a monomer.

983 THE USE OF MERCURATED NUCLEOTIDES TO STUDY DNA REPLICATION IN PERMEABLE CELLS OF B. SUBTILIS, Nilima Sarkar and Sudha Bhattacharya, Boston Biomedical Research Institute and Harvard Medical School, Boston, MA. 02114

As a prerequisite for analyzing replication intermediates where sequence of interests represent only a small fraction of the total cellular DNA, we have used a new approach utilizing mercurated nucleotides which have been such a useful tool in the study of eukaryotic transcription. The selective isolation of newly synthesized mercurated DNA is achieved with a thiol affinity matrix. We have studied DNA replication in vitro in B. subtilis cells made permeable by treatment with toluene. ^{203}Hg-dCTP was incorporated into DNA with an apparent Km of 40 μM, compared to a Km of 22 μM for dCTP and had only a slight effect on the initial rate of DNA synthesis at saturating concentration. The incorporation of ^{203}Hg dCTP was inhibited by ara-CTP, novobiocin and 6-hydroxyphenylazouracil, implicating the involvement of DNA polymerase III. Sedimentation analysis of the products revealed that the incorporation of Hg dCTP occurred primarily into short fragments of 29 S, whereas normally in vitro synthesized DNA is distributed over the same size range as prelabeled chromosomal DNA. This suggests that the mercurinucleotide may interfere with the ligation of nascent DNA fragments. Pyknographic analysis of CS_2SO_4 gradients of DNA labeled with both ^{3}H-dTTP and ^{203}Hg dCTP showed that most of the ^{3}H-label banded at the heavy density region corresponding to ^{203}Hg containing DNA. Permeable bacterial cells utilizing mercurinucleotides may thus represent a useful experimental system for the analysis of DNA replication, specifically to analyze Okazaki fragments.

984 EVIDENCE FOR BASE SUBSTITUTIONS IN SYNTHESIS OF INITIATOR RNA, Ben Y. Tseng and Mehran Goulian, University of California, San Diego, La Jolla, CA 92093

A nuclear in vitro system prepared from cultured lymphoblasts was used to study the effect of ribonucleotide omission on synthesis of initiator RNA (iRNA) for discontinuous synthesis of DNA. α-^{32}P-rNTPs were incorporated and nearest neighbor frequencies in iRNA were analyzed. It was found that omission of a rNTP resulted in substitution of deoxynucleotide into iRNA in place of the cognate ribonucleotide (as previously described for E. coli primase product and polyoma iRNA), but, the dN was incorporated less frequently than the cognate rN. In addition, when rUTP or rGTP was omitted, only the nearest neighbor frequency of rA was increased. This suggests the unusual possibility that base substitution, i.e. rA for rU or rG, may occur in iRNA synthesis. When rATP was omitted, although the amounts of DNA and iRNA synthesis were reduced, nearest neighbor frequencies were not altered. However, when rCTP was omitted, alterations in all nearest neighbor frequencies were observed, suggesting that all three (rATP, rGTP and rUTP) were substituting for the limiting rCTP. Under the conditions used (dNTPs, 10 μM; rNTPs, 0.1-2 mM) the amounts of DNA synthesized in all of these experiments were essentially the same (except when rATP was omitted). In addition, in all of the experiments the size of iRNA (9 nucleotides) was not affected. The properties demonstrated by these results indicate remarkably relaxed nucleotide requirements for synthesis of iRNA, not only for sugar but for base, as well.

Site-Specific Recombination Mechanisms

985 STUDIES ON EXCISIVE RECOMBINATION OF BACTERIOPHAGE λ, Kenneth E. Abremski and Susan Gottesman, National Cancer Institute, Bethesda, Maryland 20205.

In vivo we find that an *E. coli* strain containing an int-c plasmid can carry out more excisive recombination in the absence of Xis than strains which have an int-c prophage, or a defective prophage which produces Int on induction at 41°C. However, the amount of integrative recombination is very high and similar in these strains. We are investigating this difference by purifying Int protein from these strains and looking at their effects on excision *in vitro*.

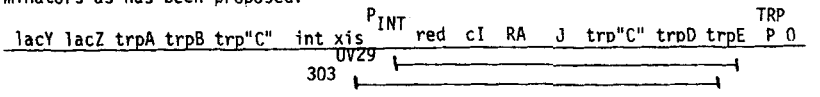
We have also examined various methods for overproducing Xis to facilitate its purification and study.

986 GENETIC ANALYSIS OF INTEGRASE REGULATION OF BACTERIOPHAGE LAMBDA, Michael J. Benedik, Desmond D. Mascarenhas, and Allan M. Campbell, Department of Biological Sciences, Stanford University, Stanford, CA. 94305

Regulation of integrase expression from the integrase promoter is mediated by the cII and cIII gene products of the phage. They have been shown to stimulate integrase expression, but in their absence low level constitutive transcription is detected from the int regulatory region.

A secondary site lysogen of lambda in the trpC gene of a trp-lac fusionstrain shows expression of the distal genes trpBA and lacZY from the integrase promoter. Xis gene expression does not occur from this promoter, although deletion analysis and nucleotide sequence studies demonstrate that essential regulatory elements of the integrase promoter lie upstream from xis.

In one such deletion termed UV29, transcription initiated at the Trp promoter can read through the integrase regulatory region, allowing expression of all distal genes under trp operator control. Under conditions of trp operator repression, this deletion still demonstrates cII/cIII activation of the integrase promoter. From this we can conclude that the cII and cIII gene products act to stimulate de novo transcription and do not act as antiterminators as has been proposed.



987 REGULATION OF BACTERIOPHAGE λ INTEGRATION BY A SITE DISTAL TO pI AND pL PROMOTERS, G. Guarneros, C. Montañez, L. E. Sosa, J. M. Galindo, and T. Hernández, Department of Genetics, Centro de Investigación, IPN, México City.

We have previously found that a cis-acting site (sib) in bacteriophage λ b-region inhibits integrative recombination under cII⁻ conditions. It is likely that sib affects int expression by pL promoter, one of the two promoters which control int, since promoter pI es virtually inactive in the absence of cII protein. New observations on this subject have been made in our laboratory: 1) Regulation by sib may be at the transcriptional or translational levels because under cII⁻ conditions Int protein seems to be synthesized only in the absence of the b-region; 2) We have located sib within about 350 base pairs adjacent to the integration site in lambda DNA by functional mapping with spontaneous and in vitro generated deletions in the b-region; 3) We have obtained independent λsib⁻ mutants, which map in the same 350 base-pairs segment; 4) Results from experiments with phages in which int can not be expressed from pL promoter show that sib does not inhibits the expression of int by pI promoter.

988 P1 SITE-SPECIFIC RECOMBINATION, Ronald Hoess and Nat Sternberg, Cancer Biology Program, Frederick Cancer Research Center, Frederick, Maryland, 21701.

We have cloned in a λ vector a 6.5 kb EcoRI fragment (fragment 7) of P1 DNA that spans the ends of the P1 genetic map and possesses a functional site-specific recombination system. In a genetic cross between two λ-P1:7 phages, this recombination system can reassort λ genetic markers that flank the fragment with an efficiency of 5-10%. Recombination is not detected in control crosses. Recombination is independent of the bacterial recA or recB functions, the λ site-specific (int) and general recombination (red) functions, and the bacterial functions necessary for λ site-specific recombination (himA, B, C; hip). All of these properties suggest that recombination occurs at a specific site within fragment 7. In addition to recombination between two loxP sites, the P1 site-specific recombination can promote recombination between the loxP site or λ-P1:7 DNa and a specific site (designated loxB) in the bacterial chromosome. The product of this reaction is an integrated λ-P1:7 prophage flanked by two hybrid sites (loxR and loxL). We have isolated phage containing loxR, loxL, and loxB sites and have used these phages in genetic crosses to determine which DNA sequences are necessary for efficient lox recombinations. Two additional facts have emerged from our analysis of recombination processes: loxP by loxB recombination can integrate λ-P1:7 DNA into the bacterial chromosome in either of the two possible orientations with almost equal efficiency; and a fraction of the products of loxP x loxP recombination are most easily explained as having arisen by branch migration. Structural aspects of the lox sites will be presented and, time permitting, we will discuss the role of this recombination in the P1 plasmid way of life. (NCI Contract NO1-CO-75380 with Litton Bionetics, Inc.)

989 INTERACTIONS BETWEEN THE PHAGE LAMBDA INTEGRASE AND THE LAMBDA ATTACHMENT SITE, Michael L. Kotewicz, Peter H. Scheier, R. Wayne Davies, and J.C. Wang, Harvard University, Cambridge Ma. 02138

Purified lambda integrase binds to a sequence of nucleotides spanning 27 nucleotides and situated 56 base pairs to the right of the center of the common core of the phage attachment site. By reconstruction experiments we have shown that this region is both necessary and sufficient for filter binding by int. The common core per se is not required for filter retention. Exonuclease III digestion of this region in the presence of int reveals several protected bands and suggests a more complicated interaction of int with this site. Experiments on the nature of the topoisomerase activity associated with purified int protein will be presented in comparison with the topoisomerase activities of DNA gyrase and omega protein.

990 REQUIREMENTS FOR PHAGE ATT SITE FUNCTION AND ITS INTERACTION WITH INT PROTEIN, Arthur Landy, Wilma Ross and Pei-Ling Hsu, Brown University, Providence, RI 02912

Bacteriophage λ integrates into, or excises from the chromosome of its E. coli host by means of a single reciprocal recombination event that requires two phage-encoded proteins, Int and Xis, and two or more host-specified proteins. The phage and bacterial att sites as well as the left and right prophage att sites have in common a sequence that is 15 nucleotide pairs in length. The crossover event of integrative and excisive recombination must be within the limits, or at the boundaries, of this "common core" sequence. There are several distinctive features of the att sites which will be discussed in terms of possible models for the site-specific recombination.

In order to determine what features of the phage att site are essential for its role in site-specific recombination, various amounts of DNA have been resected from the right or left arms. These shortened att sites were cloned and then tested for phage att site function both in vitro and in vivo. The minimal phage site extends to +82 in the right, or P' arm, and to -160 in the left, or P arm. (The center of the 15 base pair common core is taken as 0.) This region of DNA contains at least three (and possibly four) functionally important sites which interact specifically with Int protein. Implications of this unexpectedly large phage att site will be discussed.

991 THE himA GENE REGULATES THREE PHASES OF BACTERIOPHAGE λ LYSOGENY, Harvey I. Miller National Cancer Institute, Bethesda, Maryland 20205

The product of the E.coli himA gene is required for bacteriophage λ site-specific recombination as well as for the growth and excision of bacteriophage Mu and the precise excision of transposable antibiotic resistance elements. In addition to the requirement for himA gene product for λ integration, two other processes necessary for λ lysogeny appear to be affected by himA mutations namely, the synthesis of both λ repressor and λ Int protein.

An effect of himA mutations on the synthesis of λ repressor and λ Int protein was not evident in previous studies using himA point mutants. However, recent studies with himA gene deletion strains indicate a strong requirement for the himA gene product in the synthesis of these two proteins necessary for λ lysogeny. Chemical measurements of λ Int protein after infection of himA deletion strains indicate an almost total lack of Int protein synthesis. Wild-type λ forms clear plaques on himA deletion strains. However, genetic measurements of repressor activity in lysogens of these strains indicate a normal level of repressor activity. Thus, only repressor synthesis from the establishment mode but not the activity of repressor nor its synthesis from the maintenance mode are affected by himA mutations.

Therefore, the himA gene appears to coordinate three phases of λ lysogeny: λ Int protein synthesis, λ repressor synthesis and λ site-specific recombination. Regulation of the himA gene may provide a mechanism for the host control of λ lysogeny.

992 INTEGRATIVE RECOMBINATION OF PHAGE λ: DNA SEQUENCE REQUIRED FOR ATTACHMENT SITES, Kiyoshi Mizuuchi and Michiyo Mizuuchi, National Institutes of Health, Bethesda, Maryland 20205

To determine the minimum length of DNA sequence required for the function of attachment sites, we trimmed and re-cloned DNA fragments carrying the phage or bacterial attachment site of bacteriophage λ. A functional phage attachment site (attP) requires about 200 base pairs of its original sequence. On the left side, essential sequence extends beyond 115 base pairs from the center of the 15 base pair common core sequence but not beyond 152 base pairs. On the right side, the required sequence extends beyond 68 base pairs but not beyond 99 base pairs from the center of the core. When parts of this sequence are lost, the site still can act as one of the prophage attachment sites (attR or attL) or the bacterial attachment site (attB), depending on which part is still present. On the other hand the bacterial attachment site (attB) requires only the 15 base pair common core sequence and a few base pair sequence adjacent to it which is also common among the attachment sites.

993 SITE-SPECIFIC RECOMBINATION WITH SUPERTWISTED AND NONSUPERTWISTED DNA, Thomas J. Pollock and Howard A. Nash, LNC/NIMH, Bethesda, Md. 20205

Both intramolecular and intermolecular integrative recombination for DNA molecules carrying the bacteriophage λ attachment site (attP) and/or the host E. coli attachment site (attB) can be accomplished in vitro with extensively purified proteins. The reaction requires the λ int gene product and host factors. In this system naturally supercoiled DNA is a more effective substrate for recombination than nonsupercoiled molecules regardless of the ionic strength of the reaction. But at salt concentrations above 50 mM KCl the nonsupercoiled substrates yield little if any recombinant products. Binding studies show no distinctive differences between the affinities of supercoiled and nonsupercoiled DNA for the recombination proteins. Moreover, preincubation of nonsupercoiled substrate (attB, attP or attBattP) with proteins (Int, host factors or a mixture) produced no stimulation of the inefficient recombination for nonsupercoiled DNA when the missing component was added back. But from physical analysis of the DNA during active recombination we conclude that the rate-limiting step for recombination of nonsupercoiled DNA occurs before the first strand is cut; figure-8 molecules or specifically cut strands do not accumulate. These results suggest that the advantage for supercoiled DNA is not because it serves to store energy later used to reorient DNA strands by rotation. We propose that supercoiling promotes the assembly of recombination proteins and the two attachment sites into a productive complex. For example, an assembly in which the first cuts are possible.

994 ACTIVATION OF A CRYPTIC OPERON IN E. COLI BY INSERTION OF DNA, Ann E. Reynolds, Andrew Wright, and Jeffrey Felton, Tufts University School of Medicine, Department of Molecular Biology and Microbiology, Boston, Mass. 02111.

Wild-type E. coli K12 strains are unable to utilize β-glucosides as a carbon source. Genes for the catabolism of these compounds are present in the cell in the cryptic bgl operon. Cis-dominant mutations (bgl R^-) which activate the bgl operon arise spontaneously at a fairly high frequency (10^{-5} to 10^{-7}). Expression of the bgl operon in the bgl R^- (Bgl^+ phenotype) cell is regulated, requiring β-glucoside as inducer. Transcription of the operon appears from genetic studies to proceed divergently from the R region (1).

We have studied the bgl R^- activating mutation by comparing isogenic lambda specialized transducing phages which carry Bgl^- and spontaneously derived Bgl^+ operons. Analysis of restriction fragments from these phages indicates that the activating mutation is correlated with the insertion of DNA. In every Bgl^+ operon studied, either a 1250 or 780 base pair insertion was observed. The larger insertion has been identified as IS5 by Southern hybridization. There may exist a region of preferential integration in the bgl operon for these two insertion sequences. It is not yet known whether the insertions contribute elements necessary for gene regulation, act as spacers in the R region, or inactivate a negative control system. The correlation of insertion of DNA with activation of the cryptic bgl operon suggests a novel function for insertion sequences in gene control.

(1) Prasad, I. and S. Schaefler. 1974. J. Bacteriol. 120:638.

995 *XIS*-INDEPENDENT EXCISION OF BACTERIOPHAGE λ: ISOLATION AND CHARACTERIZATION OF AN *XIN* MUTANT. C. Gritzmacher, L. Enquist, and R. Weisberg, NIH, Bethesda, Md. 20205

*Int-c*226, a mutation in the *xis* gene of phage λ, confers two phenotypes: constitutive expression of the *int* gene and inability to produce active *xis* protein. We have isolated a pseudorevertant of λ *int-c*226 that retains *int* constitutivity but now promotes excisive recombination, a reaction that normally requires active *xis* protein. The new mutation is called *xin* (*xis*-independent). *Xin* is located to the right of a deletion, *b*538, that removes all of *int* and part of *xis*. It is therefore within or to the right of *xis*. *Xin* does not, however, restore the production of active *xis* protein: an *int*$^-$ derivative of λ *xin int-c*226 did not complement known *xis* mutants (including λ *int-c*226). We suggest that *xin* compensates for reduced *xis* activity by increasing the expression of *int*. This hypothesis can also account for four other properties of λ *xin int-c*226: (1) It is partially independent of the host *hip* gene for both integrative and excisive recombination; (2) it integrates more efficiently than its parent into secondary attachment sites in the bacterial chromosome; (3) it no longer requires a wild type phage attachment site for efficient integration; and (4) it promotes the formation of phage deletions at a high rate when the wild type phage attachment site and the host *hip* and *himA*$^+$ gene products are present. We have separated *xin* from *int-c*226 by recombination. λ *xin int-c*$^+$ retains property (1) and, to a reduced extent, property (4), but no longer expresses *int* constitutively. Therefore, *xin* does not create a constitutive promoter. It might instead increase transcription from the existing promoter.

Procaryotic Replication Systems

996 MEASURING THE RATE OF REPLICATION FORK MOVEMENT THROUGH DOUBLE HELICAL DNA IN THE T4 BACTERIOPHAGE IN VITRO DNA REPLICATION SYSTEM, Jack Barry and Bruce Alberts, Dept. of Biochemistry, University of California, San Francisco, CA 94143

The minimal system capable of extensive DNA synthesis on double-stranded DNA templates contains the T4 DNA polymerase (gene 43 protein), the T4 helix destabilizing protein (gene 32-protein) and the T4 "polymerase accessory" proteins (gene 44/62 and 45 proteins). When the synthesis takes place on a cytosine-containing DNA with 5- hydroxymethyl dCTP substituted for dCTP as a substrate, the newly synthesized radioactive product DNA can be sized after digestion of the unreacted template with a mixture of restriction endonucleases which only cut DNAs containing cytosine in both strands. For the above described "leading strand model reaction" containing 5 T4 gene products, fork rates of 40 to 80 nucleotides/sec are obtained. These rates are unaffected by varying the concentrations of the T4 DNA polymerase, indicating that the same polymerase molecule proceeds continuously for thousands of nucleotides. When the T4 gene 41 protein is added to the above reaction a four-fold increase in fork movement rate is observed for a discrete fraction of the replication forks. This increased rate of fork movement requires the continued hydrolysis of rGTP by the 41 protein. These data reveal a role for the gene 41 protein in the leading strand reaction, separate from its role in RNA primer formation on the lagging strand. Considering also other results, we suggest that the 41 protein uses its GTP hydrolysis energy to walk rapidly down the lagging strand DNA template at the fork, where it helps to open up the parental DNA helix; simultaneously, it marks the spot where RNA primers are to be made on the lagging strand in conjunction with the T4 gene 61 protein.

997 PURIFICATION AND PROPERTIES OF THE T4 GENE 61 RNA PRIMING PROTEIN, R. L. Burke, C.-C. Lui, and B. M. Alberts , Dept of Biochemistry, University of California San Francisco, San Francisco, CA 94143

We have purified a protein from T4 bacteriophage-infected E. coli which is essential for the de novo initiation of DNA chains in an in vitro T4 DNA replication system. In a reaction requiring single-stranded DNA, and purified T4 gene 41 protein, this protein catalyzes the synthesis of pentaribonucleotides which are used as RNA primers for Okazaki fragment synthesis. Two dimensional PAGE analysis of the proteins made by T4 mutants indicate that the RNA priming protein is the product of gene 61, a gene shown by others to be necessary for normal T4 DNA replication at early times after infection. The gene 61 protein, which represents 0.01-0.001% of the total protein of the infected cell, has been purified to greater than 80% homogenity. The 61 protein binds to both ss and ds DNA, is extremely basic, and has a molecular weight of 44,000 as judged by SDS PAGE. The major contaminant in our preparations is the T4 helix-destabilizing protein, the gene 32 protein. T4 mutants which overproduce 32 protein show a concomitant increase in the activity of isolatable 61 protein, an effect which is due to its stabilization. Sedimentation and gel filtration analyses show that the 61 protein binds tightly to 32 protein. The association of 61 with 32 protein, like that of 32 protein with T4 DNA polymerase, is lost upon the proteolytic removal of 58 to 60 amino acids at the carboxyl terminus of 32 protein; the proteolysis generates 32*I, a cleavage product which retains many of the original 32 protein activities in the T4 in vitro DNA replication system.

998 DNA POLYMERASE I: THE GENE SEQUENCE, Catherine M. Clarke, William S. Kelley* and Nigel D.F. Grindley, University of Pittsburgh and Carnegie-Mellon University*, Pittsburgh, PA 15260

As part of a study of the structure and function of DNA polymerase I of E. coli, we are determining the nucleotide sequence of the polA gene. DNA for sequencing has been isolated from a small multicopy plasmid carrying the polA1 amber mutation. We have constructed a detailed restriction map of the cloned DNA and are carrying out DNA sequencing by the method of Maxam and Gilbert (Proc. Natl. Acad. Sci. 74, 560). The sequence of a substantial portion of the gene, including the region encoding the N-terminus of the protein, has been determined. We expect to present the sequence of the entire coding region.

999 HIGH FREQUENCY GENERALIZED TRANSDUCTION BY BACTERIOPHAGE T4. Gordon J. Edlin Karen K. Y. Young and Geoffrey G. Wilson. Genetics Dept. Univ. of Calif. Davis Davis, Ca. 95616.
Studies have recently shown that a mutant of bacteriophage T4 is capable of generalized transduction in E. coli (Nature 280: 80, 1979). Certain early phage gene functions are essential for transduction. Transducing particles do not shutoff host metabolism as has been demonstrated for wild-type T4 phage and phage "ghosts". Entire plasmids can be packaged by T4.

1000 EFFECT OF NOVOBIOCIN AND NALIDIXIC ACID ON ØX174 VIRAL DNA SYNTHESIS, Robert K, Hamatake, Ryozaburo Mukai, and Masaki Hayashi, Univ Calif, San Diego, La Jolla, CA 92093
The role of DNA gyrase in the synthesis of bacteriophage ØX174 viral DNA has been investigated using an in vitro system. When ØX174 proheads are added to a prohead deficient mutant cell extract, radioactive dNTP's are incorporated into DNA of phage particles and into intermediates of phage morphogenesis. Novobiocin, an inhibitor of the B subunit of gyrase, inhibits DNA synthesis into phage particles and intermediates. However, this inhibition is seen only at high concentrations of the drug (50% inhibition is seen with a novobiocin concentration two orders of magnitude higher than the amount necessary for 50% inhibition of gyrase supertwisting activity). Nalidixic acid, whose target protein is the A subunit of gyrase, specifically inhibits incorporation of label into phage particles. The amount of label in intermediates is slightly increased and the drug concentrations necessary for these effects is comparable to those necessary for inhibition of gyrase in vitro. These results suggest that gyrase, or the A subunit of gyrase, is necessary for elongation of DNA chains to occur. Our results support the model of gyrase action during DNA synthesis in which gyrase relieves the positive superhelical strain that accumulates ahead of the replication fork by the introduction of negative supertwists.

1001 THE ROLE OF T4 GENES 44/62 AND 45 IN DNA REPLICATION *in vitro*, Chiao-Chain Huang and John E. Hearst, University of California, Berkeley, CA 94720 and Bruce M. Alberts, University of California, San Francisco, CA 94143
Genes 44/62 and 45 code for essential protein components of the T4 bacteriophage DNA replication apparatus. The 44/62 protein complex has a DNA-dependent ATPase activity which is strongly stimulated by the presence of 45 protein. Although ATP hydrolysis *in vitro* is associated with the accessory proteins, exactly how the 44/62 and 45 proteins function in T4 DNA synthesis has yet to be determined. We have recently demonstrated that DNA is synthesized at variable rates by T4 DNA polymerase on a circular, single-stranded fd DNA template (Huang and Hearst, in press). In this report we investigate the efficiency of fd DNA synthesis by T4 DNA polymerase when supplemented by the presence of various combinations of T4 auxiliary replication proteins. We find that the combination of 44/62 and 45 proteins stimulates T4 DNA polymerase in an ATP-utilizing reaction. The enzymatic action of T4 DNA polymerase alone is not highly processive and 44/62 and 45 proteins together are shown to enhance this processivity. Using the inhibitor of ATP hydrolysis, ATPγS, we are able to demonstrate that ATP hydrolysis is involved with the formation of a 44/62 protein·45·protein·DNA template complex which then allows T4 DNA polymerase to be more processive.

1002 SUBUNITS OF E. COLI DNA POLYMERASE III HOLOENZYME, Ulrich Hübscher and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305
Enzymatic conversion of primed single-stranded φX174 and G4 DNA to the duplex form and enzymatic replication of duplex φX174 circles require DNA polymerase III holoenzyme. Eight polypeptides are identified in SDS-polyacrylamide gels of nearly homogeneous holoenzyme preparations: (i) DNA polymerase III, the core which contains three polypeptides, α, ε, and θ of 140, 25, and 10 k daltons, respectively; (ii) β, 40 k daltons; (iii) γ, 52 k daltons; (iv) δ, 32 k daltons; and (v) τ, 83 k daltons (DNA-dependent ATPase). The core, β, γ and δ components are essential for φX replication, but δ appears to be dispensable for G4. The γ and δ subunits have been extensively purified and established as the products of dnaZ and dnaX genes, respectively. Extracts of recombinant lambda lysogens carrying the dnaZ gene contain 90-fold increased levels of the γ subunit. Studies of the physical and functional features of the γ and δ subunits will be presented.

1003 MULTIPLE FORMS OF DNA POLYMERASE III OF ESCHERICHIA COLI: PURIFICATION AND IDENTIFICATION OF SUBUNITS. Charles S. McHenry. Department of Biochemistry and Molecular Biology, University of Texas Medical School, P.O. Box 20708, Houston, Texas 77025.

DNA polymerase III, the core of the DNA polymerase III holoenzyme, has been purified 28,000-fold to 97% homogeneity from E. coli HMS-83. The enzyme contains three subunits: α, ε and θ of 140,000, 25,000 and 10,000 daltons, respectively. The α subunit has been previously shown to be a component of both DNA polymerase III and the more complex DNA polymerase III holoenzyme (Livingston, D., Hinkle, D., and Richardson, C. (1975) J. Biol. Chem. 250, 461-469; McHenry, C. and Kornberg, A. (1977) J. Biol. Chem. 252, 6478-6484). It is demonstrated here that the ε and θ subunits are also subunits of the DNA polymerase III holoenzyme. Additionally, we have isolated a new form of DNA polymerase III, termed DNA polymerase III'. DNA polymerase III' can be distinguished from DNA polymerase III by its functional and chromatographic properties. An 83,000 dalton protein, assigned as the τ subunit, is present in highly purified preparations of both DNA polymerase III and the DNA polymerase III holoenzyme. Thus, the DNA polymerase III holoenzyme contains at least seven different subunits.

1004 REPLICATION OF PHAGE fd DNA WITH PURIFIED PROTEINS, T.F. Meyer and K. Geider, Max-Planck-Institut für medizinische Forschung, Abteilung Molekulare Biologie, Jahnstr. 29, 6900 Heidelberg, West Germany

Conversion of viral single strands to double-stranded DNA requires *E.coli* DNA binding protein I, *E.coli* RNA polymerase holoenzyme and *E.coli* DNA polymerase III holoenzyme. The initiating ori-RNA is formed at a specific site (1).

Supercoiled fd double strands are specifically cleaved by gene II-protein (2) and unwound in the presence of gene II-protein, *rep* protein and DNA binding protein I. DNA polymerase III holoenzyme performs the extension of the nick at the gene II-protein cleavage site. The topoisomerase activity of gene II-protein is presumably involved in cleaving the replicated viral strands and their circularization. In the cell the single-stranded circles are then converted into double strands or packaged into the phage particles.

(1) Geider, K., Beck, E., and Schaller, H. (1978) Proc. Natl. Acad. Sci. (USA) 75, 645-649
(2) Meyer, T.F. and Geider, K. (1979) J. Biol. Chem. 254, in the press

1005 GENE 2 MUTANTS OF BACTERIOPHAGE T7, Ian J. Molineux and P. Mooney, University of Texas. Austin, Texas 78712.

Gene 2 amber mutants of bacteriophage T7 do not make progeny if plated on the non-suppressing (sup^+) E. coli B or K12. They do however grow on E. coli C sup^+. Analysis of replicative DNA intermediates has shown that infection of E. coli B by T7-2^{am} does not lead to the formation of the fast-sedimenting "flower" structure DNA characteristic of $T7^+$ infections whereas these structures are seen in infections of E. coli C strains. Maturation to unit length of the concatemeric DNA is dependent on the T7 gene 3 endonuclease. Infection of E. coli sup^+ hosts by T7-3^{am} leads to the stable formation of fast-sedimenting DNA. Further analysis has shown that in both E. coli B and C sup^+ hosts, infection by the double mutant T7-$2^{am}3^{am}$ leads to the formation of fast-sedimenting, stable DNA. It thus appears that the gene 2 protein is not required for the formation of fast-sedimenting DNA, but at least in E. coli B strains, is necessary to prevent premature maturation of the replicating concatemeric DNA by gene 3 endonuclease. Current studies indicate that mutants of T7 deleted for at least part of the gene 2 protein can be isolated and propagated on E. coli C. The characterization of the deletion mutants will be presented.

1006 STRUCTURAL ANALYSIS OF E. coli DNA POLYMERASE I, William E. Brown, Karen H. Stump and William S. Kelley, Carnegie-Mellon University, Pittsburgh, PA 15213

DNA polymerase I from wild type and several polA⁻ E. coli mutants has been purified and chemically characterized according to the procedure of Kelley and Stump (J. Biol. Chem. 254, 2306 (1979)). Peptide mapping of the tryptic and cyanogen bromide peptides of the whole enzyme and of the Klenow large (75,000 MW) and small (35,000 MW) fragments indicate that there is significant internal sequence homology in the molecule. The extent of the homology supports the model of a three domain structure with each domain containing one of the enzymatic activities (Que, Downey and So, Biochemistry 18, 2064 (1979)). Similar mapping of polymerases from polA⁻ mutants yield the details of the structural defects resulting from the loss or modification of enzymatic activities caused by genetically characterized mutations. Crystals of associated large and small fragments (75,000 plus 35,000 MW) from wild type polymerase have been grown and analyzed by X-ray diffraction, optical diffraction of electron micrographs and gel electrophoresis.

Enzymology of General Recombination in E. Coli

1007 THE ROLE OF NUCLEOSIDE TRIPHOSPHATES IN THE ACTION OF THE recA PROTEIN OF E. COLI, G. M. Weinstock, K. McEntee, and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

The recA protein of E. coli plays a fundamental role in DNA repair and recombination in vivo. In vitro, the purified recA protein promotes renaturation of single-stranded DNA and assimilation of a single strand into duplex DNA to produce "D loop" structures. Both of these reactions utilize ATP and are accompanied by ADP generation. Hydrolysis of ATP is an intrinsic activity of the recA protein as demonstrated with purified mutant recA proteins. Three recA ATPase activities can be operationally defined: single-stranded DNA-dependent and double-stranded DNA-dependent ATPases, distinguished by pH and salt sensitivity and a DNA-independent ATPase with a significantly lower rate of hydrolysis. Consistent with these multiple activities, equilibrium dialysis indicates there are two types of ATP binding sites in the recA protein.

What is the role of ATP in the renaturation and assimilation reactions? ATP facilitates binding of recA protein to DNA and dissociation of recA-DNA complexes is accompanied by ATP hydrolysis. In addition, ATP promotes aggregation of the recA protein. This suggests that ATP promotes formation of a complex between recA protein and two DNA molecules which is ultimately dissociated by ATP hydrolysis. Complexes formed in the presence of the analog ATP-γ-S, which behaves like an irreversible inhibitor of the ATPase activity, do not dissociate. Since non-hydrolyzable analog inhibitors do not promote complex formation, it appears that ATP hydrolysis is required for complex formation. Possibly a phosphorylated recA protein is the active intermediate.

RecA protein also hydrolyzes UTP. Although UTP stimulates DNA binding, it is not as effective as ATP in renaturation or assimilation. This indicates that in vivo the activities of the recA protein are influenced by other nucleoside triphosphates.

1008 GENERAL RECOMBINATION IN DNA⁻ STRAINS OF E. COLI. M. Capage and B. Low. Yale University School of Medicine, New Haven, Conn. 06510.

We are investigating the question of whether or not enzymes involved in DNA replication, for example various dna functions, are involved in general genetic recombination in E. coli. By assaying the levels of active enzyme (β-galactosidase) produced from newly formed transcribable recombination products of a cross between two non-complementing mutants (lacZ118 x lacZ813), a measure of recombination can be obtained while under Dna⁻ conditions, using temperature-sensitive Dna⁻ mutants. So far, this approach has been used with the particular cross of λlacZ118 x FlacZ813 in strains carrying dnaZ⁻, dnaC2 or dnaA508. With dnaZ⁻ or dnaA508, the production of active β-galactosidase is virtually undiminished under conditions where the rate of DNA synthesis (uptake of ^{3}thymine) is less than 5% of that in isogenic dna+ strains. In the case of dnaC2, a 15-fold reduction of recombination products is observed, and this is correlated with a drastic inhibition of replication and/or expression of an Flac^+ for which recombination is not required for lacZ⁺ expression. dnaC2 had virtually no effect on the rate of recombination between λlacZ118 and a chromosomal lacZ813. We conclude that in those cases examined so far, recombination is not dependent on dnaZ, dnaA or dnaC function.

1009 A SINGLE-STRAND DNA-BINDING FORM OF THE PUTATIVE NEUROSPORA REC-NUCLEASE (ENDO-EXO-NUCLEASE) AND ITS RELATIONSHIP TO OTHER NUCLEASES OF NEUROSPORA, M.J. Fraser and T.Y.-K. Chow, McGill Univ., Montreal, CANADA H3G 1Y6

Neurospora endo-exonuclease, which was implicated previously in recombination, occurs in the cytosol of log phase wild-type mycelia in at least three forms, an inactive precursor (a single 90K polypeptide) present in an amount three times that of total active enzyme and two active forms which are resolved by chromatography on DEAE-Sepharose. One of the active forms (D1) binds to single-strand DNA (ss-DNA) cellulose in the presence of EDTA at low ionic strength and is insensitive to ATP. The other active form (D3) does not bind to ss-DNA and is inhibited by ATP. D1, but not D3, is found also in isolated nuclei, where it constitutes the major DNase activity. Both the inactive precursor and D1 are converted in vitro to a D3-like enzyme by treatment with trypsin. D1 also undergoes a dramatic conversion to another form (D2) when it is chromatographed on phosphocellulose. D2 strongly resembles the previously described mitochondrial nuclease and, like D3, does not bind to ss-DNA. Some evidence has been obtained that these enzymes are also closely related to both the ss-exonuclease and ss-endonuclease previously isolated from Neurospora. It now appears that all five enzymes listed above (the major nucleases in this organism) may be derived from the same inactive precursor, possibly by different routes of proteolysis at different sites in the cell or under different physiological conditions. An additional control on the active forms of endo-exonuclease (D1 and D3) is exerted:the exonuclease activities are completely suppressed in crude extracts by protein inhibitor(s). (Supported by M.R.C., Canada).

1010 REGULATION OF lexA GENE EXPRESSION, Joan E. Harper and David W. Mount, University of Arizona College of Medicine, Tucson, AZ 85724

The recA protein is essential for homologous recombination in E. coli, and plays a major role in the response to conditions which lead to the arrest of DNA synthesis. The level of recA gene expression is regulated by the lexA protein. We present evidence that the lexA protein participates in the negative regulation of its own synthesis, as well as that of recA gene expression.

The protein products of recombinant plasmids carrying various alleles of the lexA gene were examined using minicells. The plasmid carrying the mutant lexA3 allele directed the synthesis of the 24,000 dalton lexA3 protein, and the plasmid carrying the wild type allele directed the synthesis of the 22,000 dalton protein believed to be the $lexA^+$ product. Both the wild type and the lexA3 proteins were made in small amounts. The products of plasmids carrying three different amber mutations in the lexA3 gene were examined. In each case the lexA3 protein was not synthesized; and a new polypeptide, smaller than the lexA3 protein,was made. The new polypeptides, apparently amber fragments of the lexA3 protein, were greatly over-produced relative to the lexA3 product. A plasmid carrying a new mutation causing reduced lexA function, isolated following mutagenesis of the $lexA^+$ plasmid, was found to direct synthesis of a large amount of the putative $lexA^+$ protein in minicells.

Self regulation of lexA gene expression provides a possible mechanism for maintaining a constant level of recA protein during normal growth, while allowing rapid fluctuation of recA protein concentration following perturbation of DNA metabolism.

1011 λ PHAGES GROWN ON *E. coli arl* MUTANTS: RECOMBINOGENICITY AND S-1 SITES, John Hays and Brent Korba, University of Maryland Baltimore County, Catonsville, Md. 20228

A special subclass of *E. coli* hyper-rec mutants exhibits elevated recombination (but normal yields) of λ Red^- duplication phages. The mutants have been designated *arl* (accumulation of recombinogenic lesions), since substantially enhanced phage recombination requires prior growth on the mutant bacteria for 2 or 3 lytic cycles, yet phage recombinogenicity persists for 1 or 2 cycles in wild-type bacteria. "Arl^-" λ phages (propagated on *arl* bacteria) exhibit normal plating efficiencies, burst sizes, and mutation frequencies. Arl^- phage DNA sediments normally in alkaline sucrose, yet is cleaved by the (single-strand-specific) S-1 nuclease. S-1 treatment reduces the single-strand molecular weight of Arl^- λ DNA 8-fold, but does not cleave Arl^+ phage DNA. During successive rounds of phage growth in *arl* cells, recombinogenicity and S-1 susceptibility increase comitantly; limiting values (5-fold enhancement of recombination, about 18 S-1 sites per duplex) are attained after 5-6 cycles. Both Arl^- phage properties are lost in parallel during 2-3 growth cycles in arl^+ cells. Recombination of Arl^- phages is elevated in rec^+ and *recB recC sbcA* bacteria, but not in *recA, recB recC* or *recB recC sbcB* strains. Phages grown on *arl recA* double mutants and on *arl* rec^+ strains have similar "Arl^-" properties. Chromosomal DNA and plasmid DNA (pBR322) from *arl* mutants display enhanced cleavage by S-1. Mutation frequencies of *arl* bacteria are normal. By P-1 transduction, *arl* mutations are located near 2 min. on the *E. coli* genetic map.

1012 INITIATION OF GENETIC RECOMBINATION AND POSTREPLICATION REPAIR IN E. coli. Paul Howard-Flanders, Stephen West and Era Cassuto. Yale University, New Haven, CT.
A series of rec genes (A,B,C,F,L) have been identified by means of Hfr crosses as controlling recombination in E. coli. Investigations into λ phage-prophage recombination under repressed conditions have shown that disappointingly few of the available mutations affect this form of homologous recombination. Neither recB nor recF, either singly or together, cause any substantial reduction in the yield of recombinants. Evidently genetic recombination between homologous molecules can take place at a normal frequency without the need for the recB,C gene product (exonuclease V) or the recF gene product and the concepts of recB and recF pathways are of limited applicability. However, a lexC mutant showed only 5% of normal phage-prophage recombiation. Because of the difficulty in finding other mutations that block homologous recombination, the depression to 5 % by lexC (which controls single strand binding protein) may be significant. Experiments on cutting in trans (a related phenomenon) show a similar response to these mutations.

RecA protein recognises duplex DNA containing single stranded regions, binds cooperatively to this DNA showing an enhanced ATPase activity (West et al. these abstracts) and promotes homologous pairing with intact duplex molecules (Cassuto et al, these abstracts). Since the addition of recA protein tends to increase neutral sedimentation rates of duplex DNA molecules with single stranded regions, we conclude that recA protein binds extensively but does not cause extensive strand separation and hence might promote four strand hydrogen bonding between duplexes as well as other forms of pairing.

1013 RECOMBINATION OF PLASMID DNA IN VIVO AND IN VITRO, R. Kolodner, J. Joseph, A. James, F. Dean and M.J. Doherty, Sidney Farber Cancer Institute, Boston, Mass. 02115
A circular tetramer of pMB9 DNA containing a single Eco R1 site per tetramer was constructed and used to investigate intramolecular recombination in E.coli. In vivo, the tetramer was converted into a mixture of circular trimers, dimers and monomers in wild type strains, recB recC strains and recF strains. Conversion was blocked in recA strains and recB recC recF strains. Cell-free extracts of wild type E.coli converted the tetramer into circular dimers, trimers and monomers with "figure-8" molecules and catenanes of two dimers or a trimer and a monomer being observed as minor products. The total proportion of product molecules observed ranged from 7% to 18%. Formation of product molecules in vitro was blocked in extracts of recA strains and recB recC recF strains but was not blocked in extracts of recB recC strains or recF strains. Addition of homogeneous RecA protein restored activity to recA extracts while activity in recB recC recF extracts could be restored by the addition of either Exo V (RecBC nuclease) or a RecF protein donor extract. Novobiocin and oxolinic acid, specific inhibiters of DNA gyrase, inhibited the reaction catalyzed by wild type extracts by 70-80%. Studies on the structure of the tetramer substrate DNA and on other related plasmid DNAs suggested that the intramolecular recombination reaction observed in vivo and in vitro was dependent on the presense of a recombination hotspot in the substrate DNA. These hotspots appear to be different from λ Chi recombination hotspots in that they appear to stimulate recombination by the RecF pathway as well as by the RecBC pathway. Unlike λ Chi sites, they are also active when present in the plasmid DNA in either of the two possible orientations.

1014 GENETIC AND BIOCHEMICAL ANALYSIS OF THE recB and recC LOCI OF ESCHERICHIA COLI, Sidney R. Kushner, Mark Fortson, Peter Jensen, Paul Cook and Pau-hwa Chang, University of Georgia, Athens, Georgia 30602
The recB and recC loci of E. coli encode the multi-functional enzyme exonuclease V. Mutants in either loci are sensitive to uitraviolet light, DNA crosslinking agents and are markedly deficient in genetic recombination. In addition, in a growing culture a large fraction of the cells are inviable. Purified exonuclease V has a molecular weight of 270,000 daltons and is composed of two subunits of unequal size as based on SDS-polyacrylamide gel electrophoresis. We have recently isolated mutants of E. coli which are conditionally lethal for growth and which appear to map within the recC subunit of exonuclease V. At the non-permissive temperature DNA, RNA and protein synthesis appear to simultaneously shut-off within one generation of the temperature shift. At intermediate temperatures there is a reduction in recombination proficiency. Three factor transductional crosses indicate that the mutations are flanked by known recC alleles. Stable double mutants containing either recB or recC and the temperature sensitive locus can be constructed. The mutations are recessive and are not suppressed by either sbcB or sbcA as are the traditional recB and recC mutations. ATP-dependent degradation of double-stranded DNA in crude lysates seems to be higher in the mutants when grown at 44° C. Recently a large EcoRl fragment(approximately 20 kb) has been cloned from F'15 which appears to complement the temperature sensitive mutations. This fragment is currently being investigated for the presence of either recB or recC. (This work was supported in part by NIH grants GM00048 and GM21454.)

1015 Regulation of recA protein synthesis in E. coli, J.W. Little, J.E. Harper, S.H. Edmiston, L. Pacelli and D.W. Mount, University of Arizona, Tucson, Arizona, 85724.

The rate of transcription of the recA gene in E. coli is regulated by the lexA gene. A low level of recA protein is synthesized in normal cells, whereas in cells induced by treatment with a DNA-damaging agent, a very high level of recA protein is synthesized. Some mutations in the lexA gene block the induction of recA protein while others (including amber mutations) allow constitutive synthesis of recA protein. recA protein must also be functional for its own induction. These properties of mutant strains have led us to formulate a model in which the lexA protein is a repressor of the recA gene, and in which the recA protein is "activated" by DNA damage and cleaves the lexA protein, similar to the cleavage of λ phage repressor demonstrated by Roberts. This cleavage inactivates the lexA repressor and induces the recA gene. The lexA product has now been identified (J. Little and J. Harper, in press), and cleavage of the wild type $lexA^+$ protein demonstrated in vitro in an ATP, DNA and recA protein-dependent reaction. The mutant lexA product which prevents recA induction in vivo is not cleaved in vitro, suggesting this mutant makes a repressor which is, by analogy with the phage c1 ind^- mutant, not destroyed in the induced cell.

1016 MUTATIONS AFFECTING THE REC F PATHWAY OF RECOMBINATION, Susan T. Lovett and Alvin J. Clark, University of California, Berkeley, CA 94720.

In E. coli K12 recombination can be mediated via the Rec BC or the Rec F pathway. We have made insertion mutants in rec genes using the transposon Tn10. One such mutation, rec-284::Tn10, defines a gene which, like recF, is specific for Rec F pathway recombination in that it confers a recombination deficient, UV sensitive phenotype in a $recBC^-$ $sbcB^-$ genetic background and not in a $recBC^+$ background. This mutation has been mapped to 62 minutes on the E. coli genetic map, between lysA and serA, and is therefore distinct from those in recF which map at 82 minutes.

Subsequently we have found that six other mutations, including one designated as recJ, also map to 62'. Like rec-284::Tn10, these mutations affect only the Rec F pathway of recombination. Some of these mutations, unlike rec-284::Tn10, have no effect on UV repair. We are using genetic complementation to determine if these mutations all lie within the same gene.

1017 THE E.COLI RECOMBINATION SYSTEM DISCRIMINATES BETWEEN TRANSDUCING DNA ORIGINATING FROM GENE DENSE REGIONS AND THAT ORIGINATING FROM GENE SPARSE REGIONS, Millicent Masters and Barbara Newman, Department of Molecular Biology, Edinburgh University, Edinburgh, Scotland.

The genes of E.coli are not evenly distributed along the chromosome but tend to be grouped so as to form gene dense and gene sparse regions (1). Markers located within gene dense regions are transduced by the phage P1 with higher frequency than those located within gene sparse regions (2). We have evidence to show that this discrimination occurs in the recipient cell by, presumably, the recombination system, rather than in the donor cell through the action of the P1 packaging system. We find that UV-irradiation of the transducing lysate preferentially stimulates the transduction of those markers previously transduced at low frequency, indicating that these markers, although present in the recipient cells, had failed to be integrated. Irradiation of the recipient cells before transduction results in a similar preferential stimulation of transduction. We interpret these results to mean either that some component of the recombination system discriminates amongst transducing fragments or their chromosomal homologs unless either the synthesis of this component is stimulated or its substrate altered by damage to DNA. In either case, the result is a selection of gene dense regions as the preferred sites of recombination.

(1) Bachmann, B.J., Low, K.B., Taylor, A.L. Bact.Rev. 40, 116-166 (1976)

(2) M. Masters, Molec.Gen.Genet. 155, 197 (1977)

1018 DNA SYNAPTASE: AN ENZYME THAT FUSES DNA MOLECULES AT A REGION OF HOMOLOGY, Huntington Potter and David Dressler, Harvard University, Cambridge, Mass. 02138

DNA synaptase is a new Escherichia coli protein which has been purified to apparent homogeneity. The native protein has a molecular weight of 33,000 daltons, and appears to be composed of a single subunit. The enzyme functions without a high energy cofactor. DNA synaptase fuses genomes at a region of homology. This reaction is observed electron-microscopically as the conversion of monomer-size plasmid DNA circles to dimeric figure-8 molecules. Cleavage of the plasmid DNA with a restriction enzyme, prior to electron microscopy, leaves the fusion point in the figure-8 intact, and generates a structure snaped like the Greek letter chi. The geometry of the chi form with its two pairs of equal length arms, indicates that the enzyme has fused the two genomes at a region of DNA homology.

Purified synaptase also catalyzes an apparent partial reaction in which a free single strand is partially assimilated into duplex DNA at a region of homology. In this partial reaction, DNA synaptase shows a functional similarity with the Rec A protein of E. coli, which has been purified and studied by McEntee, Weinstock, and Lehman and by Radding and his colleagues. Genome fusion reactions will be discussed in terms of the molecular splicing mechanisms which have been proposed for DNA recombination.

1019 NUCLEOTIDE SEQUENCE OF CHI RECOMBINATIONAL HOTSPOTS. Gerald R. Smith, Dennis Schultz and Jean Crasemann, Inst. of Molecular Biology, Univ. of Oregon, Eugene, OR 97403.

Chi sites stimulate generalized recombination catalyzed by the RecA-RecBC dependent pathway of E. coli. Stimulation is greatest near the locus but is detectable 10^4 bp away. Presumably, these sites are recognized by a protein involved in a rate-limiting step of recombination. Chi sites are present in the chromosome of E. coli, but not of phage λ. χ^+ mutations at four loci in λ create Chi sites. [See Stahl, Ann. Rev. Genetics 13, 7 (1979) for a review.]

We have determined the nucleotide sequence at the χ^+B locus of λ (between xis and red) and compared this sequence with that at the χ^+C locus (in cII) [Sprague, Faulds, and Smith, Proc. Nat. Acad. Sci. 75, 6182 (1979)]. We find sequence homology between these loci as follows:

Site of χ^+B mutations creating Chi↓

χ^+B 5' A-C-A-A-T-G-A-G-T-G-G-C-A-G-A-T-A-T-A-G-C-T-G-G-T-G-G-T-T-C-A-G-G-C-G-G 3'

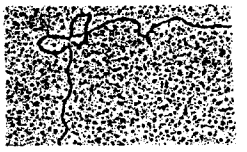
χ^+C 5' T-T-G-A-T-A-A-G-T-C-G-C-A-G-A-T-C-A-G-C-T-G-G-T-G-G-A-A-G-A-G-G-G-A-C 3'

Site of a χ^-C mutation destroying Chi↑ ↑Site of χ^+C mutations creating Chi

The extensive homology between the χ^+B and χ^+C loci supports the view that these sites are recognized by the same protein. The extent of the homology (23 bp) is much greater than we anticipated from the observation by Stahl and coworkers that Chi sites occur in the E. coli genome once every 5000 bp and from the assumption that these sites occur at random. To determine whether Chi sites have been selectively maintained in the E. coli genome or whether the Chi sequence is a subset of the homologous sequence shown above, we are determining the following sequences: 1) the χ^+A and χ^+D loci in λ, 2) the site of mutations creating Chi in the plasmid pBR322, and 3) the Chi site in the lacZ gene of E. coli.

1020 PHYSICAL AND GENETIC STUDIES OF THE ROLE OF EXONUCLEASE V IN GENETIC RECOMBINATION IN ESCHERICHIA COLI, Andrew Taylor and Gerald R. Smith, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403.

The structures shown in the electron micrograph result from the action of purified RecBC nuclease (Exo V) from E. coli on linear double stranded DNA. Their formation requires DNA binding protein, ATP and Mg^{++} ions in addition to the RecBC nuclease.



These "rabbit's ears" appear preferentially in certain regions of λ or T7 DNA, and we consider them candidates for an intermediate in genetic recombination. Their appearance on circular DNA and on DNA bearing Chi recombinational hotspots will be discussed.

N. Kleckner and V. Lundblad (Harvard University) have recently isolated mutants of E. coli in which the frequency of precise excision of the Tn10 transposon is greatly enhanced. We have confirmed that the lesions in two of these mutants map in or near the recBC genes. We have shown that the activity of Chi recombinational hotspots is greatly reduced in these mutants, which are still proficient in genetic recombination. We are testing the possibility that the RecBC nuclease is directly involved in Chi-mediated recombination by a comparison of the RecBC nuclease isolated from wild-type and mutant strains. (Supported by USPHS Grant AI 13079.)

1021 ELECTRON MICROSCOPY OF *E. COLI RECBC* ENZYME REACTION INTERMEDIATES, Karen M. Telander-Muskavitch and Stuart Linn, University of California, Berkeley, CA 94720

Reaction intermediates formed by the *E. coli recBC* enzyme with linear, duplex DNA have been spread for electron microscopy using polylysine. When spread immediately after the reaction, three types of single-stranded structures are observed at the termini of duplexes: (i) single tails, (ii) double tails, generally of unequal length (forks), and (iii) loops associated with one or two tails [loop + tail(s)]. Terminal, single-stranded loops appear always to be associated with one or two tails. Post-reaction incubation of the intermediates for several hours at 0° or for 10 minutes at 23° or 37° causes a loss of the loop + tail(s) and forks with a concomitant increase in the frequency of tails. These represent the tails observed by MacKay and Linn [J. Biol. Chem. 249, 4286 (1974)]. If *E. coli* DNA binding protein is included in the post-reaction incubation, loop + tail(s) are lost, while the frequencies of forks and tails both increase. Evidently the dissociation of the loop + tail(s) creates forks which can reanneal in the absence of DNA binding protein.

These results lead us to refine the model for *recBC* enzyme action on linear, duplex DNA proposed by MacKay and Linn, since that model is inconsistent with the observation of either large forks or of loop + tail(s). Therefore, we propose that the enzyme acts by (1) binding to the terminus of a linear, duplex DNA molecule; (2) unwinding the two strands of the double helix forming a loop with one and a tail with the other; (3) degrading the loop from its terminus toward the single-stranded: duplex junction; then (4) degrading the tail, presumably toward the terminus, but in a manner which allows the enzyme to stay bound to the duplex. This cycle is repeated until the entire DNA molecule is eventually degraded.

1022 TEMPERATURE DEPENDENT RESTORATION OF RECOMBINATION PROFICIENCY IN A recB21 recC22 sbcB15 recF143 MUTANT STRAIN BY THE tif-1 MUTATION OF THE recA GENE. Michael R. Volkert, Linda J. Margossian, and Alvin J. Clark, Department of Molecular Biology, University of California, Berkeley, California 94720.

In wild type strains genetic recombination occurs primarily via the recB recC pathway since mutations in either of these genes reduces recombination proficiency to less than 1%. In recB, recC, or recB recC double mutant strains, genetic recombination can be restored to wild type levels by sbcB mutations. Recombination in a recB recC sbcB triple mutant strain is dependent upon the recF gene. The recF143 mutation causes recombination proficiency to be reduced to less than 0.1% when it is introduced into a recB recC sbcB mutant strain. We can restore recombination proficiency to a recB21 recC22 sbcB15 recF143 strain by introducing the tif-1 mutation. The tif-1 mutation lies in the recA gene; in wild type strains it has little or no effect on recombination proficiency but causes the temperature dependent expression of SOS functions (SOS functions are those cellular processes which are induced after UV treatment in recA$^+$ lexA$^+$ strains of E. coli). In a recB21 recC22 sbcB15 recF143 strain some recombination proficiency is restored at 30°C by the tif-1 mutation; upon incubation at 42°C recombination proficiency is restored to wild type levels. This indicates that in this strain the mutant recA protein produced by the tif-1 allele is able to suppress the recombination deficiency conferred by the recF143 mutation by one of three types of mechanisms: interaction of the mutant recA and recF proteins, functional substitution of recA for recF, or by allowing recA to function independently of recF.

1023 SITE-SPECIFIC RECOMBINATION OF THE PLASMID COL E1, Gareth J. Warren and Alvin J. Clark, University of California, Berkeley, CA 94720

Conjugal transmission of ColE1 is thought to occur by transfer of a linear DNA strand. A breakage event is postulated to create this linear in the conjugal donor, and a ligation event is postulated to recircularize it in the recipient. We expected this breakage and reunion to result in a system capable of recA-independent recombination.

We were able to demonstrate such a system: recombination occurred only upon conjugal transmission, and it was specific to the genetic site bom and physical site nic (bom and nic probably represent the transfer origin of ColE1). To show recA-independent recombination, derivatives of ColE1 containing tandem duplications were transmitted by conjugation from recA$^-$ donors to recA$^-$ recipients. Recombination between the duplications could be detected because it reduced the size of the plasmids appearing in the recipients. Such a size reduction occurred only when the region to be duplicated contained a bom site.

This recombination in cis occurred at high frequency: 10-70% of the recipient clones contained the plasmid products of recombination. We also detected bom-site-specific recombination in trans, when two different plasmids each carrying a bom site were transmitted in the same cross. The recombination in trans was less frequent (1-5%) than in cis. This contrasts with the preponderance of trans- over cis-recombination of plasmids during vegetative growth of recA$^+$ cells.

1024 CLONING AND STUDY OF THE recA and ssb GENES OF E. coli, W. Dean Rupp and Aziz Sancar, Yale University, New Haven, CT 06510.

The recA gene of E. coli is of much interest because of its role in recombination, repair and mutagenesis. The recA gene is regulated at the transcriptional level and the recA protein itself is fascinating because of its diverse enzymatic activities including a protease activity highly specific for repressors, a DNA-dependent ATPase and its ability to catalyse the pairing of homologous DNA's. In an effort to better understand its regulation and function, we have cloned and sequenced the recA gene and its regulatory region. The sequence of the promoter closely resembles other very strong E. coli promoters. The amino acid sequence derived from the DNA sequence is of interest because a sequence similar to that observed in a variety of serine proteases is present in the recA protein. Comparison with the sequences of recA mutants is now in progress to determine specific sites of alteration and to correlate them with phenotypic properties of the various mutants.

The ssb gene of E. coli coding for the DNA single-strand binding protein has also been cloned and experiments are now in progress to determine the DNA sequence of the gene.

Genetic Analysis of DNA Replication

1025 STUDIES WITH THE ESCHERICHIA COLI dnaJ and dnaK GENES, Costa P. Georgopoulos[1], J. Yochem[2] and Mike Feiss[2], Department of Cellular, Viral and Molecular Biology, University of Utah, Salt Lake City, Utah 84132[1] and Department of Microbiology, The University of Iowa, Iowa City, Iowa 52242[2]. Analysis of E. coli mutants unable to propagate bacteriophage λ has revealed the existence of dnaK and dnaJ bacterial genes. These two genes have been shown to be closely linked and to map at 1' on the E. coli genetic map between the thr and leu loci. They have also been shown to constitute an operon with the relative order being promoter-dnaK dnaJ. DnaK and dnaJ bacterial mutants were found to block λ propagation at the level of λ DNA replication by specifically interfering with the function of the λ gene P product. In addition, some of these bacterial mutants were shown to be temperature sensitive for bacterial growth at high temperature. Some dnaK mutations result in a complex phenotype at high temperature, inasmuch as both bacterial DNA replication and RNA transcription seem to be affected. The dnaK and dnaJ genes have been cloned onto bacteriophage λ vectors and amber mutations in these genes have been isolated. The dnaK and dnaJ gene products have been identified as polypeptides with apparent molecular weight of 93,000 and 37,000 respectively. It has been shown that the dnaK756 mutation results in the overproduction of the dnaK756 product at high temperature.

1026 dnaA AND THE REGULATION OF INITIATION, L. Caro, J. Frey, G. Kellenberger -Gujer and M. Chandler, Dpt. Molecular Biology, University of Geneva, Switzerland

The dnaAcos mutations are phenotypic suppressors of dnaAts which retain the dnaAts allele, are co-transduced with dnaA, render the cell cold sensitive, and cause an overinitiation of chromosome replication when the cells are shifted from 42° to 30°.

The plasmid pSC101 requires dnaA for its replication. This requirement is not satisfied when a dnaAts is phenotypically suppressed at 42° by integration of R100 into the chromosome but it is satisfied by cos suppression. A shift of a dnaAcos strain from 42° to 30° stimulates pSC101 replication. DNA-DNA hybridization studies on the replication of various plasmids and of the chromosome in dnaA and dnaAcos strains indicate that dnaA plays a role in the regulation of both the chromosome and pSC101 but that additional factors are involved in chromosome regulation.

1027 REGULATION OF CHROMOSOME COPY NUMBER IN A *dnaA*^{ts} MUTANT. Joe A. Fralick, Texas Tech Health Sciences Center, Lubbock, Texas 79430. We have previously demonstrated that under balanced, steady-state growth conditions, the *dnaA*^{ts} mutant of *Escherichia coli*, N167, maintains, on the average, two replicating chromosomes per cell at the permissive growth temperature of 30°C and only one per cell at 38°C. When the growth temperature of this mutant is changed from 30°C to 38°C or *vice versa* the cells rapidly adjust their chromosome copy number to a number characteristic of the temperature to which they have been transferred. We have examined the kinetics of this transition with reference to DNA replication and cell division. Our results demonstrate that this mutant can uncouple cell division and chromosome replication transiently in order to achieve the appropriate chromosome composition suggesting that the *dnaA* gene product may be involved in controlling the coordination between these two cellular events.

1028 THE ISOLATION OF DUPLICATIONS IN GENE 32 OF PHAGE T4 AND OF INTERACTING MUTATIONS IN THREE DIFFERENT GENES, Debabrota Ghosal, Gisela Mosig and Susan Bock, Vanderbilt University, Nashville, Tennessee 37235

To better understand the multiple interactions of gene-32 protein (HDP) in complexes of DNA replication and recombination we have tried (unsuccessfully) to construct gene-32 double mutants. We found no stable gene-32 double mutants in the progeny of appropriate crosses, even though wild type recombinants were formed. (Presumably most true double mutants are not viable.) However, two kinds of secondary mutations appear with rather high frequencies in such crosses. Numerous genetic tests together with PAGE of the proteins synthesized in various strains, suggest that many of these mutants contain duplications of a large segment of gene 32. As expected, the duplications are unstable; they generate single copy derivatives as well as higher copy numbers and compensating deletions in non-essential regions, particularly in the rI region. They are easily recognized when they segregate both *am* and *am*$^{+}$ progeny. Apparently the duplications also generate *ts-am* double mutants which contain a parental gene-32 *am* mutation together with a new *ts* mutation elsewhere on the genome. The *ts* mutations map in three different regions: in gene 41, in or near gene v; the third cluster is not yet mapped. - We have concentrated on those that map in gene 41 which is involved in synthesis of RNA primers. These gene 41 mutations interact with other phage and host mutations affecting membrane functions. These results suggest that precise interactions of gp32 and gp41 are essential in initiation of DNA replication and for membrane properties.

1029 DECREASED INITIATION OF DNA SYNTHESIS IN A TEMPERATURE-SENSITIVE MUTANT OF HAMSTER CELLS. Roger Hand, Eric Eilen and Claudio Basilico, McGill Cancer Center, Montreal, P.Q. H3G 1Y6 and New York University School of Medicine, New York, N.Y. 10021.

We have analyzed ongoing DNA replication in ts BN-2, a *dna*$^{-}$ mutant of BHK-21 cells. At the non-permissive temperature of 39.5°C, inhibition of ^{3}H-thymidine incorporation into acid-precipitable material begins 2 hr after the cells are released from a block at the start of the S-phase. The fraction of nuclei incorporating ^{3}H-thymidine is similar to that of wild-type cells through the synchronized S-phase of 8 hr. Alkaline sucrose gradient analysis shows that pulse-labeled DNA from mutant cells is incorporated into high molecular weight material after 3 hr at either the permissive or non-permissive temperature. DNA fiber autoradiograms reveal that, at 39.5°C, the rate of replication fork movement is about 30% increased in the mutant as compared to wild-type cells. On the other hand, in the mutant cells the interval between adjacent initiation sites is increased and the relative frequency of initiation events is decreased at the restrictive temperature. The results indicate that there is a block to ongoing replication in ts BN-2 at the level of initiation of synthesis on individual replication units; elongation of nascent chains is not inhibited.

1030 GENETIC REGULATION OF THE T4 REPLICATION FUNCTIONS. Jim Karam, William Gerald, and Myra Dawson. Dept. of Biochem., Med. Univ. of S.C., Charleston, SC 29403

Control of function of the T4 DNA replication proteins is being studied by the use of regulatory phage mutants and cloned fragments of T4 DNA. We are focusing on the T4 chromosomal segment that encompasses genes 43 (DNA polymerase), regA (mRNA-inactivation protein), 62/44 (DNA-dependent ATPase), and 45 (45-protein, which stimulates the 62/44-ATPase and also plays a role in transcription of "late" phage genes). This segment has been cloned in a lambdoid phage vehicle by N. Murray. All 5 T4 proteins are synthesized in E. coli infected with the clone and transcription of the cloned T4 segment appears to be primarily under control of the lambdoid P_L promotor. Attempts are being undertaken to subclone the genes of this segment in plasmids carrying the E. coli lac promotor/operator element. Hitherto uncharacterized DNA fragments in some subclones have been shown to be lethal to the cells when induced with IPTG. Measurements of the relative levels of 43-, regA-, 62-, 44- and 45-protein in infections with the lambdoid clone and normal T4 phage suggest that both transcriptional and translational mechanisms control these levels. In related work, we have identified a region on T4 rIIB mRNA that is probably the target site for the regA protein, and similar regions are being sought for the gene 45, 44, and 62 transcripts. [Supported by GM18842, NIGMS].

1031 FINE STRUCTURE OF THE E. coli polA GENE, William S. Kelley, Dept. of Biological Sciences, Mellon Institute of Science, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213

The fine structure of the E. coli polA gene has been determined by generalized transductional analysis utilizing phage P1. The close genetic linkage of the polA gene to the glnA gene provides a more powerful transductional selection than other markers used previously. This updated genetic map includes nine different polA alleles, seven missense and two nonsense mutations. Map positions of the seven missense mutations correlate well with known enzymatic defects observed in the mutant enzymes. The products of the nonsense mutations have been analyzed by gel filtration chromatography and identified using anti-Pol I antibodies. Derivatives of lambda polA specialized transducing phages carrying the nonsense mutations have been used to amplify the amber peptides produced by these alleles.

1032 ISOLATION AND CHARACTERIZATION OF A CONDITIONAL-LETHAL Sdr MUTANT OF Escherichia coli, Tokio Kogoma, University of New Mexico, NM 87131.

Previously we isolated Sdr^C mutants capable of stable DNA replication (SDR - DNA replication in the absence of protein synthesis) without inducing treatments (Kogoma, 1978). These mutants were not conditional-lethal and could grow at 37°C although their growth rates were slightly slower than that of the parent. To determine whether or not the sdr gene function is indispensable for normal cell growth, we have made attempts to isolate an Sdr mutant which is temperature-sensitive for growth. We will report the isolation and partial characterization of such an Sdr^C mutant. The temperature sensitivity of the mutant is cell-density dependent. Whereas stable DNA replication (iSDR) induced in a wild type strain is resistant to UV irradiation (Kogoma et al., 1979), constitutive SDR of the ts mutant is not. Temperature-insensitive spontaneous revertants isolated from the mutant have lost the SDR capability, suggesting that a single mutation is responsible both for the temperature sensitivity of growth and for the SDR phenotype.

Kogoma, T. (1978). J. Mol. Biol., 121, 55-69.
Kogoma, T., Torrey, T.A., Connaughton, M.J. (1979). Mol. Gen. Genet. (in press)

1033 The capR (lon) GENE OF E. coli K-12 CODES FOR A DNA BINDING PROTEIN
Alvin Markovitz, Barbara Zehnbauer, Gordon W. Henderson and Edward C. Foley, University of Chicago, Chicago, Illinois 60637

Bacteria, mutant in the capR (lon) gene, are UV-sensitive and mucoid. An 8.2 Mdal EcoRl DNA fragment that contains the $capR^+$ gene was cloned, in both orientations, using EcoRl-digested pSC101 as a cloning vehicle. Minicells and maxicells (recA uvrA phr) containing the $capR^+$ plasmids, pBZ201 or pBZ203, synthesized two new plasmid-coded polypetides of approximately 92 and 67 Kdal. capR mutant derivatives of pBZ201 were isolated and characterized as (1) recessive to $capR^+$and specified 10% as much 92 Kdal polypeptide or (2) dominant to $capR^+$, with expression of mucoidy and UV sensitivity, and specified increased quantities of the 92 Kdal polypeptide (pBZ201M9). These results indicated that the 92 Kdal polypeptide was the translation product of the capR (lon) structural gene. Cell extracts containing pBZ201 or pBZ201M9 were fractionated on phosphocellulose and then DNA-cellulose columns and assayed for DNA binding activity by a nitrocellulose filter assay. The 92 Kdal polypeptide (90% purity) contained DNA binding activity. DNA binding activity was also demonstrated by retention on a DNA-cellulose column; the protein did not bind to cellulose. The ^{35}S-labeled, plasmid-specified 92 Kdal polypeptide and the purified 92 Kdal DNA binding polypeptide were identical by peptide mapping and by antibody precipitation. Thus the cloned capR (lon) gene specified a DNA binding protein with monomer molecular weight of 92,000.

1034 TWO DIFFERENT MODES OF INITIATION OF PHAGE T4 DNA REPLICATION, Gisela Mosig, Stephen Benedict, Andreas Luder and Susan Bock, Vanderbilt University, Nashville TN 37235

Our previous studies have suggested that phage T4 uses two different modes to initiate DNA replication: (i) initiation from defined origin sequence(s) which generates loops in double stranded DNA and presumably requires RNA primers and (ii) initiation from branched recombinational intermediates which can use 3'OH ends of parental DNA molecules as primers (Mosig et al, Microbiology 1980, in press).

After appropriate density labeling, progeny DNA which is initiated de novo (presumably RNA primed) and has not recombined can be separated from progeny DNA which is covalently linked to parental DNA. In "Southern hybridizations" progeny DNA initiated de novo early after infection, hybridizes preferentially to restriction fragments containing an origin region near (but not in) gene 43 (DNA polymerase). In contrast, the DNA which is covalently linked to parental DNA hybridizes to all restriction fragments in nearly equimolar proportions. (Fragments containing a hot spot of recombination, gene 34, are slightly preferred.) We are extending these studies using appropriate mutants to determine which of the T4 initiation proteins are involved in de novo (RNA primed) initiation and which initiation proteins are required for converting recombination forks into replication forks.

1035 SUPPRESSION OF THE $polC_{ts}$ MUTATION IN Escherichia coli, Osami Niwa, Sharon L. Bryan and Robb E. Moses, Baylor, Houston, TX 77030

Spontaneous temperature-resistant (TR) revertants or methyl methanesulfonate (MMS)-resistant revertants were isolated from E. coli HS432 ($polA1^-$, $polB^-$, $polC_{ts}$). Many of the revertants manifest $PolA^+$ (MMS-resistant) and $PolC^+$ (TR) phenotype despite the selective pressure. Genetic analysis of several TR revertants revealed that they still contained $polA^-$ and $polC_{ts}$ mutations. Partially purified DNA polymerase III from them is as temperature-sensitive as that of HS432. This suggests that an extragenic suppressor (sac, suppressor of polA, polC) may allow DNA replication in the presence of inactive DNA polymerase III. The suppression appeared to be accompanied with the occurrence of N-ethylmaleimide (NEM)-resistant DNA polymerase activity, which is characteristic of DNA polymerase I . The NEM-resistant polymerase has been purified several hundred fold, but requires a different purification from authentic DNA polymerase I. The purified polymerase shows the same inactivation by anti-polymerase I antibody, elution from phosphocellulose and heat inactivation as DNA polymerase I. To investigate the nature of the polymerase, we introduced a $polA^+$ gene into one of the TR strains by P1 transduction. The transductants contained $polC_{ts}$ as well as $polA^+$ and they remained TR. This indicates the TR suppression is not interfered with by the wild type polA gene. Moreover, the transductants contained a normal level of NEM-resistant polymerase and it could be purified by an ordinary purification for DNA polymerase I. Therefore, it is unlikely that the sac mutation generally affects the activity level and the purification properties of DNA polymerase I. (Supported by grants from the U.S.P.H.S., the American Cancer Society and the Robert A. Welch Foundation).

1036 ABERRANT DNA SYNTHESIS IN MUTAGEN SENSITIVE STRAINS OF DROSOPHILA MELANOGASTER, Robert Shenkar, Jeannette Robertson and James B. Boyd, University of California, Davis, CA 95616.

Drosophila melanogaster is being employed as a model for analysis of DNA metabolism in higher eukaryotes. In preparation for biochemical analyses over 100 mutants have been recovered which are hypersensitive to mutagens. These mutants have been assigned to about 30 genetic loci on the basis of complementation and mapping studies. Thus far, primary cell cultures have been prepared from representative mutants of 19 complementation groups. Such cultures have been incubated with ^{3}H-thymidine for 30 min and in its absence for an additional 3 hrs. Alkaline sucrose gradient analysis of the labeled product has revealed an altered pattern of synthesis in mutants at 6 different genetic loci. Experiments in which the concentration of the radioactive precursor was varied largely rules out artifacts due to hypersensitivity of the mutants to ^{3}H-thymidine. Parallel analyses which involve only a 20 min pulse have revealed synthetic defects in an additional 3 mutants from this collection (data of T. C. Brown).

Cell cultures derived from the mei-41^{D5} mutant have previously been shown to exhibit a reduced capacity to synthesize DNA on a damaged template. We now report that larvae of the homozygous mei-41^{D5} stock are highly sensitive to killing by hydroxyurea. In addition, the DNA synthetic capacity of these mutant cells is hypersensitive to this inhibitor. This mutant may, therefore, influence normal DNA synthesis as well as replication on a damaged template.

1037 GENETIC ANALYSIS OF dnaB MUTANTS OF E. COLI, Robert A. Sclafani and James A. Wechsler, University of Utah, Salt Lake City, UT 84112

An analysis of the nascent DNA intermediates synthesized in vitro by wild-type, dnaB, dnaG and dnaB, dnaG mutants will be discussed. The intermediates examined are 5.4s, observed after synthesis by dnaB and detectable after synthesis by dnaG extracts; 10s, observed after synthesis by all but dnaG extracts; and 35s DNA. Genetic studies of dnaB amber and dnaB::Tn10 mutants discussing the interaction of dnaB, P1 ban + λP gene products will be presented. A fine-structure map of dnaB alleles using a series of deletions within the dnaB gene is currently in progress. The partial fine-structure map will be analyzed with respect to position of the alleles causing various DNA synthesis defects and groP phenotypes characteristic of mutations in this gene.

1038 RECOMBINATION ASSOCIATED DNA SYNTHESIS INDUCED BY T4 BACTERIOPHAGE, Susan S. Wallace and Robert J. Melamede, New York Medical College, Valhalla, NY 10595.

Escherichia coli cells were infected with wild-type T4 bacteriophage or recombination deficient T4 mutants and examined 15 minutes after infection both in vivo or after sucrose plasmolysis for their ability to incorporate into the acid-insoluble fraction low concentrations of several radioactive DNA precursors. Both plasmolysed and non-plasmolysed infected cells showed similar rates of incorporation of 1μM ^{3}HTdr and ^{3}HTMP, while non-plasmolysed cells exhibited a distinct lag in incorporation of label from TTP. T4x-infected cells showed reduced incoporation of 1μM ^{3}HTdr both in vivo and after plasmolysis, which effect was obviated in both cases by either the addition of hydroxyurea at the onset of infection, or when the Tdr concentration was raised to 10μM. A reduced rate of incorporation of 1μM ^{3}HAdr was also observed in T4x-infected cells when compared to wild-type. In contrast, T4w-infected cells showed an increase in incorporation of ^{3}HTMP. Using thin layer chromatography, comparisons were made between mutant- and wild-type-infected cells with respect to the distribution of exogenously added ^{3}HTdr and ^{3}HTMP immediately and 5 minutes after addition of label, and no obvious differences were observed. These and other results will be discussed with reference to the possible involvement of DNA precursor complexes in recombinational DNA synthesis.

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1039 EFFECT OF N^6-METHYLADENINE CONTENT IN DNA ON FREQUENCY OF SPONTANEOUS MUTATION IN NON-GLUCOSYLATED PHAGE T2gt⁻, Stanley Hattman and Anna Iannotti, University of Rochester, Rochester, N.Y. 14627.

Derivatives of non-glucosylated phage T2gt⁻ differing genetically in their DNA content in N^6-methyladenine (MeAde) were compared with respect to their frequencies of spontaneous mutation. The frequency of spontaneous reversion from gt⁻ to gt⁺, as well as for an amber mutation in gene 42, was analyzed. We observed that the unmethylated (< 0.05 mole % MeAde) and partially methylated (0.7 mole % of MeAde) phage strains exhibited fairly similar reversion indices for the α gt-1 and am 42 markers; in contrast, the hypermethylated form (2.2 mole % MeAde) showed significantly elevated reversion indices compared to the other two phages. It is concluded that "methylation-instructed mismatch repair" is not operative on the T2gt⁻ phages. Furthermore, we propose that hypermethylated phage has a higher incidence of mutation because during DNA replication MeAde has a higher probability of base-mismatching than Ade.

1040 EVIDENCE FOR A SET OF GENES INDUCED BY DNA DAMAGE, Graham C. Walker and Cynthia J. Kenyon, Biology Department, Massachusetts Institute of Technology, Cambridge MA 02139

The Mud(Ap, lac) operon fusion vector (Casadaban and Cohen, 1979) was used to insert the lactose structural genes randomly into the Echerichia coli chromosome. Fusions were identified which showed increased lac gene expression in response to treatments with the DNA-damaging agent mitomycin C. Induction of β-galactosidase in these din (damage-inducible) strains was i)triggered by ultraviolet light as well as by mitomycin C and ii)abolished by either a recA⁻ or lexA⁻ mutation. In these respects din gene expression resembles the expression of prophage λ genes in a λ-lac fusion which was also generated by Mud(Ap, lac). The din genes map at five distinct bacterial loci. One din::Mud(Ap, lac) insertion results in a UV-sensitive phenotype and may be within the uvrA transcriptional unit. Another din::Mud(Ap, lac) fusion maps very close to polB by three factor crosses. We are determining whether this insertion has any effect on polymerase II levels. These results indicate that E. coli contains a set of genes whose expression is stimulated by DNA-damaging agents and regulated by the recA and lexA gene products.

1041 COLD-SENSITIVE EXTRAGENIC SUPPRESSORS OF AN ESCHERICHIA COLI DNAZ TS ALLELE, Joan M. Henson, Joyce A. Ramsey, William G. Haldenwang, and James R. Walker, University of Texas, Austin, Texas 78712

Temperature-sensitive (ts) dnaZ mutants of Escherichia coli are defective in chromosome polymerization at elevated temperatures. In an effort to identify other components of the chromosome replication mechanism, temperature-insensitive "revertants" were selected and screened for the acquisition of cold-sensitivity (i.e., the loss of ability to grow at 20°C). Approximately 2% of the "revertants" concomitantly became cold-sensitive (cs). Half the cs revertants had acquired second mutations which are tightly linked to the original dnaZts mutation and might be in the dnaZ gene (near minute 10.5).

Some of the remainder were mapped near minute 82 in the sequence cou cs tna by P1 transduction; they are, therefore, near the dnaA gene. Transduction experiments with a set of λtna phages (from Kaspar von Meyenburg) are in progress to determine if the cs mutations are in or near dnaA. A cs mutation, when moved into a dnaZ⁺ genetic background, retained its cold-sensitivity and acquired a ts phenotype. If these cs mutations are in dnaA, they indicate an additional role for wild-type dnaA protein. If they are in a gene near, but separate from, dnaA, they define a previously undiscovered DNA synthesis gene.