MECHANISMS OF DNA REPLICATION AND RECOMBINATION

Thomas Kelly and Roger McMacken, Organizers

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Plenary Sessions

Poster Sessions

Enzymology of DNA Synthesis

I1 ANALYSIS OF METHYL-DIRECTED MISMATCH REPAIR IN VITRO. Robert S. Lahue, Shin-san Su, Katherine Welsh, A-Lien Lu, and Paul Modrich, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710.

We have developed an in vitro assay to study DNA mismatch repair in cell-free systems (1,2). Repair of single base pair mismatches with efficiencies up to 80% is observed using wild-type E. coli extracts but not with extracts from strains deficient in mutH, mutL, mutS, or uvrD. The mutant extracts can be complemented which has provided an assay for the purification of milligram quantities of each of these proteins in biologically active forms. Footprinting experiments showed that mutS protein specifically binds to DNA regions containing a single base pair mismatch with a specificity of GT GA CA CT. Each of these mismatches can be repaired efficiently in vitro. Preparations of mutH protein exhibit an endonuclease activity which cleaves the unmethylated strand at the sequence GATC in hemi-methylated substrates. Unmethylated duplexes are cleaved more slowly and fully methylated molecules not at all. The activity of mutL protein has not yet been elucidated. Previous studies (3,4) have identified uvrD protein as DNA helicase II.

Reconstitution experiments with pure proteins have not succeeded, prompting a search for other components. Single-stranded binding protein (ssb) has been identified as a necessary factor for repair (2). Other proteins which are not required include DNA polymerase I and recBC and recF proteins. A number of other enzymes involved in DNA metabolism are under investigation.

Mismatch correction in vitro also requires d(GATC) sequences. Earlier experiments showed that not only does the state of methylation at these sequences direct repair both in vivo (5) and in vitro (1,2) but also that DNA synthesis associated with the repair process appears to require an endonucleolytic event at or near d(GATC) sites (2). A substrate lacking d(GATC) sequences is not repaired in our system whereas very similar DNA molecules containing one, two, or four d(GATC) sites are repaired with increasing efficiencies. These results and those observed for the activity of mutH protein suggest a direct role for these sequences in the repair process.

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I2 DNA REPLICATION IN EMBRYOS OF DROSOPHILA MELANOGASTER, Susan Cotterill, Robert DiFrancesco and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

We have undertaken the analysis of DNA replication in embryos of Drosophila melanogaster. Our approach has been to purify enzymes analogous to those known to be essential for DNA replication in prokaryotes, and with the purified enzymes, to reconstitute a replication complex that can function at a replication fork in vitro. Thus far, we have purified a polymerase-primase and a RNase H, both to near homogeneity. The polymerase-primase consists of four subunits with molecular weights of 182,000, 73,000, 60,000 and 50,000. Polymerase activity is associated with the 183 kdalton subunit; primase is associated with the 60 and/or the 50 kdalton subunit. The subunits can be effectively separated by treatment with 3.4 M urea, followed by a combination of Biorex 70 and DNA-cellulose chromatography. Attempts to reconstitute fully active enzyme from the separated subunits are in progress with the aim of determining the factors that regulate coupling of primase and polymerase action. A clone containing all or a large part of the gene coding for the 73 kdalton subunit has been isolated; isolation of clones for the genes coding the other three subunits is under way.

The Drosophila RNase H has a molecular weight of 180,000 and consists of two 49,000 and two 39,000 dalton polypeptides. It can remove RNA primers that are synthesized and subsequently elongated by the Drosophila polymerase-primase. Preincubation of the Drosophila RNase H with the polymerase-primase results in an increased rate of DNA synthesis. The DNA chains synthesized under these conditions are shorter than those synthesized in the absence of the RNase H and the rate of primer synthesis is increased. These findings suggest that the RNase H forms a complex with the polymerase-primase, increasing its recycling capacity and thereby increasing the frequency of chain initiation.

13 **MECHANISM OF THE DNA POLYMERASE III HOLOENZYME CATALYZED REACTION**, Charles S. McHenry, Kyung O. Johanson and Robert Oberfelder, Department of Biochemistry, Biophysics and Genetics, University of Colorado School of Medicine, Denver, Colorado 80262

The DNA polymerase III holoenzyme of *Escherichia coli* is the complex form of DNA polymerase III that is responsible for the synthesis of the bacterial genome. We have shown it to be a dimeric enzyme that contains at least seven different subunits (α, ε, θ, τ, γ, δ, and β)[1]. The enzyme forms a tight initiation complex with primed single-stranded DNA that is encoated by the *E. coli* single-stranded DNA binding protein in the presence of ATP, dATP or ATPγS[2]. Replication proceeding from this initiation complex is exceedingly rapid, approximating the rate of movement of the replicative complex at replication forks *in vivo*. However, the recycling of holoenzyme from one DNA template to the next presents a strong kinetic barrier on single-stranded DNA templates[3]. Using the ATP analog, ATPγS, we have demonstrated a functional asymmetry between the two halves of the dimeric holoenzyme[2]. This observation has been extended to build a model for a replicative complex that has distinguishable leading and lagging strand polymerases with properties that permit overcoming the afore-mentioned kinetic barrier on natural replication forks[4].

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14 BACTERIOPHAGE T4 DNA REPLICATION SYSTEM, Nancy G. Nossal, Deborah M. Hinton, and Ross W. Richardson, National Institutes of Health, Bethesda, MD 20892

Seven bacteriophage T4 proteins are required for DNA synthesis at a replication fork in vitro. T4 DNA polymerase carries out strand displacement synthesis on the leading strand only with the assistance of the gene 44/62 and 45 polymerase accessory proteins and the gene 32 helix destabilizing protein. The gene 41 and 61 proteins together function as a primase which makes ribonucleotide primers initiating discontinuous DNA synthesis on the lagging strand. In addition, the primase complex acts as a 5' to 3' helicase that unwinds the DNA duplex ahead of the growing leading strand.

In order to determine the functions of the 41 and 61 proteins in the primase complex, we have produced large quantities of each protein from plasmids in which the primase genes are controlled by the P_{tac} or λP_L promoters. Although previous studies showed no primer synthesis without both 41 and 61 proteins, we now find that very high concentrations of 61 protein alone can catalyze some oligonucleotide synthesis on single-stranded DNA templates. 41 protein alone has no primer synthesis activity. Oligonucleotides made by 61 without 41 serve as primers for DNA synthesis by either T4 DNA polymerase (with the three polymerase accessory proteins) or by E. coli DNA polymerase I. The dinucleotides pppAC and pppGC are the major products made by 61 protein, in contrast to the pentanucleotides made by the 41 and 61 proteins together. However, it is not yet clear whether it is these dinucleotides or the trace quantities of larger oligonucleotides that act as primers in the absence of 41 protein. Even at the highest levels of 61 protein, both oligonucleotide and DNA synthesis are markedly stimulated by 41 protein. Similarly, the 41 protein helicase activity is increased more than 30-fold by 61 protein, which has no independent unwinding activity. Thus we conclude that within the primase complex, 61 protein is primarily responsible for primer synthesis, and 41 protein for DNA unwinding, but that the 41/61 complex catalyzes each reaction much more efficiently than the individual proteins.

The T4 uvsX gene product has been shown by other investigators to be required for repair, recombination, and sustained late DNA replication in vivo. Our plasmid with the T4 DNA upstream of gene 41 produces a biologically active uvsX protein which increases the survival of a UV-irradiated T4 uvsX mutant in vivo. UvsX protein purified from cells with this plasmid catalyzes ATP-dependent annealing of ssDNA, D-loop formation, and, in the presence of T4 32 protein, strand exchange between homologous circular ssDNA and linear dsDNA, in agreement with the recent characterization of the uvsX protein from T4 infected cells by Yonesaki et al. and by Formosa and Alberts. In addition, we find that at higher ratios of dsDNA to ssDNA, uvsX protein catalyzes some strand exchange in the absence of 32 protein .

Detailed Studies of DNA Replication

15 DNA POLYMERASE III HOLOENZYME: SUBUNITS AND FUNCTION, Hisaji Maki, Satoko Maki, Roger Lasken and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

DNA polymerase III holoenzyme, the principal replicative enzyme in E. coli, catalyzes DNA synthesis with high processivity and fidelity. These distinctive properties of the holoenzyme are thought to be based on its multi-subunit structure (Table 1). However, precise composition and structure of the holoenzyme are largely unknown. To define the holoenzyme structure and to elucidate individul functions of the subunits, a project to isolate each subunit from strains overproducing individual polypeptides and to reconstitute the active holoenzyme from highly purified subunits has been started.

The α subunit was purified to homogeneity from a strain overproducing the dnaE gene product and was identified as the polymerase subunit devoid of nuclease activities. The ε subunit was identified as the 3'→5' exonuclease subunit by Echols and coworkers. An α-ε complex formed from purified α and ε subunits revealed their functional cooperation (Table 2). This cooperation is mainly due to the increased affinity of the ε subunit for the DNA 3'-OH terminus by the DNA binding of the α subunit. It seems likely that the α subunit destabilizes 3'-OH terminus of the paired substrate. The α-ε complex showed high fidelity of DNA synthesis indistinguishable from the Pol III core subassembly.

The α subunit and Pol III core showed very low processivity of DNA synthesis, and excess β subunit increased the processivity of the α subunit. This stimulation of processivity requires neither ATP nor dATP and suggests that the β subunit interacts directly with the α subunit and acts as the processivity subunit. Other subunits required for reconstitution of the holoenzyme activity were found to be amplified in a strain overproducing the dnaX-Z gene product, and were purified from this overproducer strain. Properties of these subunits and the reconstituted holoenzyme will be discussed.

Table 1

	Subunit	Size	Gene	Function
Core	α	140(kd)	dnaE	polymerase
	ε	27	dnaQ	3'→5' exo
	θ	10	?	?
Pol III*	γ	52	dnaZ	?
	δ	32	dnaX	?
	τ	78	dnaX-Z	?
	β	37	dnaN	processivity

Table 2

	Efficiency (ntd/sec/molecule)		
		3'→5' Exo	
	Pol	mispaired	paired
Pol III core	20	2.3	0.24
α subunit	7.7	-	-
ε subunit	-	0.063	0.0014
α-ε complex	13	0.70	0.058

16 NMR STUDIES OF THE CONFORMATIONS AND INTERACTIONS OF SUBSTRATES AND RIBONUCLEOTIDE TEMPLATES BOUND TO THE LARGE FRAGMENT OF DNA POLYMERASE I, Lance J. Ferrin and Albert S. Mildvan, Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205

The large fragment of Pol I effectively uses oligoribouridylates and oligoriboadenylates as templates, with kinetic properties similar to those of poly U and poly A, respectively, and has little or no activity in degrading them. The conformations and binding site environments of substrates and ribonucleotide templates bound to this enzyme have been studied by proton NMR. The effect of the large fragment of Pol I on the NMR line widths of the protons of Mg^{2+}TTP detected one binding site for this substrate and established simple competitive binding of the other deoxynucleoside triphosphate substrates at this site. The nuclear Overhauser effect (NOE) which measures interproton distances, was used to determine the glycosidic torsional angle (χ) and the sugar pucker of the bound substrates. The conformations of the enzyme bound substrates dATP and TTP, and of the substrate analog AMPCPP, are similar to nucleotides of B-DNA and differ greatly from those of A- and Z-DNA. Templates and primers exert little or no further effects on these conformations. In contrast, bound dGTP exists in at least two conformations in the absence of template, one of which is syn-, while in the presence of oligo(rU)$_{43+9}$ only a single B-like conformation is detected possibly due to the formation of a G-U wobble base pair. Despite this, guanine deoxynucleotides are not misincorporated by Pol I when oligo(rU) is used as the template. Templates and primer do not alter the relative affinities of the enzyme for complementary and non-complementary substrates. Hence, a verification step, subsequent to substrate binding and prior to DNA chain elongation, is necessary to explain the high fidelity of template replication by Pol I. Like the substrates, the enzyme-bound oligoribonucleotide templates oligo(rU) and oligo(rA) are partially immobilized, and are held in a conformation such that their average nucleotides are more B-like than A- or Z-like. Intermolecular NOE studies indicate that the enzyme binds substrates using at least two hydrophobic residues including ile, and an aromatic residue, probably tyr. The enzyme binds templates using cationic arg and/or lys residues, and possibly a hydrophobic residue.

17 SOME FUNCTIONAL IMPLICATIONS OF THE STRUCTURE OF THE KLENOW FRAGMENT, T.A. Steitz, P.S. Freemont and C.M. Joyce, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

The structure of the Klenow fragment of E. coli DNA polymerase I and its complex with dTMP has been determined at high resolution and led to the hypothesis that the smaller 200 amino acid domain contains the 3'-5' exonuclease active site whereas the larger domain forms the active site for polymerase.[1] About 10 to 12 base pairs of a duplex DNA product of DNA synthesis can be fitted into the crevice in the large domain. Co-crystallization experiments with deoxyoligonucleotides are in progress. Extensive amino acid sequence homologies exist between the large domain of the Klenow fragment of T7 DNA polymerase, particularly in those polypeptides that form the putative DNA binding cleft.[2] Crosslinking of 8-azido-dATP to the large domain presumably marks the polymerase active site.[3] Most side-chains facing the cleft in the vicinity of the crosslinked Tyr are identical in both polymerases, consistent with an important role for this region. About 50 amino acid residues that must lie above the cleft are disordered in the crystal. It is possible that when DNA binds, the protein completely surrounds the DNA which may be important in both the processivity and fidelity of the enzyme. The small domain is seen to bind a metal-nucleoside monophosphate to a site having residues consistent with it being a nuclease site. To test the hypothesis that each domain catalyses a separate activity, DNA coding for the large and small domains have been cloned in high expression vecters and the large domain has been purified. The 45 kdal large domain has polymerase but no 3'-5' exonuclease activity. Purification of the small domain is in progress.

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18 DYNAMIC AND STRUCTURAL INTERACTIONS IN THE BACTERIOPHAGE T4-CODED DNA REPLICATION COMPLEX. Peter H. von Hippel, Joel W. Hockensmith, Frederic R. Fairfield, Mary K. Dolejsi, John W. Newport, Thale A. Cross, and Leland S. Paul. Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, Oregon 97403 U.S.A.

In this lecture we will describe recent studies on molecular aspects of the protein-protein and protein-DNA interactions involved in processive DNA synthesis, carried out by the bacteriophage T4-coded DNA replication system at model [oligo(dT)-poly(dA)] and the "real" (natural DNA) primer-template junctions. Experiments on molecular mechanisms involving both synthesis and "editing" (3' to 5' exonuclease activity) will be discussed. We will consider how the T4 DNA polymerase binds at the primer-template junction, and how the polymerase accessory proteins complex and the single-stranded DNA binding proteins collaborate to form the five protein complex. Results of laser-induced protein-DNA crosslinking experiments on the replication complex will be presented, and a tentative molecular model of the processive synthesis "cycle" will be proposed. (Supported by USPHS Research Grant GM-29158 and USPHS National Research Service Award GM-07759.)

General Recombination Mechanisms

19 *RECA PROTEIN-PROMOTED BRANCH MIGRATION*, Michael M. Cox, Sandra K. Neuendorf, B. Franklin Pugh, Brian C. Schutte, Janet E. Lindsley, and Scott W. Morrical, Department of Biochemistry, School of Agriculture and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706.

Spontaneous branch migration in vitro is random; there is no force or factor which favors one direction of branch movement over the other. As the final phase of DNA strand exchange reactions, filamentous complexes of recA protein and DNA promote a branch migration which exhibits a unique polarity. Considerations of microscopic reversibility are sufficient to indicate that this polarity cannot be achieved without expenditure of chemical energy. Correspondingly, this is the only phase of recA protein-promoted DNA strand exchange which exhibits a requirement for ATP hydrolysis. Mechanisms by which ATP hydrolysis can be coupled to unidirectional branch migration by a recA complex can be divided loosely into two classes: those which involve association and/or dissociation of recA protein at the head or tail of a filament, and those in which the filamentous recA protein complex remains intact throughout the reaction.

Several results, obtained under conditions which are optimal for recA protein-promoted DNA strand exchange, favor the latter class of model. ATP hydrolysis is not restricted to recA monomers at the ends of filamentous complexes, but instead appears to occur throughout the complex. More than 1000 ATP's are hydrolyzed for every observable movement of a single monomer between a complex and a pool of free recA protein, so that no tight coupling exists between ATP hydrolysis and association/dissociation of recA protein with/from the complex. Under optimal conditions, increases in the concentration of recA protein or in the concentration of complete recA/DNA complexes have no effect on the rate of strand exchange or ATP hydrolysis. Similarly, ongoing strand exchange reactions are insensitive to a sudden dilution involving all components (except buffer and ATP). These and other results indicate that recA protein-promoted branch migration does not require association and/or dissociation of recA protein at the ends of a complex. Instead, each recA/DNA complex appears to act as an independent unit which remains largely intact throughout DNA strand exchange.

110 GENETIC RECOMBINATION AND Z-DNA, Eric B. Kmiec[1] and William K. Holloman[2], [1]Department of Biology, University of Rochester, Rochester, NY, 14627, and [2]Department of Microbiology, Cornell University Medical College, New York, NY 10021

The rec 1 protein from the lower eukaryote Ustilago maydis is a recombinase that, like E. coli rec A protein, can promote homologous pairing of DNA molecules in vitro. We have been interested in the role of left-handed Z-DNA in the pairing reaction promoted by rec 1 protein. Z-DNA is generated during formation of the paranemic joint. When stable heteroduplex regions are topologically barred from forming, either by use of a circular combination of homologous DNA molecules, or by use of a single stranded circle and a linear duplex molecule with long nonhomologous blocks on either end, a paranemic joint with paired but nonintertwined complementary strands is formed. The presence of Z-DNA in the paranemic joint supports the idea that the joint may be stabilized by formation of alternating stretches of right-handed and left-handed regions. The paranemic joint appears to be a true intermediate in the homologous pairing reaction since it is formed rapidly and at high frequency in pairing reactions. Thus it is not surprising that rec 1 protein binds strongly to left-handed Z-DNA. The binding reaction parallels the reaction leading to paranemic joint formation, suggesting that Z-DNA may be a kinetic analog of the paranemic joint structure. Pairing between two completely duplex DNA molecules can be promoted by rec 1 protein if each DNA molecule contains a stretch of Z-DNA. Even when flanking sequences are nonhomologous, rec 1 protein can pair two DNA molecules at Z-DNA sites. One final area of study involves generation of Z-DNA during DNA strand exchange. After synapsis of a single stranded circle and a homologous linear duplex molecule, rec 1 protein promotes strand exchange of the circle with its homolog strand in the duplex. The reaction is directional. If complete exchange is prevented by presence of a nonhomologous stretch in the duplex, some Z-DNA is generated presumably at the junction between the homologous and nonhomologous regions.

This work was supported by NIH grant GM 27103 and GM 36327.

I11 INTERMEDIATES IN HOMOLOGOUS PAIRING AND STRAND EXCHANGE PROMOTED BY REC A PROTEIN

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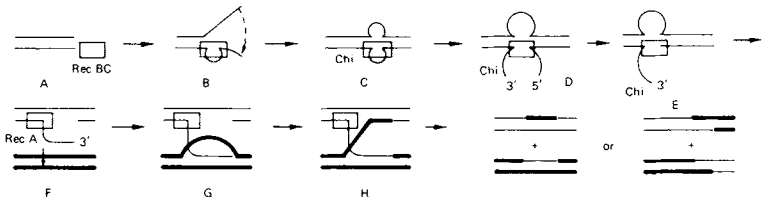
RecA protein, composed of a single polypeptide chain of MW 38,000, promotes homologous pairing and strand exchange, by building higher order structures. The first detectable PRESYNAPTIC INTERMEDIATE, which is formed in the presence of ATP, is a helical nucleoprotein filament 93 Å in diameter that contains about 22 nucleotide residues and 6 molecules of recA protein per 90 Å repeat. The phosphodiester backbone of the single-stranded DNA in the nucleoprotein filament is in contact with the protein whereas the bases are exposed. From the measured parameters we infer that the axial separation of bases in the filament is greater than that in B form DNA, and that the single-stranded DNA is not wrapped around the outside of the filament, but probably follows a helical path whose diameter can not exceed 40 Å in diameter. The first detectable SYNAPTIC INTERMEDIATE is one in which naked duplex DNA makes multiple non-homologous contacts with presynaptic filaments to form condensed nucleoprotein networks that sediment at more than 10,000 S. Networks form faster than homologously paired joint molecules and give rise to joint molecules more rapidly than they exchange DNA with the surrounding medium or with added exogenous networks. The recA nucleoprotein networks play an instrumental role in homologous pairing by facilitating diffusion within concentrated domains. The next detectable synaptic intermediate is one in which the homologous alignment of single-stranded DNA and duplex DNA results in the instantaneous removal of 10 to 20 turns from the duplex DNA, reflecting presumably the matching of the axial separation of bases in duplex DNA to that of single-stranded DNA in the presynaptic filament. The nature of the POSTSYNAPTIC INTERMEDIATES that accomplish strand exchange is adumbrated by studies on the resistance to DNAses of intermediates undergoing exchange. A circular plus strand in the presynaptic filament is resistant to cleavage by DNAse I and remains so during subsequent strand exchange; the plus strand that it displaces from the parental duplex becomes progressively resistant to DNAse I as the point of strand exchange proceeds 5' to 3'. Surprisingly, the minus strand of the parental duplex, which changes pairing partners, remains sensitive to DNAse I throughout the exchange, suggesting that it is unhindered by any long-lived contact with protein. Since recA protein can push strand exchange past heterologous insertions that are hundreds of base pairs in length, it must exert a helicase action at the point of strand exchange. A more condensed conformation of the single-stranded nucleoprotein filament that has been observed on removal of ATP from a filament formed in the presence of ATP provides evidence of a major conformational change that may be related to the equally striking conformational change in duplex DNA that accompanies its pairing and subsequent reaction with the single-stranded nucleoprotein filament.

I12 MECHANISM AND CONTROL OF HOMOLOGOUS RECOMBINATION, G.R. Smith, S.K. Amundsen, K.C. Cheng, N.H. McKittrick, A.S. Ponticelli, D.W. Schultz, A.F. Taylor and S. Thibodeaux, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

Our previous studies on RecBC enzyme and Chi sites of Escherichia coli, plus studies by others, led us to propose the model of recombination shown below (1).



RecBC enzyme binds specifically to duplex DNA ends (step A) and produces ss DNA loops and tails as it moves along the DNA (steps B-C). During unwinding from right to left, RecBC enzyme cleaves the DNA strand containing the Chi hotspot sequence 5'G-C-T-G-G-T-G-G3' (steps D-E). RecA and SSB proteins pair homologous ss DNA and ds DNA (steps F-G). The formation and resolution of the postulated Holliday junction (steps H-I) have not yet been demonstrated for this pathway of recombination. This proposed mechanism is supported by enzymological, genetic, and physiological evidence (2,3).

Recently, we have found a requirement for the E. coli ssb, polA, lig, and gyrAB gene products in this pathway of recombination; possible roles for these functions will be discussed. We have found that exonuclease V, previously designated RecBC enzyme, contains a third polypeptide of about 58 kDa coded by a newly designated gene recD located between recB and argA; the recD polypeptide is required for nuclease activity and Chi activation. This mechanism of recombination appears to operate in diverse enteric bacteria: most tested species contain Chi-dependent cleavage activity; where tested, DNA clones of recBCD-like genes from other species confer Chi stimulatable recombination-proficiency on E. coli deleted for recBCD. The possibility that other organisms utilize other sequences as their recombinational signal will be discussed.

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Recombination Systems

I13 **PARTIAL PURIFICATION AND CHARACTERIZATION OF A RECOMBINASE FROM HUMAN CELLS**, R. Daniel Camerini-Otero, Peggy Hsieh and M. Stephen Meyn, Genetics and Biochemistry Branch, NIADDK, National Institutes of Health, Bethesda, MD 20892

We describe the partial purification and characterization of a human recombinase activity from RPMI 1788 B lymphoblasts. Stoichiometric amounts of recombinase carry out a strand transfer reaction between linear duplex DNA and homologous circular single-strand DNA. This is the first demonstration of a strand transfer activity from a high eukaryote. This activity also promotes the reannealing of complementary single-strand DNA.

The product of strand transfer by the recombinase is a joint molecule composed of a single-strand circle joined to one end of the linear duplex molecule by a region of DNA heteroduplex at least 150 bp in length. To exclude the formation of joint molecules by sequential activities of an exonuclease followed by a reannealing protein, we demonstrate the existence of a displaced strand by several independent means. Examination of various parameters affecting the formation of joint molecules and characterization of the physical structure of the product reveal that strand transfer involves a stoichiometric interaction between single-strand DNA and recombinase. Strand invasion initiates at the ends of the linear duplex and is blocked by as few as 61 bp of nonhomologous DNA at the ends of an otherwise homologous linear duplex molecule. Strand displacement by human recombinase is polar and proceeds in a 3' to 5' direction whereas RecA protein carries out strand displacement in a 5' to 3' direction. In this regard, the human recombinase is similar to recI, the only other eukaryotic recombinase characterized to date. The presence of a strand transfer activity from human B lymphoblasts provides biochemical support for models which invoke strand exchange in recombination in mammalian cells.

114 THE LOX-CRE SITE-SPECIFIC RECOMBINATION SYSTEM OF BACTERIOPHAGE P1, Ronald H. Hoess, Kenneth Abremski, Beth Frommer, Anna Wierzbicki, and Marvin Kendall, Central Research & Development Department, E. I. Du Pont de Nemours and Company, Inc., Experimental Station, Wilmington, DE 19898

The lox-Cre site-specific recombination system affords a simple system by which the detailed mechanism of site-specific recombination can be studied. The system is comprised of two components, a site on the DNA where crossing over takes place called loxP, and a protein which mediates the reaction, Cre.

The loxP site is defined by two 13 bp inverted repeats separated by an 8 bp spacer region. It is within the spacer region that the DNA is cleaved and strands exchanged during Cre mediated recombination. We have systematically altered the sequence of bases within the spacer region and asked how these mutant lox sites are affected during recombination.

We have also begun to isolate and analyze mutants within the Cre gene. Mutant proteins have been purified and assayed for a number of functions pertaining to those found in the wild-type Cre protein, i.e. DNA binding. These will be discussed with regard to a structure-function relationship between the activities of Cre and its primary sequence.

115 GENETIC RECOMBINATION AND MISMATCH CORRECTION CATALYZED BY A SACCHAROMYCES CEREVISIAE CELL-FREE SYSTEM. R. Kolodner, D. Bishop, D. Evans, C. DeLuca, P. T. Morrison and C. Muster-Nassal. Laboratory of Molecular Genetics, Dana-Farber Cancer Institute and Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

We have developed a cell-free system that uses extracts of mitotic Saccharomyces cerevisiae cells to catalyze homologous recombination of plasmid substrates and repair of substrates containing mismatched nucleotides. Recombination between plasmid substrates containing different mutant tet alleles or mutant arg4 alleles was detected using a transformation assay. The frequency of recombinants obtained ranged from 1×10^{-3} to 2×10^{-2} depending on the substrate used. Six different DNA species that were formed during the reaction and were enriched in recombinant DNA molecules were purified using agarose gel electrophoresis. These DNA species were identified by electron microscopy and were found to be circular dimers, figure-8 molecules, sigma-forms, alpha-forms, catenated dimers and circular monomers containing a single-strand break. Kinetic studies suggested that the figure-8 molecules were being processed during the recombination reaction in vitro. Using model substrates containing Holliday junctions we have detected and purified an endonuclease that cleaves Holliday junctions and appears to be responsible for the processing of figure-8 molecules in vitro. Structural studies indicated that heteroduplex recombinant molecules were formed in vitro and in some cases were further processed by mismatch repair. Using M13 DNA substrates containing defined mismatches we have demonstrated that the in vitro recombination system will catalyze mismatch correction. Transition mismatches and 4bp insertion/deletion mismatches were repaired most efficiently and transversion mismatches were repaired poorly or were not repaired. Mismatch correction was found to involve specific repair synthesis resulting in the incorporation of less than 50 nucleotides at the site of the mismatch. Selected experiments on recombination in E. coli will also be discussed.

116 MECHANISMS OF DIRECTIONALITY IN SITE-SPECIFIC RECOMBINATION

Arthur Landy, John Thompson, Lina Vargas and Sarah Skinner, Division of Biology and Medicine, Brown University, Providence, RI 02912

The simple relationship between the substrates and products of site-specific recombination raises interesting questions about the control of directionality often observed in this class of DNA transactions. For the Int-dependent pathway of bacteriophage lambda it is shown that integrative and excisive recombination proceed along discrete pathways and it is possible to construct sites with the intrinsic property of recombining in only one direction. The two pathways differ not only in their dependence on the presence or absence of Xis but also in their response to changes in the concentration of Int and IHF. The E. coli protein IHF plays a key role in directionality by differentially affecting integrative and excisive recombination. A 4-8 fold increase in intracellular IHF levels coincides with the transition from exponential to stationary phase and provides a mechanism for growth phase-dependent regulation of recombination.

The two recombination reactions also differ significantly in the set of protein binding sites required for recombination: attP requires the full extent of P arm binding sites for integrative recombination while an attR lacking the outermost binding sites for Int and IHF is undiminished in excisive recombination efficiency. The regulation (and mechanism) of recombination involve striking cooperative and anti-cooperative interactions that we are beginning to decipher. These include interactions between adjacently bound proteins and "long range" interactions between proteins bound at sites that are not adjacent on the linear att site DNA.

As a consequence of the fact that the "forward" and "reverse" recombination reactions proceed along discrete pathways, this site-specific recombination reaction functions well as a long term mode of genetic regulation that can be altered only after significant physiological changes have taken place.

Multienzyme Replication Systems

117 IN VITRO REPLICATION OF DNA CONTAINING THE SV40 ORIGIN. C. R. Wobbe, Y. Murakami, F.B. Dean, L. Weissbach and J. Hurwitz. Sloan-Kettering Institute, New York, NY 10021

Extracts prepared from exponentially growing HeLa cells catalyze extensive, T antigen (TAg)-dependent replication of DNA containing the SV40 origin sequence. Replication is dependent on closed circular ori$^+$DNA, dNTPs, ATP and an ATP regenerating system and is inhibited by aphidicolin and camptothecin (inhibitors of DNA polymerase α and topoisomerases, respectively), RNase A and pretreatment of extracts with micrococcal nuclease. The products include RFI, RFII, topoisomers and discrete high molecular weight bands when analyzed by agarose gel electrophoresis.

Extracts depleted of DNA polymerase α-primase complex by immunoaffinity chromatography are inactive. Replication activity is restored by the addition of purified human and monkey, but not mouse or calf thymus, DNA polymerase α-primase. Both the polymerase and primase activities are required for restoration and the products formed are identical to those made by non-depleted extracts. We have also isolated four protein fractions which, in addition to DNA polymerase α-primase and TAg, are required for replication. Replication carried out by these combined fractions exhibits the same requirements and yields the same products as the crude extract and is sensitive to aphidicolin and RNase A.

At 25° there is an extended lag preceeding DNA synthesis which can be eliminated by preincubation of crude extract, TAg and ori$^+$DNA in the absence of dNTPs, suggesting that a pre-elongation complex is formed. A complex has been isolated by gel filtration chromatography and is dependent on ATP, TAg, ori$^+$DNA and crude extracts or certain of the protein fractions mentioned above. Complex formation, but not elongation, is sensitive to RNase A. This observation, taken together with the data above, suggests that RNA is involved in the early stages of SV40 DNA replication in vitro.

118 ENZYMOLOGY OF INITIATION OF BACTERIOPHAGE λ DNA REPLICATION, Roger McMacken, Kojo Mensa-Wilmot, Jonathan LeBowitz, Bruce Gomes, Christine L. Alfano, John D. Roberts and Marc S. Wold, Dept. of Biochemistry, The Johns Hopkins University, Baltimore, MD 21205

We have established a crude soluble enzyme system that specifically replicates supercoiled plasmid DNA molecules (λdv plasmids) that contain a bacteriophage λ replication origin (oriλ) to produce supercoiled daughter molecules. λdv DNA replication in vitro appears to be identical in every respect to the early, circular phase of λ DNA replication in vivo. Initiation of λ DNA replication in the soluble enzyme system requires transcription of the origin region by RNA polymerase (i.e., transcriptional activation) and is, therefore, blocked by the presence of either the λ cI repressor or rifampicin.

The λ O and P initiators, in conjunction with several host proteins, stimulates the replication of single-stranded (ss) DNA molecules. We have recently reconstituted this λ ss replication reaction with a set of eight purified λ and E. coli proteins. The rate-limiting step of this reaction involves the ATP-dependent transfer of the bacterial dnaB protein onto ss DNA that is coated with the ss DNA binding protein (SSB). This critical prepriming step requires the function of the λ O and P initiators and the E. coli dnaJ and dnaK heat shock proteins. Once assembled on the template DNA strand, the bacterial dnaB protein migrates along the SSB-coated strand, serving as a mobile promoter for the synthesis of multiple primers by primase. In related studies we have found that the dnaB protein is a DNA helicase that moves 5' to 3' on the template DNA strand. From this and other data, we conclude that the mobile, processive apparatus that primes synthesis of lagging strands during replication of both the E. coli and λ chromosomes is simple, consisting solely of a helicase (dnaB protein) and a primase (dnaG protein).

We have recently reconstituted, with 9 purified proteins, a system that specifically replicates supercoiled λdv plasmid DNA. A combination of electron microscopic and biochemical studies have helped identify some of the early steps in the initiation of DNA replication at oriλ. The λ O initiator wraps oriλ into a specific configuration. This nucleosome-like complex is converted into an even larger structure by the addition of the λ P protein and the E. coli dnaB protein. The addition of SSB and the bacterial dnaJ and dnaK proteins to this structure apparently stimulates the dnaB protein helicase to unwind the two DNA strands in the region of the λ replication origin. A stable replication fork in the λ chromosome is established by the subsequent action of primase, DNA polymerase III holoenzyme and DNA gyrase. A striking difference in the behavior of the reconstituted enzyme system was uncovered by the discovery that transcriptional activation of oriλ is not required for initiation of λ DNA replication. Our finding that the histone-like E. coli HU protein inhibits λ DNA replication in a transcription-sensitive fashion suggests that chromatin structure in the region of the λ replication origin is a critical factor in vivo.

119 BACTERIOPHAGE T7 DNA REPLICATION, Charles C. Richardson, Benjamin B. Beauchamp, Hans Huber, Richard A. Ikeda, Jill A. Myers, Hiroshi Nakai, Samuel D. Rabkin, Stanley Tabor, and John White, Dept. of Biological Chemistry, Harvard Medical School, Boston, MA 02115

Studies with phage T7 have revealed the minimal requirements for the replication of a duplex DNA molecule. DNA replication is initiated 15% from the left end of the 39,936 bp chromosome. This site, the primary origin, consists of a 200-bp intergenic segment from 14.5 to 15.0% within which are located two T7 RNA polymerase promoters (ϕ1.1A and ϕ1.1B) followed by a 61-bp AT-rich region. Initiation of DNA synthesis can be reconstituted using T7 RNA polymerase, T7 DNA polymerase, and T7 origin-containing plasmid DNAs. The initial products of DNA synthesis have 10 to 60 ribonucleotides covalently linked to their 5'-termini. These RNA primers arise by transcription from each of the two promoters, ϕ1.1A and ϕ1.1B, located within the primary origin. In the presence of T7 gene 4 protein, extensive unidirectional DNA synthesis occurs, resulting in Y-form molecules. Initiation of DNA synthesis in the opposite direction requires the addition of a single-stranded DNA-binding protein.

Three proteins, T7 gene 5 protein, E. coli thioredoxin, and T7 gene 4 protein account for the basic reactions at the replication fork. The T7 gene 5 protein is a DNA polymerase, albeit extremely non-processive (less than 15 nucleotides polymerized). Thioredoxin forms a tight complex with T7 gene 5 protein in 1 to 1 stoichiometry. In this role thioredoxin serves as an accessory protein to increase the processivity of DNA synthesis by the gene 5 protein (greater than 10,000 nucleotides polymerized). We have analyzed a number of mutationally altered thioredoxins for their ability to interact with T7 gene 5 protein. Both cysteines at the active site can be replaced without affecting the maximum polymerase activity; only the affinity of thioredoxin for gene 5 protein is altered.

The gene 5 protein/thioredoxin complex cannot polymerize nucleotides at a preformed replication fork. DNA synthesis requires the presence of gene 4 protein to function as a helicase. In this reaction the three proteins catalyze processive DNA synthesis along a duplex DNA molecule; the microscopic rate of fork movement is 300 nucleotides per sec at 30°C. In addition to its helicase activity the gene 4 protein also catalyzes the synthesis of primers, predominantly pppACCC and pppACCA in response to the template recognition sequences 3'-CTGGC-5' and 3'-CTGGT-5'. Both helicase and primase activities are dependent on the ability of the gene 4 protein to bind to single-stranded DNA and to translocate 5' to 3' along the DNA, a reaction coupled to the hydrolysis of NTPs. A second protein interaction consists of an association of gene 4 protein with the gene 5 protein/thioredoxin complex. Such an interaction leads to the formation of a complex consisting of all three proteins with single-stranded M13 DNA. Additions of rATP and rCTP to the multiprotein complex allows for primer synthesis and the subsequent isolation of a preprimed M13 DNA molecule.

120 STUDIES ON THE T4 BACTERIOPHAGE DNA REPLICATION SYSTEM, Harold E. Selick, Jack Barry, Tai-An Cha, Maureen Munn, Mikiye Nakanishi and Bruce M. Alberts, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

In vitro studies of DNA replication using the purified T4 replication proteins are contributing to a more refined understanding of the mechanistic aspects of DNA replication. Previous studies have led to a model in which leading and lagging strand DNA syntheses are mediated by a single complex of proteins consisting of the gene 41-encoded helicase as well as 2 copies of the gene 43 DNA polymerase with its associated accessory proteins. Dilution experiments have revealed that the lagging strand DNA polymerase molecule is recycled to synthesize successive Okazaki fragments at a replication fork. One recycling model proposes that all of the Okazaki fragments made on an individual lagging strand should be similar in size to one another, as determined by the length of the first Okazaki fragment to be made on that strand. As a test, we have limited the availability of rNTPs for primer synthesis, so that abnormally long Okazaki fragments (6-7 kb) are initially synthesized. Subsequent addition of the missing rNTPs results in an immediate shift to the synthesis of normal length, 1.5 kb Okazaki fragments. These observations suggest that, rather than a templating mechanism for determining the size of successive Okazaki fragments, the lagging strand DNA polymerase rapidly synthesizes each Okazaki fragment and then "waits" for the next primer from which it initiates another cycle of rapid polymerization and "waiting". Our related studies show that the trinucleotide sequence GTT is necessary and sufficient to specify RNA primer synthesis by the gene 61 primase on T4 DNA. On average, however, only one in every thirty of these sites is utilized, and no very short Okazaki fragments are made. The minimum length for Okazaki fragments is most likely set by a "timing" mechanism during DNA synthesis that is based upon the completion of synthesis of the previous Okazaki fragment.

In order to better understand the molecular topography of the replication complex, DNA footprinting analyses have been performed using the replication proteins with a defined DNA molecule containing a primer-template junction. In the presence of ATP and the gene 32 helix-destabilizing protein, the polymerase and its accessory proteins form a specific complex, as detected by protection of the DNA from DNase I digestion.

While these studies are providing insight into the events involved in the propagation of replication forks, we have not yet reconstituted the origin-specific initiation of replication on an intact, double-stranded template. Our DNA sequence analysis of the region of the T4 chromosome encompassing the helicase and primase genes reveals several open reading frames (orfs) suspected to encode the T4 equivalent of the λ O and P proteins or the E. coli dnaA protein. In order to test whether these and other orfs are required for normal DNA synthesis, we have developed an insertion/substitution vector system that has allowed us to alter each orf sequence and determine the effect of these mutations on replication in vivo.

Initiation of DNA Replication

121 YEAST DNA REPLICATION, Judith L. Campbell, Martin Budd, Mary Gilbert, Ambrose Jong and Kevin Sweder, Divisions of Chemistry and Biology, California Institute of Technology, Pasadena, CA 91125

One of the most important but least well understood aspects of eukaryotic replication is the question of what constitutes a chromosomal origin of replication. In yeast, ARS sequences seem to function as origins of replication on plasmids, but have not been shown to correspond to sites of initiation within chromosomes. We have studied the question of ARS function in two ways. By mutational analysis, we have defined three domains of function within ARS1. Recently, we have purified a protein that binds specifically to ARS1-containing DNA. Characterization of this protein and the gene encoding it should lead to a clearer understanding of the role of ARS elements in yeast.

The eventual goal of our studies is to reconstitute a chromosomal initiation event from purified components. In order to accomplish this, a better understanding of the proteins, in addition to the ARS-specific binding protein, that are involved in initiation is necessary. Our approach is a combined genetic and biochemical one. For example, yeast DNA polymerase I has been purified and antibody has been used to clone the gene encoding DNA polymerase I. The gene is located on chromosome XIV. Gene disruptions show that the polymerase is essential and temperature sensitive mutants have been constructed to study residual polymerases and the role of DNA polymerase I in replication, recombination and repair. A second activity thought to be involved in replication is that of a single-stranded DNA binding protein (SSB). We have purified three SSBs from yeast. SSB-1, previously thought to be required for replication, has been shown to be non-essential by isolation of the gene and gene disruption. The gene for SSB1 maps on chromosome V or VIII.

122 REPLICATION OF SV40 DNA, Joachim Li[1], Keith Peden[2], Marc S. Wold[1], David H. Weinberg[1], Pamela Simancek[1], and Thomas Kelly[1],
[1]Johns Hopkins University School of Medicine, Baltimore, Md. 21205
[2]Howard Hughs Medical Institute Laboratory of Genetics, Baltimore, Md. 21205

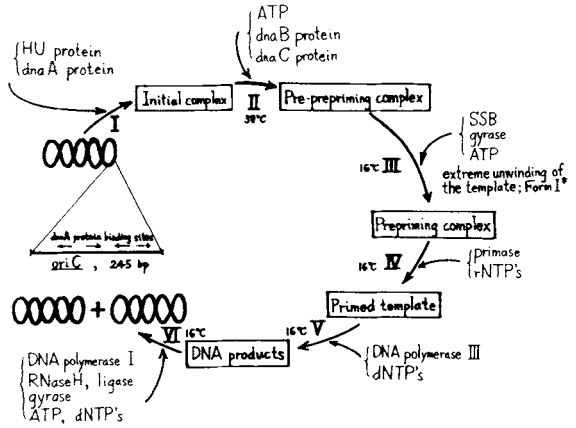
We have developed a cell-free system that is capable of replicating plasmid DNA containing the SV40 origin of replication. Replication requires extracts from cells permissive for SV40 DNA replication in vivo (e.g. human, simian), and is strictly dependent on the addition of SV40 T antigen. DNA synthesis, which ocurs most efficiently on circular templates, is initiated within the viral origin and proceeds bidirectionally via theta structures to produce fully separated daughter molecules.

We have examined the sequence requirements for replication by quantitating the level of replication in vitro of templates containing a variety of mutations within the origin region. For purposes of comparison, we measured the relative replication efficiency of the same templates in vivo. The minimal origin of DNA replication defined in both assays is comprised of the 15-bp imperfect inverted repeat, the 27-bp perfect inverted repeat, and the AT-rich region. T antigen binding site I is not required for replication, but its presence increases replication efficiency several fold both in vivo and in vitro, and increasing T antigen concentration in vitro will not compensate for its absence. The presence of late side transcriptional elements (either the SP1 binding sites or the 72-bp enhancers) increase replication efficiency in vivo at least 10-fold, but has no detectable effect in vitro. Although the mechanism by which the transcriptional elements stimulate replication in vivo is not yet clear, one possibility is that they facilitate the access of initiation factors to the origin of SV40 minichromosomes.

The replication activity of crude extracts from HeLa cells can be reconstituted from inactive fractions obtained by standard chromatographic procedures. This provides an approach to characterize cellular proteins involved in DNA replication.

123 ENZYMATIC STUDIES OF REPLICATION OF oriC PLASMIDS, Arthur Kornberg, Tania A. Baker, LeRoy L. Bertsch, David Bramhill, Barbara E. Funnell, Roger Lasken, Hisaji Maki, Satoko Maki, Kazuhisa Sekimizu and Elmar Wahle, Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

More than ten proteins are known to participate in replication of plasmids bearing the unique origin of the E. coli chromosome (oriC). Initiation of replication of oriC plasmids has been resolved into five separable stages (Scheme, below). An initial complex formation (Stage I) requires an oriC plasmid, dnaA protein and HU protein. In the presence of ATP at a temperature >28°C, a dnaB-C protein complex interacts to form a prepriming complex (Stage II). This is followed by extensive unwinding of the template that depends on the further addition of gyrase and SSB (Stage III). Hydrolysis of an rNTP by dnaB protein (a helicase action) and of ATP by gyrase (a swivelling action) drives the extreme unwinding of the template. This unwound template-protein complex is the substrate for priming by primase (Stage IV) and elongation by DNA polymerase III holoenzyme (Stage V). Priming of all DNA chains is done by primase; RNA polymerase functions in template activation rather than priming. DNA polymerase III holoenzyme, composed of at least seven subunits, synthesizes the DNA chains. The α subunit is the polymerase, the ε subunit is the 3'→5' exonuclease; α + ε is the proofreading activity. Following the synthesis of new DNA chains, DNA polymerase I and ribonuclease H remove the RNA primers, polymerase I fills the gaps, and ligase seals the daughter strands (Stage VI). Replication produces plasmids identical in structure and sequence to the initial template.



Stages of oriC plasmid replication

124 INITIATION OF PHAGE Ø29 DNA REPLICATION PRIMED BY THE TERMINAL PROTEIN, Margarita Salas, Ignacio Prieto, Julio Gutiérrez, Luis Blanco, José M. Lázaro, Gil Martín, Angel Zaballos, Antonio Bernad, Cristina Garmendia, Rafael P. Mellado, Cristina Escarmís and José M. Hermoso, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

Bacteriophage Ø29 has a linear, double-stranded DNA of 18 kbp with a protein, p3, covalently linked to the 5' ends by a phosphodiester bond between serine and 5'dAMP (1). The linkage of the terminal protein to the DNA occurs through the serine residue at position 232 of a total of 266 amino acids. Deletions at the carboxyl end of the terminal protein were shown to affect its activity in the initiation reaction. Initiation of Ø29 DNA replication starts at either end of the DNA by a protein-priming mechanism. In the presence of Ø29 DNA-protein p3 complex as template, free protein p3, the Ø29 DNA polymerase p2 and dATP, a protein p3-dAMP initiation complex is formed (2,3). This in vitro initiation reaction is stimulated by NH_4^+ ions, which are needed for the formation of a complex between the terminal protein and the Ø29 DNA polymerase. In these conditions the p3-dAMP initiation complex is elongated by the DNA polymerase activity of protein p2 to produce full-length Ø29 DNA, although at a low rate, suggesting the need of accessory proteins.

To study the role of other viral proteins involved in Ø29 DNA replication we have cloned genes 5 and 6 and the overproduced proteins have been highly purified. Protein p6 stimulates the formation of the initiation complex as well as a limited elongation reaction in the presence of ddCTP. In addition, it binds specifically to terminal, but not internal, Ø29 DNA fragments.

The replication origins of Ø29 DNA have been cloned. Fragments of the recombinant plasmids containing the Ø29 terminal sequences at the DNA end have ~20% the template activity of Ø29 DNA-protein p3 in the initiation reaction. The precise location of the Ø29 terminal sequences at the DNA end is required for template activity. The in vivo role of the parental terminal protein in Ø29 DNA replication is also being studied.

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Site-Specific Recombination

125 INTEGRATION AND EXCISION OF PHAGE LAMBDA: MECHANISTIC STUDIES, Howard A. Nash, Laboratory of Molecular Biology, NIMH, Bethesda, MD 20892

The site-specific recombinations that join and disjoin the chromosomes of E. coli and bacteriophage lambda are now well-characterized biochemical reactions. Integration occurs when two proteins, Int and IHF, cooperate to cause the breakage and rejoining of two special sites, attP and attB. Excision of the integrated viral DNA, bounded by the rearranged sites attL and attR, requires Int and IHF plus a third protein, Xis. Supercoiling of attP strongly activates integrative recombination but excisive recombination is little affected by the state of the prophage DNA.

We have studied how supercoiling influences the recombination process. Several possibilities - rotation of DNA strands during exchange, melting of DNA sequences at the region of crossing-over, binding of recombination proteins to DNA, assembly of protein-DNA complexes into a higher order structure - have been explored. Previous studies on the topological outcome of integrative recombination indicated that higher order structures are essential for recombination and that formation of these structures would be favored by supercoiling (1). To examine this and other possibilities more directly we have developed a method that permits chemical protection experiments on supercoiled DNA. We have used this method to assess differences in the way recombination proteins bind to supercoiled versus non-supercoiled DNA; a summary of recent progress will be presented.

To explore the relationship between integration and excision, we have compared the way each recombination depends upon homology within the crossover region. Earlier work had shown that, in vivo, efficient integrative and excisive recombination requires that two att sites have the same 7 bp crossover sequence (2). In vitro experiments show that integrative recombination works well even if only one of the two strands of attB matches attP (3); this indicates that during integration homology recognition is redundant. To test whether a similar redundancy operates during excision, we constructed variants of attL that match their partner attR site in 0, 1 or 2 strands. The requirement for homology proved to be identical to that seen for integrative recombination. This suggests that the basic mechanism for synapsis during excision may be identical to that used during integration.

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126 THE FLP RECOMBINASE OF THE 2-MICRON PLASMID OF YEAST, Paul D. Sadowski, Brenda J. Andrews, Donna Babineau-Clary, Linda Beatty, Richard M. Gronostajski, Gerald Proteau and Deborah Sidenberg, Department of Medical Genetics, University of Toronto, Toronto M5S 1A8, Canada.

Most strains of Saccharomyces cerevisiae harbor several copies of a 6318 bp plasmid, the '2-micron circle DNA'. This DNA undergoes site-specific recombination across two limited sequences (FLP sites) that reside within two identical 599 bp inverted repeats. This recombination event is promoted in vivo by a plasmid-encoded function named 'FLP' and results in the presence within the yeast cell of approximately equal amounts of two isomeric forms ('A' and 'B') of the 2-micron plasmid.

We have cloned the FLP gene under the control of a strong E. coli promoter and have purified the FLP activity from bacterial cells expressing the gene. The protein promotes highly efficient recombination in vitro and we are using this protein to study the mechanism of its action and the sequence requirements of the FLP target site.

The FLP protein promotes efficient intramolecular recombination between FLP sites whether they be present in direct or inverse orientation. Furthermore the enzyme does not display a requirement for supercoiling of the substrate. However when supercoiled circular substrates are used, the products are complex knots or catenanes that result from random entrapment of interdomainal supercoils during the reaction. The enzyme can promote iterative rounds of recombination with the accumulation of products with further complexity. Finally, the enzyme exhibits a site-specific topoisomerase activity.

Although the plasmid contains two 599 bp inverted repetitions, the actual sites required for recombination are much smaller. In vivo studies suggested the FLP site was <60 bp and was centered near an XbaI site. Destruction of this site abolishes recombination in vivo and in vitro. The FLP protein protects from DNase digestion about 50 bp surrounding this XbaI site and encompassing an 8 bp core region surrounded by two 13 bp inverted symmetry elements. A third 13 bp symmetry element is also protected by the FLP protein.

We have used deletional analysis and synthetic oligonucleotides to define the minimal sequence necessary for efficient recombination with a wild-type site. These analyses show that the minimal site is quite small, about 22 bp. While such sites can recombine well with a complete site, they do so poorly with themselves, suggesting that sequences outside the minimal duplex site may enhance the efficiency of recombination.

We have studied two point mutants in the FLP site that were isolated by McLeod and Broach. One mutation drastically reduces binding of FLP to its target. A second mutation in the 8 bp core region severely impairs recombination with a wild-type site but recombination between two mutant sites is efficient. Supported by M.R.C., Canada.

127 DETERMINATION OF THE MECHANISMS OF GENETIC RECOMBINATION BY NEW TOPOLOGICAL METHODS. Steven A. Wasserman, James B. Bliska, Howard W. Benjamin, Jan M. Dungan, Robert L. Weinberg, and Nicholas R. Cozzarelli, Department of Molecular Biology, University of California, Berkeley CA 94720.

The two stages in site-specific recombination are the bringing together of the target DNA sites on the recombinase surface and the breakage and crossed reunion of DNA at each site. Using topological methods, we determined important features of the mechanisms of these processes of synapsis and strand exchange for site-specific recombination events mediated by either the Tn3 resolvase or the phage λ Int system. Irrespective of substrate supertwist density, the resolvase synaptic mechanism ensures that there are three (-) supercoils between the resolvase sites. Two are metamorphosed into the links of the single linked catenane product whereas the third is cancelled by the single (+) supercoil introduced by strand exchange. In contrast, synapsis in the λ system is essentially by random collision, giving a variety of product knots and catenanes both in vivo and in vitro. The topological methods are rapid and simple and can be used to study a wide range of other problems including the cellular level and structure of supercoils, the mechanism of strand passage by topo-isomerases, the properties of paranemic recombination joints, and the untangling of daughter chromosomes at the termination of DNA replication.

DNA Transposition

I28 Ty ELEMENT TRANSPOSITION IN YEAST: FUNCTIONAL AND NON-FUNCTIONAL ELEMENTS.
Jef D. Boeke, Diego H. Castrillon, David J. Garfinkel, and Gerald R. Fink.
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Ty elements are transposons of the yeast, Saccharomyces cerevisiae. We have shown that these elements transpose via an RNA intermediate[1] and that overproduction of a certain Ty element, TyH3, results in the accumulation of intracellular virus-like particles. These particles contain Ty RNA, at least one Ty-specified protein antigen, and a reverse transcriptase activity not normally found in yeast cells[2]. Finally, such cells undergo very high rates of transposition, both of TyH3 itself and of other, chromosomal Ty elements. In contrast, another Ty element, Ty173, which was overproduced in the same way, does not stimulate high frequency Ty element transposition, suggesting that Ty173 is non-functional. Ty173 is very similar in structure to TyH3. By making hybrid constructs containing part of TyH3 and part of Ty173, we are mapping the Ty 173 defect, which lies in open reading frame 2 (thought to encode reverse transcriptase[2,3]. We have determined the DNA sequence of TyH3, and we are sequencing the defective segment of Ty173 in order to determine the molecular nature of the defect. During transposition of TyH3, occasional progeny transposons arise which are apparently rearranged or otherwise altered. These obsevations suggest that the population of Ty elements in a yeast genome (typically composed of about 30-35 members) contains both transpositionally active and inactive members.

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I29 MECHANISM OF Tn10/IS10 TRANSPOSITION IN VIVO AND IN VITRO, Nancy Kleckner, Judy Bender and Donald Morisato, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

Three lines of evidence have suggested previously that transposition of Tn10, and its component IS10 sequences, occurs by a "cut-and-paste" mechanism that does not involve replication of the transposing segment. (1) Genetic analysis of transposition by a Tn10 element carrying a single basepair heteroduplex mismatch reveals that both strands of information are recovered in the transposition product, as expected from a non-replicative mechanism. All mechanisms involving replication prior to insertion are excluded. Additional data either exclude or render highly unlikely mechanisms involving formation and reduction of cointegrates. (Bender and Kleckner, in preparation). (2) The array of DNA rearrangements promoted by Tn10 does not include the occurrence of adjacent deletions promoted by a single IS10 element (as opposed to adjacent deletions promoted by the combined action of two IS10 elements). (Weinert et al., 1984, CSHSQB 44: 251; Shen, Raleigh and Kleckner, in preparation). (3) In vivo, mini-Tn10 elements on multi-copy plasmids, when provided with high levels of transposase function, undergo transposase-dependent double strand excision from the plasmid molecule followed by circularization to an open circular form by closure of one single strand (Morisato and Kleckner, 1984, Cell 39: 181). Efficient double-strand breaks at the termini of the transposon would be required for an element that undergoes non-replicative transposition.

Recent experiments provide new information which supports and extends the previous picture.
In vivo. Transposition by cut-and-paste mechanisms is presumed to involve the formation of single strand joints between transposon and target molecules at each end of the element, and must involve at least a small amount of DNA repair synthesis to restore the integrity of the second strand at each end. Analysis of genetically marked transposons carrying three single base heteroduplexes, one of which is located close to one end of the element, suggests that in most transposition events, DNA repair does not extend as far as 70 basepairs into either end of the element.
In vitro. We (D.M.) have been able to observe both mini-Tn10 excision/circle formation and inter-molecular Tn10 transposition in cell-free extracts. Circle-formation is quite efficient and does not require ATP. Thus, IS10 transposase resembles site-specific recombination proteins in its ability to promote break/join events in the absence of any high energy co-factor. Transposition is presently much less efficient than circle-formation.

130 MECHANISM OF TRANSPOSITION OF BACTERIOPHAGE MU, Kiyoshi Mizuuchi, Robert Craigie, Anthony Maxwell, Laboratory of Molecular Biology, NIADDK, National Institutes of Health, Bethesda, Maryland 20892

Phage Mu uses transposition throughout its life cycle (1). A replicative transposition reaction is used during lytic growth to replicate the phage DNA. On the other hand, the reaction which results in insertion of the infecting phage genome into the host chromosome at the beginning of infection has been shown to be non-replicative (2), leading to an integrative transposition event called simple insertion which is essential for the stable lysogenization of the infecting phage.

The mechanism of the transposition reaction of phage Mu has been studied by making use of a cell-free reaction system and has been shown to involve a branched DNA structure as a reaction intermediate (3). The intermediate is formed by a pair of DNA strand transfer events involving the 3' ends of the Mu sequence and the 5' ends of the target site generated by a staggered cut. The branches in the intermediate can become replication forks by recruiting the host replication proteins to replicate the Mu sequence, or they can be resolved without DNA replication to form a simple insert.

The DNA strand transfer reaction that forms the transposition intermediate requires Mu A protein, Mu B protein and host protein HU as the protein factors for an efficient reaction (4). Efficient reaction also requires ATP and Mg^{++} (3). The ATPase activity of the Mu B protein is responsible for the ATP requirement. In the absence of ATP or Mu B protein or both, a low level of DNA strand transfer can still be observed. This residual reaction mostly involves intramolecular transposition to target sites within the mini Mu transposon donor. Under these reaction conditions, a nicked circular form of the mini Mu donor molecule also accumulates. The cuts in these molecules are localized at the ends of the Mu sequence. The role of Mu B protein in the transposition reaction will be discussed.

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Replication of Extrachromosomal Elements

131 INITIATION AT THE TWO DISTINCT ORIGINS OF MITOCHONDRIAL DNA REPLICATION, David A. Clayton, David D. Chang and Tai Wai Wong, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

Mammalian mtDNAs have two separate and distinct origins of replication. Initiation of heavy (H)-strand DNA synthesis represents the first event in mtDNA replication. The primer RNA for H-strand replication is generated from a transcriptional promoter involved in the expression of the light (L)-strand encoded genes (the L-strand promoter, LSP) (1,2). The transition from RNA to DNA synthesis occurs within short, conserved sequence blocks (CSB-I, II, III) and is mediated by specific endonucleolytic cleavage of the primary transcript from the LSP. An enzymatic component involved in the generation of a primer RNA in mouse mitochondria has been identified. It is a sequence-specific ribonuclease that cleaves single-strand RNA substrate precisely at one of the H-strand replication origins near CSB-II.

The initiation of L-strand synthesis occurs only after H-strand synthesis is at least two-thirds complete. A characteristic stem and loop structure is found at the L-strand origin. Specific L-strand replication of a single-strand template can be accomplished in vitro using DNA primase, DNA polymerase, and other accessory proteins isolated from human mitochondria (3,4). Replication begins with the synthesis of primer RNA along a T-rich sequence in the loop of the dyadic structure and absolutely requires ATP. The transition from RNA to DNA synthesis occurs near the base of the stem. Deletion and site-directed mutagenesis of the template indicate that the nucleotide sequence near the transition sites, as well as the stem and loop structure, are important in replication.

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132 Reconstitution of pBR322 DNA Synthesis with Purified Proteins, Jonathan S. Minden, Camilo Parada, Minsen Mok, and Kenneth J. Marians, Graduate Program in Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

pBR322 DNA replication has been reconstituted in vitro using purified proteins from Escherichia coli (1). Initiation of the leading-strand requires RNA polymerase holoenzyme, DNA polymerase I, RNase H and DNA gyrase. Initiation of the lagging-strand requires the primosomal proteins and SSB. Low levels of topoisomerase I are required to confer pBR322 template specificity and to generate the synthetic strand specificity inherent in the different mechanisms of initiation of the nascent DNA strands.

In the presence of DNA ligase, the aforementioned proteins initiate DNA replication correctly but yield replicative intermediate as the major product-little form I DNA is produced. It was determined that 30 to 50-fold higher levels of topoisomerase I will catalyze the segregation of this replicative intermediate and allow completion of the molecules, yielding primarily form I DNA product (2). Evidence is offered in support of a mechanism to account for segregation of the daughter DNA molecules that involves unwinding of the final nonreplicated region of parental DNA by the primosome coupled to topoisomerase I catalyzed strand passage at a gap just 5' of the origin of DNA replication. A possible mechanism to account for the ability of topoisomerase I to act as a discriminatory factor will also be discussed.

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133 CHARACTERIZATION OF A PLASMID REPLICON DERIVED FROM EPSTEIN-BARR VIRUS, Bill Sugden, David Reisman, John Yates*, and Noreen Warren, McArdle Laboratory, University of Wisconsin, Madison, WI 53706 and *Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, NY 14263

We have identified two genetic elements of Epstein-Barr virus (EBV) that together can act as a plasmid replicon. One element acts in cis and is termed oriP (Yates et al., PNAS 81: 3806-3810, 1984). The other element acts in trans and encodes the EBNA-1 gene product (Yates et al., Nature 313: 812-815, 1985). OriP is composed of two components, a family of repeated sequences and a region of dyad symmetry, both of which are required for plasmid replication (Reisman et al. Mol. Cell. Biol. 5: 1822-1832, 1985). We have inserted the family of repeated sequences derived from oriP into constructions containing either the SV40 early promoter or the Herpes Simplex virus-1 thymidine kinase (HSV-1 TK) promoter and the chloramphenicol acetyl transferase (CAT) gene. When these constructions are introduced into EBV-transformed lymphoblasts or lymphoblasts that express only the EBNA-1 gene of EBV, the family of repeated sequences enhances transcription of the CAT gene. The transcriptional enhancement by the family of repeated sequences requires the EBNA-1 gene product. No enhanced expression of the SV40 early promoter by the family of repeated sequences is detected in the adherent cell lines, 143 and D98, in the presence of EBNA-1, although EBV-derived plasmids do replicate in these cells. However, the family of repeated sequences does enhance expression in both transient and long-term experiments from the HSV-1 TK promoter in the lymphoid and adherent cells that express EBNA-1 that have been tested. Substitution of the family of repeated sequences in oriP with the 72 bp enhancer of SV40 does not permit replication of this chimeric plasmid in EBV-transformed lymphoblasts. We are now trying to determine if DNA synthesis initiates within the region of dyad symmetry of oriP.

We have tested if multiple copies of oriP yield a selective advantage by constructing plasmids with one or two copies of oriP that carry different selective markers, introducing them into the same cell, and measuring their rate of loss from the derived cell population. Both plasmids are maintained at similar average copy numbers under selection; and both are lost independently and at similar rates under non-selective conditions. These findings indicate that multiple copies of oriP do not confer a selective advantage like that observed with the defective molecules of SV40 and HSV-1 which carry multiple origins of replication.

134 MECHANISM OF COLE1 PLASMID DNA REPLICATION, Hisao Masukata and Jun-ichi Tomizawa, NIADDKD, NIH, Bethesda, MD 20892

Transcription by E.coli RNA polymerase provides the precursor to primer for ColE1 DNA replication. The transcript (RNA II) starts 555 bp upstream of the replication origin. More than half of the nascent RNA II molecules that extend beyond the origin form a persistent hybrid with the template DNA near the origin. The hybridized RNA II is cleaved by RNase H at the origin and then serves as a primer for DNA synthesis by DNA polymerase I.

Primer formation is negatively regulated by RNA I. Binding of RNA I to the complementary region of RNA II prevents cleavage of RNase H at the origin. It has been thought that the binding of RNA I prevents folding of RNA II into a structure that is necessary for hybridization of RNA II to the template DNA.

Using limmited digestion of RNA II by RNase T1 or RNase A, we have demonstrated that binding of RNA I to RNA II causes structural changes in various regions of RNA II, even in the region far downstream of the site of binding of RNA I. We have found that a single base-change in RNA II by a mutation in the segment complementary to RNA I prevents hybridization of RNA II to the template DNA. The mutation itself causes conformational changes of RNA II quite similar to those caused by binding of RNA I. A suppressor mutation restores both structure and function of RNA II. These results indicate that folding of a certain structure by RNA II is required for RNA II to hybridize to the template DNA at the origin.

However, a region of about 200 nucleotides proximal to the 5'-end of RNA II that includes the segment complementary to RNA I can be deleted with little effect on primer formation. This result shows that the region is not directly involved in primer formation. By forming an alternative structure, this dispensable region affects folding of a downstream region and thus participates in regulation of primer formation. The downstream region of functional RNA II forms characteristic tertiary structure.

Chromosome Structure and Topology

135 DNA GYRASE AND THE REGULATION OF BACTERIAL DNA SUPERCOILING, Martin Gellert[1], Anthony Maxwell[1], Donald C. Rau[2], and Rolf Menzel[1], [1]Laboratory of Molecular Biology and [2]Laboratory of Chemical Biology, NIADDK, National Institutes of Health, Bethesda, MD 20892

We have been studying both the mechanism and the regulation of the synthesis of DNA gyrase. Complexes between gyrase and specific DNA fragments have been examined by the method of transient electric dichroism. Both the DNA dichroism (extrapolated to infinite field) and the rotational diffusion coefficient of these complexes suggest that the DNA is wrapped around the enzyme in a single turn with the entry and exit points of the DNA located near each other. About 105 base pairs of DNA are wrapped on the enzyme; the rest of the DNA is extended as tails which make an angle of about 120°. If the non-hydrolyzable ATP analog (β,γ-imido)-ATP is added to complexes between gyrase and DNA fragments of 172 or 207 bp, the tails are no longer apparent. This suggests that ATP binding causes the association of a considerably greater length of DNA with the enzyme.

Synthesis of DNA gyrase is largely controlled by DNA supercoiling; expression of the gyrA and gyrB genes is greatest when the template DNA is relaxed. A deletion analysis of both promoter regions shows that this relaxation-activation is a very local property of the promoter sequence. Only 20 base pairs of sequence, extending from just upstream of the -10 region to just beyond the transcription start, are sufficient to retain full activation. The -35 consensus region modulates the overall strength of the promoters but is apparently not involved in this control mechanism. These results severely limit possible explanations for the effects of supercoiling on gene expression.

136 CHROMATIN ASSEMBLY AND DNA REPLICATION IN XENOPUS EGG EXTRACTS, R.A. Laskey, J.J. Blow, A.D. Mills, and J.A. Kleinschmidt, CRC Molecular Embryology Unit, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, England. Xenopus oocytes replicate their chromosomes faster than E.coli replicates its smaller chromosome. Xenopus eggs contain a stockpile of materials for many rounds of chromosome replication. We have used egg extracts to study chromatin assembly and DNA replication. We have found that nucleosomes are assembled by a family of acidic proteins which bind histones and transfer them to DNA. Their properties and functions will be discussed. Extracts from activated eggs are able to initiate DNA replication in vitro and to synthesize complete DNA strands semi-conservatively. Specific DNA sequences are not required for replication in this system or in injected Xenopus eggs, posing questions about possible functions for specific replication origins.

137 THE ROLES OF DNA TOPOISOMERASES IN SV40 DNA REPLICATION, Leroy F. Liu and Liu Yang, Department of Biological Chemistry, Johns Hopkins University, School of Medicine, Baltimore, MD 21205

Both the topoisomerase I inhibitor (camptothecin)[1] and the topoisomerase II inhibitor (epipodophyllotoxin VM-26)[2] inhibit ^{3}H-thymidine incorporation into SV40 DNA and host chromosomal DNA. Direct analyses of the SV40 DNA in the infected BSC-1 cells by Southern blotting also indicate that DNA replication stopped upon treatment with inhibitors. Analyses of the fluorographic results after pulse labeling show that the major effect of the topoisomerase inhibitors is the reduction of DNA synthesis. Using the in vitro SV40 DNA replication system developed by Li and Kelly[3], we have also observed that both topoisomerase inhibitors reduce the extent of DNA synthesis of exogenously added plasmid DNA. The topoisomerase I inhibitor is more effective than the topoisomerase II inhibitor in inhibiting DNA synthesis in vitro. Inhibition by anti-topoisomerase I and anti-topoisomerase II IgGs in this in vitro system was also observed. The patterns of inhibition by inhibitors and antibodies were identical. Our results suggest that both DNA topoisomerases are involved in SV40 DNA replication.

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138 ROLES OF YEAST DNA TOPOISOMERASES I AND II IN DNA REPLICATION AND TRANSCRIPTION, Rolf Sternglanz, Steven J. Brill, Catherine Thrash, Stephen DiNardo and Karen Voelkel-Meiman, SUNY, Stony Brook, NY 11794

We have identified yeast strains with mutations in the genes for DNA topoisomerases I and II. The topoisomerase II mutant is a conditional-lethal temperature-sensitive mutant. It is defective in the termination of DNA replication and the segregation of daughter chromosomes, but otherwise appears to replicate and transcribe its DNA normally (PNAS 81, 2616 (1984)). Topoisomerase I mutants have been identified and mapped to the gene previously called MAK1 (JBC 259, 1375 (1984)). The topoisomerase I structural gene (TOP1) has been cloned from two yeast genomic plasmid banks. Integration of plasmids carrying the gene and subsequent genetic mapping shows that TOP1 is identical to MAK1. A 3787 bp DNA fragment including the gene has been sequenced. The protein has 769 amino acids and a molecular weight of 90,020. Seven top1 (mak1) mutants including gene disruptions are viable and exhibit no obvious growth defects, demonstrating that DNA topoisomerase I is not essential for viability in yeast (PNAS 82, 4374 (1985)).

In contrast to the single mutants, top1 top2 double mutants grow poorly at the permissive temperature and shut down DNA and bulk RNA synthesis at the restrictive temperature. Ribosomal RNA synthesis is severely affected whereas tRNA and mRNA synthesis are relatively unaffected. This suggests that DNA replication and at least ribosomal RNA synthesis require topoisomerase action but that either topoisomerase can perform the required function (except for termination of DNA replication where topoisomerase II is essential).

Regulation of DNA Replication

139

MUTATIONS WHICH ENHANCE CHROMOSOME STABILITY IN YEAST, Stephen Kearsey, Department of Zoology, South Parks Road, Oxford, OX1 3PS, England.

In an attempt to identify proteins involved in the initiation of DNA replication, mutations which enhance the stability of minichromosomes containing defective origins of replication have been isolated (Rar^- mutations). The genetic screen uses CEN plasmids containing "weak" ARS elements, such as certain mutant alleles of the HO ARS[1]. These minichromosomes are mitotically unstable (segregation frequency = 0.2-0.5) presumably because DNA replication initiates with low efficiency on these molecules, and their stability can be greatly increased by introduction of a "strong" ARS in cis. The minichromosomes also contain a SUP4 gene which changes the colour of ade2-1 yeast cells and allows for rapid detection of changes in plasmid stability (see References 2 & 3 for description of analogous systems).

Twelve independent Rar^- mutants have been isolated from a screen of about 5×10^4 mutagenized colonies. These mutations are second-site, and typically show a 2 to 5 fold reduction in segregation frequency. Preliminary genetic data indicates that these mutations are recessive or co-dominant, and define at least 5 complementation groups. The phenotypic effect of the mutations on plasmid stability does not appear to be specific to the weak ARS used in the original screen. At least one Rar^- mutation affects an essential gene (RAR1) since the mutation also confers temperature-sensitivity. The RAR1 gene has been cloned and its function is being investigated.

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140

REGULATION OF DNA REPLICATION INITIATION IN YEAST. Bik-Kwoon Tye, Clarence Chan, Gregory Maine, Pratima Sinha, Steve Passmore, Susan Gibson and Shlomo Eisenberg. Cornell University, Ithaca, NY 14853.

We have previously reported the isolation of yeast mutants that are defective in the maintenance of circular minichromosomes. The minichromosomes are mitotically stable plasmids, each carrying an ARS and a centromeric sequence. We characterized 40 minichromosome maintenance defective (Mcm^-) mutants which comprise 16 complementation groups. These mutants can be divided into two classes, specific and nonspecific, by their differential ability to maintain minichromosomes with different ARSs. The specific class of mutants is defective only in the maintenance of minichromosomes that carry a particular group of ARSs. Three specific mutants, Mcm9, Mcm46 and Mcm61 have been analyzed further. Mcm46 shows temperature sensitivity for ARS specificity. At low temperatures, it affects the stability of some but not all minichromosomes, depending on the ARS present. At high temperatures, it affects all minichromosomes tested. The homozygous diploid of this mutant also loses its natural chromosomes at an abnormally high frequency. The mcm9 mutation, besides giving an Mcm^- phenotype, results in sterility in α cells. These two phenotypes are caused by a single mutation mapped at chromosome XIII, 2cM from LYS7. The MCM9 gene has been cloned and its activity is localized within 400 bp of a DNA fragment. The Mcm61 mutant, unlike the other specific mutants, affect all ARSs tested, but some more than others. The functions of the products of these MCM genes will be discussed. To investigate the element responsible for the differential behavior of the different ARSs in the specific mutants, we determined the nucleotide sequence of 5 telomeric ARSs, which are highly homologous and yet were affected differently in the specific mutants. We will report on the controlling element, which seems to be the site of interaction with the products of the MCM9 and MCM46 genes, within these ARSs. These results will be discussed in relation to the *in vitro* studies of the ARS-binding protein reported by Eisenberg and Tye in this same volume.

DNA Replication I: Eukaryotes

I41 ISOLATION AND CHARACTERIZATION OF cDNA CLONES CONTAINING E1 OPEN READING FRAME SEQUENCE OF BPV-1, N. Arai, P. Hoy, K. Yokota, Y. Takebe, DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94303-1104.

Bovine papilloma virus type-1 (BPV-1) can transform certain mouse cells in vitro and the DNA is maintained as an extrachomosomal multicopy plasmid in the transformed cells. Eight open reading frames (ORFs) have been located in 69% transforming region of BPV genome (BPV69T).

Mutational analyses have indicated that the longest ORF, E1, is essential for plasmid replication and/or maintenance. The analysis of E1 mRNA has been hampered by the difficulty in isolation of nearly complete E1 cDNA clone. We have isolated several cDNA clones which contain E1 ORF from BPV transformed mouse cell line (ID13) cDNA libraries. The libraries were constructed in a pcD vector which permits the expression of cDNA inserts in mammalian cells.

One class of cDNA clone (pCD•E1-1), about 2kb in length, started 250 bp upstream from second ATG in the E1 ORF and was terminated outside of the transforming region. Another class of cDNA clone (pcD•E1-2), carried about 3.4 kb insert, contained the entire E1 ORF and was terminated within the 69% transforming region. The detailed structural analysis of these E1 cDNA clones and their function is under investigation.

I42 IN VITRO REPLICATION OF SV40 DNA BY YEAST EXTRACTS , Josef Arendes, Michael Oed , Kai Lüdemann and Anne Rohrbacher, Institut für Physiologische Chemie, Johannes-Gutenberg-Universität, D-6500 Mainz, Fed. Rep. Germany

Soluble extracts prepared from exponentially growing yeast Saccharomyces cerevisiae cells catalyze efficient replication of exogenously added 2-µm and ARS (autonomously replicating sequences) containing plasmid DNAs. Initiation of DNA replication starts at the in vivo origin and proceeds bidirectionally as it does in vivo. In this study, we examined the template activity of SV40 DNA and SV40 containing plasmids in the yeast in vitro replication system. The SV40 DNA is efficiently replicated by yeast extracts. The products of the reaction consist of full length DNA molecules as shown by restriction analysis. The in vitro replication of SV40 DNA is sensitive to aphidicolin but relatively resistant to dideoxy-thymidine triphosphate. Linear molecules also supported a low levelof DNA synthesis, suggesting that superhelical density is not absolutely required for replication in vitro. The results suggest that the yeast replisome recognizes SV40 DNA sequences and initiates DNA replication in vitro, although there are no sequence similarities between the origins of replication of 2-µm DNA and SV40 DNA. Wether in the yeast system the SV40 replication origin or other domains on the plasmid are used as initiation site will be determined and the precise location of the initiation site on SV40 DNA in the yeast system will be described.

I43 REPLICATION CONTROL MUTANTS OF INCQ PLASMIDS,
Peter T Barth, ICI Corporate Bioscience Group, Runcorn, Cheshire, England

The prototype IncQ plasmid R300B is relatively small (8.7kb), has a copy number of about 30 per cell in E.coli, is readily mobilizable into a broad range of Gram-negative bacterial species and is stably maintained in most of these. We have therefore used it to derive cloning and expression vectors for use in various Gram-negative species. To isolate replication control mutants, the bla gene from pBR322 was first inserted into R300B between its existing sul and aphC antibiotic resistance genes. The resulting plasmid, pTB200, retains its previous properties but the bla gene permits the selection of mutant plasmids that confer resistance to high levels of ampicillin in the presence of clavulanic acid. The majority of such mutants were found to have a raised plasmid copy number. However only three out of 430 mutants tested were temperature-sensitive for plasmids copy control (cop^{ts}) and the increase in copy number at high temperature was relatively small (about 3 fold) for all three mutants. Further rounds of mutagenesis and selection on these has given secondary mutants with a more significant cop^{ts} response. Also, one isolate, pTB225, has a very high constitutive copy number (about 30 fold higher than its parent) giving about 1000 copies per cell and a significant depression of growth rate of its host. The multiple restriction site cloning sequence from M13 mp18 has been inserted upstream of bla in several of these mutant plasmids to facilitate the cloning of foreign genes into these vectors. Using restriction fragment exchange experiments, the cop mutations are being localized and will then be sequenced in an attempt to understand the nature of the replication control.

144 INFLUENCE OF DNA SEQUENCE AND POLYMERASE ACTIVE SITE ENVIRONMENT ON THE NATURE OF MISPAIRING DURING DNA SYNTHESIS, Kenneth L. Beattie and Ming-Derg Lai, Baylor College of Medicine, Houston, Texas 77030

The specificity of nucleotide incorporation during DNA synthesis, which is essential for accurate duplication of the genetic material, results from selective forces that occur at the active site of the enzyme, including formation of hydrogen-bonded Watson-Crick base pairs between template residue and incoming nucleotide, and interactions involving active site residues in the polymerase. Incorrect incorporation, though rare, may be specified to some degree by additional interactions, involving the nearest neighbor sequences in the primer-template. To investigate this possibility, we have directly determined the identity of dNMPs misincorporated in place of each of the 4 dNTPs at many positions along a bacteriophage template, during DNA synthesis catalyzed by the "large fragment" form of E. coli DNA polymerase I. The results indicate that the nature of misincorporation during DNA synthesis is indeed influenced by the DNA sequence. Furthermore, the results or experiments carried out with different DNA polymerases strongly suggest that the type of mispairing that occurs during DNA synthesis is also influenced by the active site environment of the polymerase. Thus, the nature of mispairing during DNA synthesis is at least partially specified by interactions both within the DNA and with active site residues in the DNA polymerase.

145 NUCLEAR MATRIX-BOUND DNA POLYMERASE ALPHA: AN IN VITRO MODEL FOR EUCARYOTIC REPLICATION AND DNA LOOP TOPOLOGY, Ronald Berezney and Ross Allen Tubo, Department of Biological Sciences, State University of New York, Buffalo, NY 14260

As a step toward the molecular elucidation of the putative replicational machinery associated with nuclear matrix, we have developed a procedure for solubilization of matrix-bound complexes containing DNA polymerase alpha and potentially other replicational related components. Nuclear matrices isolated from regenerating rat liver during maximal in vivo replication (22 h post-hepatectomy) were exposed to gentle sonication. Up to 80% of total matrix-bound DNA polymerase alpha activity was released and resolved on 5-20% sucrose gradients. Three major peaks of polymerase activity were detected at 10S, 100S, and 150S. A survey of other replicational related components associated with nuclear matrix revealed significant levels (20-50% of total nuclear activity) of DNA primase, DNA methylase, 3′-5′ exonuclease, RNase H, and diadenosine tetraphosphate binding. At least a portion of these components were found to be associated with the complexes separated on sucrose gradients. DNA polymerase alpha complexes extracted from isolated nuclei during matrix preparation consisted of 10S complexes and were totally devoid of 100S and 150S complexes. Similarly, complexes from nuclear matrix prepared immediately preceding the onset of in vivo replicaton (14 h post hepatectomy) sedimented entirely at 10S. These relationships lead us to propose a dynamic assembly of the eucaryotic replisome which is initiated pre-replicatively as a 10S complex and functionally expressed during in vivo replication as large 100S to 150S complexes. A possible relationship of the large 100S and 150S complexes to replicon clusters is also suggested by experiments which demonstrate the in vitro conversion of those larger forms to the 10S species. (Supported by NIH grant GM 23922).

146 REPLICATION OF KDNA IN KINETOPLASTS ISOLATED FROM CRITHIDIA FASCICULATA: IDENTIFICATION OF MINICIRCLE DNA REPLICATION INTERMEDIATES
Larry Birkenmeyer and Dan S. Ray, UCLA, Los Angeles, CA 90024

The kinetoplast DNA (kDNA) or trypanosomes is comprised of thousands of DNA minicircles and 20-50 maxicircles catenated into a single network. We show that kinetoplasts isolated from the trypanosomatid species Crithidia fasciculata incorporate labeled nucleotides and support minicircle DNA replication in a manner which mimics two characteristics of minicircle replication in vivo: 1) The minicircles are replicated as free molecules and subsequently reattached to the kDNA network, and 2) a replication intermediate having a structure consistent with a highly gapped minicircle species is generated. In addition, a class of minicircle DNA replication intermediates is observed containing discontinuities at specific sites within each of the newly synthesized DNA strands. By using a strain of Crithidia fasciculata possessing nearly homogenous minicircles, we were able to map the discontinuities to two small regions situated 180 apart on the minicircle. Each region has two sites at which a discontinuity can occur, one on each strand and separated by approximately 100 base pairs. These sites may represent origins of minicircle DNA replication.

147 CLONING OF THE GENE ENCODING THE β SUBUNIT OF YEAST DNA POLYMERASE I, Alan Blowers and David C. Hinkle, Deptartment of Biology, University of Rochester, Rochester, NY 14627

We have purified yeast DNA polymerase I to near homogeneity using procedures designed to minimize proteolysis. A DNA primase activity is associated with the DNA polymerase throughout purification. SDS-PAGE analysis shows four major polypeptides (175 kDa, 70 kDa, 52 kDa and 48 kDa) associated with the DNA polymerase-primase complex. An immunological response in rabbits was elicited against the 70 kdal protein purified by SDS-PAGE. The antiserum does not neutralize either polymerase or primase activity but it will immunoprecipitate both activities. This result supports the idea that the 70 kdal protein is a component of the polymerase-primase complex, although it seems likely that neither polymerase nor primase activity is contained within this subunit. Based on Western blots, this antiserum, and also monoclonal antibodies, react with both the 70 kDa and an additional 85 kDa polypeptide. This suggests that the 70 kDa (B) subunit may be derived from an 85 kDa precursor by proteolysis.

We have screened a lambda gt11 yeast DNA library with the antiserum against the 70 kDa protein and have isolated a clone which produces antigen which reacts with this serum. The complete gene has been isolated from another yeast library and produces an 85 kDa protein in E. coli "maxi-cells". Northern analysis of yeast RNA shows a single 2300 nucleotide transcript complementary to the cloned DNA fragment. This length is consistent with a protein of 85 kDa. A gene disruption was performed by replacing a portion of the B subunit gene with URA3 and integrating the disrupted gene into a ura3-52 diploid strain. Upon sporulation only ura3 haploid cells were obtained, indicating that the URA3 DNA has disrupted an essential gene.

148 CHARACTERIZATION OF A PROTEIN COVALENTLY BOUND TO THE DNA OF MINUTE VIRUS OF MICE, John W. Bodnar[1], Marie Chow[2], Maryellen Polvino-Bodnar[3], and David C. Ward[4]. 1-Northeastern University, Boston, MA 02115, 2-Massachusetts Institute of Technology, Cambridge, MA, 3-Dana-Farber Cancer Institute, Boston, MA, 4-Yale University, New Haven, CT.

We have identified a protein which is covalently bound to a fraction of the DNA synthesized in minute virus of mice (MVM) infected cells. This protein is specifically linked to the 5' terminus of the extended terminal conformers of the MVM replicative form DNA species and a variable fraction of the single-stranded viral DNA. The terminal protein (TP) bound on all DNA forms has a molecular weight of 60 Kd; it is also seen free in extracts from infected cells. Immunological comparison of the TP with other known viral proteins suggest that the TP is not related to the capsid proteins or to NS-1 and may be a host encoded protein induced by viral infection.

149 INHIBITION OF DNA REPLICATION BY UV AND BPDE IN XP VARIANT AND NORMAL HUMAN FIBROBLASTS, J. Boyer, W. Kaufmann, M. Cordeiro-Stone and B. Smith, Pathology Dept and Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC 27514

Xeroderma pigmentosum variant (XPV) fibroblasts are characterized by an abnormal pattern of replication of DNA damaged by 254 nm radiation (UV). These cells display enhanced sensitivity to inhibition of DNA chain elongation by UV. We have compared the effects of UV and benzo[a]pyrene-diol-epoxide-I (BPDE) on DNA replication in XP variant and normal skin fibroblasts. The XPV phenotype was confirmed by the mutant cells' decreased capacity to replicate UV-damaged DNA. Doses of UV (1-3 J/m^2) that only affected replicon initiation in normal human fibroblasts (NHF) inhibited DNA chain elongation in XPV cells. Identical analyses using BPDE (0.1-0.3 uM), however, indicated that the two cell types were equally sensitive to inhibition of both initiation and elongation. The rate of DNA strand growth was analyzed in cells synchronized at the beginning of the S phase. After exposure to low UV fluences, XPV cells displayed a lower rate of DNA strand growth than did NHF cells. However, both cell types responded similarly to low and high concentrations of BPDE. After low doses that primarily inhibited replicon initiation, nascent strands seemed to grow at a rate greater than that seen in untreated cells. Higher doses produced a dose-dependent inhibition of strand growth. These results suggest that XPV cells are not capable of rapidly replicating past certain UV lesions, as are normal cells, but, both cell types appear to be unable to rapidly by-pass BPDE adducts. Supported by NIH grants CA35657 and ES07126.

150 A GENETIC ANALYSIS OF THE STRUCTURE AND FUNCTION OF THE DNA REPLICATION GENE, dnaA, IN E.COLI K-12, Robert E. Braun, Kathy O'Day and Andrew Wright, Tufts University, 136 Harrison Ave., Boston, MA. 02111

The dnaA gene product is required for the initiation of DNA replication in E. coli K-12. We have previously reported that the dnaA gene is autogenously regulated (1), and that one of the two dnaA promoters is also regulated by Dam methylation (2). We are currently investigating the dual role of the dnaA protein in its own regulation and in the initiation of DNA replication. To this end we have genetically defined two domains in the dnaA protein; one involved in autogenous regulation (DNA binding), and the other involved in the initiation of DNA replication. We have also cloned and sequenced the dnaAcos gene (a cold-sensitive suppressor of the dnaA46(Ts) mutation) and shown that it contains three point mutations in addition to the original dnaA46 mutation. Preliminary results suggest that the dnaAcos protein has a greater affinity for its DNA binding site (the dnaA box), than does the wild-type dnaA protein.

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151 2-(BUTYLANILINO) PURINE DEOXYRIBONUCLEOSIDE 5'-TRIPHOSPHATES: EXPLOITATION AS DNA POLYMERASE ALPHA-SPECIFIC AFFINITY TOOLS, Neal C. Brown, Lech W. Dudycz, George E. Wright, Marjorie H. Barnes and Naseema N. Khan, Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605

BuPdGTP and BuAdATP, the respective N^2-p-n-butylphenyl (i.e. C_4H_9-C$_6$H$_4$-) forms of dGTP and 2-amino dATP, are very potent and selective inhibitors of mammalian DNA pol α (Khan et al., Nucl. Acids Res. 12, 3695, 1984; Ibid, in press). We seek to exploit these inhibitors as affinity reagents with which to both purify pol α and to dissect its nucleotide binding sites(s). Our first step in this search has been to incorporate in the inhibitor molecule an appropriate bridge by which it can be linked to various "reporter" molecules and macromolecular media. Among the several sites we have examined thusfar, the 5' α, β, and γ phosphoryl substituents show excellent potential as minimally intrusive bridgeheads. The 5'-γ aminohexyl phosphoroamidates, BuPdGTP-C_6-NH_2 and BuAdATP-C_6-NH_2 (i.e. NDP-PO_2-NH-$(CH_2)_6$-NH_2), display inhibitory potency, base analogy, and pol α specificity nearly indistinguishable from the corresponding property of their respective unsubstituted analogs. Further, the reaction of BuPdNTP-C_6-NH_2 derivatives with CNBr-activated agarose yielded substituted matrices which reversibly bind pol α from crude extracts with high affinity. The properties of these affinity matrices and related soluble derivatives of variously γ-substituted BuPdNTPs will be presented. (Supported by NIH grants GM28775 to NCB and GM21747 to GEW).

152 CHARACTERIZATION OF ORI^{dhfr}, A CHROMOSOMAL ORIGIN OF REPLICATION FROM CHINESE HAMSTER CELLS, William C. Burhans, Jane E. Selegue, Richard H. Lussier, and Nicholas H. Heintz, University of Vermont College of Medicine, Burlington, Vermont 05405.

Pulse labeling studies in whole or permeabilized CHOC 400 cells has shown that replication of the CHOC 400 amplified DHFR domain is initiated within a 4.3 kb XbaI fragment that is located 14 kb 3' to the DHFR gene. We have begun biological and physical characterization of the origin fragment. Directed Bal31 deletions of this fragment have been subcloned in M13 vectors, and partial DNA sequence analysis shows this region to be very AT rich, with prominent tracts of As (6-15 residues), frequent stretches of alternating AT residues (12-20 residues), and several simple direct repeat motifs, i.e. $(AGTAAA)_4$, $(ATT)_7$. Although the 4.3 kb fragment does not contain either type I or type II AluI family elements, it does contain as yet uncharacterized repetitive DNA sequences. When deproteinized, ds genomic CHOC 400 DNA is incubated with either Klenow enzyme or T4 polymerase, synthesis of sequences homologous to the 4.3 kb fragment occurs 150-200 fold more frequently than does synthesis of other random or repetitive sequences. The preferential labeling of the origin-related sequences is not the result of a persistant nick in the genome. Studies with purified T4 replication proteins, and electron microscopy of amplified restriction fragments isolated from synchronized CHOC 400 cells, suggests that preferential labeling of origin-related sequences may result from the presence of unusual structures that promote site-specific chromosomal DNA replication.

153 MOLECULAR CHANGES OF DNA POLYMERASE-α's ISOLATED FROM TRYPSIN AND TUNICAMYCIN-TREATED NEUROBLASTOMA CELLS IN CULTURE, Stephen R. Campion and Subhash Basu, Biochem. Biophys. & Mol. Biol. Prog., Dept. of Chem., Univ. of Notre Dame, Notre Dame, IN 46556

A qualitative change in the replicative enzyme DNA polymerase-α activity occurs upon induction of DNA synthesis by trypsinization of neuroblastoma (N-18 and IMR-32) cells in culture. These observed changes included a decrease in the sedimentation coefficient of pol-α (including any associated subunits) from a high molecular weight 10S complex to a lower molecular weight complex of 7.5S. This change in sedimentation is accompanied by a change in the isoelectric pH values of the pol-α activities. In nonsynchronous cultures pol-α activities with three acidic pI values (4.2, 4.8 and 5.5) were observed, whereas during synchronous DNA replication less acidic pI=5.5 and pI=6.5 pol-α forms were detected. SDS-PAGE activity gel analysis showed the molecular weights of acidic (pI=4.25) and less acidic (pI=5.5) DNA pol-α activities as >160 Kdal and 145 Kdal, respectively. The change of pol-α isoelectric points could also be induced by addition of tunicamycin (the glycosylation inhibitor) to cultures of density-dependent growth-inhibited cells. The tunicamycin-induced change in the pol-α structure is similar to that found upon induction of replication by trypsinization. This indicates that the appropriate cell surface glycoproteins are necessary for maintenance of cell-cell contact inhibited growth. Tunicamycin (1-3 μg/ml) also inhibits ^{3}H-TdR incorporation in nuclear DNA and lowers pol-α_2 activity in IMR-32 cells (Bhattacharya, P. and Basu, S. (1982) Proc.Natl.Acad.Sci., 79, 1488-1491). However, the direct involvement of a glycoprotein subunit in the DNA chain elongation process has yet to be determined. (NS-18005/S.B.)

154 MAMMALIAN MITOCHONDRIAL DNA TOPOISOMERASE I. Frank J. Castora, J. Philip Henrich, William G. Kelly, and Gary M. Lazarus, Univ. of MD Balto. Co., Catonsville, MD 21228

A type I topoisomerase has been purified to apparent homogeneity from calf thymus mitochondria. *This ATP-independent enzyme relaxes both positively and negatively supercoiled DNA* with a ΔL_K=1. Gel permeation chromatography and glycerol gradient centrifugation studies indicate that the native molecular weight is 130,000 daltons, while electrophoresis in the presence of sodium dodecyl sulfate suggests a denatured molecular weight of 60,000 daltons. Thus the mitochondrial topoisomerase appears to be a dimer of identical monomer subunits. The enzyme is inhibited by the trypanocidal drug Berenil as well as by elevated concentrations of the bacterial DNA gyrase inhibitors, novobiocin and coumermycin but not nalidixic or oxolinic acids. Sensitivity to N-ethylmaleimide indicates the importance of cysteine for the catalytic activity of the enzyme. It is estimated that there are approximately six copies of topoisomerase I per mammalian mitochondrion or approximately 1-2 per mitochondrial genome.

The mitochondrial enzyme is immunologically distinct from the nuclear topoisomerase I based upon results with nuclear anti-topoisomerase I antibodies. In a manner similar to that observed with leukemia (nuclear and mitochondrial), calf thymus (nuclear) and HeLa (nuclear) cell type I topoisomerases, the calf thymus mitochondrial enzyme is inhibited by physiological concentrations of ATP. These results further support the generality of the hypothesis that ATP regulates type I topoisomerase activity in the cell.

This work was supported by a grant from the March of Dimes Foundation (#5-402).

155 **PROPERTIES OF A SEQUENCE FROM THE HUMAN HPRT LOCUS THAT FUNCTIONS AS AN *ARS* ELEMENT IN YEAST, A.C. Chinault, P.E. Framson, R.C. Sykes, D. Lin and H.C. Smith, Baylor College of Medicine, Houston, TX 77030**

The temporal and spatial control of replication timing in mammalian chromosomes has been suggested to play an important role in gene regulation. One of the most dramatic changes in replication timing is associated with the developmental phenomenon of X-chromosome inactivation, where the X chromosome that is transcriptionally inactivated also shows a relatively late pattern of replication. We have tested cloned sequences from the human X-linked hypoxanthine phosphoribosyltransferase (HPRT) locus for ability to function as autonomously replicating sequences (*ars*) in yeast, when incorporated into the YIp5 integration vector. From the 60 kilobase region tested, a specific one kb fragment from the first intron of the gene was found to function efficiently in this assay. We are presently characterizing this sequence in more detail, and are also comparing intrinsic properties of the sequence, including chromatin configuration and nuclear matrix association, on active and inactive chromosomes to try to establish potential factors involved in the control of replication timing. (Supported by the American Cancer Society and the Howard Hughes Medical Institute.)

156 Thymine glycol lesions in DNA arrest synthesis by DNA polymerase I in vitro
James M. Clark & G. Peter Beardsley, Yale University, New Haven, CT 06510

The ability of DNA polymerases to replicate DNA containing structural abnormalities plays an important role in determining the biological consequences of exposure to radiation or chemical carcinogens. We studied the ability of E. coli polymerase I (Klenow fragment) to replicate DNA containing cis-thymine glycol (cis-5,6-dihydroxy-5,6-dihydrothymine), an oxidative damage produced in DNA by ionizing radiation. Single-stranded DNA from bacteriophage M13 was treated with osmium tetroxide to selectively oxidize a fraction of the thymine bases to the cis-glycol derivative. Analysis of the reaction products synthesized in primer extension experiments showed that the polymerase stopped specifically at sites corresponding to thymine bases in the osmium tetroxide-treated template; synthesis on undamaged templates generated much longer fragments. Thus synthesis by pol I (Kf) was arrested at sites corresponding to putative thymine glycol lesions. Primer extension experiments carried out in the presence or absence of dATP indicated that dAMP was incorporated opposite the lesion. These data suggest that thymine glycol in DNA can form a proper base pair with adenine and can serve as an appropriate template residue for the incorporation of the correct nucleotide at the site of the lesion. The incorporation of additional nucleotides appears to be inhibited, however, suggesting that the distortions of overall geometry and loss of base stacking interactions with the flanking bases generates a template/primer substrate that is not utilized efficiently by the polymerase. Also models show that the glycol hydroxyls project into the major groove of duplex DNA, suggesting that they may hinder the relative progression of the enzyme.

157 PREFERENTIAL REPLICATION OF EBV (HR-1) HETEROGENEOUS DNA.
J. COUNTRYMAN, L. HESTON AND G. MILLER

Variants of the Epstein-Barr virus strain HR-1 were used to superinfect either Raji or X50-7 cells. The fate of input and endogenous viral DNA sequences were analyzed by Southern blotting. One variant, which contained heterogeneous (het) DNA, induced significant viral replication. By exploiting the restriction site polymorphisms that existed between the endogenous viral and standard infecting genomes, it was determined that there was preferential replication of the infecting het DNA. There did not appear to be replication of the incoming standard DNA, however replication of the endogenous virus was observed.

Present in the het DNA are restriction fragments of unique mobility, as compared with the standard genome. These fragments have been shown to possess homologies with standard EBV fragments that are not contiguous on the standard genome (Miller et al, J. Virol. 1984). Three of these unique Bam HI fragments have been cloned, and one has been shown to be specifically responsible for the interruption of a normally latent EBV genome (Countryman and Miller, PNAS, 1985). It is likely that this same 2.7 Kbp fragment from the het DNA or the gene product that it encodes is also responsible for the stimulation of replication of both the endogenous viral genome and the het DNA. Data will be presented here to support this hypothesis

158 PURIFICATION AND CHARACTERIZATION OF TWO NEW FORMS OF DNA POLYMERASE DELTA,
James J. Crute*, Alan F. Wahl+, and Robert A. Bambara, University of Rochester School of Medicine and Dentistry, NY 14642. Present address: *DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, Ca 94304-1104, +Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

Two high molecular weight DNA polymerases, which have been designated delta I and delta II, have been purified from calf thymus tissue. DNA polymerase delta I and DNA polymerase delta II were purified 1400 and 4100 fold, respectively. The molecular weight of DNA polymerase delta I is 230,000 and that of DNA polymerase delta II is 280,000. Both enzymes display several similarities to previously reported delta polymerases. One of these characteristics is the ability to degrade single-stranded DNA in a 3' to 5' direction. These two forms of DNA polymerase delta also share several common features with alpha type DNA polymerases. Both calf DNA polymerase delta I and DNA polymerase delta II are similar to calf DNA polymerase alpha. Molecular weight, inhibition by the alpha polymerase inhibitors aphidicolin and N-ethylmaleimide, the presence of an active DNA dependent RNA polymerase or primase activity, a similar extent of processive DNA synthesis, and the ability to be stimulated by millimolar concentrations of ATP, are characteristics of both enzymes. This work was supported by NIH Grants GM2441,5-P30-CA 11198-14, Public Health Service Grant T32-GM07101-08, and ACS grant FRA220.

159 REPLICATION AND CHROMATIN STRUCTURE. A.M. De Recondo, C. Bonne-Andréa, F. Harper and E. Puvion. Groupe de Biologie et Génétique Moléculaires. Institut de Recherches Scientifiques sur le Cancer - B.P. N°8 - 94802 Villejuif Cédex- FRANCE.

The protein HMG1 purified by affinity chromatograhy was characterized in our laboratory as a single-stranded DNA binding protein, able to interact with the four core histones, to rapidly mediate in vitro a correct assembly of nucleosomes under physiological conditions (C. Bonne et al., 1984. The EMBO J. 3, 1193-1199). These properties suggested that HMG1 might be involved in the in vivo assembly and segregation of nucleosome during genetic readout (replication and/or transcription). In order to verify this hypothesis, we studied the subcellular location of HMG1 using specific antibodies in exponentially growing or confluent CV1 cells, infected or not by SV40. Immunofluorescence and immunoelectron microscopy studies demonstrate clearly that HMG1 migrates from the cytoplasm into the nucleus in relation to DNA and/or RNA synthesis. Moreover, the combination of the immunostaining for HMG1 with ultrastructural autoradiography revealed that the areas of the nucleoplasm which accumulate HMG1 are the sites of active SV40 DNA synthesis.

160 INITIATION OF POLYOMA DNA REPLICATION, M. DePamphilis, D. Wirak, L. Chalifour, R. Perona, S. Fields-Berry, P. Wassarman[1], J. Hassell[2], E. Hendrickson, C. Fritze, U. Heine, and W. Folk[3]. Harvard Medical School, Boston, MA; [1]Roche Institute, Nutley, NJ; [2]McGill Univ., Montreal, Canada; [3]Univ. Texas, Austin, TX.

What constitutes the polyoma (PyV) origin of replication (ori), how does it differ from that of SV40, and how does initiation of DNA replication in differentiated mammalian cells compare with embryonic mammalian cells? We have provided some insight to these questions by measuring DNA replication and gene expression following microinjection of various plasmids into the nuclei of preimplantation mouse embryos, and by mapping the sites of DNA synthesis in wild-type and mutant PyV(RI) DNA. The principle conclusions at this time are: (i) DNA replication in mouse embryos requires specific cis-acting sequences such as the PyV ori, in contrast to sequence-independent replication of DNA injected into Xenopus eggs. (ii) Mouse embryos are permissive for PyV DNA replication, in contrast to mouse embryonal carcinoma cells. (iii) The PyV ori requires an enhancer element, in contrast to the SV40 ori, and some of these elements can activate ori-core in differentiated cells but not in embryos. (iv) The role of enhancer elements in activating ori-core can be distinguished from their role in activating gene expression. (v) The arrangement of DNA synthesis initiation sites that define the origin of bidirectional replication (OBR) is distinctly different in PyV compared with SV40, although both OBRs lie within the ori-core sequence.

161 COMPARATIVE ENZYMOLOGY OF DNA REPAIR SYNTHESIS AND DNA REPLICATION IN PERMEABLE HUMAN FIBROBLASTS, Steven L. Dresler, Washington University School of Medicine, St. Louis, MO 63110

In nongrowing mammalian cells, DNA repair patch synthesis following high doses of UV damage is mediated by DNA polymerase α. Using permeable human fibroblasts, such repair synthesis has been compared with DNA replication, which is also mediated by polymerase α. The two processes have similar apparent K_i's for aphidicolin and butylphenyl-dGTP, however, their K_i's for butylphenyl-dGTP are several hundred-fold higher than that of isolated polymerase α. The apparent K_m's of repair synthesis for dCTP and dGTP are both 0.15 μM, much lower than those of replication, for which the K_m for dCTP is 2.0 μM and the K_m for dGTP is 1.4 μM. The K_m of repair synthesis for dTTP is 1.2 μM, much greater than the K_m's for dCTP and dGTP. Clearly, the characteristics of polymerase α as it participates in cellular nucleic acid metabolism are significantly different from those of the isolated enzyme and the characteristics of the polymerase as it participates in DNA repair synthesis are different from the characteristics of its involvement in DNA replication. The differences between DNA repair and replication might arise from utilization of different polymerase α subfractions, different primer-template structures, or different polymerase accessory proteins. The remarkably high K_m of repair synthesis for dTTP suggests the possibility that the repair synthesis complex incorporates some of the distal steps of dTTP biosynthesis and utilizes a precursor of dTTP as its preferred substrate.

I62 DNA TOPOISOMERASES AND THE BACTERIAL CHROMOSOME, Karl Drlica, Public Health Research Institute, 455 First Avenue, New York, NY 10016

Two enzymatic activities, DNA gyrase and DNA topoisomerase I, appear to control DNA supercoiling in bacterial cells. Mutations in the gene encoding topoisomerase I lead to abnormally high levels of supercoiling, and mutations in the genes encoding gyrase lead to abnormally low levels. Inactivation of gyrase through conditional lethal mutations or treatment of cells with specific antibiotics reduces titratable supercoiling, inhibits DNA replication, and alters expression from some genes. We currently feel that the topoisomerases maintain superhelical tension in the chromosome at a fixed level; determination of the precise level of superhelical tension requires additional information about bacterial chromatin structure and its contribution to the constraint of DNA supercoils. Division of the chromosome into topologically independent domains allows most of the chromosome to remain supercoiled even though replication and repair may introduce localized strand scissions. Another function of gyrase appears to involve resolution of catenated daughter chromosomes. A temperature-sensitive gyrB mutation has been found which causes nucleoids isolated from cells incubated at nonpermissive temperature to have a doublet morphology, and the doublet nucleoids from these cells can be resolved into singlets by purified gyrase.

I63 ISOLATION OF GENES INDUCED DURING LIVER REGENERATION, Michel Duguet, Joëlle Sobczak, Anne-Marie Lotti, Philippe Tarroux*, Université Pierre et Marie Curie, 96, Bd. Raspail, 75006 Paris, FRANCE. * Ecole Normale Supérieure, 46, rue d'Ulm, 75005 Paris,FRANCE.

Regenerating liver is a model for studying replication and cell proliferation. In this system, shortly after partial hepatectomy, the hepatic cells are triggered to divide, until the mass of liver is restored. The most interresting feature is that the dividing cells are in a "physiological" environment which controls the cell division and stops it at the end of the regeneration process. The first cell type undergoing DNA synthesis is the hepatocyte. It enters synchronously the first cell cycle; DNA synthesis begins at 16h and peaks at 22-24h post-hepatectomy.

In order to isolate genes which are induced during liver regeneration, we have constructed a cDNA library from 16h-regenerating liver poly(A)+ RNA. By differential plaque hybridization with cDNA from normal or 16h-regenerating liver, and analysis of the autoradiograms with a computer, we have isolated several recombinant clones representing induced sequences. The size and the kinetics of expression of the corresponding RNAs have been determined for three of the clones, showing that their induction is correlated with the burst of DNA synthesis in the hepatocytes. One recombinant clone ($\lambda\bar{7}$) which appears to be regeneration-specific, exhibits a sequence identical to that of rat fibrinogen α chain. It is speculated that this molecule may be involved in the growth process or in its regulation.

I64 ULTRAVIOLET LIGHT BLOCKS REPLICATION FORKS IN MAMMALIAN CELLS. Howard J. Edenberg and Cheryl A. Berger, Dept. of Biochemistry, Indiana University School of Medicine, Indianapolis IN 46223.

Ultraviolet light introduces lesions which inhibit DNA replication in mammalian cells. To elucidate the mechanism of this inhibition we have isolated and studied Simian virus 40 replication intermediates. In unirradiated cells, SV40 replication is bidirectional at approximately equal rates from a fixed origin. The two replication forks are usually at equal distances from the origin. We are interested in the question of whether UV lesions block replication fork movement, or whether the forks continue to unwind despite overall inhibition of synthesis. If UV lesions block replication fork movement the forks should be at different distances from the origin (i.e. asymmetrical), since in any one molecule lesions are introduced at random sites. If replication forks progress beyond UV lesions (whether or not they leave gaps) the fork positions will be symmetrical. Electron-microscopic examination of Simian virus 40 replication intermediates revealed asymmetrical molecules, demonstrating that UV lesions block replication fork movement.

165 MOLECULAR AND GENETIC CHARACTERIZATION OF A YEAST MUTATOR ALLELE, Eric Eisenstadt, Giulia Michelini, Helen Kim and R.C. von Borstel*, Harvard University, Boston, MA 02115 and University of Alberta*, Edmonton, Canada

The mut7 allele of Saccharomyces cerevisiae confers increased spontaneous mutation frequencies and temperature sensitive growth. At non-permissive temperatures (37 C), mut7 cells show a cdc-like terminal phenotype, arresting as singly budded cells. We have isolated DNA from a YEp13 library which complements the temperature sensitive phenotype of mut7. Genetic analysis reveals that the complementing sequence maps to the right arm of chromosome 10 and is centromere linked. The genetic and physical characterizations performed to date suggest that this is a previously undescribed essential yeast gene which may play a role in DNA replication.

166 ISOLATION OF DNA SYNTHESIS MUTANTS OF YEAST. Michael J. Engler, Anne DeChavigny, and F. Janine Abbott, University of Texas Medical School, Houston, TX 77225

A number of temperature sensitive DNA synthesis mutants of yeast have been isolated using an enrichment procedure based on the incorporation of the thymidine analog 5-bromodeoxyuridine (BUdR) into DNA. Yeast lack thymidine kinase and are unable to incorporate thymidine and its analogs. However, introduction of the herpes simplex virus thymidine kinase gene into yeast via a replicating plasmid gave expression of the thymidine kinase gene[1]. In this genetic background, a mutant auxotrophic for thymidine was isolated. Mutagenized cells are shifted to 37° and BUdR substituted for TdR. Mutants with temperature sensitive DNA synthesis phenotypes incorporate less BUdR than wild type cells and are resistant to 300 nm light. Temperature sensitive mutants isolated by this enrichment procedure were screened for defects in the rates of in vivo DNA synthesis and protein synthesis at the restrictive temperature. Those with primary defects in DNA synthesis are under further study. The focus of current work is the cloning of genes by complementation of these DNA synthesis mutants. Isolation of clones of genes essential for DNA synthesis should aid in the isolation, identification and characterization of proteins involved in the replication of the yeast genome.

1. McNeil, J.B., and Friesen, J.P. (1981) Mol. Gen. Genet. 184, 386-393.

167 A REPLICATION ORIGIN IN TRYPANOSOMA EQUIPERDUM KINETOPLAST MINICIRCLES, Paul Englund, James Ntambi and Kathleen Ryan, Johns Hopkins Medical School, Baltimore, MD 21205

In Trypanosoma equiperdum, some newly replicated kinetoplast DNA minicircles contain a small gap, of roughly 10 nucleotides, in the newly synthesized strand. This gap overlaps the GGGGTTGGTGTAA sequence found in all minicircles, from several species, which have been sequenced (J. Biol. Chem. 260, 5574, (1985)). We have found that ribonucleotides are associated with this gap. The evidence for ribonucleotides includes: (1) alkali lability of minicircles in which the gap had been repaired by T4 DNA polymerase and T4 DNA ligase; (2) susceptibility of alkali-treated minicircles to phosphorylation with polynucleotide kinase and $\gamma[^{32}P]$ATP; (3) detection of $[^{32}P]$ribonucleotides on DNA which had been dephosphorylated with phosphatase, rephosphorylated with polynucleotide kinase and $\gamma[^{32}P]$ATP, and then hydrolyzed with NaOH. Using sequencing methodology, we mapped the ribonucleotides to the 5' end of the newly synthesized strand. There are one or two ribonucleotides present, and the RNA:DNA junction is at one of two neighboring positions in the DNA sequence. It is likely that these ribonucleotides are remnants of a replication primer. It is possible that most of the primer had already been excised, to create the gap, and that only a remnant remained. Subsequent processing would release these remaining ribonucleotides. The presence of ribonucleotides at the 5' end of the newly synthesized strand provides strong evidence that the GGGGTTGGTGTAA sequence is conserved in minicircles of all species because it is a replication origin.

168 CLONING OF PUTATIVE DNA REPLICATION ORIGINS FROM HUMAN CELLS. A.Falaschi, G.Biamonti, C.Tribioli, G.Della Valle, S.Riva. Istituto di Genetica Biochimica ed Evoluzionistica, C.N.R., Pavia, Italy.

In order to selectively label DNA sequences replicating at the onset of S phase in human cells,(HL-60) we have applied a synchronization procedure which involves two subsequent blocks with aphidicolin. The cell population, thus accumulated at the G1/S boundary,was stepped down to a low drug concentration in order to allow a slow rate of fork advancement. DNA was pulse-labelled with ^{3}H-Thd for periods of 10 to 60 min. and shown not to be covalently linked to the parental one and to increase in size with time. The synchronized cells were pulse-labelled with ^{3}H-BudR for 60 min (corresponding to 3 min of normal DNA synthesis). Genomic DNA was extracted and fractionated on CsCl density gradient. Newly synthesized DNA was found to band at a hybrid density (HL) with an (HH) shoulder.The HL and HH fractions were separately pooled and rebanded on CsCl gradient. (HH) and (HL) DNA was digested with Mung Bean nuclease and cloned by blunt end ligation into pAT153. We obtained over 200 plasmids containing human DNA inserts with an average length of 300 bp; several inserts from 1000 to 1600 bp were also obtained. The longest insert belongs to a moderately repetitive family (approximately 10.000 copies per genome) and does not contain Alu, mitochondrial, or rDNA sequences. This and other long inserts are being characterized for their ability to perform origin function in vivo or in vitro.

169 ARS ELEMENTS AND CHROMOSOME REPLICATION IN YEAST, Walton L. Fangman, Robert M. McCarroll, Bonita J. Brewer and Stephan G. Zweifel, Department of Genetics, University of Washington, Seattle, WA 98195.

Several approaches are being used to understand autonomous replication sequence (ARS) elements and their role in DNA replication in Saccharomyces cerevisiae. Different chromosomal ARS elements replicate at different times during the S phase. If ARS elements are replication origins then these results are consistent with the idea that origins are activated at different times. Each of four contiguous ARS elements along a 190 kb section of chromosome III replicates in early S (collaboration with Carol S. Newlon) suggesting that large chromosomal domains may represent a temporal unit. Analysis of four centromeres (CENs 3, 4, 6 and 11) indicates that their adjacent origins are activated in early S phase. Therefore, unreplicated centromere DNA does not account for the adherence of chromatids at metaphase.

The 75 kb yeast mitochondrial (mit) DNA has been shown to contain a high density of ARS elements, a finding that is consistent with the observation that natural deletion variants can retain many different segments of the mit genome. We have found that spontaneous deletion genomes consisting of as little as 35 bp of sequence information are maintained stably in the mitochondrion as large head-to-tail tandem repeats. Fewer than four copies of a 64 bp repeat do not confer ARS activity on a plasmid, whereas greater than eight tandem copies does. These results are consistent with the hypothesis that a single repeat is a weak replication initiator but that multiple copies in a single recombinant plasmid raise the efficiency of initiation sufficiently to allow plasmid maintenance.

170 RESTRICTION FRAGMENTS DERIVED FROM A MAMMALIAN INITIATION LOCUS PROMOTE HIGH FREQUENCY TRANSFORMATION IN TRANSFECTED CELLS, Pamela K. Foreman and Joyce L. Hamlin, Dept. of Biochemistry, University of Virginia, Charlottesville, Va. 22908

Our laboratory has shown that DNA replication initiates preferentially within a 10 Kb sequence in the amplified dihydrofolate reductase domain in methotrexate-resistant Chinese hamster ovary cells. This region has been cloned in the cosmid cS21. In order to determine whether DNA synthesis initiates at a fixed origin of replication, we are testing several restriction fragments from this region for their ability to replicate autonomously in mammalian cell lines. Several overlapping fragments have been cloned into a vector that contains the neomycin resistance gene. These clones were then introduced into either CHO or murine cell lines by spheroplast fusion, and stable transformants were selected by the drug, G418. Fragments that overlap the left end of cosmid cS21 consistantly give a 10-30 fold increase in the number of G418-resistant colonies. We are presently attemptingto delineate the minimum boundaries of the sequence that potentiates transformation to resistance, and to determine whether this effect isthe result of replication of these fragments to high copy number in the early stages after transfection. Results of the analysis of Hirt supernatants prepared within the first two days after transfection will be presented.

171 MONOCLONAL ANTIBODIES PRODUCED AGAINST CRUCIFORM DNA STRUCTURES THAT CONTAIN THE 27-BP PALINDROME OF THE SV40 REPLICATION ORIGIN. Lori Frappier*, Gerald B. Price*, Robert G. Martin+ and Maria Zannis-Hadjopoulos*.
*McGill Cancer Center, McGill University, Montreal, Quebec, Canada and +Laboratory of molecular Biology, NIADDK, National Institutes of Health, Bethesda, MD.

Inverted repeat (palindromic) DNA sequences have the potential to form cruciform (hairpin or stem-loop) structures. Inverted repeats are a common feature of initiation sites of DNA replication in prokaryotes, eukaryotes and viruses. Although the existence of stem-loop structures in vivo has not been demonstrated directly, it has long been postulated that they may play a role in the function of origins of replication. We have raised two monoclonal antibodies, an IgG1 and an IgM, against a cruciform structure in a heteroduplex DNA molecule. The cruciform was created by reannealing a single strand of the wild-type SV40 Hind III to Sph I fragment, which comprises the 27-bp palindrome, with a single strand of a synthetic molecule, consisting of an EcoRI linker flanked on either side by a SalI linker, that contains a 26-bp palindrome of an unrelated sequence to that of SV40 (Nobile and Martin, J. Int. Virol., in press). These two antibodies may recognize conformational determinants specific to stem-loop structures as they bound, in addition to the cruciform , a modified DNA cruciform with one shortened arm, a hairpin DNA molecule and yeast tRNA, whereas they did not bind the respective linear double-stranded DNA homoduplex molecules. Antibody binding to DNA fragments was determined by a radioimmunoassay in which DNA-antibody complexes were separated from unbound DNA by polyacrylamide gel electrophoresis. These monoclonal antibodies will be used to detect stem-loop structures in vivo.

172 INTERACTIONS OF ESCHERICHIA COLI AND BACTERIOPHAGE λ PROTEINS IN REPLICATION, D. Ang, C. Johnson, G.N. Chandrasekhar, M. Zylicz and C. Georgopoulos, Department of Cellular, Viral and Molecular Biology, University of Utah Medical Center, Salt Lake City, Utah 84132

The grpE gene product of E. coli has been previously shown by Saito and Uchida [J. Mol. Biol. 113, 1-25 (1977)] to be essential for bacteriophage λ DNA replication in vivo. We have extensively characterized the properties of the bacterial grpE280 mutation and have found the following: (a) a Fraction II extract from mutant bacteria does not replicate λ DNA in vitro; (b) the mutation results in host temperature sensitivity at temperatures above 43.5°C. By means of P1 transduction analysis we have shown that the grpE280 mutation is responsible for both the phage and host phenotypes; (c) the dnaK+ protein of E. coli interacts with the grpE+ but not with the grpE280 protein in vitro; (d) the grpE protein has been purified on the basis of its association with the dnaK protein; (e) the grpE protein has been shown to be identical to the B25.3 heat shock protein of E. coli.

173 TEMPLATE REQUIREMENTS FOR SV40 DNA REPLICATION Robert D. Gerard and Yakov Gluzman Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

In order to assess the contributions of various sequence elements to SV40 DNA replication, we have constructed a series of deletion mutations in the origin region and analyzed their effect on replication *in vivo*. Deletions into the *ori* from both sides showed a sharp decrease at a specific boundary in the ability to replicate DNA. On the early side, little effect was noted when T antigen binding site 1 was deleted. Rather, the boundary correlates with the transition point of continuous to discontinuous strand synthesis. Deletion of sequences past this point resulted in a total loss of the ability to replicate. On the late side, the 21bp and 72bp repeats comprising the promoter/enhancer elements had little effect on DNA replication. However, deletion of only a few bases into the the AT region adjacent to the 27bp palindrome resulted in a DNA template which was defective for replication.

The late side mutations defective for replication were reconstructed into the viral genome containing an intact early promoter/enhancer region. Even though the mutants synthesized wild type levels of T antigen, they were still defective for replication. However, transfection of the defective viral DNAs into monkey cells yielded phenotypic revertants which were replication competent. The DNA structure of these revertants were analyzed and found to contain insertions or point mutations which at least partially regenerate the AT stretch. These results indicate the importance of the AT region, which perhaps serves as a binding site for a specific protein involved in papovavirus DNA replication.

174 Mutant Cell Lines with a"Hyperamplifiable"Phenotype. Elena Giulotto, Catherine Knights and George R. Stark. The Imperial Cancer Research Fund, Lincoln's Inn Fields London WC2A 3PX.

Amplification of several different loci has been obtained in drug-resistant cells at frequencies that are typically between 10^{-4} and 10^{-6}. These frequencies might be observed because any cell in the population has a probability of 10^{-4} to 10^{-6} to amplify the selected gene. Alternatively, there might be a subpopulation of cells with a very high probability of undergoing amplification

In an effort to distinguish between these two possibilities, we treated BHK cells simultaneously with N-phosphonacetyl-L-aspartate (PALA) and methotrexate (MTX). Resistance to these drugs is usually due to gene amplification. If the probability of being resistant to PALA were independent of the probability of being resistant to MTX, the frequency of cells resistant to both drugs should be equal to the independent frequency of cells resistant to PALA multiplied by the independent frequency ofcells resistant to MTX. However, our results show that the frequency of colonies resistant to both drugs simultaneously is 250 to 500 times higher than the frequency actually determined for independent events in the same experiment.

We have isolated several colonies resistant to simultaneous treatment with PALA and MTX. Preliminary results suggest that some of these cell lines have a stable " hyperamplifiable" phenotype. When challenged with an additional selective drug, coformycin, they show a 100 times higher frequency of resistant colonies than the parental cell line.

175 MURINE GENOMIC DNA REPLICATING AUTONOMOUSLY IN MOUSE L CELLS, Friedrich Grummt, Andreas Holst, Friedemann Muller and Erika Dinkl, Institut für Biochemie, Rontgenring 11, D-8700 Wurzburg, FRG.

We have isolated putative origins of replication from chromosomal mouse DNA. In shot-gun experiments we have cloned random genomic mouse DNA fragments into a vector ptk consisting of pBR327 and the Eco R1 fragment of HSV-1 containing the viral tk gene. The chimeric vectors containing the shot-gun fragments were tested for autonomous replication in L-tk^--cells. No stable transformants to the tk^+ phenotype of L cells were obtained with the vectors ptk alone. So far 10 chromosomal mouse DNA fragments were identified that lead to stable transformation and four of them were structurally and functionally characterized. The vectors containing these four genomic DNA fragments replicate and amplify in the stably transformed mouse L cells as circular extrachromosomal DNA with 40 to 800 copies per cell. In contrast to the original transfecting DNA the circular DNA molecules extracted from transformed mouse cells by the Hirt procedure were unmethylated at A as demonstrated by Sau 3a/Mbo restriction analysis. Sequence data and deletion mutagenesis demonstrated boxes of homology in these murine ars elements that differ from those described from yeast ars elements.

176 Origin specific DNA binding proteins from mammalian cells, Friedrich Grummt, Jürgen Reinhold, Brigitta Faber, Angelika Lux, and Andreas Holst, Institut für Biochemie, Röntgenring 11, D-8700 Würzburg, F.R.G.

Initiation of DNA replication in mammalian cells probably requires proteins that specifically recognize chromosomal origins of replication. We attempted to isolate origin specific DNA binding proteins from calf thymus and mouse liver using yeast 2 μ circle origins and chromosomal yeast ars 1 elements as models for eukaryotic origins. Filter binding assays demonstrated a protein fraction from calf thymus that eluted from dsDNA cellulose at 150-175 mM NaCl and binds preferentially to both the 2 μ circle origin and ars 1 under stringent conditions. Restriction mapping of the preferentially bound DNA fragment revealed that the origin containing sequences are required for the specifity of binding. In the case of the 2 μ circle origin exonuclease III footprint analysis demonstrated two different 24 bp long sequences to be protected one within the AT rich region of the origin the other one immediately flanking this AT rich sequence. The protein fraction binding specifically yeast type origins of replication also binds one of four ars elements isolated from mouse genomic DNA. Western blotting revealed one polypeptide of Mr 32 000 interacting strongly with yeast ars elements. A second protein of Mr 27 000 gave only a very weak signal.

177 HYDROXYUREA DOES NOT INDUCE REREPLICATION OF DNA IN A SINGLE CELL CYCLE: IMPLICATIONS FOR GENE AMPLIFICATION, Peter Hahn, Leon N. Kapp, William F. Morgan, and Robert B. Painter, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143

It has been reported that a 6-h incubation of early S phase Chinese hamster cells with hydroxyurea promotes rereplication of DNA within a single cell cycle. When we incubated methotrexate-resistant Chinese hamster cells that were approximately 2 h into the S phase with hydroxyurea for 6 h, DNA that had been rereplicated before the incubation with hydroxyurea (early S DNA) was replicated again within 11 h after the hydroxyurea treatment. However, incubation with colchicine or Colcemid after hydroxyurea treatment virtually abolished this rereplication, as well as that of the amplified dihydrofolate reductase genes in these cells, indicating that the rereplication was in a second cell cycle. Cells collected in the first mitosis after incubation with hydroxyurea and Colcemid contained no rereplicated early S DNA, but did contain abundant chromosome aberrations. Early S DNA did rereplicate in the presence of Colcemid during the next few hours after mitosis. Asymmetric segregation of chromosome fragments resulting from these hydroxyurea-induced aberrations may be the actual basis for amplification in drug-treated mammalian cells.

178 DNA POLYMERASE FROM HERPES SIMPLEX VIRUS TYPE 1 (HSV-1): STRUCTURAL AND FUNCTIONAL DOMAINS, J.D. Hall and D.W. Mount, University of Arizona, Tucson, Arizona, 85721; D.M. Coen and J.S. Gibbs, Harvard Medical School, Boston, Massachusetts, 02115.

We have used a combined approach of computer and genetic analysis to investigate the structure and function of the HSV-1 DNA polymerase. First we have compared the DNA sequence from the HSV-1 polymerase with that of other polymerase genes. We find regions of striking homology with sequences from Epstein Barr and adeno viruses. We will discuss probable structural similarities between these proteins and the use of these homologies to define structural and functional domains within the polymerase. Second, we have attempted to identify amino acid residues at the active site of the polymerase by isolating mutants with altered recognition of nucleoside triphosphates. These mutants are fully functional and, therefore, are likely to be altered in recognition specificity. We will describe a new class of mutants resistant to 2'-nor-2'deoxyguanosine, a nucleotide analogue missing the 2'residue. These mutants appear to have altered interactions with this 2' group.

179 CHARACTERIZATION OF A MAMMALIAN CHROMOSOMAL ORIGIN OF REPLICATION, Joyce L. Hamlin, Mark S. Gray, Lorne F. Erdile, and Pamela S. Foreman, University of Virginia School Medicine, Charlottesville, VA 22908.

Our laboratory is interested in the control of DNA replication in mammalian cells. In In vivo labelling studies, we have shown that DNA synthesis initiates preferentially within or close to a 6 kb EcoRI fragment in the amplified dihydrofolate reductase domain in methotrexate-resistant CHO cells. We have therefore suggested that the origin of replication in this domain is a fixed genetic element analogous to origins in microorganisms. We have cloned the majority of the amplified sequence from this cell line, including the initiation locus itself. In recent studies, we have been attempting to identify the precise locus of initiation in vivo, and several different experimental approaches will be presented in which preferential initiation at a defined locus will be demonstrated. In addition, we will present the results of experiments that suggest that the cloned 6 kb EcoRI fragment is able to replicate autonomously when introduced into CHO or mouse cells by spheroplast fusion.

180 ROLE OF NUCLEAR FACTOR I IN ADENOVIRUS DNA REPLICATION. Ronald T. Hay, Department of Biochemistry & Microbiology, St. Andrews University, Scotland.

An in vivo assay is described which detects a single round of initiation and DNA synthesis directed by a linear molecule containing an exposed single copy of an adenovirus (Ad) origin of replication. This and an assay, which measures multiple rounds of DNA replication were used to identify DNA sequences within the Ad2 and Ad4 origins of replication that are important for ori function. Linear DNA molecules containing sequences from the Ad2 or Ad4 genome termini were cotransfected with homologous and heterologous helper virus and net amounts of DNA synthesis were determined. Linear molecules containing the Ad4 inverted terminal repeats (ITRs) were replicated efficiently in the presence of Ad4, but not Ad2 helper, whereas both Ad2 and Ad4 ITRs were utilised efficiently by Ad4. DNA sequence analysis of the Ad2 ori and the corresponding region in Ad4 indicated that, although there are only 10 variant base pairs, 8 are located within the Ad2 DNA sequence recognised by the cellular protein nuclear factor I (NFI). A consequence of these changes is that the AD4 ITR does not contain a functional NFI binding site, and deletion analysis demonstrated that this region of the Ad4 genome was not required for ori function. In contrast to Ad2 the DNA sequences required for the initiation of Ad4 DNA replication were shown to reside entirely within the terminal 18 base pairs of the Ad4 ITR. The biochemical basis for this difference is being investigated.

181 IDENTIFICATION OF THE ORIGIN OF REPLICATION ASSOCIATED WITH THE AMPLIFIED CHINESE HAMSTER DIHYDROFOLATE REDUCTASE DOMAIN, Nicholas H. Heintz, William C. Burhans and Jane Selegue, University of Vermont College of Medicine, Burlington, VT 05405.

Replication of the amplified 180 kb dihydrofolate reductase (DHFR) domain of CHOC 400 cells is initiated within a region 10-30 kb 3' to the last exon of the DHFR gene. In order to localize the origin of replication associated with this domain, the replication timing of amplified sequences was studied in synchronized CHOC 400 cells by three different methods. First, replication intermediates formed during the onset of S phase were isolated by BND cellulose chromatography, labeled with ^{32}P in vitro, and hybridized to Southern blots of CHOC 400 DNA. Secondly, cells were pulse labeled with ^{3}H-thymidine at various times during S phase, and synthesis of amplified sequences was quantitated by hybridization of the replication products to dot blots of cloned restriction fragments. Finally, CHOC 400 cells were permeabilized with NP-40 and incubated under conditions that support limited chromosomal DNA replication. Synthesis of amplified sequences in permeabilized cells was detected by hybridization of the in vitro replication products to Southern blots of recombinant cosmid clones that span 70 kb of the amplified unit. At the G1/S boundary, the labeled products derived from the replication of amplified sequences, either in whole or permeabilized cells, are symmetrically distributed about a 4.3 kb XbaI fragment that maps 14 kb distal to the DHFR gene. As cells progress through S phase, bidirectional replication away from this site is observed. Thus, the 4.3 kb XbaI fragment contains a functionally-defined mammalian chromosomal origin of replication.

182 DIFFERENTIAL STABILITY OF AN AMPLIFIED DNA SEQUENCE CARRYING A SPECTINOMYCIN RESISTANCE DETERMINANT IN STREPTOMYCES ACHROMOGENES SUBSP. RUBRADIRIS, Ulfert Hornemann, Christopher J. Otto, and Guy G. Hoffman, University of Wisconsin, Madison, WI 53706

S. achromogenes shows a progressive growth lag on increasing levels of spectinomycin and yields initially bald colonies on 1000 µg/ml spectinomycin which carry an 8 kb highly amplified DNA sequence as 100 to 200 copies per genome. This DNA sequence is present as a single copy in wild type cells, most likely located on the chromosome. The amplified DNA has been shown to carry a spectinomycin resistance gene. Upon prolonged incubation, the bald colonies give rise to secondary cells which are able to proliferate rapidly, form white aerial mycelium, sporulate, and also carry the reiterated DNA sequences. Separate subculturing of cells derived from the bald and the white cells for five sporulation stages in spectinomycin-free medium yields bald cell derived cells devoid of amplified DNA. These cells grow on 1000 µg/ml spectinomycin with a 4 day growth lag. White cell derived cells, in contrast, show the reiterated DNA at nearly the same amplitude as control cells raised on 1000 µg/ml spectinomycin. These cells grow on 1000 µg/ml spectinomycin without any lag. Protoplast regenerants prepared from bald cells raised on 1000 µg/ml spectinomycin yield progeny at a frequency of 5% which are unable to grow on spectinomycin and which by Southern blot analysis can be shown to lack any sequences homologous to the 8 kb amplified DNA. In contrast, protoplast regenerants obtained from white cells yield progeny at a frequency of 77% which are unable to grow on spectinomycin. The reasons for the emergence of the white cells which carry the amplified DNA stably, yet lose it readily upon protoplasting, are presently obscure.

183 SPECIFIC PRIMING BY DNA POLYMERASE α HOLOENZYME ON THE NATURALLY OCCURRING MAMMALIAN SINGLE-STRANDED PORCINE CIRCO-VIRUS DNA, Ulrich Hübscher, Michael Hässig and Hansjörg Buhk*. Department of Pharmacology and Biochemistry, University of Zürich, CH-8057 Zürich, Switzerland and *Robert Koch-Institute, D-1000 Berlin, Germany.

The single-stranded DNA genome of the porcine circovirus (PCV) is a covalently closed circle (Tischer et al., Nature 295, 64-66, 1982). We have used this DNA template to study in vitro initiation of DNA replication. A DNA polymerase α holoenzyme form, that has been isolated by the criterion of efficient priming on single-stranded M13 DNA, contains polypeptides of Mr 198,000, 97,000, 63,000, 57,000, 52,000 and 47,000, respectively. This holoenzyme form is able to specifically prime the single-stranded PCV DNA at one preferred site in the genome. The region of in vitro initiation contains potential to form secondary structures and sequences related to the binding site of nuclear factor I. The DNA polymerase α holoenzyme on the other hand contains a polypeptide of 47,000 dalton corresponding to the same Mr as nuclear factor I. This in vitro DNA replication-initiation system can be used in analogy to small bacteriophages DNA's in Escherichia coli to identify and to purify proteins and enzymes involved in mammalian DNA replication.

184 REPLICATION BLOCKS INDUCED BY AFLATOXIN B_1: DNA SEQUENCE AND CONFORMATION SPECIFICITY AND EFFECT OF Mn^{2+}, J. Steven Jacobsen, Lawrence M. Refolo, Kumar Sambamurti and M. Zafri Humayun, Department of Microbiology, New Jersey Medical School, 100 Bergen Street, Newark, New Jersey 07103.

The activated form of the human carcinogen aflatoxinB_1 (AFB_1) reacts with DNA predominantly at guanine residues to form (7-guanyl)-AFB_1. We have previously suggested that AFB_1 preferred double-stranded (ds) DNA over single-stranded (ss) DNA and that within ds DNA, it reacted with G residues in a highly sequence-specific manner. In this report, we show that in an in vitro DNA replication system utilizing Escherichia coli DNA polymerase I and M13mp8 ss DNA template, significant AFB_1-induced replication blocks occur predominantly at basepaired G residues, as revealed by several types of experiments. Thus, AFB_1 blocks are predominantly in the stem regions of putative secondary structures. The existence of the secondary structures is independently confirmed by using chemical probes (2-chloroacetaldehyde and chloroperbenzoic acid) which preferentially attack non-basepaired residues to create replication blocks. Furthermore, acid-depurinated DNA and DNA modified by dimethyl sulfate give rise to distinctly different patterns of replication blocks. In a more direct strategy, creation of partial duplexes by annealing synthetic complementary oligonucleotides results in specifically enhanced, sequence specific AFB_1 blocks within the duplex regions. Remarkably, unlike other bulky adducts, the substitution of Mn^{2+} for Mg^{2+} has not significant effect on the AFB_1 blocks under a variety of experimental conditions used, suggesting that AFB_1 adduct sites do not permit significant incorporation and are not bypassable. In addition to the implications of these findings to the mechanisms of AFB_1 genotoxicity, these data show that a complete set of chemical probes for experimental verification of the formation of intrastrand secondary structures at the sequence level is available.

185 INTERACTIONS OF THE REPLICATION CONTROL ELEMENTS OF p15A AND pUC8. Syd Johnson and Robert E. Bird, Genex Corporation, Gaithersburg, MD. 20877

Replication of ColE1-type plasmids is negatively regulated by an interaction of RNAI with a complementary region of the nascent pre-primer transcript. This interaction is incompatibility specific. The copy number of pUC8 is reduced four fold when the rom gene from the compatible plasmid p15A is present, in either a cis or trans configuration. This indicates that the activity of the p15A Rom protein is not incompatibility specific.

When a rom$^-$ derivative of p15A is present with pUC8, its copy number is increased by a factor of two. This suggests that non-productive interactions between heterologous RNAI-preprimer pairs compete with binding of the RNAI molecules to homologous preprimer transcripts. This affect is alleviated when the p15A rom gene is present on either plasmid. These results and their implications on models for the mode of Rom action will be presented.

186 **APHIDICOLIN RESISTANT EXTENSION OF RNA PRIMERS BY CA. 20–30 dNMPS OCCURS DURING SV40 DNA REPLICATION IN ISOLATED NUCLEI** Gail Dinter Gottlieb, Tamar Natanel & Gabriel Kaufmann. Dept. of Biochemistry, Tel Aviv University, Israel 69978.
DNA polymerase alpha, considered solely responsible for replicative SV40 and nuclear DNA synthesis, is competitively inhibited by aphidicolin. Yet, in vivo, the drug irreversibly impairs replicating SV40 chromosomes (RSC). To explain this apparent discrepancy, we propose that occurrence of aphidicolin resistant replicative reactions, uncoupled from the sensitive, underlies the permanent inactivation of RSC. We have detected aphidicolin resistant incorporations of iRNA and DNA precursors into RSC in isolated nuclei. The label was distributed between long nascent DNA chains and iRNA containing Okazaki piece precursors of less than 40 nucleotides. Strikingly, Okazaki pieces in the 40 to 200–300 nucleotide range were absent. We interpret the data as follows. There exists an aphidicolin resistant DNA polymerase whose role is to extend the iRNA by ca. 20–30 dNMPs (**iRNA extension**). This activity corresponds perhaps to the aphidicolin resistant DNA polymerase found associated with DNA primase. Accordingly, the role of DNA polymerase alpha is to elongate the extended iRNA, both on leading and lagging templates, while aphidicolin inhibits the transition from iRNA extension to further elongation.

187 REDUCED DNA TOPOISOMERASE I IN ATAXIA-TELANGIECTASIA CELLS, Martin F. Lavin, Rahmah Mohamed and Sharad Kumar, University of Queensland, Brisbane 4067, Australia

A number of characteristics observed in the human genetic disorder ataxia-telangiectasia (A-T) include radioresistant DNA synthesis, chromosomal instability and abnormalities in gene expression. It has been proposed that these may arise due to alterations in chromatin structure or to changes in proteins that recognize chromatin structure. It has not been possible to detect changes in the major histone and non-histone proteins or alteration to gross chromatin structure in A-T cells. DNA topoisomerases are enzymes defined by their *in vitro* reactions that cause the interconversion of various topological forms of DNA. A defect in ability to alter the superhelicity of DNA or modulate chromatin structure with defective topoisomerase I could explain some of the abnormalities observed in A-T cells.

Indeed abnormal distribution of DNA topoisomerase I has been described in placental cells in Fanconi's anaemia, a genetic disease also characterized by chromosomal instability (Wunder *et al.*, 1981). In this study we have examined Topoisomerase I activity in control and A-T lymphoblastoid cells by measuring relaxation of supercoiled DNA. Six A-T cell lines were demonstrated to have a 5-10 fold reduction in topoisomerase I activity compared to control cells. This data with increasing protein concentration was also supported by time course experiments. The defect in topoisomerase I represents the first description of a defective protein in A-T cells.

Wunder, E., Borghardt, V., Lang, B. and Hamilton, L. (1981) Human Genet. *58*, 149-155.

188 CHARACTERIZATION OF THE HUMAN MITOCHONDRIAL DNA POLYMERASE. Patrick P. Lestienne and David A. Clayton. Stanford University School of Medicine. Stanford CA 94305

The DNA polymerase from KB cell mitochondria was purified 360 times with a yield of 47 %. The native enzyme is formed by the homologous association of four identical subunits of 54,000 d. Addition of micromolar range concentrations of the Rifampicin derivate AF 013 inhibits the formation of the initiation complex between the DNA polymerase, the TTP and the poly (rA) dT_{12-18}. The addition of dATP, dGTP and dCTP competitively inhibit the dTMP incorporation using the poly(rA) dT_{12-18} and are required for the dTMP incorporation with the primed M13 MP8 single stranded DNA. Antibodies prepared against the 54,000 d peptide inhibit the dTMP incorporation using either the poly (rA) dT_{12-18}, or a replication assay directed by a plasmid DNA containing the origin of replication of the heavy strand of the human mitochondrial DNA.

189 MUTANTS OF YEAST WITH ALTERED TELOMERE LENGTH, Victoria Lundblad and Jack W. Szostak
Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Our laboratory has characterized two DNA processing activities in yeast involving telomeric sequences. The first reaction, termed telomere elongation, occurs at the termini of linear molecules which contain certain CA-rich repeated sequences and results in the addition of $(C_{1-3}A)$ repeats. In the second reaction, inverted repeats composed of CA-rich repeated sequences residing on a circular plasmid are resolved to yield linear plasmids with two functional telomeres. We have developed an assay for mutants of yeast which incorporates both the elongation and resolution reactions. This is a plasmid assay which measures the frequency with which a circular plasmid containing the URA3 gene between inverted repeats of telomeric DNA is resolved into a linear molecule with termini which have been elongated and transformed into telomeres. Using this assay, we have isolated 25 mutants of yeast. Two of these mutants are altered for telomere length: one mutant is decreased by 200 bp and one is increased by 650 bp, relative to wild type. One of these two mutants displays other phenotypes consistent with a defect in telomere replication.

190 CHARACTERISTICS OF A DNA SEQUENCE RECOGNITION PROTEIN ASSOCIATED WITH HeLa DNA POLYMERASE ALPHA, L.H. Malkas, J.K. Vishwanatha and E.F. Baril, Worcester Foundation for Experimantal Biology, Sherwsbury, MA 01603

A multiprotein form of DNA polymerase α is readily isolated from HeLa cells. This particular form of polymerase is designated DNA polymerase α_2 by this laboratory. Experiments performed to map DNA replication initiation sites by DNA polymerase α_2 on the Simian virus 40 (SV40) replication origin demonstrated two strongly preferred sites. However, if a form of DNA polymerase α (from either HeLa or monkey cells) containing only a primase activity was used numerous initiation sites were observed throughout the DNA template. The two preferred initiation sites of the DNA polymerase α_2 on the SV40 replication origin occurred in regions rich in adenosine (A) and thymidine (T) residues. These results suggest a factor(s) or protein (5) is present in the multiprotein form of the HeLa DNA polymerase α which helps position the replication complex at specific sites on the SV40 origin.

To isolate the protein from the multi-protein form of HeLa DNA polymerase α, advantage of the apparent affinity of the protein for DNA sequences rich in A and T residues was taken. Preliminary evidence obtained using poly (dA)-cellulose, as measured by the ability to bind ^{3}H-poly (dA) and ^{3}H-poly(dT) in a nitrocellulose filter binding assay, at 0.35M - 0.4M KCL. Further properties of this protein and its sequence specific binding to DNA will be discussed. Supported by NIH Grant CA15187.

191 AN UNUSUAL SEQUENCE ELEMENT FOUND AT A TERMINATION SITE FOR DNA REPLICATION, Haim Manor, Nava Baran, and Aviva Lapidot, Technion, Haifa, Israel.

In the LPT line of polyoma (Py) transformed rat cells several Py genomes are tandemly integrated into a single chromosomal site. Multiple rounds of bidirectional replication of the integrated Py genes and the flanking cellular sequences can be induced in these cells by treatment with carcinogens. We have partially mapped the "onion skin" intermediates that are thus generated. Our data indicate that the replication proceeding in one direction ends within a specific cellular DNA segment. This segment includes a cruciform structure, whose hairpins consist of five dA:dT base pairs and one dC:dC base pair, followed by $d(GA)_{27}$-$d(CT)_{27}$. Similar sequence elements may serve as signals for termination of "onion skin" replication, or of normal replication, at other cellular sites.

192 CHANGES IN DNA POLYMERASE ALPHA AND DELTA ACTIVITIES WITH CELL CYCLE, R.L.Marraccino, A.F.Wahl, L.W.Harwell, P.C.Keng, E.M.Lord, and R.A.Bambara, University of Rochester School of Medicine and Dentistry, N.Y. 14642.

Chinese hamster ovary (CHO) cells were used to examine the regulation of α and δ DNA polymerases during cellular proliferation and cell cycle. Exponential CHO cells were synchronized by centrifugal elutriation to obtain cell populations enriched for G_1,S and G_2 phases of cell cycle. Cytosolic extracts of synchronized cells were assayed for DNA polymerase activity. These results were compared to the activities found in extracts from confluent and exponential, asynchronous populations. The total activity of DNA polymerases, per mg protein, was approximately 2-fold greater in proliferating (log phase) cells than non-proliferating (confluent) cells. In synchronous cells, total DNA polymerase activity increases gradually throughout the cell cycle rather than peaking sharply in S phase. In these crude extracts from either G_1, S, or G_2 cells, inhibitors of DNA polymerases were used to distinguish the α from the δ DNA polymerase activity. The results of these experiments show that the amount of α polymerase activity increases, and the amount of δ activity decreases in cellular extracts from S phase cells. This difference in the relative activities of α and δ DNA polymerases suggests that a mechanism of entry into S phase may involve a coordinate regulation of a specific species of DNA polymerase, required for a specialized function. Supported by NIH grants: GM2441,CA11198, AND CA28322, and ACS grant FRA220.

193 MONKEY DNA ORIGIN-ENRICHED SEQUENCES CONTAIN EXTENSIVE NUCLEOTIDE HOMOLOGIES, Robert G. Martin, Bachoti S. Rao and Maria Zannis-Hadjopoulos, National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases, Bethesda, Md. 20892, and McGill Cancer Center, McGill University, Montreal, Canada H3G1Y6.

Monkey DNA origin-enriched sequences (*ors*) were obtained by physical-chemical means and cloned (Kaufmann et al., Molec. Cell. Biol. 5, 721, 1985; Zannis-Hadjopoulos et al., Molec. Cell. Biol. 5, 1621, 1985). One of the sequences, *ors7*, was shown to be derived from the bona fide origin of mitochondrial DNA. An additional eleven *ors* fragments have been sequenced and analyzed. *Ors6* (196 bp in length), selected for analysis because of its homology to *ors2* (778 bp in length), differs from the central portion of *ors2* by only two nucleotides. The remaining nine *ors* fragments were independently isolated. All contain extended AT-rich regions. Eight of the ten (nine of eleven if *ors6* is included) share extensive homology to a 21 bp consensus sequence.

194 RESOLUTION OF RECOMBINANT PLASMIDS CONTAINING THE TERMINAL SEQUENCES OF VACCINIA VIRUS INTO REPLICATING LINEAR MINICHROMOSOMES.
Mike Merchlinsky and Bernard Moss, NIH, LVD, Bethesda, MD 20892
Vaccinia virus is a large double-stranded DNA virus with covalently continuous hairpin ends. During the early stages of viral DNA synthesis replicative intermediates can be detected which contain the genomic termini as inverted repeats in head to head concatemeric junction fragments. These junction fragments were isolated from infected cells, cloned into plasmid vectors and the resultant chimeric plasmid was stably propagated in E. coli. The recombinant plasmids were also used to transfect vaccinia virus infected CV-1 cells where the presence of newly replicated DNA was detected by Southern blotting for plasmid DNA resistant to digestion by DpnI. Replication was observed only with constructs containing at least 252 base pairs of the intact inverted repeat. In each case the supercoiled plasmids were converted into linear molecules in which the plasmid vector was flanked by vaccinia virus DNA with covalently continuous hairpin telomeres. An enzyme partially purified from vaccinia virions is also able to convert the supercoiled plasmids into linear molecules in which some ends have been cross-linked. The dependence of this activity on superhelical density suggests the enzyme nicks the cruciform structure formed when the inverted repeat is extruded from the recombinant plasmid.

195 IMPORTANCE OF TRANSCRIPTS IN AND AROUND oriC FOR ORIGIN FUNCTION IN E. COLI, Walter Messer, Claudia Kücherer, Helmut Kunze and Heinz Lother, Max-Planck-Institut für molekulare Genetik, Ihnestrasse 63/73, D-1000 Berlin 33, Germany

Directly adjacent to the minimal replication origin, oriC, of E. coli is a coding region for a 16 kd protein. The transcript of this gene is of importance for correctly regulated origin function. Deletions affecting the 16 kd promoter have negative effects on copy number and segregational stability of minichromosomes.

The 16 kd transcript is negatively regulated by the dnaA protein. More than 60 % of the transcripts traverse the minimal origin. Part of the transcripts are processed within oriC by RNaseH. This suggests that the 16 kd transcript is the natural primer for DNA replication.

Within oriC are two promoters, Pori-1 and Pori-r, which transcribe outward. Transcription from Pori-r results in a 110 nucleotide transcript which is complementary to part of the 16 kd transcript and may be important for its processing.

The observations that initiation in minichromosomes lacking the 16 kd transcript is possible, albeit at a reduced rate, suggests that alternative priming systems must exist. Thus could be the transcript from Pori-1 or primers synthesized by the dnaG product.

196 STUDIES ON THE PROCESSIVITY AND 3'→5' EXONUCLEASE ACTIVITIES OF THE DNA POLYMERASE ENCODED BY HERPES SIMPLEX VIRUS 1, Michael E. O'Donnell, Per Elias and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

We are attempting to establish an in vitro system for the study of the mechanism of HSV-1 DNA replication. As an initial step we have undertaken the purification and characterization of some of the proteins essential for DNA synthesis in vivo. The DNA polymerase encoded by HSV-1 appears to be a monomeric protein (150 kDa) in highly purified preparations. In this form the polymerase is processive and will efficiently replicate a uniquely primed circular ssDNA without dissociation from its template. After completing the synthesis, the polymerase remains bound to its original template. The rate of DNA synthesis is, under these conditions, stimulated about 20-fold by E. coli single-strand binding protein. A 3'→5' exonuclease activity appears to be a part of the polymerase and further evidence for its role as a proofreader will be presented. Another HSV-1 protein essential for viral replication, ICP8 protein, has been highly purified and some of the properties of ICP8 in these assay systems will be discussed.

197 MECHANISM OF SELF-PRIMED REPLICATION OF ADENO-ASSOCIATED VIRUS DNA: TENTATIVE IDENTIFICATION OF AN ORIGIN-SPECIFIC PROCESSING FACTOR, Seigo Ohi, Bahige M. Baroudy, and James A. Rose, Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

The AAV type 2 genome (4.7 kb) contains palindromic inverted terminal repeats of 145 nucleotides which can form hairpin structures, and viral DNA synthesis proceeds via a self-priming mechanism with the 3' terminal hairpin serving as the primer. This mechanism requires origin-specific, single-strand cleavages in hairpin termini to yield unit-length duplex templates from which progeny strands can be generated by displacement synthesis. To identify the predicted factor(s) responsible for the site-specific processing reaction, we analyzed (i) DNA products synthesized in cell-free extracts with DNA templates obtained from purified AAV2 virions and (ii) the molecular construction of template molecules. When AAV DNA was released in a 4M guanidinium chloride-sucrose gradient, a DNA-protein template component (P2) was obtained which directed synthesis of both unit-length hairpins and completely denaturable nonhairpin duplexes. Upon treatment with proteinase K, a fraction of the P2 molecules released terminal palindromic fragments, suggesting that these fragments may be linked by a protein at the putative processing site. SDS-PAGE analysis of the P2 molecules revealed two associated proteins, M_r=25,000 and 22,000. These proteins appear to represent new AAV virion polypeptides, and correspond in size to previously observed nonstructural components of AAV and autonomous parvoviruses. We propose that one or both of these proteins may play a role in origin-specific processing during replication of AAV DNA.

198 IDENTIFICATION OF MAMMALIAN CELLS TEMPERATURE-SENSITIVE FOR CELLULAR AND VIRAL DNA SYNTHESIS, Harvey L. Ozer, James J. Dermody, Brian Wojcik, and Hong Du, Hunter College, City University of New York, New York, NY 10021

The papovaviruses (SV40 and polyoma) and human adenoviruses (Ad2) infect cells in culture from several species. We have recently demonstrated that both polyoma (Py) and Ad2 replicate their DNA genomes efficiently in established Chinese hamster (CHO, V79) as well as mouse (3T3) cell lines. We have isolated over 200 mutants which are temperature-sensitive (ts) for cell growth at 39°C in these cell lines using a variety of selection and non-selection techniques. Approximately 10% were tentatively defined as consistent with a ts defect in DNA synthesis ($tsDNA^-$) based on preferential inhibition of thymidine incorporation upon shift to the restrictive temperature. We have examined the efficiency of replication of Py and Ad2 DNA in 14 such ts DNA^- cell mutants at 33°C and 39°C in an effort to facilitate further definition of the biochemical bases for the mutant phenotypes and identify those mutants likely to be defective in macromolecules involved in DNA synthesis. All mutants support viral DNA synthesis at 33°C. Five mutants (3T3 mutants ts2 and ts20, CHO mutants JB3-O, JB7-K, JB8-D) restrict Py but not Ad2. This differential effect would appear to eliminate a defect in deoxynucleotide pools as responsible. One CHO mutant (JB3-B) restricts both Py and Ad2. The other 8 restrict neither, suggesting that their ts DNA^- phenotypes are due to secondary effects as cell cycle progression. Further studies with three of the mutants (ts2, ts20, and JB3-B) suggest that defects principally affecting various aspects of strand elongation are involved.

I99 MOLECULAR ANALYSIS OF THE DNA POLYMERASE-DNA PRIMASE COMPLEX FROM YEAST, Mark H. Pausch, Byran C. Peterson, and Lawrence B. Dumas, Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, IL 60201

Recent observations of phylogenetically diverse eukaryotic organisms suggest that DNA polymerase and DNA primase are subunits in a functional complex. We have partially purified this enzyme complex from S. cerevisiae, an organism particularly well suited to genetic analysis and molecular cloning technology. This enzyme fraction was used to develop a collection of monoclonal antibodies selected for their ability to recognize DNA polymerase-DNA primase in solution. Two classes of these monoclonal antibodies, each recognizing a different size polypeptide chain on Western blots, immunoprecipitate equivalent amounts of DNA polymerase and DNA primase activity. This supports the notion that these enzymes exist in a functional complex. Another class, represented by the 21A6 monoclonal antibody, also immunoprecipitates both enzyme activities. However, 21A6 specifically inhibits DNA primase in solution demonstrating that the antigenic polypeptide is necessary for its catalytic activity. The 21A6 antibody has been used to probe a λgtll-yeast genomic DNA library. The yeast DNA sequences identified are being characterized.

I100 STUDIES IN VIVO AND IN VITRO ON THE ORIGIN OF REPLICATION OF TETRAHYMENA THERMOPHILA rDNA, Ronald E. Pearlman, Anthony A. Amin and Kim Riekki, Dept. of Biology, York University, Toronto, Ontario, Canada, M3J 1P3.

The origin of bidirectional replication in the extrachromosomal palindromic rDNA of the ciliated protozoan T. thermophila is 600 ± 300 bp from the center of the molecule in the 5' non transcribed spacer (NTS), likely in a region of chromatin hypersensitive to digestion by a number of nucleases. Using in vitro deletion analysis, we have identified an 82 bp sequence in restriction fragments encompassing the in vivo replication origin, which allows autonomous replication of plasmids in yeast; i.e. an ARS. Within this 82 bp DNA segment is a 39 bp sequence which appears to be a core sequence required for ARS function in yeast. The 39 bp sequence contains the 11 bp A+T rich consensus sequence identified in ARS elements from yeast chromosomal DNA. Although this T. thermophila ARS element is near the replication origin of the palindrome, it is unlikely that the ARS and the in vivo replication origin are coincident. In order to identify functional sequences at the rDNA replication origin, we have initiated a series of in vitro experiments. We have identified in nuclear extracts of T. thermophila protein(s) which bind to a restriction fragment from the region of the origin of rDNA replication. Sequence specificity and other characteristics of the binding reaction will be presented.

I101 FLP-mediated recombination between inverted repeats causes 2um circle amplification Ann Reynolds[1], Andrew Murray[2], Jack Szostak[2] [1] Biology Dept., Mount Holyoke College South Hadley, Ma. [2] Dept. Molecular Biology, Mass. General Hospital, Boston, Ma.

The 2um circle of S. cerevisiae is normally present in about 50 copies per cell, each of which replicates once per cell cycle. If the copy number drops, amplification can occur to restore the original high copy number. We have tested a model (proposed by Bruce Futcher) in which FLP-mediated recombination between inverted repeat sequences (IRS) of 2um is responsible for this amplification. Recombination between a replicated and an unreplicated IRS creates a structure in which the two replication forks now run in the same direction. If replication is normally terminated by the collision of forks travelling in opposite directions, this recombination event leads to multiple replication of the 2um circle.

To test this model, we have used plasmids which contain a direct repeat of the IRS integrated into cir° strains. When crossed to cir° strains containing an integrated copy of the FLP gene, the sequences between the IRSs are excised as a circle as a result of FLP action. By analysing the DNA from mass matings, we can show that an excised circle containing an inverted pair of IRSs and ori amplifies; one which has only a single IRS and ori does not.

Since overreplication does not occur at normal copy number we would expect FLP activity to be low when the copy number is high. We have examined the regulation of FLP activity by constructing a lacZ fusion to the FLP gene. The fusion was integrated into a cir° strain and the β-galactosidase activity was measured in this strain and in isogenic cir° and cir⁺ diploids. The activity in the cir° haploid and diploid were identical but that in the cir⁺ diploid was reduced about 13-fold showing that FLP expression is sensitive to 2um copy number.

I102 IN VITRO REPLICATION OF THE ORIGIN OF BACILLUS SUBTILIS CHROMOSOME. N. Sarkar[1], M. Sasvari-Szekely[1], G. Banfalvi[1], and K.F. Bott[2]. [1]Boston Biomedical Research Institute and Harvard Medical School; [2]University of North Carolina.

Using a new methodology which involves the use of 5-mercuri-dCTP as substrate for the isolation of nascent DNA chains by affinity chromatography on thiol-agarose, DNA replication in Bacillus subtilis cells permeabilized with toluene was investigated. Our earlier studies have characterized the partial sequence of RNA primers for the Okazaki fragments involved in B. subtilis chromosome replication and the processing of RNA primers. We have extended this approach of pulse-labeling with Hg-nucleotides to study the nearly replicative intermediates during the initiation of origin of chromosomal replication of B. subtilis. Synchronous initiation of DNA replication was achieved by using outgrowing B. subtilis spores and germinated cells were permeabilized with toluene for in vitro DNA synthesis with mercurated nucleotide substrates. Newly synthesized DNA was isolated by affinity chromatography on thiol agarose. The purified labeled DNA was characterized by two methods: (A) By hybridization with plasmid probes containing various parts of "ori," it was observed that replication of a sequence containing recF and gyrB occurred earlier than the sequence containing gyrA and rRNA operon rrnO. (B) The isolated nascent DNA contains mostly RNA primer at their 5-end as judged by the alkali and RNAse sensitivity of the 5'-^{32}P-end labeled nascent DNA. In conclusion, we have a replication system which primarily synthesized "ori" region and could be used to study the role of various physiological parameters on chromosomal initiaiton.

I103 GENETIC STUDIES OF CDC7 PROTEIN IN YEAST DNA REPLICATION

Robert A. Sclafani and Lee Niswander, Dept. of BBGN, UCHSC, Denver, CO 80262

The CDC7 protein of S. cerivisiae is required for the initiation of DNA replication because cdc7ts mutants under restrictive conditions are unable to enter the S phase of the cell cycle. In an effort to determine the biochemical function of the CDC7 protein we have cloned and sequenced the CDC7 gene and several cdc7ts alleles. The cloned sequences were used to construct an insertion mutation (cdc7::URA3) and to measure the level of CDC7 protein in mitotic (vegatative cells) and post-meiotic germinating spores. The following results were obtained: 1) The cell cycle phenotype of the cdc7::URA3 mutant is the same as for the cdc7ts mutants under restrictive conditions. Therefore, this phenotype is the result of a loss of a function. 2) By performing pedigree analysis on a strain carrying the cdc7::URA3 insertion on the chromosome and CDC7$^-$ on a single copy CDC7$^+$ CEN3$^+$ plasmid, we have observed the cells which lose the plasmid can still undergo 8-10 divisions. Since the steady-state mRNA level is about 1-0.5 per cell and the CDC7 protein functions each and every cell cycle, the CDC7 protein must be present in vast excess (200X) of that required for a single cell division. 3) Following sporulation of a CDC7$^-$/cdc7::URA3 strain, spores which receive the insertion mutation germinate but arrest in the first mitotic cell division with a large bud. The CDC7 protein present in the original diploid cell either must be preferentially proteolysed or excluded from these spores. Similar experiments with the CDC8 protein (dTMP kinase) indicate that not all CDC proteins behave in this manner. 4) Computer analysis has shown that the CDC7 protein has significant homology with the CDC28 protein which is a ser-thr protein kinase, and with the src family of protein kinases. Since the CDC7 protein is present in vast excess in mitotic cells, it is unlikely that the CDC7 gene is cell-cycle regulated. Therefore, we propose that the CDC7 protein acts by phosphorylating other protein(s) which are produced at the G1/S boundary.

I104 DUAL FUNCTION OF A DNA REGION IN COPY NUMBER CONTROL IN THE PLASMID PROPHAGE P1,

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The plasmid prophages P1 and P7 are both in the same incompatibility group and therefore share some components of replication regulation. Three P1 copy mutants maintained at about 5-8 copies per cell all have single base changes within the open reading frame encoding the Rep protein, which is required for replication of mini-P1. Using a multicopy vector containing just the P1 Rep open reading frame, from which all vestiges of the Inc repeats have been deleted, we find that all three P1cop mutants are recessive for high copy number. However, in dominance tests with P7cop+, P1copE38 is recessive, and P1copN3 and N22 are cis-specific. The P1 copy mutants show no change in type-specific ("homoplasmid") incompatibility, but show reduced group-specific ("heteroplasmid") incompatibility. Thus, the two different phenotypes of P1cop mutants, recessive high copy number and reduced incompatibility, show different specificities: the former is type-specific for copN22 and N3 and the latter is group-specific for all three mutants. This suggests that each single cop mutation may alter more than one genetic function and thus that there is another function encoded in the same DNA region as the open reading frame for Rep.

I105 INITIATION OF CHROMOSOMAL REPLICATION IS SUBJECT TO STRINGENT CONTROL IN B. SUBTILIS

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In B. subtilis, we have recently shown that the synthesis of a specific RNA-DNA copolymer containing ribosomal RNA and DNA sequences is correlated with the transcriptional step required for initiation (1, 2). In addition a new ribosomal RNA operon, rrnO, has been identified in the region of the origin (3). These results suggested to us that chromosomal initiation which is correlated to the synthesis of a stable RNA was also likely to be subject to stringent control.

Using an initiation mutant of B. subtilis, dna37, we were able to study the effect of the stringent system upon the transcriptional step required for initiation, independently of any requirement for protein synthesis. The results obtained demonstrated that, under conditions which mimic aminoacid starvation, initiation of chromosomal replication was blocked in a relA$^+$ but not in the isogenic relA$^-$ strain. In that case, initiation was shown to occur in the same origin region as defined by the earliest fragments to be replicated. Preliminary experiments indicate that this effect is directly due to the action of ppGpp. We propose that control of initiation of replication, at least in B. subtilis, is part of a global metabolic coordination system.

1. G. Henckes, F. Vannier, A. Buu & S.J. Séror-Laurent. J. Bacteriol., 1982, 144, 79-91.
2. S.J. Séror-Laurent & G. Henckes. Proc. Natl. Acad. Sci. U.S.A., 1985, 82, 3586-3590.
3. G. Henckes, F. Vannier, M. Seiki, N. Ogasawara, H. Yoshikawa & S.J. Séror-Laurent. Nature, 1982, 299, 268-271.

I106 STABILITY DETERMINANTS OF ColE1 AND RELATED PLASMIDS

David Sherratt, Gill Stewart, Colin Stirling and David Summers
Institute of Genetics, Glasgow University, Glasgow, Scotland.

Plasmid ColE1 has a determinant, cer, that is required for its stable maintenance. It acts by maintaining the pool of ColE1 molecules in a cell as monomers; multimers of ColE1-related plasmids are not stably maintained. In cer$^+$ ColE1 derivatives, any multimers that arise through generalized recombination or replication, are efficiently converted to monomers by site-specific recombination between cer sites. A function that acts at cer to mediate recombination is chromosomally encoded, mapping at about 90 min on the E. coli chromosome. No other characterized host recombination gene products are required. Mutants defective in this function do not stably maintain ColE1-related plasmids (eg ColE1, ColK, pMB1, CloDF13) even when they are cer$^+$. By sequencing of hybrid recombination sites generated by recombination between the ColE1 and ColK stability determinants, we have localised the position of the site-specific recombination event.

I107 DETERMINATION OF THE EFFICIENCY AND SPECIFICITY OF EDITING OF BASE MISPAIRS BY THE 3'→ 5'- EXONUCLEASE ACTIVITY OF A DNA POLYMERASE.

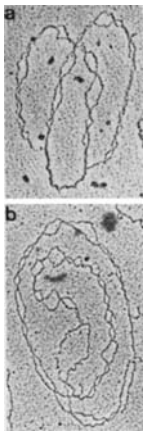
Navin K. Sinha, Waksman Institute of Microbiology, Rutgers University, Piscataway, N.J. 08854.

A series of oligodeoxynucleotide primers was synthesized chemically. These primers, when hybridized to ØX174 single-stranded DNA circles containing an amber mutation, result in a mispaired nucleotide at the 3'-termini of the primers, thus, mimicking the effect of 100% error in the deoxynucleotide insertion step by a DNA polymerase. DNA synthesis on these primer-templates without the removal of the mispaired terminal nucleotide results in the formation of heteroduplex molecules which give viable revertants upon transfection into an amber-restrictive host. This method permits the measurement of editing by a DNA polymerase of a mismatch over a 10^6-fold range for all 8 mispairs capable of yielding a viable revertant at an amber codon. Studies with mismatched primers at the ØX174 am16 and am3 codons suggest that the order of editing of mispairs by phage T4 DNA polymerase is G.T< A.G< T.C< T.T= G.A= G.G≤ A.C≤ A.A. The efficiency of editing depends upon the mispair as well as the neighboring DNA sequence. The editing nuclease activity, depending upon the mispair, contributes from 6×10^3 to greater than 10^5-fold to the accuracy of T4 DNA polymerase. Variations in the concentration of the next correct deoxynucleotide substrate (which might affect the time available for editing), change the efficiency of editing for most, but not all, mispairs. Addition of the polymerase accessory proteins (32, 44/62 and 45) increases the efficiency of editing. DNA polymerase I of E. coli has a very different specificity of editing for these same mismatches, suggesting that the specificity is determined by the nature of the active site of the enzyme rather than by the inherent stability of a mispair.

1108 INITIATION AT E. COLI ORIC AND DAM METHYLATION OF GATC SITES, Douglas Smith, Della Garland, Karen Geller and Piotr Jonczyk, Univ Cal, San Diego, La Jolla, CA 92093

The 245 bp minimal E. coli-type origin of replication, oriC, contains 11 GATC sites. These sites are methylated by the Dam methylase, product of the dam gene. Recent evidence shows that methylation of one or more of these sites is important, if not essential, for the initiation process: residual methylation of specific GATC sites persists in dam mutants, transformation of these mutants by oriC plasmids is reduced 5 orders of magnitude, and oriC DNA isolated from these mutants functions poorly in oriC-specific in vitro initiation systems. We have isolated insertion and deletion mutants of the dam gene by in vitro construction prior to transformation and recombination into the chromosome. These mutants are viable, but grow very poorly and die easily. They are unable to harbor ColE1-type plasmids, but can maintain F and be complemented by F-prime plasmids harboring dam. During growth, they rapidly accumulate suppressor mutations which alleviate the poor growth phenotype. The nucleotide sequence of the dam gene shows the presence of a putative DnaA binding site proximal to the N-terminal end. Fusion studies of this region to galK expression vectors indicate that DnaA protein acts negatively in regulation of dam gene expression. Similar studies show that the major promoter activity for dam is about 1.5 kb upstream. A noncoding region of much potential structure and an open reading frame potentially coding for a 46 kD protein are present between dam and this promoter. Thus, the dam gene appears to be one of a two gene operon, under negative control by DnaA protein. These results together indicate an important role of GATC methylation in oriC function different from its role in mismatch repair.

1109 THE STRUCTURE OF REPLICATING SV40 MINICHROMOSOMES, J.M.Sogo, H.Stahl*, Th.Koller, and R.Knippers*, Fed.Inst.Techn., 8093 Zürich, Switzerland, *University Konstanz, FRG



SV40 replicating minichromosomes were studied by DNA crosslinking with Trimethylpsoralen.The spreading of the crosslinked DNA molecules under denaturing conditions revealed single-stranded bubbles at positions where nucleosomes were sitting.This technique has allowed us to analyze the detailed structure of replicating intermediates at the chromatin level with unusual clarity (a), e.g.:

1) In the unreplicated parental strand nucleosomes are present up to the replication fork,possibly penetrating into the nucleosomal DNA before the histone octamer is removed.
2) Nucleosomes reassociate on the newly synthesized strands at a distance from the branch point of 225 ± 145 bases on the leading strand and of 285 ± 120 bases on the lagging strand.
3) The segregation of the old histone octamers is dispersive,random, and in clusters. Finally,
4) A novel structure of interlocked dimers with a single-stranded bridge is found (b). This type of molecule probably represents the last intermediate in the SV40 replication before segregation of the daughter strand takes place.

1110 ADENOVIRUS MAKES DETERGENT SOLUBLE DNA LATE IN INFECTION, Phyllis R. Strauss* and John W. Bodnar, Northeastern University, Boston,MA 02115

Detergent soluble(DS) DNA is that fraction of DNA which is released into the supernate upon lysis of eukaryotic cells with nonionic detergents. It is non-mitochondrial in origin. It labels efficiently with (^{3}H)deoxyribonucleosides and the labeling is prevented by inhibitors of polymerase alpha and ribonucleotide reductase. Furthermore, release of (^{3}H)thymidine-labeled DNA into the DS fraction requires the action of topoisomerase II. Recently we examined the DS fraction from uninfected HeLa cells and from HeLa cells late in infection with adenovirus type 2 for the presence of rapidly labeled DS DNA. Both uninfected cells and cells late in infection incorporated (^{3}H)thymidine into DNA which was recovered from the DS fraction. The molecular weight distribution of (^{3}H)thymidine-labeled DS DNA from both sources was the same, namely, a sharply defined ladder of size classes, the smallest of which was 190 base pairs in length. Incorporation of (^{3}H)thymidine was inhibited in a complex fashion by novobiocin, which inhibits topoisomerase II. Therefore, adenovirus replication may involve a previously overlooked intermediate step in common with its host cell.
*Supported by funds from Northeastern University and ONR contract 14-82-K-0283.

I111 CELL FREE DNA REPLICATION DEPENDENT ON ENDOGENOUS TEMPLATE IN YEAST, Randy Strich and John F. Scott, Department of Microbiology, University of Illinois, Urbana, IL, 61801.

Yeast Acentric Ring plasmid 1 (YARp1) is a 1453 base pair synthetic plasmid derived from yeast chromosome IV. This plasmid contains a functional *TRP1* gene and Autonomous Replicating Sequence 1 (*ARS1*) which directs the replication of the plasmid. Previous studies have been described using *ARS* containing plasmid DNA purified from *E. coli* as template for replication in cell free extracts. We are using YARp1 in a partially purified chromatin form to serve as template for *in vitro* replication reactions.

Exponentially growing cultures containing YARp1 were harvested and subjected to bead beat lysis and high speed centrifugation to remove most of the high molecular weight cell components. The resulting fraction alone was capable of incorporating radioactively labeled deoxynucleotides upon addition of normal DNA synthesis substrates and an ATP regenerating system. The replication products of these reactions were of low molecular weight, about 150 base pair in length, and not covalently attached to template DNA. Analysis of replication reactions revealed that YARp1-hybridizable DNA exhibited slower mobility in an agarose gel in a time dependent fashion.

I112 SV40 LARGE T ANTIGEN STRUCTURE AND REPLICATIVE FUNCTION, L.C. Tack[1], G.N. Proctor[1], J.H. Wright[1] and E. Gurney[2], [1]The Salk Institute, San Diego, CA 92138 and [2]University of Utah, Salt Lake City, UT 84112.

During lytic infection, simian virus 40 (SV40) replicates as a minichromosome in the nucleus of permissive host cells, utilizing both viral-specified proteins and normal cellular processes. Binding of SV40 large T antigen (T Ag) to the SV40 ori is required to initiate viral DNA synthesis. We have observed that a major subclass of replicating SV40 chromosomes whose replication forks progress synchronously have bound T Ag during elongation. Another subclass, lacking T Ag during elongation, replicates asynchronously. These two replicating classes have altered chromatin structures at ori. Further analysis indicates that T Ag may be involved in the selection of either of two different sets of start and termination sites during bidirectional replication. To determine if there are distinct sites in T Ag or several functional classes of T Ag active in the initiation, elongation and, perhaps, termination steps of SV40 replication, we are utilizing twenty-one monoclonal antibodies directed against different structural domains in T Ag. We are analyzing several T Ag-dependent activities important for SV40 replicative function, including adenylation and ATPase activity. Ten antibodies recognize stable determinants and react with total T Ag. Six others recognize a T Ag subclass (20%) active for ATP hydrolysis, including two inactivating antibodies. The ATPase-active T Ag subclass preferentially associates with the cellular tumor protein, p53, during lytic infection. Other antibodies recognize an adenylated T Ag subclass distinct from that active in ATP hydrolysis. We are studying the effect of these antibodies on the in vitro replication of SV40 DNA compared to chromosome templates.

I113 REPLICATION OF ADENOVIRUS DNA: INTERACTION OF CELLULAR PROTEINS WITH THE ORIGIN, Peter C. van der Vliet, Erik de Vries, Peter A.J. Leegwater, Ger J.M. Pruijn and Wim van Driel, Laboratory for Physiological Chemistry, State University of Utrecht, The Netherlands

We have studied Adenovirus DNA replication in vitro using a system of purified viral and cellular proteins and origin containing plasmid DNAs as templates. Optimal replication required the viral DNA binding protein, precursor terminal protein and DNA polymerase, HeLa cell cytosol, nuclear factor I and an additional nuclear protein which we tentatively designate nuclear factor III.

Nuclear factor (NF I) has a minimal DNA binding site of 15-16 nucleotides in the origin which contains two conserved nucleotide blocks. Reversion of this binding sequence did not diminish its stimulating activity in DNA replication. However, its position relative to the first nucleotide is rather critical. From a detailed analysis of DNA contact points, including G, A, T and phosphate contacts, we conclude that NF I binds in the large groove with all contacts at one side of the helix. The chicken TGGCA protein can functionally replace the human NF I. NF III binds to a region containing another conserved block and stimulates initiation only in the presence of this sequence. A point mutation in this region destroys its stimulating activity.

I114 ADENOVIRUS SEQUENCES REQUIRED FOR REPLICATION IN VIVO. Kai Wang, Kevin G. Ahern, and George D. Pearson. Oregon State University, Corvallis, OR 97331.

Specially constructed plasmids containing adenovirus terminal sequences can replicate in cells cotransformed with helper adenovirus DNA. pIR10, a 3.2-kb plasmid, contains the leftmost 358 bp of adenovirus DNA flanked by 90-bp inverted repeats (IR). EcoRI cleavage yields a linear molecule with adenovirus sequences at one end and one copy of the IR at the other. Replication of linearized pIR10 generates a new, linear 3.6-kb molecule with adenovirus sequences at both ends. We have used this assay to determine that the adenovirus minimal origin as well as the adjacent binding site for nuclear factor I are required for efficient replication in vivo (K. Wang and G. D. Pearson, Nucl. Acids Res. 13, 5173, 1985). Recent work has focused on the role of IR sequences in adenovirus replication. IR sequences are presumably required to form "panhandle" intermediates for complementary strand synthesis. Less than 45 bp are required although no minimum size has yet been determined. We will present work on the minimum size of the inverted repeats, the selection between multiple copies of the inverted repeats, and the ability of one copy of the inverted repeats to repair mutations in the other copy.

I115 STUDIES OF DNA REPLICATION BY HERPES SIMPLEX VIRUS TYPE-1 DNA POLYMERASE, Y-S. Wang & J.D. Hall, University of Arizona, Tucson, AZ 85721

Experiments have been conducted to investigate the interaction of DNA polymerase from herpes simplex virus type-1 (HSV-1) with defined DNA templates (poly(dA) and M13mp8 single-stranded DNA). Potential pausing sites of the polymerase on the M13mp8 DNA template and the processivity of the enzyme on both templates will be described. The major DNA binding protein (ICP8), a single stranded DNA binding protein from HSV-1, is required for DNA replication. The effect of ICP8 on processivity and pausing sites in the above replication systems will be presented. Finally, comparisons with heterologous single stranded DNA binding proteins, such as E. coli ssb, will be described to distinguish specific and indirect interactions between the DNA polymerase and binding proteins.

I116 CLONING AND CHARACTERIZATION OF A DELETION-PRONE REGION OF HSV-1 DNA WHICH CONTAINS ORI_L, AN HSV-1 ORIGIN OF DNA REPLICATION. Sandra K. Weller and Ellen M. Petkaitis, University of Connecticut Health Center, Farmington, CT 06032.

A seven kilobase region of the HSV-1 genome between map coordinates 0.385 and 0.434 contains three essential DNA replicative functions: the gene for the major DNA-binding protein, an origin of DNA replication (ori_L), and the HSV DNA polymerase gene. Sequence analysis of an 870-base pair fragment from the origin containing region has revealed a perfect 144-bp palindrome. In an in vivo assay, we have mapped the origin to a 308 bp fragment containing the palindrome. Clones with 55 bp deletions, removing the center of symmetry of the palindrome, have lost origin function. Precise localization and further characterization of ori_L have been severely hampered by the inability to clone sequences which contain it in an undeleted form in bacteria. We reported previously the successful cloning of these sequences in an undeleted form, using a yeast cloning vector (Weller et al., 1985. Mol. Cell Biol. 5:930-942). However it has been difficult to obtain large amounts of origin-containing DNA from yeast cells. We have recently been able to circumvent this problem by propagating ori_L clones in a recombination deficient strain of E. coli, CES 201 (recA, recBC, sbcB). Although deletions of the palindrome still occur in this strain, the frequency is much reduced. Attempts to define the minimal sequence requirements for origin function will be discussed.

I117 SUBSTRUCTURE AND CONTEXT OF *ARS1* EFFECT REPLICATION EFFICIENCY OF PLASMIDS IN YEAST, Michael Woontner, Randy Strich, and John F. Scott, Department of Microbiology, University of Illinois, Urbana, IL 61801.

Previous studies in our laboratory as well as others have shown that an *ARS* consensus sequence requires a proper context for full function. The sequence surrounding the *ARS1* consensus plays an important role in plasmid replication. We have cloned a series of deletions on one side of the *ARS1* consensus ("region C", Celniker *et al.*, Cell **31**:201-213) into the replication/segregation test vector YRp14/CEN4 (Hieter *et al.*, Cell **40**:381-392). Our measurements of plasmid loss rate using Hieter *et al.*'s "half-sector" method show a single small step decrease in replication efficiency as the deletion endpoint approaches within approximately 200 base pairs of the consensus. Additionally, we see small differences in loss rates when our inserts are cloned in opposing orientations, indicating a long-range effect of plasmid structure on replication efficiency. In order to carry our deletion series as far as possible, we have had synthesized an oligonucleotide containing only the *ARS1* consensus sequence flanked by BglII and EcoRI "sticky ends". This fragment has been cloned and sequenced, and will be subcloned along with various amounts of "region B" in YRp14/CEN4 for testing.

I118 INITIATION OF CHLOROPLAST DNA REPLICATION IN CHLAMYDOMONAS REINHARDTII, Madeline Wu, J.K. Lou, D.Y. Chang and Z.Q. Nie, University of Maryland Baltimore County, Baltimore, Maryland 21228

Chloroplast DNA replication in *Chlamydomonas reinhardtii* is initiated by the formation of displacement loops (D-loop) at specific sites. Two D-loop sites were mapped by electron microscopy. One D-loop site and its adjacent region were cloned in recombinant plasmid R-13 and SC3-1. The DNA sequence of the 1.05 Kb chloroplast DNA insert in SC3-1 has been determined. This includes the 0.42 Kb D-loop region, 0.2 Kb to the left and 0.43 Kb to the right of the D-loop region. The sequence is characterized by the presence of 4 large stem-loop structures. Two back to back promoters are mapped within the stem region of a large stem-loop structure which is located in the D-loop region. The 5.5 Kb EcoRI fragment cloned in R-13 contains the SC3-1 insert and its flanking regions. In the flanking regions, a yeast ARS sequence and an ARC sequence which promotes autonomous replication in *Chlamydomonas* were mapped (1). Both R-13 and SC3-1 are better templates than pBR325 in a crude protein mixture which support *in vitro* DNA synthesis. Preliminary data indicates the DNA synthesis starts near the D-loop site in this *in vitro* system. Protein gel analyses show a 18 KD chloroplast encoded polypeptide and 2 nuclear encoded polypeptides bind preferentially to the double stranded DNA of SC3-1.

1) Vallet, J.-M., and Rochaix, J. -D, (1985) Current Genetics 9: 321-324

I119 CONTROL OF PLASMID STABILITY AND COPY NUMBER IN THE YEAST PLASTMID, 2 MICRON CIRCLE, Lih-Juan Young, Jose L. Revuelta and Makkuni Jayaram, Scripps Clinic and Research Foundation, La Jolla, CA 92037

Two aspects of the physiology of the 2 micron circle plasmid ensure its stable propagation at its normal copy number. These are: (a) the amplification potential of the plasmid, that is, its ability to undergo more than one round of replication in a single cell cycle when the copy number drops below steady-state level; (b) the stability system encoded by the plasmid which ensures that, following replication, the plasmid molecules are partitioned equally between the mother and daughter cells at mitosis. The stability system consists of two plasmid-coded proteins, the products of the REP1 and REP2 genes and a cis-acting locus REP3. By placing the expression of the REP1 and REP2 genes under the yeast GAL10 promoter, we have constructed 2 micron circle derivatives in which the stability can be turned off by glucose and turned on by galactose. The 5' proximal sequences of the REP1 gene suggest that in the native 2 micron circle its expression is under translational control. Our preliminary results from deletion analysis as well as oligonucleotide directed mutagenesis support the idea of such a regulation. We have also developed convenient assay systems to test whether the stability system is active on 2 micron circle-derived linear plasmids and whether some or all of the components of the stability system partake in plasmid amplification as well.

I120 IDENTIFICATION OF SEQUENCES INVOLVED IN INITIATION OF YEAST CHROMOSOMAL DNA REPLICATION. V.A. Zakian and M.N. Conrad, Hutchinson Cancer Res. Cen., Seattle, WA. A variety of data are consistent with ARSs being origins of replication for plasmid DNAs in yeast. However, there is no direct evidence for a role of ARSs in the initiation of chromosomal DNA replication. The DNA contained within a replication bubble, by definition, contains a replication origin. We have developed procedures which allow the isolation of DNA from small replication bubbles, DNA which should be highly enriched in replication origins. 4-Thiourdine (TU) is used to label newly replicated DNA. Asynchronous cells go through about one doubling in TU; whereas, cells synchronized at the beginning of S phase appear to undergo a full round of DNA synthesis in its presence. Thio-substituted DNA is denatured and then purified by affinity chromatography on Hg-agarose. Because small DNA molecules bind far more efficiently to the column than large ones, DNA from small bubbles is preferentially isolated. Using asynchronous cells, about 30% of the DNA synthesized during a ^{32}P 10' period in the presence of TU is recovered from the column. This DNA was labeled with ^{32}P and hybridized to restriction digested recombinant DNA plasmids containing specific yeast DNAs. In a first experiment, fragments containing the ARS from both 2 um and ribosomal DNAs appeared to be enriched in thio-substituted DNA compared to non-ARS fragments from the two DNAs (although all fragments were represented in the probe). Our current goal is to use thio-substituted DNA prepared from cells synchronized in early S phase to screen a library derived from yeast chromosome III in which each chromosome fragment has been tested for ARS function (library constructed and characterized by C.S. Newlon). If ARSs are chromosomal replication origins, they should be enriched in early replicated DNA.

DNA Replication II: Prokaryotes and Systems

I121 INITIATION OF BACTERIOPHAGE λ DNA REPLICATION WITH 9 PURIFIED λ AND E. COLI REPLICATION PROTEINS, Christine L. Alfano, Kojo Mensa-Wilmot, Marc S. Wold and Roger McMacken, Johns Hopkins University, Baltimore, MD 21205

We have previously established a crude soluble enzyme system that specifically replicates supercoiled λdv plasmid DNA (Wold et al., PNAS 79, 6176-6180, 1982). The properties of plasmid DNA replication in this in vitro system closely mimic those found for the early circle-to-circle phase of λ DNA replication in vivo. Replication is initiated at or near the λ replication origin (oriλ) and proceeds bidirectionally through theta-structures to produce multiply catenated daughter circles that are eventually segregated into supercoiled plasmid monomers. In the crude system, as in vivo, the λ cI repressor directly blocks initiation of replication at oriλ by preventing transcriptional activation of the origin region by the bacterial RNA polymerase.

A system that supports the initiation and propagation of λdv DNA replication has now been reconstituted with nine purified λ and E. coli proteins. Electron microscopic and biochemical evidence indicates that an early step prior to the initial priming event is the formation of a nucleoprotein complex containing the λ O and P initiators and the bacterial dnaB protein helicase at oriλ. Movement of dnaB protein away from oriλ is accompanied by unwinding of DNA and requires the action of the bacterial dnaJ and dnaK heat shock proteins and the presence of the single-stranded DNA binding protein. Primase functionally interacts with dnaB protein to synthesize all RNA primers for DNA synthesis by the DNA polymerase III holoenzyme. Extensive propagation of a replication fork on the λdv chromosome requires the action of DNA gyrase.

I122 DNA UNWINDING AS A KEY STEP IN INITIATION OF REPLICATION FROM THE Escherichia coli ORIGIN, Tania A. Baker, Barbara E. Funnell, Kazuhisa Sekimizu and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

Initiation of enzymatic replication of plasmids containing the unique origin of the E. coli chromosome (oriC) contains a stage during which the parental DNA duplex is unwound. This unwinding reaction is driven by the coordinate actions of dnaB protein and gyrase, both of which hydrolyze ATP. DnaB protein appears to provide the helicase function while gyrase serves as the swivel. SSB is also required to coat the single-stranded regions. This unwinding reaction is specific for plasmids containing the oriC sequence and requires the initiation proteins dnaA, dnaC and HU (see Sekimizu et al.) in addition to the unwinding enzymes. Unwinding of the parental DNA is required for priming by primase and elongation by DNA polymerase III holoenzyme. Thus a mechanism by which the initiation reactions act to set up replication forks is emerging: dnaA and HU at oriC provide a gate for the entry of a dnaB-dnaC complex. Unwinding by dnaB protein and gyrase in turn allows the entry of primase to prime DNA chains at oriC and the replication forks and holoenzyme to synthesize the chains.

I123 GENETIC STRUCTURE OF THE E. COLI dnaZX REGION, Aleksandra Blinkowa, Kuo-Chang Yin, and James R. Walker, The University of Texas, Austin, TX 78712

The E. coli 2.2 kb dnaZX region, which has a maximum coding capacity of 77,000 daltons of protein in one reading frame, directs the synthesis of two proteins-one of about 75,000 and the other of about 56,500 daltons. This ZX region is required for the polymerization phase of replication and it is possible that the larger and smaller products are the τ and λ subunits of DNA polymerase III holoenzyme. To investigate the mechanism by which the ZX region is expressed, the 2218 base pairs have been sequenced. There is only one long open reading frame which could encode a 71,100 dalton protein-presumably the one previously measured as 75,000 daltons. All other possible reading frames contain multiple stop codons. The ZX structural gene is preceded by a potential promoter consisting of reasonable -35 (TCGCCG) and -10 (TAGCAT) sequences separated by 17 base pairs. A potential Shine-Dalgarno sequence (AGAG) is located seven base pairs upstream of the ATG initiator. The deduced amino acid sequence indicates a slightly acidic protein of pI 6.92. The codon usage is characteristic of a weakly expressed gene. Analysis of the nucleotide sequence provides two models for the expression of ZX. Possibly, the larger protein is cleaved to generate the smaller; alternatively, the two proteins can be translated from different length messengers, using one reading frame. The possibility of two reading frames is excluded.

I124 EFFECT OF dnaA AND recA MUTATIONS ON THE TIMING OF INITIATION IN E.coli,
Erik Boye, Kirsten Skarstad, Harald B. Steen, and Kaspar von Meyenburg
Dept. of Biophysics, Norsk Hydro's Institute for Cancer Research, 0310 Oslo 3, Norway and Dept. of Microbiology, The Technical Univ. of Denmark, 2800 Lyngby, Denmark.
A flow cytometric method has been devised to measure the degree of synchrony in initiation of multiple origins within one and the same E. coli cell (see accompanying abstract). While initiation occurs almost simultaneously in wild type cells, the timing appears to be severely disturbed in dnaA(ts) mutants grown at the permissive temperature. One class of mutants initiated almost randomly in time (all mapping in the central part of the gene between dnaA46 and dnaA602) while the other class retained some degree of synchrony (mapping in the distal parts of the gene). For the dnaA46 mutant, the degree of asynchrony was independent of temperature between 25 and 37°C. Introduction of a plasmid carrying the dnaA$^+$ gene partly restored synchrony in dnaA mutants, but reduced synchrony in the wild type cell. Introduction of minichromosomes containing oriC or plasmids containing dnaA-boxes and no dnaA gene had only small effects on synchrony in the wild type. We believe that the lack of functional DnaA-activity is limiting even at permissive temperatures causing scattered initiations from single origins. With too much DnaA-activity overinitiations occur. The data indicate that initiation is extremely sensitive to the concentration of DnaA protein free to bind to the oriC region. Initiation in recA mutants were also found to be extremely asynchronous suggesting a role for the RecA protein in control of chromosome initiation.

I125

DNA POLYMERASE I AND DNA PRIMASE COMPLEX IN YEAST. LUCY M. S. CHANG, F. CHERIATHUNDAM, P. PLEVANI AND G. BADARACCO, DEPARTMENT OF BIOCHEMISTRY, UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES, BETHESDA, MARYLAND, AND DEPARTMENTO DI BIOLOGI, SEZIONE DE GENETICA, UNIVERSITA DEGLI STUDI DI MILANO, ITALY.

An immunoaffinity chromatographic procedure was developed to purify DNA polymerase -DNA primase complex from crude soluble extracts of yeast cells. Analysis of peptides in the complex by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed the presence of five major peptides: p180, p140, p74, p58 and p48. Separation of the polymerase and primase activities was accomplished by ion exchange chromatography and immunoaffinity chromatography. Enzyme activity gel and immunoblot analyses showed that p74 cofractionated with p180 and p140, the catalytic peptides of DNA polymerase. p58 and p48 cofractionated with the DNA primase activity. DNA primase isolated from the polymerase-primase complex sedimented on sucrose gradients with an estimated molecular weight of 110,000.

In the presence of rATP and dNTPs, the yeast polymerase-primase complex converts the RFI form of single-stranded circular DNAs to the RFII form. Replication of polydeoxynucleotides showed the yeast primase in the complex can initiate with ribopurine nucleoside triphosphates but not with ribopyrimidine nucleoside triphosphates. Addition of E. coli DNA polymerase I to the poly (dT) replication mixture resulted in 7 to 8-fold stimulation of dNTP polymerization activity.

I126 INSERTION MUTAGENESIS AND TRANSCRIPTION MAPPING OF PLASMID pSC101 REPLICATION GENES
Gordon Churchward, Elisa Izaurralde & Danielle Manen, Department of Molecular Biology, University of Geneva, Geneva, Switzerland.

We have isolated a series of insertions of omega, a DNA fragment carrying an antibiotic resistance gene, into the pSC101 replication genes cloned on a pBR322 vector plasmid. From these insertions, we have constructed a number of deletions by recombining fragments from different insertion plasmids in vitro. The characterization of these deletion mutants shows that the segment of DNA containing the par locus is required for normal pSC101 replication. Deletion of this segment abolishes replication activity and also defines a new replication gene, repB. The insertion of omega results in the introduction of new restriction sites. These have been used to clone segments of DNA from the insertion mutants into a promoter-probe plasmid where a galactokinase gene is expressed from promoters within the cloned segments. Analysis of galactokinase expression reveals a complex pattern of transcription, and suggests that both strands of the inc locus, involved in the regulation of plasmid replication, are transcribed.

1127 SYNTHESIS OF LINEAR PLASMID MULTIMERS IN E. coli K-12, Amikam Cohen and Alvin J. Clark, Department of Medical Genetics, The Hebrew University Hadassah Medical School, Jerusalem, Israel, and Department of Molecular Biology, University of California, Berkeley CA 94720

Linear plasmid multimers were identified in extracts of recB and recC mutant strains containing derivatives of the ColE1-type plasmids pACYC184 and pBR322. A mutation in sbcB increases the proportion of plasmid DNA as linear multimers. A model to explain this is based on proposed roles of RecBC enzyme and SbcB enzyme (DNA exonuclease I) in preventing two types of rolling circle DNA synthesis. Reinitiation of rolling circle DNA synthesis was proposed to occur by $recA^+$ dependent intermolecular recombination between linear multimers and circular monomers and by $recA^+$ independent intramolecular recombination of linear multimers potentiated by an sbcA mutation. By using a plasmid carrying a shortened but functional form of lambda's gam gene under thermosensitive repression, we were able not only to measure the kinetics of formation of linear multimers but also to observe a dramatic increase in the proportion of cell DNA found as plasmid DNA. This supports the hypothesis of rolling circle DNA synthesis because such synthesis is expected to be insensitive to RNA-I control of initiation of monomer circle replication. The presence of linear plasmid multimers in recB and recC mutants sheds new light on plasmid recombination frequencies in various mutant strains.

1128 INITIATION OF SV40 DNA REPLICATION IN VITRO, R.S. Decker, M. Yamaguchi, R. Possenti, B. Weiner, M.K. Bradley* and M.L. DePamphilis, Dept. Biological Chemistry, and Dana Farber Cancer Center*, Harvard Medical School, Boston, MA.

Efficient initiation of bidirectional SV40 DNA replication was obtained using a low-salt whole-cell extract supplemented with a high-salt nuclear fraction from SV40-infected CV-1 cells. Purified SV40 T-antigen could substitute for the high salt nuclear fraction. In addition to providing active T-antigen, the high salt fraction also contained a second stimulatory factor of initiation. This system initiated replication in either SV40 chromosomes or plasmid DNA containing a functional SV40 ori. Competition between plasmids containing SV40 ori and plasmids carrying deletions in and about ori served to identify a sequence that binds the rate-limiting factor(s) required to initiate DNA replication. This site included the late-gene side ori-auxillary sequence (21 bp repeats) plus half of ori-core, but excluded the high affinity T-antigen DNA binding sites. Both initiation and elongation of replicating intermediates was resistant to α-amanatin and ddTTP. In contrast, aphidicolin (Aph) had little effect on the rate of initiation under conditions where elongation was 80-90% inhibited. Initiation of CV-1 DNA primase-DNA polymerase α on ssDNA templates was also insensitive to Aph compared with initiation on preformed DNA-primers, consistent with the proposed role of this enzyme in initiation of DNA synthesis at SV40 ori. The relative insensitivity of initiation to Aph permitted identification of the sequence in the ori region where DNA synthesis began.

1129 IN VIVO AND IN VITRO STUDIES OF RNA SPECIES WHICH ARE INVOLVED IN PLASMID NR1 REPLICATION REGULATION, Xinnian Dong, David D. Womble, and Robert H. Rownd Northwestern Unveristy, Chicago, IL 60611

The replication frequency of plasmid NR1 is determined by the rate of synthesis of its replication initiation protein repA1, whose synthesis is regulated at both the transcriptional and the translational levels. The translation of repA1 mRNA can be inhibited by a 91 base RNA molecule (RNA-E) which is transcribed from the complementary DNA strand. Both this RNA inhibitor and its target sequence in repA1 mRNA have potential stem-loop structures which contain 6-base single-stranded loops. According to free energy calculations of stable secondary structure, the 6-base loop target site for RNA-E is a transient structure during synthesis of repA1 mRNA. When transcription continues past a critical point, the longer mRNA transcripts will refold into either of two more-stable forms. Without the binding of RNA-E to its target site, the mRNA will refold into a form that favors the initiation of repA1 protein translation. If RNA-E binds to its target, the mRNA will refold into a different form which is less favorable for translation. By Northern blot analysis of in vivo transcripts, we discovered a mRNA species which ends at a position just downstream from the RNA-E target site. In vitro single-round transcription experiments have shown that this RNA species is a transcription intermediate instead of a degradation product. Our evidence suggests that the transcription of repA1 mRNA pauses after the RNA-E target site has been synthesized. The pause of RNA polymerase may prolong the existence of the transient target structure and be essential for the RNA-RNA interaction.

I130

IDENTIFICATION AND PARTIAL ISOLATION OF A PROTEIN FACTOR FROM SACCHAROMYCES CEREVISIAE THAT BINDS SPECIFICALLY TO AN ARS SEQUENCE. Shlomo Eisenberg and Bik Tye. Department of Biochemistry, Cornell University, Ithaca, NY 14853.

All known initiation proteins in DNA replication share a common property; they recognize and bind tightly to a nucleotide sequence at their respective origins of replication. This prompted us to search for protein(s) in S. Cerevisiae that will bind tightly to a nucleotide sequence present in the yeast ARS element.

A protein fraction was prepared by gently lysing the yeast cells with zymolyase, ammonium sulfate precipitation and ion exchange chromatography. The incubation of this protein fraction with [^{32}P]-labelled DNA restriction fragments caused a specific shift in mobility of a DNA fragment containing the ARS activity. This shift in mobility was sensitive to prior incubation with either SDS or a protease, indicating that the effect on the migration of the DNA fragment is due to the formation of a protein-DNA complex. The binding site has now been localized to a 200 bp. nucleotide region within the ARS element, and DNA binding competition experiments suggest that this site is present in a large number of ARSs. We are now determining the nucleotide sequence involved in the protein binding and are in the process of isolating the protein. The possible involvement of this protein in the initiation of DNA replication at the yeast ARS sequence will be discussed.

I131

USE OF M13-oriC CHIMERIC PHAGE: AMPLIFICATION OF HIGH COPY LETHAL GENETIC ELEMENTS and OLIGONUCLEOTIDE MUTAGENESIS OF dnaA BINDING SITES IN oriC, Robert E. Enns and Douglas W. Smith, University of California, San Diego, La Jolla, CA 92093

High copy lethal (HCL) DNA sequences are a heterologous group of structural genes and controlling elements that for any of a number of reasons kill host cells when present above a certain copy number. This presents serious problems both for constructing complete genetic libraries and studying individual, known HCL genes. We have developed a system involving M13 viruses mp8 and mp9 that overcomes this problem. Transfecting host cells with M13-oriC vectors developed in our laboratory and isolating both double-stranded recombinant form DNA and single-stranded viruses during timed intervals after transfection shows elevated copy number and a much greater extent of overall transfection, with the added bonus of single-stranded DNA for both sequencing and mutagenesis studies.

The minimum origins of DNA replication in Escherichia coli and related bacteria have highly conserved regions referred to as "R" sequences that may serve as dnaA binding sites and may also be involved in intrastrand pairing and subsequent secondary structure formation. The relative importance of this hypothesized pairing was investigated using nucleotide-specific mutagenesis to initially inhibit, then restore, potential base-pairing relationships between R1 and R4 in the Escherichia coli origin of replication, oriC. Our results indicate that a single transversion (A to T) in R1, or the analogous one in R4, renders oriC completely nonfunctional. Combining both mutations on the same molecule should restore any hypothetical R1-R4 pairing, but the double mutant is also nonfunctional.

I132

P4 PHAGE DNA REPLICATION. John Flensburg, Mark Krevolin, Richard Calendar and Rosemarie Christian. University of California, Berkeley, CA 94720

The P4 α gene, which is required for DNA replication, has been cloned into a plasmid under the control of the λ CI857 repressor. Upon heat induction, the M_r=86,000 α protein is overproduced, and we have purified it to about 99% purity. This P4 α protein causes origin-specific DNA replication of P4 supercoiled DNA in the presence of E. coli DNA polymerase III holoenzyme and E. coli single-stranded DNA binding protein, four deoxyribonucleoside triphosphates, ATP and GTP. The DNA sequences required for replication include the origin and a second region (crr=cis replication region), that lies 4 kb from the origin, just beyond the transcription terminator for the α gene. Crr consists of about 300 bp that include two direct repeats of 120 bp, which match one another at 96 positions. Each repeat in crr contains two copies of an octamer that is found five times in ori, as well as an 11-mer that is found once in ori. The ability of 99% pure α protein to synthesize polyriboguanylic acid from GTP in the presence of poly dC is reduced 1000-fold, compared to the 30-fold purified α protein fraction prepared from P4-infected cells (1). This "primase" activity can be regenerated by addition of RNA polymerase core in the presence of rifamycin. 99% pure α protein can hydrolyze ATP and GTP to their diphosphate forms in a reaction that does not depend upon DNA.

1. M. Krevolin and R. Calendar. J. Mol. Biol. 182:509, 1985.

1133 ANALYSIS OF COLE1-Type PLASMID CONFORMATIONS IN E. coli rnh MUTANTS, Wendy Fuge and Tokio Kogoma, University of New Mexico, Albuquerque, NM 87131

ColE1-type plasmids replicate in rnh mutants deficient in RNase H in an altered manner which requires neither RNase H nor DNA polymerase I activity. Previously we reported that ColE1-type plasmids replicating in rnh mutants exist as a highly concatemeric form, i.e. circular multimers of unit plasmid joined in a head-to-tail fashion. Further detailed analysis has revealed that plasmid preparations from rnh mutants contain varied amounts of monomers, dimers, etc. up to octamers. Transformation of rnh$^-$ cells with a pair of plasmids which can be distinguished by size, restriction endonuclease specificity and genetic markers results in concatemers of a hybrid between the two plasmids. Our analysis has indicated that plasmid preparations from such transformants contain a small but significant amount of catenates in addition to various sizes of concatemers.

1134 RECONSTITUTION OF THE COMPLETE REPLICATION CYCLE OF PLASMIDS CONTAINING THE ORIGIN OF THE Escherichia coli CHROMOSOME, Barbara E. Funnell, Tania A. Baker and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

A complete cycle of oriC plasmid replication, beginning and ending with supercoiled molecules, has been reconstituted with pure proteins. The first step in initiation, formation of an active prepriming complex by dnaA, dnaB, dnaC and HU proteins, requires a supercoiled template. Nicked and linear DNAs are completely inactive; relaxed covalently closed molecules must be supercoiled by gyrase prior to complex formation. DNA synthesis proceeds via theta-structure intermediates. Elongation to near completion is rapid, but subsequent termination and segregation steps are slow, resulting in an accumulation of nearly completed, late theta-structures. The predominant products are nicked monomers and catenated pairs of daughter molecules. Processing steps required to produce supercoiled daughters include removal of ribonucleotide residues (primers) by DNA polymerase I and RNase H, ligation of nascent strands by DNA ligase and supercoiling by gyrase. Covalently closed molecules are supercoiled rapidly; decatenation of intertwined daughters to monomers is significantly slower. Thus, replication in this purified protein reconstitution system produces supercoiled daughter molecules identical in sequence and topology to their parents.

1135 DIRECTED MUTAGENESIS OF THE S. TYPHIMURIUM ORIC BGLII FRAGMENT, Della Garland, Henry Barnes, Sandra Groos and Douglas Smith, Univ Cal, San Diego, La Jolla, CA 92093

The plasmid pJZ34 contains the "minimal" origin (oriC) from Salmonella typhimurium as two BamHI fragments, "BamD" and the smaller "BamF". Two BglII sites are present in pJZ34, both in the oriC BamF fragment. A completely nonfunctional oriC plasmid, pJZ37, is obtained by deletion of the small 16 bp BglII fragment. Sau3A fragments resulting from a total digest of either pJZ34 or of pJZ37, serving as sources of different sized Sau3A fragments, were inserted into the BglII site of pJZ37, followed by transformation of JZ294 polA1 cells, to regenerate and select for phenotypically OriC$^+$ derviative plasmids. Sequence analysis of many of the resulting recombinant origins showed that the new origins fall into seven classes of identical sequence, with as many as four plasmids in each class. One class is wildtype pJZ34 although this class contains only one member. Thus, specificity for functional Sau3A fragments was observed, and there is no strong selection for the wild type fragment. Two of the classes contain no GATC site comparable to the second BglII site, and one class appears to contain the pJZ37 origin. Functionality of the mutant origins has been assayed in several ways: 1) growth rates under selective conditions; 2) plasmid stability under nonselective conditions; 3) copy number determinations; and 4) ability to serve as template for the Fraction II oriC-specific in vitro initiation system. This region of oriC is important for transcription termination events in oriC (J. W. Zyskind, personal communication), and we are currently examining the effects of the new inserted sequences on such termination events.

I136 PROCESSIVE DISCONTINUOUS DNA SYNTHESIS BY MAMMALIAN DNA POLYMERASE α/PRIMASE, M. Goulian, C. Carton, L. De Grandpre, C. Heard, B. Olinger, S. Richards, University of California, San Diego, La Jolla, CA 92093

We have purified a pol α/primase from cultured mouse cells. Used alone, this enzyme converts fd single stranded circular DNA to duplex circles in a dispersive manner. However, in the presence of an accessory factor (AF-1) purified from the same cells, the overall mechanism appears to be processive, i.e. even with template DNA in great excess, both during the reaction and after maximum synthesis has taken place, there are only two populations of molecules: completely (or almost completely) replicated circles and unreplicated template molecules. This "processivity factor", AF-1, also stimulates synthesis by the pol α/primase on long single stranded DNA and homopolymer templates. The stimulatory effect is on the polymerase rather than the primase. The product resulting from the action of pol α/primase + AF-1 on fd single stranded circles consists of multiple tandem independently initiated fragments, of 200 to 1200 nucleotides, each bearing a 5' terminal RNA primer. When the reaction mixture includes, in addition, RNase H-1 and DNA ligase, plus an additional accessory factor (AF-2), all purified from the mouse cells, the result is a continuous full length (6.4 kb) product strand. The overall reaction carried out by these five purified proteins reproduces some of the essential features required for synthesis of the lagging strand at replication forks.

(This research was supported by NIH grant CA 11705).

I137 ROLE OF dnaA IN P1 REPLICATION. Egon B. Hansen and Michael B. Yarmolinsky, Laboratory of Biochemistry, National Cancer Institute, Bethesda, Maryland 20892

The P1 prophage is a stably inherited unit copy plasmid. P1 replication origin oriR is organized similarly to ori2 of fertility-factor F and the pSC101 replication origin, although sequence homology is found uniquely at DnaA protein binding sequences in all three origins. Only pSC101 has previously been found to depend on the dnaA protein for replication.

Employing dnaA null mutant strains, we have been able to show that oriR of P1 and ori2 of F also require DnaA. One of the assays used to demonstrate DnaA dependence of oriR takes advantage of a system where replication from oriR is lethal, thus allowing us to score the absence of oriR replication in a ΔdnaA strain as the recovery of viable bacteria, rather than the failure to do so.

Surprisingly, we found that, although a basic replicon of P1 has an absolute requirement for DnaA, the intact P1 prophage does not. Strains carrying a P1 phage integrated into the chromosome at loxB tolerate insertional inactivation of the dnaA gene or deletion of the chromosomal replication origin, oriC. These strains respond differently when challenged with controlling elements from phage P1. We are currently using these differences to characterize the dnaA independent replication of P1.

I138 LOCALIZATION OF THE REPLICATION BLOCKS IN THE TERMINUS REGION OF THE ESCHERICHIA COLI CHROMOSOME. Thomas M. Hill and Peter. L. Kuempel, University of Colorado, Boulder, CO 80309

We are investigating the events which occur at the end of the E. coli DNA replication cycle, concentrating specifically on the fate of DNA replication forks as they traverse the terminus region of the chromosome. Our data indicate the following: 1) Two termination sites are present in the chromosomal terminus; located on the trp side of the terminus at kb 120-200 (Bouche map coordinates; J. Mol. Biol. 154:21) and on the man side of the terminus at kb 330-410. 2) The termination blocks impede replication forks approaching from one direction but not from the other. Therefore, the block near trp inhibits counterclockwise traveling forks and the block near man impedes clockwise traveling forks. 3) The blocks do not appear to halt DNA replication abruptly; rather, replication forks are inhibited across an 80 kb interval. We are currently testing deletion mutants in the terminus region to delineate the boundaries of the blocks. Δ1438, which maps between kb 165-290, does not remove the block for counterclockwise traveling forks, but Δ1608 (kb 120-455) does. Likewise, clockwise traveling replication forks are still impeded in Δ1738 (kb 348-387). We are also interested in a region located between kb 343-350, which has been difficult to delete. Possibly, this region encodes one of the replication blocks or some other function important for coordinating termination of replication and cell division.

1139 TRANSCRIPTIONAL ACTIVATION OF THE λ ORIGIN, G. Hobom, Fan Hua, J. Mertsching, Institut für Mikrobiologie, Justus-Liebig-Universität Giessen, 6300 Giessen

A more detailed analysis of the transcriptional activation reaction of the λ origin in plasmid model systems shows the initiation frequency to be stimulated by coupled transcription/translation across the origin sequence. This observation is in accordance with the location of *λori* within the coding sequence for *O* and is able to explain cis-effects observed for *λOam*29 and other phage mutants located in the *N*-terminal part of the *O* gene. In this interaction the palindrome sequence around the *ori-Eco*RI site serves as an RNA polymerase pausing signal and an *ori* activating element. This regular activation mode of the λ origin may be substituted by rightward transcription away from *ori* in plasmid systems serving as a model for phage mutant *λric*5b. Initiation of plasmid replication upstream from a promoter signal depends on its activity and on its distance from *ori*, it may be stimulated by the distal insertion of a terminator element. We will present evidence for our interpretation that transcriptional activation of the origin sequence in cis is in this system transmitted from the transcribed region to *ori* via local strand seperation.

1140 THE INITIATION OF DNA REPLICATION AT THE PRIMARY ORIGIN OF BACTERIOPHAGE T7, Richard A. Ikeda and Charles C. Richardson, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

T7 RNA polymerase is a single chain enzyme (MW=98,092) that catalyzes the polymerization of ribonucleotides from specific promoters--promoters characterized by a highly conserved 23 base pair sequence. The RNA polymerase is primarily responsible for the expression of the late genes of T7; however, the in vivo initiation of T7 DNA replication from the primary origin requires a competent T7 RNA polymerase promoter. Furthermore, efficient initiation at the primary origin requires both of the twin T7 RNA polymerase promoters, ϕ1.1A and ϕ1.1B, that distinguish the intergenic primary origin sequence. In vitro, truncated RNA transcripts from the T7 RNA polymerase promoters ϕ1.1A and ϕ1.1B prime DNA synthesis on plasmids containing the primary origin of T7. To begin to understand the mechanisms involved in the transition from RNA synthesis to DNA synthesis during the initiation of DNA replication, we have carefully examined the interaction of T7 RNA polymerase with its DNA template. Footprinting of T7 RNA polymerase by cleavage with methidiumpropyl-EDTA reveals protection by the polymerase on both strands of the bound DNA. In the absence of GTP T7 RNA polymerase footprints are not observable ($K_{eq}<2x10^4$ M^{-1}); however, in the presence of GTP the RNA polymerase shows a moderate affinity for the T7 promoter ($K_{eq}=1x10^6$ M^{-1}). In this initiation complex the RNA polymerase covers 28 base pairs. Once the polymerase has moved off the promoter, the protected region is reduced to 22 base pairs.

1141 REGULATION OF COLE2 AND COLE3 DNA REPLICATION, Tateo Itoh, Toshihiro Horii and Hisashi Yasueda, Dept. of Biology, Osaka University, Toyonaka, Osaka 560, Japan

ColE2 and ColE3 are small multicopy plasmids with extensive homology and require DNA polymerase I for replication. They are not amplified by treatment of plasmid-carryig cells with chloramphenicol, indicating involvement of some unstable proteins in replication. A 1.3 kb region of ColE2 sufficient for autonomous replication encodes a trans-acting positive factor (Rep) and trans-acting negative functions (IncA and IncB), and it also contains a cis-acting site (Ori). Plasmid-specific interaction of the Rep with the Ori leads to initiation of DNA replication. The IncA function common to both the plasmids negatively regulates expression of the Rep at the translational level and the IncB function specific for each plasmid is presumably titration of the Rep by binding to the cloned Ori sites. The nucleotide sequences of the regions of ColE2 and ColE3 required for autonomous replication have been determined. Extensive homology (more than 95 %) between them indicates that the replication functions identified in ColE2 are also carried by the corresponding regions of ColE3. The largest possible open reading frame of each plasmid can specify a protein of about 300 amino acids, which must be the Rep protein. By using in vitro DNA replication as an assay system we are now purifying the protein. The Ori site of ColE2 turned out to be only 47 bp with two 7-bp direct repeats and several short inverted repeats. The corresponding Ori site of ColE3 has almost identical structural features. A small RNA (RNA I, about 115 n.) is transcribed from the IncA region, which is entirely complementary to the 5'-leader region of the Rep mRNA. RNA I seems to interact with the Rep mRNA and ihibits translation of the latter.

I142 REGULATION OF T4 DNA REPLICATION GENES 45,44,62, and 43. Karam, J., Hughes, M., Hsu, T, Dawson, M., and Alford, C. Department of Biochemistry, Medical University of South Carolina, Charleston, SC 29425

The sites for transcriptional and translational control of the T4 gene 46-45-44-62-regA-43 cluster are being investigated. We have identified several transcripts for the gene 45-44-62-regA subcluster. Two species that initiate downstream of gene 46 encompass the 45 cistron and apparently terminate near the junction between genes 45 and 44. The larger of these two transcripts includes two newly identified cistrons that map between genes 46 and 45. We have sequenced these new cistrons and overproduced their protein products under control of plasmid promoters, and are studying their possible role in T4 DNA replication. Genes 44, 62 and regA are cotranscribed and translationally coupled. In addition, gene regA is independently transcribed by two modes. All these (including the gene 45) transcripts bear translation initiation regions that are sensitive to repression by the regA protein. By using expressible, cloned $regA^+$ and $regA^-$ genetic sequences, assays for regA-mediated translational repression have been devised that allow fine structure analysis of the targets for this translational repressor.

The gene 43 (DNA polymerase) regulatory region is being studied via the construction of fusions with the E. coli lacZ gene. One fusion peptide carrying the NH_2-terminal 70 amino acids of T4 DNA polymerase and harboring β-galactosidase activity, appears to also have the autoregulatory activity of the phage polymerase. We are isolating this protein in order to study its possible DNA-binding properties in vitro. We will also report on the use of these gene 43-lacZ fusions for the isolation of DNA polymerase regulatory mutations. Supported by NIH (GM18842) and NSF (DMB-8502619).

I143 INVOLVEMENT OF A SEQUENCE-SPECIFIC ENDONUCLEASE IN THE INITIATION OF PLASMID pT181 REPLICATION, Saleem A. Khan, Richard R. Koepsel and Robert W. Murray, Department of Microbiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

pT181 is a 4437-base pair plasmid from Staphylococcus aureus, which encodes resistance to tetracycline and has a copy number of 20-25 per chromosome. Initiation of pT181 replication requires the plasmid-encoded RepC protein which acts in trans and is rate limiting for replication. Purified RepC protein was shown to be specifically required for the initiation of pT181 DNA replication in vitro. Based on filter-binding of plasmid restriction fragments, RepC protein binds to the pT181 origin of replication. Using DNAase I and neocarzinostatin foot-printing techniques, we show that the initiator protein specifically binds to a 33 base pair long sequence within the origin which is part of the initiator cistron. Furthermore, RepC protein was shown to have sequence-specific endonuclease and topoisomerase-like activities. A maximum region of 127 base pairs containing the pT181 origin of replication is required for nicking-closing by RepC protein. RepC nicks the bottom strand of DNA within the pT181 origin when either supercoiled or linear DNA is used as the substrate. The nick site has been shown by DNA sequencing to lie between nucleotides 70 and 71 in the bottom strand of the DNA within the origin sequence. The nick site probably corresponds to the start site of pT181 replication. Our results suggest that, unlike most other plasmids, pT181 replicates by a rolling circle mechanism.

I144 GENETIC ANALYSIS OF ALTERNATIVE DNA REPLICATION PATHWAYS IN E. coli rnh MUTANTS, Tokio Kogoma and Ted Torrey, University of New Mexico, Albuquerque, NM 87131

E. coli rnh mutants deficient in RNase H are capable of DNA replication in the absence of protein synthesis. This stable DNA replication (cSDR) can sustain cell viability when the $dnaA^+$ $oriC^+$-dependent initiation system is inoperational (Das^-). cSDR requires $RecA^+$ protein at an initiation step. This $RecA^+$ requirement can be suppressed by rin mutations. Thus, rnh^- rin^- strains are viable and capable of SDR despite the inactivation of both RecA and DnaA proteins. Both Rin^- SDR and Das^- phenotypes are shown to depend on some novel function of the $recF^+$ gene product. The lexA3 mutation inhibits $recA^+$-dependent, rnh^--mediated SDR via reducing the amount of $RecA^+$ activity available, and is suppressed by the recAo254 mutation. The Rin^- SDR is also inhibited by the lexA3 mutation, indicating a requirement for some other LexA-regulated gene product action in this pathway. lexA(Def) mutations also suppress the requirement for $RecA^+$ in rnh^--mediated SDR in a $recF^+$-independent manner. A model will be presented for the regulation of rnh^--mediated alternative DNA replication pathways by the products of the $lexA^+$, rin^+ and $recF^+$ genes.

1145 RELATIONSHIP OF incB AND incC INCOMPATIBILITY TO oriC REPLICATION IN E.COLI, Ralf Kölling, Walter Messer and Heinz Lother, Max-Planck-Institut, Ihnestraße 63-73, D-1000 Berlin 33, Germany

The incB-incC region directly adjacent to the minimal origin, oriC, contains reading frames for a 16kd and a 17kd protein.

We investigated the influence of these proteins on the transcriptional activity of promoters that might be important for oriC replication. Furthermore we tested the influence of dnaA protein on the activity of several promoters.

The experiments show i) that the 17kd protein, the presumed incC determinant, is an autogenously regulated activator of the adjacent asnA gene promoter, making a role in replication rather unlikely and ii) that the 16kd promoter, the presumed incB determinant, is negatively regulated by dnaA protein. The DnaA regulated transcription, which is directed towards oriC, suggests a direct role of the 16kd transcript in oriC replication.

1146 PHAGE T4 REPLICATION ORIGINS THAT COINCIDE WITH RECOMBINATION HOTSPOTS. K. N. Kreuzer, K. H. Benson, A. M. Bogerd, A. E. Menkens, R. A. Ringley and W. Y. Yap. Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710

Previous studies indicate that phage T4 uses at least three modes of replication initiation. We are currently utilizing T4-mediated plasmid transduction to study two T4 tertiary origins cloned in plasmid pBR322. Tertiary origin initiation is distinct from the other modes since it is rifampicin resistant (primary initiation is rifampicin sensitive) and does not require the phage gene 46/47 products (required for secondary initiation). Both tertiary origins coincide with recombination hotspots in the phage genome, suggesting a link between recombination and replication. There may also be a relationship between replication and transcription, since one origin maps at the middle promoter for gene uvsY and the other is at a "middle promoter-like" sequence within the coding region of the late gene 34.

The T4 insertion/substitution vector was used to delete the uvsY origin/promoter from the phage chromosome. This uvsY$^-$ phage supports tertiary origin plasmid replication, demonstrating that the uvsY protein is not required. The plasmid replicates even when it shares no homology with the deleted phage, and therefore plasmid-phage recombination is not required for origin function. Phage mutants unable to replicate tertiary origin plasmids have been isolated using a novel selective procedure. These mutants are currently being analyzed in an attempt to identify the initiation protein(s) unique to this mode of replication. (Supported by NIH Grant GM-34622)

1147 DNA HELICASE II(*UVRD*) AND THE REP HELICASE ARE REQUIRED FOR *E. COLI* DNA REPLICATION. Sidney R. Kushner, Leslie M. Fischer and Carol M. Hamilton, University of Georgia, Athens, Georgia 30602

Temperature sensitive *uvrD* (DNA helicase II) mutations (*uvrD255, uvrD256*) have been isolated by in vitro mutagenesis of the cloned structural gene. *rep-3 uvrD255* double mutants are conditionally lethal for growth in both minimal and rich media. The wild type *uvrD* gene in either single or multiple copy does not complement the *uvrD255* and *uvrD256* alleles at the nonpermissive temperature. Results from P1 transductions suggest that *uvrD255* single mutants are inviable. *uvrD255 rep-3* double mutants demonstrate a very strong antimutator effect at 30°C in marked contrast to known recessive *uvrD* alleles such as *uvrD101* and *uvrD3 uvr-257*. DNA helicase II purified from a strain carrying the *uvrD255* allele has normal levels of single-stranded DNA-dependent ATPase activity but has markedly reduced DNA unwinding activity. The DNA unwinding activity observed in vitro is less sensitive to temperature inactivation than wild type DNA helicase II. In vivo, *rep-3 uvrD255* double mutants exhibit an increase in DNA synthesis for approximately 60 minutes following shift to the nonpermissive temperature even though the number of viable cells rapidly decreases after 15-20 minutes. Southern analysis of chromosomal DNA fragments near the origin and the terminus or replication indicates a significant increase in the number of initiation events per cell after shift to the nonpermissive temperature. This evidence suggests that DNA helicase II and the Rep helicase are involved in the initiation of DNA synthesis. This does not rule out a role for the two proteins in chain elongation. (Supported by NIH GM27997 to S.R.K.)

1148 PROPERTIES OF dnaQ-DEFICIENT MUTANTS OF SALMONELLA TYPHIMURIUM, Edward Lancy, Miriam Lifsics, David Kehres, Richard Cardaman, and Russell Maurer, Case Western Reserve University, Cleveland, OH 44106

Strains of Salmonella bearing a variety of deletions and insertions in the dnaQ gene, which encodes the ε (editing) subunit of DNA polymerase III, have been constructed. Cells harboring these mutations can be maintained only if a second site suppressor is also present. Genetic mapping and cloning experiments indicate that the suppressors are located very near, possibly within, dnaE, the gene for the α'(polymerase) subunit. The strains lacking dnaQ exhibit 50 to 100 fold elevated spontaneous mutation frequency; when the wild type dnaQ gene is restored, the mutation frequency returns to wild type levels. This and other information indicates that in the suppressed mutants, an altered form of DNA polymerase III is being used for DNA replication. Since α and ε are normally found tightly associated in the core of DNA polymerase III, it is of interest to determine the structure of the altered polymerase III and characterize activity differences between the mutant and the wild type enzyme. We have detected polymerase III activity in extracts of the mutant (as well as the wild type) cells, and we will report on our characterization of these enzymes.

1149 ACTIVITY OF THE PRIMARY ORIGIN IN F PLASMID REPLICATION

D. Lane, P. Caughey, M. Kennedy and R. deFeyter. Cell Biology Department, Auckland University.

A 190 bp fragment from the primary origin (ori-1) region of the F plasmid of E. coli can initiate replication of a DNA polymerase I-dependent vector into which it is cloned provided certain mini F structural genes are also present and functional. However preventing entry of transcripts into ori-1 by linking it to transcriptional terminators diminishes its ability to replicate. The mini F gene products required for ori-1 activity are the E protein (also needed for ori-2 replication), the G protein (which also inhibits cell division when plasmid copy number falls to one per cell) and the C protein. C protein represses not only the pif operon promoter but also a nearby promoter directed towards ori-1. D protein is not required for ori-1 to initiate replication of the vector into which it is cloned, but it does repress the activity of a promoter within ori-1. The behaviour of ccdB$^-$ (=G$^-$) plasmids, and the involvement of G protein in more than one activity suggest that ori-1 is one of a set of "emergency" functions switched on when F has failed to replicate by the time cell division begins.

1150 CONJUGATIVE TRANSFER OF PROMISCUITIVE PLASMID RP4: PLASMID-SPECIFIED FUNCTIONS ESSENTIAL FOR FORMATION OF RELAXOSOMES. Erich Lanka, Werner Pansegrau, Günter Ziegelin and Jens P. Fürste, Max-Planck-Institut für Molekulare Genetik, Abt. Schuster, D-1000 Berlin 33.

The central event for initiating transfer replication during bacterial conjugation is the cleavage of a single phosphodiester bond at the *nic*-site within *oriT* of a conjugative plasmid. This process can occur after the assembly of relaxosomes (relaxation complexes) requiring the *oriT* region and plasmid-encoded transfer factors. The nicking reaction was visualized on agarose gels after isolation of relaxosomes and subsequent treatment with protein denaturing agents. The fully functional *oriT* of RP4 represents an intergenic DNA stretch of ∿320 bp which is located within TraI - one of three unlinked transfer regions. Dissection of *oriT* revealed that a portion carrying *nic* and symmetrical sequence repeats is connected contiguously to a recognition region which is essential for efficient mobilization of *oriT* plasmids. Additional features of *oriT* are promotor sites allowing divergent transcription of two operons arranged to both sides of *oriT*. Gene products encoded by these cistrons have been overproduced by applying a regulated expression vector system. Gene arrangement was deduced from analyzing the products synthesized by defined deletants with the immunoblotting technique. Formation of relaxosomes appears to depend on the presence of at least three plasmid-specified components which act in *trans*. Corresponding genes map to both sides of *oriT*. Purification of one of the products yielded an 11 kDa polypeptide - a dimer under non-denaturing conditions - which binds strongly to DNA. Striking sequence homology at *oriT* of RP4(IncPα) and R751(IncPβ) and existence of a R751 analog to the 11 kDa protein contrast the differences observed in *oriT* specificity of non-interchangeable relaxosome constituents of the two related plasmids.

1151 *In vitro* Replication of Bacteriophage N4 DNA. M. Pearle, G. Lindberg, and L. B. Rothman-Denes, University of Chicago, Chicago, Illinois 60637

An *in vitro* system consisting of three purified N4 proteins - a single-stranded DNA binding protein, a DNA polymerase, and a 5' → 3' exonuclease - has been characterized. This sytem is identical to a previously described cell-free system in both template and origin specificity. In both cases, N4 DNA is the preferred template, and replication begins within terminal restriction fragments and proceeds inward. Functionally equivalent proteins fail to substitute for the N4-specific proteins.

The structureof the *in vitro* product was analyzed. Newly synthesized DNA is covalently attached to the template, and thus a model is proposed in which the 5' → 3' exonuclease degrades the 5' ends of the DNA, allowing the 3' end to loop around, providing a primer for the DNA polymerase. Restriction analysis confirms that the two strands of the DNA are linked at the ends, forming a hairpin. Sequence analysis in the region of the hairpin reveals only one weak potential inverted repeat that could account for hairpin formation, and therefore protein-DNA interactions may be important. We are currently defining the properties of these proteins and investigating the *in vivo* mode of replication.

1152 EFFECTS OF MUTATIONS IN DNA INITIATION AND RECOMBINATION GENES ON INDUCED STABLE DNA REPLICATION IN *E. coli*, Thomas Magee and Tokio Kogoma, University of New Mexico, Albuquerque, NM 87131

Stable DNA replication (iSDR) (i.e. continued replication in the presence of chloramphenicol) can be induced in wild-type *E. coli* cells by SOS-inducing treatments (e.g. UV irradiation and incubation with nalidixic acid). iSDR appears to be error-prone and considerably more resistant to UV radiation than normal replication. It has been shown that levels of RNase H activity do not change during or after the induction despite the fact that *rnh*$^-$ mutants deficient in RNase H exhibit a similar stable DNA replication activity (cSDR). The induction of iSDR in *rnh*$^+$ cells involves RecA*, an activated form of RecA protein. We have examined induction of iSDR in *recB*, *recC*, *recF*, *recN*, and *recJ* mutants which are known to have altered SOS responses. *recF* and *recN* mutations inhibited the induction of SDR by UV irradiation but not with nalidixic acid. *recBC* mutations abolished the induction with both agents. *umuC* mutations which abolish the SOS mutagenic activity in SOS induced cells did not affect the inducibility of the cells for iSDR. We have also examined several *dnaA* mutants for the SDR inducibility. The significance of these results will be discussed in terms of the possible role of iSDR in SOS mutagenesis.

1153 MYCOPLASMA DNA REPLICATION: ORIGIN, TERMINUS, AND DIRECTIONALITY. Jack Maniloff and Saibal K. Poddar, Dept. of Microbiology, Univ. of Rochester, Rochester, NY 14642

Mycoplasmas are genome-limited organisms. Since we have shown that these cells arose by degenerate evolution from Gram-positive eubacteria, their small genomes reflect selective pressure for the loss of nonessential genetic information. To probe the molecular biological ramifications of this limited genetic complexity, we are studying mycoplasma genome structure and replication using mycoplasma virus L2 as a model. L2 is a temperate enveloped phage, containing superhelical double-stranded DNA of 11.8 kb. The origin and terminus of L2 DNA replication have been mapped with the procedure used by Nathans and coworkers for SV40. L2-infected cells were pulse labelled with ^{3}H-Thy. For times about equal to one L2 DNA replication cycle, progeny L2 DNA molecules will have a gradient of ^{3}H-Thy, with few molecules labelled near the origin and most labelled near the terminus. To measure this distribution, L2 DNA was digested with restriction endonucleases, and the fragments were separated by agarose gel electrophoresis, cut out of the gels, and assayed for ^{3}H. After normalization for the Thy content of each fragment, data analysis showed that replication proceeds bidirectionally from an origin near the single L2 BstEII site, and terminates at a site opposite the origin. When these studies were done using an L2 insertion mutant (containing a 3.1 kb insert), we mapped the same replication origin, but the terminus shifted to remain opposite the origin. Hence, termination is not site-specific, but occurs where the replicating forks meet. The L2 genome is being sequenced and the DNA replication origin will be compared to the oriC of related eubacteria.

I154 FUNCTION OF PRIMOSOME IN THE REPLICATION OF pBR322 DNA REPLICATOIN IN VITRO: INITIATION OF LAGGING STRAND DNA SYNTHESIS, Hisao Masai and Ken-ichi Arai, DNAX Research Institute, 901 California Ave., Palo Alto, CA 94304-1104.

The following evidences suggest that the primosome is involved in the initiation of lagging strand synthesis of pBR322 DNA replication in vitro: (1) The antibody against one of the primosomal proteins, i, inhibits the pBR322 DNA replication in vitro, and replication is restored by adding the purified protein i. (2) Extract from the dnaT strain, which carries a defect in the structural gene for protein i (H. Masai, M. Bond, and K. Arai Proc. Natl. Acad. Sci. U.S.A., in press), does not support the lagging strand synthesis of pBR322 DNA. (3) Deletion of the n' site on the lagging strand of pBR322 DNA, which is essential for the assembly of the primosome, reduces replication activity of the template in vitro by nearly 90%, while the leading strand synthesis is observed to some extent on these templates. (4) When introduced into those templates that lack n' site on the lagging strand of pBR322 DNA, the n' site on ϕX174 DNA can restore the replication activity. Recently Minden and Marians (J. Biol. Chem. 260 9316-9325 (1985)) reported that the lagging strand synthesis of pBR322 DNA replicaton is dependent on the primosomal proteins in a purified system. Their results are consistent with ours obtained in a crude extract.

I155 A CLONED FRAGMENT OF ESCHERICHIA COLI CHROMOSOMAL DNA WHICH PROMOTES ACCURATE PLASMID PARTITIONING. M. Masters, J.H. Pringle & I.R. Oliver, Dept. of Molecular Biology, Edinburgh University, Edinburgh EH9 3JR, Scotland.
Partitioning regions which promote stable inheritance have been identified in several low copy number plasmids (1,2,3,). In an effort to determine whether analogous regions (which may be involved in its partitioning) are contained within the chromosome, a HindIII library of E.coli DNA in pBR325 was transformed into WT286, a strain in which pBR325 has a reduced copy number of 4-5. Since pBR325 has no partitioning system of its own, it is unstably inherited in this strain, a defect we hoped to correct by the insertion of an appropriate fragment of cloned DNA. In order to enrich for plasmids with increased stability, serial passages of the transformed cells were made. After six such passages, the dominant plasmid remaining contained a small HindIII insert. Cells containing this plasmid grow at the same rate as wild type cells and do not have an increased copy number; we therefore infer that possession of the insert improves the accuracy of plasmid partitioning. The insert is 129 bp long and hybridizes with single restriction fragments in chromosomal DNA digests prepared with EcoRI, BamH, HindIII or SalI, indicating that it originates from a single chromosomal location. Its sequence does not show homology to, or structural features reminiscent of, the partitioning region of phage P1(3). Refs: (1) Ogura, T. & Hiraga, S. (1983) Cell 32, 351-360. (2) Gerdes, K., Larsen, J. & Molin, S. (1985) J.Bact. 161, 292-298. (3) Austin, S. & Abeles, A. (1985) in "Plasmids in Bacteria" ed. D. Helinski et al. Plenum Press, pp.215-226.

I156 DNA HELICASES II & III OF E.COLI; ENZYMATIC PROPERTIES AS NTPases AND HELICASES, Steven W. Matson, Dianne M. Freund, James W. George, Elaine E. Lahue, Karen R. Smith and Edgar R. Wood, Dept. of Biology, Univ. of North Carolina, Chapel Hill, NC 27514

E. coli helicase II, product of the uvrD gene, has been characterized as a DNA-dependent NTPase and a helicase. The assay we have developed to characterize the helicase reaction measures displacement of a radioactively labeled DNA fragment annealed to M13 DNA. Helicase II interacts with duplex DNA stoichiometrically and catalyzes the unwinding of duplex DNA at a rate independent of the length of duplex. A kinetic analysis of DNA effector chain length on ATPase activity suggests that helicase II translocates processively along single-stranded DNA. To determine the direction of translocation, displacement of labeled DNA fragments from a linear single-stranded DNA molecule was measured using a DNA substrate with labeled DNA fragments annealed at each end of the linear DNA molecule. Only one of the DNA fragments was displaced, the one expected if helicase II translocates in a 3′ to 5′ direction. This direction of translocation is opposite to that previously reported [Kuhn et al. (1979) J. Biol. Chem. 254: 11343]. Although E. coli SSB inhibits helicase II ATPase activity on single-stranded DNA, SSB stimulates the helicase reaction.

Antibodies directed against E. coli helicase III have been produced and used to show that the 18.5kDa helicase III polypeptide contains active sites for both ATPase activity and helicase activity. In contrast with helicase II, a processive mechanism of translocation along single-stranded DNA cannot be demonstrated for helicase III. Helicase III does, however, catalyze displacement of DNA fragments as long as 343 nucleotides in a reaction that requires ATP hydrolysis. In addition, the helicase III gene has been cloned and sequenced.

I157 SEQUENCE ANALYSIS OF PHAGE T4 TERTIARY ORIGINS. A. E. Menkens[1,2] and K. N. Kreuzer[1] 1) Dept. of Microbiology and Immunology, Duke University Medical Center 2) Dept. of Microbiology and Immunology, University of Arizona Health Sciences Center

T4-mediated plasmid transduction provides a direct means of analyzing the DNA sequence requirements of T4 replication origins. Two specific origins that operate by a novel mechanism have been isolated and are referred to as tertiary origins. One origin [ori(34)] has been mapped to a 248 bp restriction fragment within the gene 34 reading frame, and contains a sequence similar to a T4 middle promoter. The second origin [ori(uvsY)] is located on a 120 bp restriction fragment that contains the middle-mode promoter for gene uvsY. Bal31 deletions have been generated to determine the minimal sequence necessary to maintain origin function. In both cases, the minimal origin is 50-100 bp in length and includes the promoter [ori(uvsY)] or promoter-like sequence [ori(34)]. The finding that the origins coincide with promoter sequences is intriguing, since tertiary origin replication is rifampicin resistant. Studies are in progress to further characterize the minimal tertiary origin and to understand the relationship between origin activity and the presence of promoter sequences.

I158 **ENZYMOLOGY OF TRANSCRIPTIONAL ACTIVATION OF BACTERIOPHAGE λ DNA REPLICATION, Kojo A. Mensa-Wilmot and Roger McMacken, Johns Hopkins University, Baltimore, MD 21205**

Initiation of bacteriophage λ DNA replication, both in vivo and in a crude soluble enzyme system (Wold et al. PNAS 79, 6176-6180, 1982), requires transcription by host RNA polymerase at or near the λ replication origin (oriλ). The capacity of the λ cI repressor to inhibit transciption of oriλ enables the repressor to directly block λ DNA replication, even when all required replication proteins are present.

We have recently reconstituted an enzyme system with 9 purified λ and E. coli proteins that supports the specific initiation of DNA replication at oriλ. Surprisingly, in this reconstituted replication system, transcription by RNA polymerase is not required for initiation of λ DNA synthesis. It seemed likely that this rifampicin-resistant λ replication system could be exploited to elucidate the biochemical mechanisms involved in the transcriptional activation process. We reasoned that RNA polymerase was functioning to counteract the effect of an inhibitor of λ DNA replication that was present both in vivo and in the crude in vitro system, but was absent from the enzyme system reconstituted with purified proteins. Accordingly, we have purified from crude extracts of E. coli a protein that inhibits the initiation of λ DNA replication when it is added to a reconstituted λ replication system that does not contain RNA polymerase. This inhibitor appears to be identical to the E. coli HU protein, a histone-like component of the bacterial nucleoid. Our preliminary experiments indicate that HU protein functions as an inhibitor of λ DNA replication by preventing formation of a prepriming nucleoprotein complex at oriλ that contains the λ O and P replication initiators and the host dnaB protein helicase.

I159 PARTITION AND REPLICATION OF pSC101, Christine Miller and Stanley N. Cohen, Department of Genetics, Stanford University School of Medicine, Stanford, Calif. 94305

What is the relationship of the par region, which is required for stable plasmid maintenance, to the replication of the plasmid pSC101? The two functions can be separated, but the complete wild-type par region on a plasmid gives it an advantage over a plasmid with a mutant par region. This advantage is observed during incompatibility studies when the plasmid with a mutant par region is always lost preferentially to the wild-type plasmid. The rate of loss is much greater than the loss with normal incompatible plasmids; it is so fast that colonies containing two plasmids can not form when selection for both is applied. A mutant in the par region does not affect the replication of that plasmid when it is alone in the cell, i.e. the copy number is normal and two par mutants are incompatible with each-other. However it seemed possible that when a wild-type plasmid was present the plasmid with a mutant par region could not replicate as well as the wild-type. We have called the phenotype of the par mutants Cmp^- for their inability to compete for replication with a wild-type, incompatible plasmid. Experiments have been performed to see if a par mutant can replicate at all in the presence of a wild-type plasmid. This data will be presented.

Tucker, W.T., C.A. Miller, S.N. Cohen, Cell 38, 191-201, 1984.

I160 STRUCTURE AND FUNCTION OF AN ORIGIN OF T4 DNA REPLICATION AND OF TWO PROTEINS CODED BY THIS REGION, Gisela Mosig, and Paul Macdonald, Vanderbilt University, Nashville, TN 37235

We have recently characterized and sequenced an origin of T4 DNA replication, *ori*A, and a new replication gene, *69*, that spans this origin. Gene *69* codes for two proteins, which can be translated from different messages that are under different transcriptional control(1). The region spanning *ori*A has been shown previously to associate with cell envelope components via protease sensitive sites(2).

A gene *69* amber mutant which is deficient in DNA replication, lacks a protein of the predicted size of gp69, but contains the entire coding sequence for the shorter protein. We show now that gp69 has a membrane-spanning domain. We suggest that gp69 aids in assembly and in anchoring a T4 DNA initiation complex to the cell envelope. We will discuss a model correlating its structure and function with prominent signals in the *ori*A sequence.

Segments of gp69 share homologies with different patches of several other proteins, most significantly with *E. coli* DnaA protein. This homology patch is located just downstream from the membrane spanning domain of gp69 and does not include the putative DNA binding domain of DnaA protein. Its presence suggests interactions with the same cellular component(s).

1. P.M. Macdonald and G. Mosig, EMBO J. 3, 2863-2871 (1984)
2. R.C. Marsh, A.M. Breschkin and G. Mosig, J. Mol. Biol. 60, 213-233 (1971)

I161 INITIATION SITES OF DNA SYNTHESIS IN THE PRIMARY ORIGIN REGION OF T7 PHAGE GENOME Tuneko Okazaki, Kenji Sugimoto, Yuji Kohara, Nagoya University, Nagoya, Japan 464

Initiation sites of T7 phage DNA synthesis in the presence and absence of T7 phage gene 4 primase have been analyzed using E. coli cells infected with T7 phage amber mutants, $T7_3$-,6- and $T7_3$-,4-,6-, respectively. In the absence of gene 4 primase, only the L-strand of T7 DNA is synthesized to the rightward direction. This L-strand DNA synthesis has been determined to start within the 0.9 kb region of T7 genome containing the primary origin and its right outside area. RNA-linked DNA molecules have been purified from the cells infected with $T7_3$-,4-,6- or $T7_3$-,6- phages and transition sites from RNA to DNA have been mapped in the 1.9 kb region of the T7 phage genome covering the primary origin and its vicinity. With gene 4^- sample, more than 20 transition sites have been detected, all of which have been found in the L-strand and scattered widely downstream the ϕ1.1A promoter of the primary origin. These sites have been found also with the gene 4^+ sample, suggesting that the transcripts arisen from the ϕ1.1 promoters are used as primers of the rightward L-strand DNA synthesis in both conditions. In addition to these sites, many strong transition sites have been detected with gene 4^+ sample in both the H- and L-strands at gene 4 primase recognition sites.

I162 INITIATION OF BACTERIOPHAGE T7 DNA REPLICATION, Samuel Rabkin and Charles Richardson, Department of Biological Chemistry, Harvard Medical School, Boston, MA. 02115.

T7 DNA replication is initiated at a specific site, located 15% of the distance from the genetic left end of the chromosome, and proceeds bidirectionally. When this region is deleted, initiation occurs at secondary origins. One approach to analyzing DNA replication in vivo is to pulse-label replicating T7 DNA and examine the pattern of incorporation by restriction endonuclease digestion and gel electrophoresis of the fragments. This procedure distinguishes between initiation events that occur at the primary origin and the secondary origins. Using this technique we have determined the rate of elongation in vivo to be greater than 400 nucleotides/second.

In order to more finely map the positions of the secondary origins we have examined the ability of plasmids containing different regions of the T7 genome to replicate after T7 infection. Plasmids containing the primary origin region, as well as those containing T7 promoters ϕOR, ϕ13 or ϕ6.5 do replicate after T7 infection, whereas those plasmids containing the other T7 promoters do not. The cloned T7 fragments that replicate are being deleted to determine the minimum sequence necessary for initiation of T7 DNA replication.

1163 **GENETIC AND BIOCHEMICAL STUDIES OF THE BACTERIOPHAGE T4 DNA POLYMERASE**, Linda J. Reha-Krantz, Department of Genetics, University of Alberta, Edmonton, Alberta T6G 2E9 CANADA

More than 20 new bacteriophage T4 DNA polymerase mutants have been isolated by a procedure designed to select for mutants with high spontaneous mutation rates. Some of the mutants produce the highest mutation frequencies that have been observed in T4 thus far. The design of the selection procedure allows for the isolation of mutator mutants that preferentially induce certain types of replication errors, and some of the mutator mutants have mutational specificities different from wild type.

The new mutants are clustered at just two sites in the DNA polymerase gene, and this result confirms an earlier observation (Reha-Krantz & Bessman (1981) *J. Mol. Biol.* 145, 677-695). These sites may define the 3'→5' exonuclease domain, the dNMP binding site and the primer-terminus binding site.

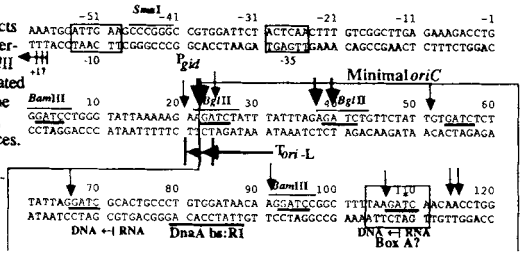
Biochemical studies of the mutator mutants will be useful in determining the molecular basis of accurate DNA synthesis, in discovering what alterations to the DNA polymerase decrease accuracy, in learning about the stuctural features of the T4 DNA polymerase and in understanding how the DNA polymerase synthesizes DNA. Because of the success of genetic and biochemical analyses in studies of the T4 DNA polymerase, the T4 DNA polymerase is a good model system for studies of prokaryote DNA polymerases in general.

Research supported by the Natural Sciences and Engineering Research Council of Canada (A8406, G1436), the Alberta Cancer Board (H-246), and the Alberta Foundation for Medical Research.

1164 TRANSCRIPTION TERMINATION EVENTS IN THE *Escherichia coli* ORIGIN OF REPLICATION, *oriC*, Luis A. Rokeach, David E. Junker Jr., Anne Chiaramello, Donna Ganea, and Judith W. Zyskind, Biology Department, San Diego State University, San Diego, CA 92182

The initiation of DNA synthesis at the chromosomal origin of replication, *oriC*, in *Escherichia coli* involves an RNA polymerase-mediated transcription step. Our studies using *galK* fusions have shown that counterclockwise transcription enters and terminates within *oriC*, with an efficiency of 92%. A similar approach led to the detection of a transcription terminator within the chromosomal replication origin of *Klebsiella pneumoniae*. The 3' termini of transcripts entering *oriC* were determined using the S1 nuclease mapping technique. The major termination event occurs at position 23, with minor terminations at positions 21, 25 ,38 ,40 ,52 , 67, 92, 107, 115, and 116 (see arrows in fig.). Most of the transcripts terminating within *oriC* contain the sequence GATC close to or at the 3' end. These results are supported by in vitro transcription experiments showing that RNA polymerase pauses at or very close to the sites identified by S1 mapping.

Analysis of putative secondary structures of *Tori*-L predicts the formation of several possible haipins, none with the properties of simple terminators, however. Deletion of a 16 bp *BglII* fragment with the potential to form a stem-loop structure located just upstream of the major 3' end of *Tori*-L, does not affect the termination efficiency nor the sites where termination occurs, indicating that termination is determined by upstream sequences. Efficient termination at *Tori*-L appears to be dependent upon Rhoand NusA proteins. Also, the appearance of shorter transcripts is observed in *dam* and *dnaA* mutants suggesting that these two gene products may be involved in termination at *Tori*-L.



1165 OVERPRODUCTION OF PHAGE T7 DNA POLYMERASE FROM RECOMBINANT PLASMIDS AND CHARACTERIZATION OF MUTANTS GENERATED *IN VITRO*, Nirmal K. Roy and David C. Hinkle, Department of Biology, University of Rochester, Rochester, New York 14627

T7 DNA polymerase contains two subunits, an 80 kDa protein encoded by the phage gene 5 and a 12 kDa host protein, thioredoxin. In the absence of thioredoxin the gene 5 protein has very low polymerase activity, but contains a 3' to 5' exonuclease which is very active with single stranded DNA. With thioredoxin this exonuclease is also active with double stranded DNA.

We have constructed a recombinant plasmid in which the T7 gene 5 is expressed from a phage lambda promoter which can be regulated by a temperature sensitive repressor. From 65 g of induced cells carrying this plasmid, 40 mg of nearly homogeneous T7 DNA polymerase are obtained in a simple two step purification. The enzyme prepared by this procedure appears to be identical to enzyme prepared from T7 infected cells by our previous methods. In particular, although we recover about twenty times more enzyme than is recovered from phage infected cells, the enzyme is saturated with thioredoxin.

To determine which regions of the T7 gene 5 protein are involved in each of the enzymatic activities associated with the DNA polymerase we have begun a project to produce and characterize altered forms of the protein. In our initial experiments we have made a series of deletions at the 3' end of the gene which result in changes to the C-terminus of the protein. All of these changes completely abolish DNA polymerase activity, but some of the altered proteins retain full exonuclease activity. This suggests that the C-terminus of the gene 5 protein may be located near the polymerase active site while the site for exonuclease activity is located elsewhere on the protein.

I166 TRANSCRIPTION SIGNALS WITHIN THE REPLICATION ORIGIN OF E. COLI, Marianna Schauzu, Walter Messer, Heinz Lother, Max-Planck-Institut, Berlin, Germany

Two promoters were detected in vitro within the minimal replication origin of E.coli (Lother and Messer, Nature 294, 376-378, 1981). We have analyzed these promoters in vivo by fusing various DNA fragments out of this region to the galactokinase gene and have detected weak promoter activities.

Transcription originated from the rightward promoter Pori-r was found to be terminated approximately 100 bp downstream from the promoter. Leftward transcription from Pori-l is not terminated to the same extent but is decreased in a stepwise manner as increasingly larger fragments are assayed.

Although promoter activities are slightly higher in a dnaA mutant strain compared to the isogenic wildtype, there is no evidence for a profound regulatory effect of the dnaA protein on the promoters within oriC.

I167 TIMING OF INITIATION OF CHROMOSOMAL REPLICATION IN E. COLI, Kirsten Skarstad, Erik Boye and Harald B. Steen, Dept. of Biophysics, Norsk Hydro's Institute for Cancer Research, 0310 Oslo 3, Norway.

Initiation of chromosomal replication is a key event in the bacterial life cycle. The frequency of initiation determines the growth rate of a steady state culture. When growth is rapid, 2 or 4 chromosomes are replicated in parallel within each cell. We have measured the synchrony of initiation at the 4 origins in individual cells. By flow cytometry we have determined the DNA contents in single cells after inhibition of initiation and run-out of replication. If initiation is synchronous all cells will end up with either 2, 4, or 8 fully replicated chromosomes, while asynchronous initiation leads in addition to cells with irregular numbers of chromosomes (3, 5, 6, or 7). The greater the degree of asynchrony the larger the amount of cells with irregular numbers of chromosomes. In strain B/r A (doubling time 27 min) initiation was found to be somewhat asynchronous (time from first to fourth initiation, J, was 3 min) when measured after rifampicin treatment, but essentially synchronous (J was less than 0.5 min) after chloramphenicol treatment. It was concluded that the DNA polymerase dependent step of initiation was less well timed than the step dependent on de novo protein synthesis. Initiation in a dnaA(Ts) mutant grown at the permissive temperature and in a recA mutant was found to be extremely asynchronous.

I168 COMPARISON OF THE dnaA GENES OF E. coli, S. typhimurium AND S. marcescens. Ole Skovgaard, Flemming G. Hansen, Technical University of Denmark, DK 2800 Lyngby.

The product of the dnaA gene, the DnaA protein, is essential for, and has a regulatory effect on, the initiation of DNA replication from oriC in E. coli. We have cloned and sequenced dnaA genes from S. typhimurium and S. marcescens, in order to obtain more information on the structure of the protein, and its autoregulated expression. Either of the genes complements the dnaA46 mutation of E. coli. The nucleotide and aminoacid sequences were compared. The three DnaA proteins are very homologous. The N-terminal 63 aminoacids and the C-terminal 333 aminoacids are identical. The region in between, corresponding to 71 aminoacids in E. coli exhibits a number of changes. This variable region coincides with the nonhomologous region found in the comparison of E. coli and B. subtilis DnaA proteins (Ogasawara et al. EMBO Journal, in press). Also the dnaA promoterregions are very homologous in E. coli, S. typhimurium and S. marcescens, suggesting, that the regulation of dnaA gene expression is identical in the three bacteria.

I169 A NEW DNA-DEPENDENT ATPase WHICH STIMULATES YEAST DNA POLYMERASE I AND HAS DNA-UNWINDING ACTIVITY MIGHT BE A RAD3 GENE PRODUCT. Akio Sugino and Toshiko Sugino, NIEHS, NIH, RTP, NC 27709

Two forms of DNA-dependent ATPase activities from yeast were previously purified and characterized (Plevani et al., 1980). Here, an additional DNA-dependent ATPase (ATPase III) has been purified from Sacchromyces cerevisiae to near homogeneity. This ATPase differs from those described previously in its chromatographic properties, molecular weight and reaction properties. Its molecular weight is about 63,000 daltons in the presence of SDS. It hydrolyzes ATP to ADP and orthophosphate in the presence of DNA as an effector. In addition, yeast DNA polymerase I, which is a true DNA replicase of yeast, is stimulated several-fold by this ATPase. Neither yeast DNA polymerase II, prokaryotic DNA polymerases, nor other eucaryotic DNA polymerases are stimulated. This stimulation is intrinsic to the ATPase activity, since both activities copurified in the last four steps of purification, showed the same heat stability and showed dependence on the hydrolysis of ATP. The ATPase III also contains a DNA-unwinding (DNA helicase) activity. In contrast to a yeast single-stranded DNA binding protein (Arendes et al., 1983), ATPase III increases the processivity of yeast DNA polymerase I only two-fold. However, it increases the accuracy of DNA polymerization several-fold. In the S. cerevisiae radiation-sensitive mutant rad3, no significant ATPase III activity could be detected, suggesting that the RAD3 gene codes or regulates the expression of ATPase III activity.

I170 SEQUENCE ANALYSIS OF THE *dnaE* GENE OF *Escherichia coli*, Henry Tomasiewicz and Charles McHenry, Department of Biochemistry, University of Colorado Health Sciences Center, Denver, CO 80262

The DNA polymerase III holoenzyme is the multi-subunit complex responsible for replicating most of the *E. coli* chromosome. The catalytic subunit, α (Mr=140,000 Da), is encoded by the *dnaE* gene. The *dnaE* gene was cloned using a selection based on its ability to complement a temperature-sensitive conditional lethal *dnaE* mutant. This plasmid, pMWE303, directs the synthesis of α in the "maxicell" system. The minimal sequence required for expression of the *dnaE* gene was determined to be 3700 base pairs by deletion mapping of pMWE303 using Bal31 nuclease. Plasmids containing deletions from one end of the *dnaE* gene direct the synthesis of shortened α proteins, while those containing deletions from the other terminus failed to direct the synthesis of any α protein. This information together with knowledge of the flanking gene allowed us to assign the direction of *dnaE* transcription toward increasing map units on the *E. coli* chromosome. To identify potential regulatory sites and to determine its amino acid sequence we have sequenced the *dnaE* gene. This sequence and the identification of the *dnaE* transcript will be presented.

I171 IN VITRO REPLICATION OF BACTERIOPHAGE λ DNA. Sue Wickner, Laboratory of Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20892

Replication of supercoiled plasmid DNA containing the λ origin of replication is catalyzed in vitro by protein fractions of uninfected E. coli supplimented with purified λ O and P replication proteins (Tsurimoto and Matsubara, 1982; Wold et al., 1982). Using this reaction as an assay I have been studying the components of the initiation reaction, including λ O and P proteins, E. coli dnaB, dnaJ and dnaK proteins and λ origin DNA. Tsurimoto and Matsubara showed that λ O protein binds to four closely spaced 19 bp repeated sequences in the origin region. The DNA sequence reveals a 40 bp region to the right of the O binding sites that is rich in adenine and thymine. To further define the limits of the λ origin, I have cloned a 354 bp piece of λ DNA containing the origin region into M13mp8 in both orientations, constructed deletions from the right and left with Bal31 nuclease and sequenced the deletions. I have tested the RF DNAs of the M13 derivatives for their ability to be replicated in in vitro reactions dependent on λ O and P proteins. M13mp8 was not replicated by these reaction mixtures but M13mp8 containing the λ origin DNA in either orientation was replicated. λ DNA between positions 39072 and 39160 was required for efficient O and P dependent replication. This piece of λ DNA includes the two right most 19 bp O binding sites and most of the adenine rich region to the right of the O binding sites.

I172 QUANTITATIVE MODELS FOR CONTROL OF PLASMID REPLICATION, David D. Womble and Robert H. Rownd, Northwestern University, Chicago, Illinois 60611

We have developed quantitative models for the regulation of replication of plasmids λdv, NR1, and mini-F in the Escherichia coli cell division cycle. The computer simulations of the control models accurately mimic the properties of each plasmid with respect to: (1) steady-state plasmid copy number; (2) recovery from unequal plasmid segregation at cell division; (3) recovery from an artificially elevated plasmid copy number; (4) response to regulatory components supplied in trans; (5) behavior of copy number mutants; (6) plasmid incompatibility; and (7) stability of plasmid inheritance in a population of cells in culture. The behaviors of the three models are quite different from each other. The models for NR1 and mini-F predict a stable pattern of plasmid inheritance despite the low copy numbers of these plasmids, whereas the λdv model predicts unstable inheritance of λdv despite its high plasmid copy number. Whereas the λdv model responds only slowly to random perturbations from the steady-state, the NR1 and mini-F models respond rapidly and recover quickly from any deviation from the steady-state. The mini-F model tends to regulate toward the normal (steady-state) plasmid copy number at the time of cell division, whereas the NR1 model regulates toward the normal (steady-state) number of initiations of plasmid replication during each cell generation.

DNA Recombination I: Prokaryotes

I173 DNA TOPOLOGY AND CHANGES IN LINKING NUMBER ASSOCIATED WITH CRE-MEDIATED LOXP SITE-SPECIFIC RECOMBINATION OF PHAGE P1. Kenneth Abremski, Beth Frommer and Ronald Hoess, E. I. Du Pont de Nemours and Company, Inc., Experimental Station 328/134, Central Research and Development Department, Wilmington, DE 19898

Site-specific recombination between two loxP sites results in changes to the DNA substrates which are observable as differences in DNA linking number and in the topological relationships of product molecules. We have analyzed these changes by gel electrophoresis and electron microscopy techniques. Our data indicate that Cre-mediated inversion reactions lead to a change in linking number of 2 between the substrate and product molecules. We have also demonstrated that supercoiling in the substrate DNA can influence the direction of this change. These results will be discussed in terms of models for synapsis of loxP sites and exchange of DNA strands during recombination.

I174 STUDIES ON THE MU TRANSPOSASE. R. ALAZARD, F. RAGUEH, M. BETERMIER, M. CHANDLER (C.R.B.G.C. 118 RTE DE NARBONNE, TOULOUSE, FRANCE); A. MARTENS, L. DESMET, M. FAELEN, A. TOUSSAINT (LAB. DE GENETIQUE, UNIVERSITE LIBRE DE BRUXELLES, RHODE ST GENESE, BELGIUM).

We are studying the product of the Mu A gene (transposase). Using a copy of the gene cloned in an expression vector we have observed that the 75kD pA can be cleaved in a semi in vitro system to yield a product of 64 kD. This truncated protein binds specifically to plasmid DNA carrying both ends of Mu and shows increased affinity for supercoiled DNA. These properties are similar to those of a protein which has been reported to occur in the phage capsid and which restrains the linear Mu genome in the form of a circle following infection.

In order to investigate the functional domains of pA, we have derived a set of Tn1000 insertion mutations in the cloned gene from which we have isolated in vitro a series of defined deletions. These are being applied to determine the DNA binding domain of the protein. We have also used these deletions to map by marker rescue a set of amber mutations carried by the phage. It is striking to note that one such mutation located in the C-terminal end does not abolish integration of the phage. This is similar to the observations of Chaconas and collaborators concerning the product of the B gene.

I175 CHARACTERIZATION OF TN3 RESOLVASE AND RES SITE INTERACTIONS, Howard W. Benjamin and Nicholas R. Cozzarelli, University of California, Berkeley, Berkeley CA 94720

The site-specific recombination enzyme, Tn3 resolvase, mediates the recombination between two directly repeated resolution (res) sites. We have characterized the interactions of resolvase with res sites by four methods: filter binding, change in linking number, gel electrophoresis and electron microscopy. Although two res sites are necessary for recombination and topoisomerase action, one is sufficient for specific binding. The binding of resolvase to a res site on a small ΔTvx plasmid containing res sites in the presence of calf thymus topoisomerase I or ligase acting on a nick introduces just less than one negative turn per site. The number of turns is proportional to the number of sites in one, two and three site plasmids and is similar for both supercoiled and nicked substrates. However, synapsis of res sites introduces about an additional turn for a total of approximately three turns as predicted by our prior studies. The synapsed complex can be cut by restriction enzymes and analyzed by both gel electrophoresis and electron microscopy. A major portion of the DNA is specifically synapsed by resolvase at the res sites before recombination and the data lead to a clear model for the structure of a synaptic intermediate.

I176 THE ROLE OF TERMINAL CONFORMATION IN ADENO-ASSOCIATED VIRUS DNA REPLICATION, Kenneth I. Berns[1] and Roy Bohenzky[2], Department of Microbiology, Cornell University Medical College, New York, NY 10021 and 2) Department of Immunology and Medical Microbiology, University of Florida, College of Medicine, Gainesville, FL 32610.

The linear single-stranded DNA genome of the defective human parvovirus adeno-associated virus (AAV)2 has an inverted terminal repetition of 145 bases. The terminal 125 bases form an overall palindrome interrupted by two internal 21 base palindromes. This potential hairpin structure is assumed to serve as the primer for DNA synthesis. As long as a potential template exists, large deletions within the terminal repeat can be repaired, but in the absence of such a template, even small deletions are lethal. However, mutants in which the potential T-shape of the hairpin are retained are viable. We have now compared chimeras with a wild type sequence at one end and a mutant sequence at the other. Progeny are found to have the wild type sequence at both ends.

I177 Recombination as a probe of DNA structure and metabolism in vivo, James B. Bliska Yishi Jin, and Nicholas R. Cozzarelli, University of California, Berkeley CA 94720

Site-specific recombination catalyzed by the phage λ Int system can be used as a probe of DNA structure and metabolism in vivo. Strains of E. coli inducible for Int expression and transformed with small multicopy plasmid substrates allow us to tightly regulate recombination. The reaction in vivo is very fast; we estimate the rate at 1 plasmid recombined per cell per minute. In vitro, product complexity is proportional to supercoil density and the structure of products suggest that free supercoils in plasmid DNA are braided, rather than solenoidally wound as in nucleosomes. We find that the reaction in vivo has these same properties, allowing us to calibrate the level of free supercoils inside the cell. This level is about one-half that found in purified DNA. We suggest that the other half of the supercoils are constrained by proteins into alternative structures. Thus there is substantially less free energy of supercoiling available to drive processes such as transcription than would be predicted from the linking deficit. Lastly, the catenated products made by recombination provide an assay for enzymes reqruired to unlink daughter molecules at the termination of replication. Our results confirm that DNA gyrase is the only enzyme required for decatenation in E. coli, and it does so extremely rapidly. We further conclude that decatenation in vivo is energetically favorable and that the final segregation of daughter plasmids does not require any specialized apparatus.

I178 TOPOLOGICAL SPECIFICITY OF TN3 RESOLVASE SUGGESTS A TWO-STOP MECHANISM FOR SYNAPSIS OF RECOMBINATION SITES, Martin R Boocock, J Lesley Brown, David J Sherratt, Inst of Genetics, University of Glasgow, Glasgow, Scotland

Processive one-dimensional diffusional "tracking" of resolvase along DNA sequences intervening between two recombination sites does not provide a satisfactory mechanistic basis for the observed topological specificity of Tn3 site-specific recombination. We have put forward an alternative "two-step" synapsis model that gives due consideration to substrate and product topology, and also accounts for the inefficiency of the intermolecular recombination and inversion reactions (Boocock et al, Biochem Soc Transac, in press). Our kinetic data on the resolution of multisite recombination substrates, and on the sequence of DNA conformational changes attendant upon resolvase binding at the res site, are fully consistent with the general consequences of a two-step synapsis mechanism. The effects of substrate topology on the course of the reaction are in accord with predictions that are unique to the two-step model. We have also shown that under certain conditions all topological linkage changes in the resolution reaction are precisely specified; these results place further constraints on the choice of models for site synapsis and the molecular mechanics of strand exchange. We are presently intrigued by the possibility that basic mechanistic features of the site synapsis and strand exchange events mediated by Tn3 resolvase are shared by integrase and other site-specific recombination enzymes.

I179 RESOLUTION OF A POLYOMA/MOUSE HYBRID DNA AND ITS DEPENDENCE ON LARGE T ANTIGEN, Pierre Bourgaux and Alain Piché, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1H 5N4.

RmI is a chimeric molecule which includes 1.03 copies of polyoma virus DNA and a segment of 1628 base pairs of mouse DNA, joined together through direct and indirect repeat sequences. It is excised from the chromosome of a transformed cell via a site-specific recombination event when the viral gene coding for large T antigen is activated. We have transfected molecularly cloned RmI into cells of various origins and analyzed its fate. We found that RmI is infectious for normal mouse cells, as it undergoes an intramolecular recombination event generating genomic viral DNA, which in turn directs the synthesis of virus progeny. Large T antigen is required for the conversion of RmI into genomic viral DNA, but replication of RmI appears to hinder rather than enhance this conversion. These findings may indicate that large T antigen mediates not only the excision but also the resolution of RmI into its mouse and polyoma constituents.

I180 THE OLIGONUCLEOTIDE LENGTH DEPENDENCE OF THE RecA ATPASE: EVIDENCE FOR HYDROLYSIS ON INTERNAL SUBUNITS IN COOPERATIVELY BOUND RecA OLIGOMERS.

Stephen L. Brenner and Richard S. Mitchell, E. I. du Pont de Nemours & Company, Central Research & Development Department, Experimental Station, Wilmington, DE 19898.

The ssDNA-dependent ATPase of RecA protein has been studied as a function of DNA chain length using homopolymer cofactors. Using poly(dT), a maximal turnover number of 30 ATP/RecA/min was found at 37°C in 10 mM HEPES, 1 mM DTT, 30 mM NaCl, 1 mM ATP, 12 mM $MgCl_2$, 50 µg/ml BSA, pH 7.5. The maximum rate was observed at a stoichiometry of 1 RecA per 4 bases. The maximum turnover number decreased with decreasing oligonucleotide length to 13, 2.0, and 0.5 ATP/RecA/min for $(dT)_{25-30}$, $(dT)_{20}$, and $(dT)_{16}$, respectively. The stoichiometry at maximal ATPase was always 1 RecA/4 bases independent of oligonucleotide length. At a fixed RecA concentration, the ATPase increased with DNA concentration until sufficient DNA was present to bind all of the RecA; increasing the DNA concentration beyond that point had no effect. A simple interpretation of these results is that only bound RecA hydrolyzes ATP, and that internal subunits on the cooperatively assembled RecA oligomers are more efficient at hydrolysis than the terminal subunits. This is in contrast to the expected result if ATP hydrolysis was coupled to RecA subunit on/off events at the end of the DNA-bound RecA oligomers. In the latter case, decreasing the oligonucleotide length would be expected to increase the ATPase due to the increased number of RecA polymer ends.

1181 COMPARATIVE PROPERTIES OF THE recA AND recA1 PROTEINS OF E. COLI, F. R. Bryant and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

We have carried out a comparative study of the properties of the wild type recA protein and the mutant recA1 protein in an effort to clarify the ATP requirement of the recA protein-promoted renaturation of complementary ssDNA molecules. Both proteins bind to ssDNA with a stoichiometry of one monomer per ~4 nucleotides as determined by a nuclease protection assay. Unlike the wild type recA protein, the recA1 protein has no ssDNA-dependent ATPase activity and, furthermore, is dissociated from ssDNA in the presence of either ATP or ADP. ATPγS appears to stabilize the binding of recA1 protein to ssDNA but does not elicit the two-fold increase in protection stoichiometry or the formation of highly condensed protein-DNA networks that was found for recA protein. The recA1 protein has no renaturation activity in the presence of ATP, consistent with the dissociation of recA1 protein from ssDNA under these conditions. However, recA1 protein does promote a pattern of Mg^{2+}-dependent renaturation reactions that is identical to that found for recA protein. These results will be incorporated into a general model for the recA protein-promoted pairing of complementary DNA strands.

1182 TRANSPOSITION OF THE INSERTION SEQUENCE IS1: ANALYSIS OF SITES AND PROTEINS. M. Chandler+, P. Gamas+, D. Zerbib+, P. Prentki* and D. J. Galas*, C.R.B.G.C. 31062 Toulouse, France+, Molecular Biology, U.S.C., Los Angeles, CA 90089-1481, U.S.A.*

IS1 carries imperfect inverted repeats of about 23 bp at its extremities and exhibits a complex array of open reading frames which may be expressed by transcripts from promoters at its ends. We have defined the minimal sequence of the left extremity necessary for transposition by successive deletion. The minimal sequence is about 23 bp. A sequence exhibiting striking homology to the consensus binding site for the protein IHF (required for integrative recombination of phage λ) abuts the inside edge of the required end sequence. This sequence is located between the -35 and -10 regions of both promoters. Footprinting experiments have demonstrated that IHF binds the extremities of IS1 and protects a region within that protected by RNA polymerase. The possible roles of IHF in the behavior of IS1 are being investigated. Studies using an expression vector have been successful in revealing the presence of only one of the six largest potential polypeptides, the product of the insA gene. Its primary structure, derived from the DNA sequence, indicates that it is a very basic protein of 9.9 kd, and exhibits properties of the αhelix-turn-αhelix motif characteristic of many site-specific DNA binding proteins. Its expression and role in IS1 transposition is under investigation.

1183 SELECTION OF RECOMBINATION-DEFICIENT MUTANTS OF *STREPTOMYCES LIVIDANS* TK64, Carton W. Chen and Fong-Ying Tsai, Institute of Microbiology and Immunology, Yang-ming Medical College & Panlabs Fermtech Div., Panlabs Taiwan, Ltd., Taipei, Taiwan, R.O.C.

A multi-copy shuttle vector, pPF132, was constructed from a *Streptomyces* plasmid vector pIJ702 (containing thiostrepton-resistance gene, *tsr*, and melanin gene, *mel*) and an *E. coli* vector pUC9. The *tsr* marker was flanked by a faulty and a functional *mel* sequence on each side in the same orientation. When this plasmid was introduced into *S. lividans* TK64 cells, intramolecular recombination occurred between the *mel* direct repeats, resulting in the deletion of a 2.7kb segment containing a functional *mel* gene and the *tsr* gene, thus rendering the host Mel^- and $Thio^S$.

This plasmid was used to transform protoplasts from mutated *S. lividans* TK64 cells. Tentative recombination-deficient (Rec^-) mutants were selected based on their persistant Mel^+ phenotype among the subcultures. Plasmid were isolated from these cultures and analyzed. From 827 transformants 2 Rec^- mutants, JT8 and JT23, exhibited drastic reduction in the intramolecular recombination. Both mutants grow poorly and produce no pigment or spores. Furthermore, JT8 is auxotrophic for arginine, typical for the so-called 'bald type' spontaneous variants resulting from chromosomal deletions. JT23 is also an auxotroph with yet unidentified nutrient requirements.

In *E. coli*, recombination between the *mel* repeats on pPF132 was rare, especially in the *recA* mutants. Oligomers of the plasmid were abundant in *E. coli* but very few if any in *S. lividans* TK64, regardless of their Rec phenotypes.

I184 DISTRIBUTION OF CHI-STIMULATED EXCHANGE IN PHAGE LAMBDA, Keith C. Cheng and Gerald R. Smith, Fred Hutchinson Cancer Research Center, Seattle, WA 98104 and Dept. of Pathology, University of Washington.

Chi recombinational hotspots stimulate recombination by the RecBC pathway of Escherichia coli. In our current model of Chi-stimulated recombination (1), RecBC enzyme unwinds DNA from right to left and, after cutting 4-6 nucleotides to the right of Chi, generates a ssDNA tail extending from Chi to the left. This model predicts that half of the Chi-stimulated genetic exchanges should be about five nucleotides to the right of Chi and half to the left of Chi. We have determined the distribution of Chi-stimulated exchange with high resolution using multiple genetic markers at known distances from Chi in phage lambda crosses in E. coli. In contrast to the prediction, about 30% of the exchanges occur to the right of Chi and about 50% occur farther than 0.6kb to the left of Chi. These results can fit the model if the "Chi tail" is degraded by a nuclease before its synapsis with a homologue. We have studied the effect of underexpression and overexpression of nucleases and other DNA metabolism functions on the distribution.

1) abstract by Smith et al., this meeting.

I185 STRAND EXCHANGE VIA CONTACTS OF RecA PROTEIN WITH DNA STRANDS OF THE SAME POLARITY Samson A. Chow, Saul M. Honigberg and Charles M. Radding, Yale University School of Medicine, New Haven, CT 06510

RecA protein polymerizes on single-stranded DNA (plus strand) to form a presynaptic nucleoprotein filament that pairs with linear duplex DNA to form a nascent heteroduplex. Under appropriate conditions, the presynaptic filament actively displaces the plus strand from the recipient duplex molecule in a polarized fashion (5' → 3'). The exact location and possible movement of RecA protein in a joint molecule during exchange process are not known. In this study, the interaction between RecA protein and DNA during strand exchange was examined by labeling different strands and probing the intermediate with pancreatic DNase I or restriction endonuclease. The incoming single strand was resistant to DNase I in the original nucleoprotein filament and remained resistant even after extensive strand exchange had occurred. Both strands of the parental duplex molecule were sensitive to DNase I in the absence of joint molecule formation; but as strand exchange progressed following homologous pairing, increasing stretches of the parental plus strand became resistant, whereas the complementary parental minus strand remained sensitive to DNase I throughout the reaction. Except for a region of 50-100 base pairs at the end of the newly formed heteroduplex DNA where strand exchange was initiated, the rest of the heteroduplex region was resistant to cleavage by restriction endonucleases. The data suggest that RecA protein promotes strand exchange by binding both the incoming and outgoing strands of the same polarity, whereas the complementary strand, which must switch pairing partners, is unhindered by direct contact with the protein.

I186 The Relationship Between sbcA mutations and recE in Escherichia coli. Charles C. Chu and Alvin J. Clark. University of California, Berkeley 94720

The product of the recE gene is involved in a recBrecC independent pathway of conjugative recombination and a recA independent pathway of plasmidic recombination. In wild type E. coli, recE is normally unexpressed. sbcA mutations turn on the recE gene, suppressing the RecBC$^-$ phenotype (recombination deficiency, UV sensitivity and MitomycinC sensitivity).

A 7.65 kb DNA fragment containing the sbcA and recE genes was cloned. As expected, sbcA$^+$ clones do not suppress the RecBC$^-$ phenotype, whereas sbcA$^-$ clones do. The sbcA mutation and the 5' end of the recE gene have been further localized to a 2.47 kb DNA fragment. The 2.47 kb sbcA$^+$ DNA fragment was sequenced. Three significant open reading frames (ORF's) were observed. One we call racC encodes a small 10 kDa protein. 103 bp downstream from racC, another ORF encodes a 68 kDa protein. We think this is sbcA. The 5'end of the third ORF overlaps 68 bp of the 3' end of sbcA. This ORF continues through the end of the fragment. The DNA from 5 small deletion mutations (de(sbc)188-192) was also sequenced. These mutations all suppress the RecBC$^-$ phenotype. Two deletions, de(sbc)189 and de(sbc)190, fuse the 5'end of the sbcA ORF with the third ORF. The other three deletion mutations, de(sbc)188, de(sbc)191, and de(sbc)192 fuse the 5'end of the racC ORF with the third ORF. These results support our hypothesis that the third ORF is recE. We predict that sbcA point mutations work by causing a frameshift between sbcA and recE in the 68 bp overlap. Experiments to test this hypothesis are underway.

1187 STUDIES ON FLP PROTEIN-PROMOTED SITE-SPECIFIC RECOMBINATION, Michael M. Cox, Janet M. Attwood, Robert C. Bruckner, Leslie Meyer-Leon, Julie F. Senecoff, and Elizabeth A. Wood, Department of Biochemistry, School of Agriculture and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706.

Utilizing extensively purified FLP protein, the kinetic and physical characterization of this eukaryotic recombination event has been initiated. Most progress has been made in defining interactions of FLP protein with its recombination site. The site consists of 24-28 base pairs including an 8 base pair unique spacer sequence flanked by inverted repeats. FLP protein binds to the DNA repeats with prominent contacts in the major groove, cleaves the DNA at the boundaries of the spacer, and becomes covalently linked to the DNA via a 3'-phosphate. Spacer sequences in two sites undergoing recombination must be homologous, but virtually any sequence is tolerated as long as this is true. Spacer size can be varied by ± 1 base pair with only modest decreases in the efficiency of recombination when the reaction is restricted to homologous sites. In each case, FLP protein cleaves the site at the boundary of the spacer leaving 9 or 7 base protruding ends instead of 8--providing further evidence that the protein interacts with the repeats rather than the spacer. The site asymmetry which determines reaction directionality (e.g., inversion vs. deletion) is located in the spacer. Recombination sites with symmetrical spacers function well in reactions with identical sites, but directionality is lost.

1188 TRANSPOSITION OF Tn7, Nancy L. Craig, Lidia Arciszewska, Robert McKown, and Candace S Waddell, Univ. of Calif., San Francisco, San Francisco, California 94143

Tn7 is a 14 kb bacterial element that encodes resistances to trimethoprim, spectinomycin and streptomycin. Tn7 has the unique feature of transposing to a specific site in the E. coli chromosome, attTn7. Transposition to this site occurs at high frequency and insertions are in a single orientation. Tn7 also transposes to plasmids at low frequency and with little site specificity. We are defining the DNA sequences required for attTn7 activity and the Tn7 sites and proteins involved in transposition. We have shown that a 0.5 kb chromosomal fragment containing the site of Tn7 insertion has attTn7 activity. Interestingly, a smaller fragment which contains about 40 bp to either side of the point of insertion has no activity. This suggests that attTn7 may be a complex site. We have shown that Tn7 derivatives containing approximately 200 bp from each end of Tn7 transpose efficently when provided Tn7-encoded proteins in trans. We have found that the ends are not functionally equivalent. Derivatives containing two Tn7 right ends transpose while elements composed of two Tn7 left ends do not. We have also defined two other activites of the ends of Tn7: 1) the ends in cis confer upon a target molecule immunity to subsequent Tn7 insertion and 2) the ends, when in trans to both Tn7 and a target molecule, inhibit Tn7 transposition. Several Tn7-encoded proteins are required for transposition. We are identifying and characterizing these proteins thru insertional mutagenesis.

1189 DNA REARRANGEMENTS IN Escherichia coli toc MUTATIONS, Richard E. Depew and Virginia Purcell, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272

Mutations that compensate for the loss of DNA topoisomerase I have been found in Escherichia coli topA mutant strains. These compensatory mutations occur in three distinct loci, gyrA, gyrB, and toc. The toc mutations map near the tolC gene at 66.5 min. on the linkage map of E. coli. Genetic analysis of the toc mutations have led our group to suggest that the toc mutations are tandem genetic duplications, and that these mutations may activate expression of the wild-type toc allele by recruiting a strong promoter. We now report preliminary results from our study of the physical structure of the toc mutations. We probed Southern blots of restriction enzyme-digested DNA with plasmids containing a cloned tolC gene (generously provided by Peter Reeves). Three of five toc mutants gave new restriction fragments in addition to the wild-type fragments. This result shows that these mutants have DNA rearrangements consistent with tandem genetic duplications, and suggests that the wild-type toc gene is located on the same EcoRI fragment as the tolC gene. The plasmid carrying this fragment does not confer a compensatory phenotype on non-compensatory host strains, showing that the compensatory phenotype provided by the toc mutations is not a simple gene dosage effect.

I190 CHARACTERIZATION OF MUTATIONS IN THE TERMINAL INVERTED REPEATS OF THE INSERTION SEQUENCE IS903. Keith M. Derbyshire, Leon Hwang and Nigel D.F.Grindley. Yale University, New Haven, CT 06510

Tn903 is a composite transposon consisting of two identical IS903 elements that lie in inverted repeat orientation on either side of a DNA segment encoding resistance to kanamycin. IS903 is 1057bp long, encodes a protein of 307 amino acids which is required for transposition, and is flanked by two 18bp inverted repeats. These two 18bp inverted repeats are the only elements required in cis for transposition. We assume that the transposase protein first specifically recognises these repeats to establish a transpositon complex, followed by cutting and ligation of the termini to the target, generating the final products of transposition. We decided to investigate this interaction further by studying the effects on transpositon of changes in the inverted repeats. To do this we have developed a novel oligo - directed mutagenesis procedure that is capable of saturating a segment of DNA with all possible nucleotide changes. This has allowed us to generate a comprehensive range of single - base substitutions in the inverted repeats. We have begun characterizing the effect of these mutations and have shown that certain mutations reduce transposition, their further characterisation will be presented.

I191 ANALYSES OF THE ROLES OF UmuDC AND MucAB IN MUTAGENESIS, Lori A. Dodson, Lorraine Marsh, David I. Sobell and Graham C. Walker, Massachusetts Institute of Technology, Cambridge, MA 02139

Many conditions that damage DNA or inhibit DNA replication induce a variety of cellular SOS responses in E. coli that include enhanced capacity for DNA repair and mutagenesis. The process of inducible mutagenesis specifically requires the products of the umuDC operon or the analogous plasmid-derived operon mucAB. Analysis of the deduced amino acid sequences of UmuD and MucA revealed that these mutagenesis proteins share homology with the C-terminal domains of LexA (the repressor of the SOS response) and the repressors of phages lambda, 434, and P22 (Perry et al., PNAS 82, 4331, 1985). We are investigating the functional significance of this homology in part by oligonucleotide-directed mutagenesis of cloned umuD/mucA. We initially changed the putative cleavage site of MucA from ala-gly to ala-glu and compared the phenotypes of strains carrying the wild type or mutant gene. The results suggest that MucA interacts with activated RecA and may contribute to the specificity of mutagenesis. Studies with umuD mutants are in progress. Our biochemical approach to this problem includes protein affinity chromatography to characterize the interactions of cellular proteins with RecA. However, we have not yet found conditions in which the Umu/Muc proteins are specifically retained by a column prepared with wild type RecA.

I192 Inhibition of synapsis of site-specific recombination by the Tn3 resolvase. Michael Fennewald and Roland Saldanha, University of Notre Dame, Notre Dame, Indiana 46556.

Models on the mechanism of site-specific recombination by the Tn3 resolvase call for resolvase to contact the DNA between the two recombination (res) sites to achieve synapsis. We have found that lac repressor protein bound between res sites inhibits both the recombinase and topoisomerase activities of resolvase in vitro. This inhibition requires a wild-type lac operator and is reversed by IPTG. We have constructed a plasmid with three directly-repeated res sites, one of which is bounded by lac operators. Site-specific recombination at the res site bounded by the lac operators is inhibited by the lac repressor, but the two other res sites are fully active. We have also detected inhibition in vivo by the lac repressor. This requires a lac operator between res sites and the overproduction of the lac repressor protein inside the cell. These results show resolvase must contact the DNA between res sites to achieve synapsis.

I193 A PARTIALLY PURIFIED ENZYMATIC SYSTEM THAT CATALYZES THE REPAIR OF HETERODUPLEX DNA FROM ESCHERICHIA COLI, Richard Fishel, Chas A Dana Res Inst & the Dept of Medicine, Beth Israel Hospital & Harvard Medical School, Boston, MA 02215.

The resolution of heteroduplex DNA generated during the process(es) of genetic recombination results in gene conversion and map expansion. We have constructed and purified heteroduplex DNA substrates that contain physical and genetic markers that can be detected in vivo and in vitro. Three pathways in E. coli have been identified that recognize and repair these heteroduplex DNA substrates. The Dam-instructed repair pathway, identified by others, appears to be uniquely responsible for the specific excision of erroneously inserted nucleotides by recognizing transient DNA adenine undermethylation following DNA replication. Additionally, there are two pathways that recognize and repair fully dam-methylated heteroduplex DNA similar in structure to the heteroduplex DNA formed during genetic recombination and mutagenesis. These two pathways are distinguished by their genetic requirements and the length of their associated excision/resynthesis tracts: (1) the co-repair pathways excises several thousand base pairs and requires the mutS and uvrD gene products, and (2) the independent repair pathway excises less than 300 nucleotides and requires the recF, recJ and recO gene products. We have developed a partially purified enzymatic system that catalyzes the repair of heteroduplex DNA and requires the mutS and recF gene products. The mutS protein has been purified and found to complement mutS extracts. Experiments detailing the heteroduplex DNA repair reaction, in vitro complementation results, some physical characteristics of the heteroduplex DNA reaction and the heteroduplex DNA substrate, and their relationship to general genetic recombination, will be presented.

I194 THERMOPHILIC DNA TOPOISOMERASE (REVERSE GYRASE) AND DNA POLYMERASE FROM THE ARCHAEBACTERIUM SULFOLOBUS ACIDOCALDARIUS. °P. Forterre, °C. Elie, †M. Nadal, †C. Jaxel, °S. Mouldy, †G. Mirambeau, †M. Duguet.
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We present purification and characterization of thermophilic DNA polymerase and topoisomerase from the archaebacterium Sulfolobus acidocaldarius. Both enzymes are monomer with size around 100 Kd for the DNA polymerase and 130 Kd for the DNA topoisomerase.

The DNA topoisomerase introduces positive supertwists in relaxed circular DNA at the expense of ATP (reverse gyration). In presence of PEG, the reaction product exhibits a very high superhelical density (positive form I DNA). Reverse gyration occurs at a low protein/DNA ratio showing that the reaction is not stoechiometric. Surprinsingly, reverse gyrase is a type I DNA topoisomerase; this is the first enzyme of this class which exhibits ATP-dependance and gyrase activity.

In vivo and in vitro studies with DNA polymerase and topoisomerases inhibitors show that several types of these enzymes exist in archaebacteria, some of them sharing features with their homologous eukaryotic or eubacterial counterparts, other with unique feature such as reverse gyrase.

I195 EFFECT OF OXOLINIC ACID ON THE CONTROL OF SUPERCOILING, Robert J. Franco and Karl Drlica, Public Health Research Institute, 455 First Ave., New York, N.Y. 10016

Oxolinic acid is a potent inhibitor of DNA gyrase and DNA replication. We have been studying a topoisomerase mutant which is partially resistant to oxolinic acid and which shows an increase in DNA supercoiling when treated with the drug. We find that gyrase expression also increases, accounting for the increase in supercoiling. Plasmids containing gyrase promoters fused to the galK structural gene also exhibit an oxolinic acid-induced increase in supercoiling, but no change in galK expression was detected. To explore the possibility that factors other than supercoiling are involved in the control of gyrase gene expression we started to search for oxolinic acid-induced DNA cleavage near the gyrase genes. Preliminary observations suggest that there may be a preferred site of cleavage upstream from the gyrB gene.

I196 THE INCLUSION OF HOMOLOGOUS DNA IN COAGGREGATES OF DNA AND RecA PROTEIN BY POLYVALENT CATIONS AND HETEROLOGOUS DUPLEX DNA: EVIDENCE THAT COAGGREGATES ARE INTERMEDIATES IN HOMOLOGOUS PAIRING. David K. Gonda, Samson A. Chow and Charles M. Radding, Departments of Molecular Biophysics and Biochemistry and Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510. Under conditions that support homologous pairing, recA protein promotes the mutually dependent aggregation of single- and double-stranded DNA to form "coaggregates" of DNA and protein which appear to be intermediates in homologous pairing (S. S. Tsang, S. A. Chow and C. M. Radding (1985) Biochemistry 24, 3226-3232; S. A. Chow and C. M. Radding (1985) PNAS 82, 5646-5650). Consistent with previous results (D. K. Gonda and C. M. Radding (1983) Cell 34, 647-654), duplex DNA 3812 bp long that shared 102 bp of homology with single-stranded DNA was paired more efficiently to the single-strands by recA protein than a DNA fragment 876 bp long that shared the same 102 bp of homology. Direct measurement of coaggregates showed that the longer duplex DNAs support a correspondingly higher level of coaggregation. The addition of heterologous duplex DNA or polyvalent cations stimulated the inclusion of DNA into coaggregates in reactions containing the small duplex DNA, and in parallel eliminated the effect of duplex DNA size on homologous pairing. The above observations support the view that coaggregates are intermediates in homologous pairing, and show that coaggregates act by concentrating the DNA molecules into a limited volume to allow the efficient pairing of homologous sequences.

I197

E. COLI MUTANTS THAT AFFECT TRANSPOSITION, Thomas J. Griffin and Mike Syvanen, Harvard Medical School, Boston, MA 02115

E. coli contributes a number of factors that promote transposition of transposons and insertion sequences. We have been identifying these factors by isolating E. coli mutants in which Tn5 transposition is deficient.

The mutant phenotype is screened by one of two papillation assays that rely on activation of silent sugar-utilizing operons by transposition of insertion sequences. 1.) The Bgl papillation assay involves transposition of either IS1 or IS5 into the promoter region of the Bgl operon causing its activation. 2.) A lactose papillation assay uses a prophage that contains a promoter-less lacZ gene. Lac$^+$ papillae arise by transposition of an IS50 into the region upstream of the lacZ gene.

We have been characterizing two classes of mutants that were isolated using these screens and are also deficient in Tn5 and Tn10 transposition. Mapping results show that two of the mutants, tnd11 and A8, are linked to the tna locus at 83.4 minutes on the E. coli chromosome. Another mutant, tnd36, is linked to ilv at 84.5 minutes.

I198 VISUALIZATION OF PARANEMIC JOINING BETWEEN TWO PARTIALLY HOMOLOGOUS DNA MOLECULES. Jack D. Griffith and Gunna Christiansen, The Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, North Carolina, 27514.

Synapsis catalyzed by the RecA protein of E. coli between two DNA molecules is believed to occur even in the absence of free homologous DNA ends, involving a metastable interaction termed paranemic joining. We have used direct electron microscopic method to visualize the joining of supertwisted M13 double stranded DNA and linear M13mp7 single stranded DNA that contains non-M13 sequences at its ends and thus would be blocked from plectonemic joining (D-loop formation). We observed joining at high frequency when the single stranded DNA was first assembled into presynaptic filaments with RecA protein, and the joints were shown to be at sites of shared homology. Examination of the topology of the double stranded DNA and single stranded DNA partners showed that the double stranded DNA in the region of joining must be unwound by 34 degrees per base pair and that this unwinding is not compensated by for by an opposite superhelical twisting of the DNAs outside tne joint. One model that would account for this new data is one in which the duplex DNA in the joint is in an alternating B and Z conformation.

1199 ANALYSIS OF MUTANTS OF γδ RESOLVASE IN VITRO, Graham F. Hatfull and Nigel D. F. Grindley. Yale University, New Haven, CT 06510

γδ resolvase mediates a site-specific recombination between the two copies of the site res in a cointegrate molecule. The 20.5 Kd resolvase protein is probably active as a dimer, one dimer binding to each of the three sites that constitute res. DNA strand cleavage and religation occurs at the crossover point at the centre of site I. We have isolated a number of mutants in the resolvase gene that are defective in the recombination function, some of which have also lost repressor activity in vivo. A number of the mutant resolvase proteins have been purified for characterization in vitro.
Analysis in vitro has shown that substitution of serine 10 by leucine results in a specific defect in the interaction of the mutant resolvase with site I. The interaction with sites II and III is similar to wild-type suggesting that serine 10 is in close proximity to the DNA in site I, perhaps at the crossover point. These results are consistent with the hypothesis that serine 10 is an active site residue.

1200 ENHANCEMENT OF HOMOLOGOUS RECOMBINATION OF THE E. COLI CHROMOSOME BY A P1 PHAGE FUNCTION. Brad Windle, Craig Laufer, Tim Schaefer, and John Hays, University of Maryland Baltimore County, Catonsville, MD 21228

A P1 bacteriophage function, designated Ref, enhanced recombination between two lac⁻ genes in the E. coli chromosome 100-fold, when expressed from a lambda-P1:ref⁺ hybrid prophage containing P1 BamHl fragment 8. Ref stimulated RecA-dependent lac⁻ x lac⁻ recombination via either the RecBC or RecF pathways, but did not affect other phage, plasmid, or transductional recombination via these pathways. ref::Tn5 mutants of P1 grew and formed lysogens normally. Although ref expression was repressed in wild-type P1 prophages, P1 cl.100 mutants showed partial expression. Substantial ref depression occurred in a P1::Tn5 mutant in which the insertion is just upstream from the ref gene, and also as a result of two P1 regulatory mutants, bof and lxc. The latter mutations also derepress a putative P1 analog of E. coli single-stranded-DNA-binding protein. The ref regulatory and coding regions are being mapped by Bal31 deletion analysis, and E. coli mutants defective in Ref-stimulated recombination have been isolated.

1201 **FRAGMENTATION OF YEAST CHROMOSOMES AT SPECIFIC SITES BY INTEGRATIVE TRANSFORMATION,** **Philip Hieter[1], Carla Connelly[1], and Douglas Vollrath[2], [1]Molecular Biology and Genetics, Johns Hopkins University Medical School, Baltimore, MD 21205; [2]Biochemistry, Stanford University Medical School, Stanford, CA 94038.**

We have developed two yeast transformation vectors that can be used to "fragment" chromosomes at specific sites. The technique involves transformation of yeast with either a linear centromeric molecule or a linear acentric molecule. One end of the molecule contains a unique DNA segment from the yeast genome, the other end contains sequences that are efficiently repaired to a functional telomere. The linear molecules will only produce transformants if a recombination event occurs between the inserted unique DNA segment and homologous sequences on the chromosome. The centromeric vector yields a stably segregating telocentric chromosome fragment - the result of addition to the linear vector of all chromosomal sequences distal to the unique DNA segment. The acentric vector results in deletion from the chromosome of all sequences distal to the unique DNA segment (and addition of a functional telomere at that site). The size of the recombinant product can be determined by pulsed field electrophoresis which allows resolution of chromosome-sized DNA molecules. Therefore, the physical distance between the cloned unique DNA segment and each chromosome end can be estimated. In addition, diploid strains heterozygous for a chromosome length polymorphism (CLP) have been used to study the fate of the chromosome with which the linear vector molecules recombine. The results suggest that recombination takes place predominantly in G2, and that the centromeric vector usually results in partial trisomy (2n + chromosome fragment) whereas the acentric vector usually results in partial monosomy (2n-1 + chromosome fragment).

1202 STRUCTURE OF RecA SPRIAL FILAMENTS AND THEIR ROLE IN HOMOLOGOUS PAIRING AND STRAND EXCHANGE IN GENETIC RECOMBINATION. Paul Howard-Flanders, Stephen C. West, Era Cassuto and Edward Egelman, Dept. Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511, USA, and Andrzej Stasiak, Institute for Cell Biology, ETH-Honggerberg, 8093 Zurich, Switzerland.

RecA protein from E. coli promotes homologous pairing and strand exchange reactions between two DNA duplexes provided one of them has a single stranded gap. RecA binds to the single stranded DNA in the gap to form a spiral filament and extends in the 5' to 3' direction along the adjoining duplex. Naked duplex DNA is able to contact the single strand in a deep spiral groove between RecA monomers, the duplex is sharply kinked and so brought into axial alignment with the ssDNA along the axis in the center of the helical RecA polymer. Base pairing presumably occurs through hydrogen bonding to the face of the wide groove of the duplex. Non-homologous contacts may be transient, but we suggest that homologous contacts extend towards the 3' terminus of the duplex. The strand exchange reaction starts most efficiently in the single stranded region and advances in the 5' to 3' direction of the single stranded region. This molecular mechanism involves reactions close to the axis of the RecA spiral filament, and is proposed on the basis of electron micrographs of RecA mediated reactions, image processing and the analysis of enzymatic reactions with defined DNA substrates.

1203 FORMATION OF HOLLIDAY STRUCTURES DURING PHAGE λ SITE-SPECIFIC RECOMBINATION IN VITRO. Paul A. Kitts and Howard A. Nash, Laboratory of Molecular Biology, National Institute of Mental Health, Bethesda, MD 20892.

Two pieces of evidence support the idea that phage λ integration proceeds via a mechanism involving a Holliday structure intermediate. First, genetic analysis of phage crosses mediated by the λ site-specific recombination system provided evidence for a single-strand exchange intermediate (1,2). Second, Hsu and Landy demonstrated that purified integrase can efficiently resolve synthetic Holliday junctions containing attachment site sequences (3). The formation of Holliday structures by phage λ site-specific recombination had, however, not been observed.

We have developed a method for synthesizing attachment sites containing modified nucleotides. A bacterial attachment site with a particular thiophosphate substitution is a poor substrate for recombination (4), but under certain conditions this substrate leads to the accumulation of a novel recombinant species. Characterization of this species reveals that it contains a Holliday junction formed by recombination between one strand of the phage and bacterial attachment sites. The significance of this finding to the mechanism of phage λ integration will be discussed.

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1204 THE ROLE OF E. COLI SSB PROTEIN IN RecA PROTEIN FUNCTION, Stephen C. Kowalczykowski, Jennifer Clow, Renee Krupp, Rahul Somani, and Abraham Varghesse, Northwestern University Medical School, Chicago, IL. 60611

We have investigated the effects of SSB protein on both the single-stranded (s.s.) DNA binding properties and the s.s. DNA-dependent ATPase activity of recA protein. The binding of SSB and recA proteins for s.s. DNA was found to be competitive, with recA protein binding preferentially in the presence of ATP-γ-S and with SSB protein binding preferentially in its absence. With ATP, the outcome of this competition was dependent on temperature, magnesium concentration and the DNA used. Under strand assimilation conditions, recA protein bound preferentially to s.s. M13 DNA and the lifetime of recA protein-s.s. M13 DNA complexes was increased ~10-fold by SSB protein. However, this same effect could be obtained by pre-incubating the recA protein-DNA complexes at low magnesium concentration, suggesting that the effect of SSB protein is indirect and is mediated through changes in DNA secondary structure. With regard to ATPase activity, SSB protein can either inhibit or stimulate the ATPase activity depending on conditions. Using DNA substrates devoid of secondary structure, maximal activity is obtained at a stoichiometry of 5 nucleotides/recA protein monomer, however, with s.s. M13 DNA at 10 mM $MgCl_2$, a stoichiometry of 12 is obtained suggesting that more than half of the s.s. DNA is unavailable to recA protein; in the presence of SSB protein or low magnesium concentration a stoichiometry of 5 is obtained. These results and others suggest that the primary role of SSB protein is to eliminate DNA secondary structure that impedes recA protein binding and function. The data also demonstrate that a unique recA protein-DNA complex is formed when secondary structure is present.

1205 CLASSICAL MULTIPLICITY REACTIVATION OF VIRUSES CAN BE QUANTITATIVELY EXPLAINED BY RECOMBINATIONAL DNA DOUBLE-STRAND BREAK REPAIR, C.S. Lange, J. DeLeon and E. Perlmutter, SUNY Downstate Medical Center, Brooklyn, New York 11203

One double-strand break (DSB) per genome has been shown to explain ca. 76% of T4 loss of plaque-forming ability (Lange et al., 1984 Rad. Res. 100:1). Infectious Center Assays were performed, at various multiplicities of infection, and their results compared with predictions based on the proportion of cells expected to be infected by: a) undamaged T4; b) ≥2 T4 containing a non-DSB potentially lethal lesion (PLL, lethal in singlet infection); c) ≥2 T4 both containing ≥1 DSB; or d) 1 T4 containing ≥1 DSB or a non-DSB PLL. The number of Infectious Centers was found to be consistent with a+b+c, at low dose (ca. 1 DSB/genome), but decreased, consistent with a lower DSB repair efficiency (ca. 1 DSB repaired/genome), at higher dose (ca. 2, 3.5, 5.5 DSB/genome). Therefore, DSB repair takes place during multiplicity reactivation (MR) and is quantitatively consistent with being the only significant cause of MR. Experiments with genetically identical recA⁻ and recF⁻ deletion mutant hosts showed that the MR observed is independent of the lost recA and recF systems and may depend only on phage-coded enzymes.

This work is supported by grants from USDOE (AC0280EV10503) and the Mathers Charitable Foundation.

1206 TOPOGRAPHY OF THE INTERACTION OF RecA PROTEIN WITH SINGLE-STRANDED DEOXYOLIGONUCLEOTIDES M. C. Leahy and C. M. Radding, Dept. of Human Genetics, School of Medicine, Yale University, New Haven, CT 06510

We have probed the interaction of recA protein with single-stranded DNA in experiments with a model system consisting of oligonucleotides as substrates, and ATPγS as cofactor. Since the helical repeat of the presynaptic filaments of recA protein and single-stranded DNA contains about 20 nucleotide residues, we studied oligonucleotides of 20 residues or less. RecA protein formed stable complexes with oligonucleotides between 9 and 20 residues but not with those only 8 residues long. The binding of recA protein to a 15mer and 20mer completely protected the sugar-phosphate backbone of the nucleic acids from digestion by DNAseI and protected the 5' terminal phosphate from cleavage by calf intestinal alkaline phosphatase. Ethylation of the phosphate backbone at any position by ethylnitrosourea blocked the binding of recA protein to the 15mer but not to the 20mer. Ethylation of phosphates near the ends of the 15mer interfered less with binding, suggesting a minimum binding site requirement. In contrast to the protection of the nucleic acid backbone, recA protein did not protect the N-7 position of guanine or the N-3 position of adenine from methylation by dimethylsulfate, but rather enhanced the methylation of guanine. Extrapolation of these results to conclusions about the interaction of recA protein with single strands in the presence of ATP may be justified by the observations that presynaptic filaments formed in the presence of ATPγS are active in homologous pairing and are morphologically indistinguishable from ATP filaments. The results indicate that recA protein binds primarily to the phosphate backbone of single-stranded DNA, leaving the bases free for homologous pairing.

1207 PLASMID RECOMBINATION BY THE E. COLI SBCA-INDUCED RECOMBINATION SYSTEM. Cynthia Luisi-DeLuca and Richard Kolodner. Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA 02115.

Recombination of circular monomer or dimer plasmids occurs at least 10-fold more efficiently in recBrecCsbcA E. coli than in wild-type cells. Linearized plasmid dimers recombine at least 50 times more efficiently in recBrecCsbcA cells than in wild-type cells by a recA-independent intramolecular recombination mechanism. A phenotype associated with an sbcA mutation is the induction of an ATP-independent, 5' to 3' exonuclease, exo VIII. Exo VIII, the recE gene product, has been overproduced and purified. Double-stranded linear dimer molecules incubated in vitro with exo VIII underwent intramolecular recombination after transformation into recA cells 22 times more efficiently than untreated linear dimers. Electron microscopic analysis of the exo VIII treated linear dimers revealed that at least 20% of the dimers had been converted into circular monomeric molecules containing single-stranded tails. These observations support a recA-independent recombination model proposing limited 5'-end degradation of the linear dimers and subsequent intramolecular circularization by homologous pairing. Cell-free extracts prepared from strains containing either a point, insertion, or deletion sbcA mutation were analyzed using monospecific antibodies directed against exo VIII. Western blot analysis revealed that sbcA point or insertion mutants induce the synthesis of the 140Kd polypeptide of wild-type exo VIII; the plasmid-encoded sbcA deletion mutants each direct the synthesis of a lower molecular weight polypeptide. Exo VIII assays and the western blot analysis suggest that 41Kd of the 140Kd exo VIII subunit is all that is essential for the protein to have exonuclease activity and function in genetic recombination. The identification of other gene products involved in plasmid recombination by the sbcA-induced system is currently underway. Two phenotypically distinct mutants of recArecBrecCsbcA cells that decrease the frequency of plasmid recombination at least 20-fold and, at least three mutants that increase the frequency of plasmid recombination 10 to 50 fold have been identified to date.

1208 Site-Specific and Illegitimate Recombination in the oriV1 Region of the F factor. Michael H. Malamy and Michael B. O'Connor, Tufts University, Boston, MA 02111

We have defined some of the sequences involved in high frequency recA-independent recombination at the oriV1 region of the F factor. Using a mobilization assay, we determined that a pBR322 derivative (pMB080) bearing the PvuII-BamHI (45.43-46.0) fragment from the oriV1 region of F, contained all sequences necessary to undergo efficient site-specific recombination with the F derivative pOX38. We constructed a series of pMB080 deletions in vitro using exonucleases S1 and Bal31. Deletions removing a 10 bp sequence, which forms part of an inverted repeat segment located 62 bp to the left of the NcoI site (45.87), totally eliminated the recA-independent recombination reaction. Other deletions differentially affected both the frequency and stability of cointegrate molecules formed by the site-specific recombination system. The F factor oriV1 region is also involved in low frequency recombination with several sites on pBR322 and related plasmids. We have determined the precise location of these recombination sites within oriV1. Recombination always took place within an 8 bp spacer region between the 10 bp inverted repeats found to be important for oriV1-oriV1 interactions. We propose that the low efficiency recombination between pBR322 and pOX38 results from the ability of the F site-specific recombination apparatus to weakly recognize and interact with sequences which bear some resemblance to the normal oriV1 recognition elements. Furthermore we suggest, by analogy to the lambda paradigm, that the nucleotide sequences at the junctions of secondary site recombinants define at least one crossover site used during the normal site-specific recombination process.

1209 EFFECTS OF ATP ON THE TRANSFER OF recA PROTEIN FROM ONE POLYNUCLEOTIDE TO ANOTHER. Joseph P. Menetski and Stephen C. Kowalczykowski, Northwestern University Medical School, Chicago, Illinois 60611

We have shown that the major protein involved in recombination in E. coli, recA protein, transfers from one single-stranded DNA molecule to another through a ternary intermediate. The intermediate contains recA protein, etheno-M13 DNA (fluorescently modified single-stranded M13 DNA), and poly(dT) (a high affinity competitor). In the presence of ATP, the reaction exhibits several interesting properties. First, the reaction time course is affected by pre-incubation of recA protein-etheno M13 DNA complex with ATP, that is the time course shows a distinct linear region after short periods of incubation; no linear region is obtained after preincubation for 15 minutes. Secondly, the rate of transfer is dependent on ATP concentration in a biphasic manner; increasing ATP concentrations increase, then decrease the transfer rate. Finally, the processivity of recA protein ATP hydrolysis on etheno M13 DNA was found to increase with ATP concentration, varying from one to 100 ATP molecules hydrolyzed per transfer event. The results presented suggest that it may be necessary for recA protein to be in a specific structure in order to transfer efficiently (i.e. a specific cluster formation). The processivity of the reaction appears to be governed by an ATP binding step in the binding cycle of recA protein for single-stranded DNA (Menetski and Kowalczykowski (1985) J. Mol. Biol. 181, 281-295) Therefore, at low ATP concentrations the rate of ATP binding to recA protein may be the factor determining processivity. Also, ATP was found to have two effects on recA protein in the transfer reaction, and may also be involved in determining the processivity of ATP hydrolysis.

1210 ACTION OF 4-QUINOLONES DERIVATIVES ON MAINTENANCE OF PLASMIDS IN ESCHERICHIA COLI, Yvon Michel-Briand and Valérie Ucelli, Faculté de Médecine, 25030 Besançon, France.

4-quinolones are antibacterial agents which inhibit the α sub-unit of DNA gyrase and which could eliminated plasmidic DNA from their bacterial host (which are cured).

In the present study we compared the curing effect of eleven 4-quinolones on the maintenance of 10 plasmids harboured in the same host (Escherichia coli J53). The aim was to determine whether all quinolones act by the same mechanism on a particular plasmid and whether plasmid maintenance and segregation mechanisms may be different according to the nature of plasmid. The quinolones tested belonged to 4 groups 1- (nalidixic acid, enoxacin), 2- (oxolinic acid, rosoxacin, norfloxacin, pefloxacin, flumequin, ofloxacin), 3- (pipemidic and piromidic acids), 4- (cinoxaxin). All curing assays were conducted simultaneously with novobiocin, a curing agent acting on gyrase β-sub-unit.

The plasmids used were R386 (Inc-FI), pIP24 (Inc-FII), pIP129 (Inc-FIV), R64 (Inc-I), R446b (Inc-M), N3 (Inc-N), RP4 (Inc-P), pYMB1 (Inc-P1), S-a (Inc-W), R6K (Inc-X).

The most important factor in the interaction of 4-quinolones with plasmid maintenance was the nature of plasmid. Only three plasmids were eliminated (R446b, R386 and S-a) at a rate between 0.5 to 16 per cent bacteria in antibiotic containing medium — The quinolone nature does not seem to be a critical factor as a plasmid was cured by all quinolones although at different rate. Novobiocin cured bacteria from R446b, R386, pIP24 plasmids —These results might give some insight in the mechanism of maintenance and segregation of plasmid in E. coli, via the superhelicity and the balanced equilibrium between topoisomerase I and II activities.

1211 TARGET SITE SPECIFICITY IN BACTERIOPHAGE MU TRANSPOSITION, Michiyo Mizuuchi, Robert Craigie and Kiyoshi Mizuuchi, Laboratory of Molecular Biology, NIADDK, National Institutes of Health, Bethesda, MD 20892

The sites of insertion of a large number of mini Mu transpositions have been sequenced. The 5 bp duplication at the site of insertion had a consensus sequence 5'-N(Py)$\binom{C}{G}$(Pu)N-3'. The C or G at the third position appeared to be the most highly conserved base; of 52 independent insertions only two did not have a C or G at this position. The second and fourth base agreed with the consensus sequence in approximately 90% of the insertion sites. Bacteriophage Mu therefore has a quite strong target site sequence specificity, but since this sequence specificity is limited to only 3 bp, the sites of insertion are essentially random at the macroscopic level.

The protein requirements for the Mu DNA strand transfer reaction, which is an early step in Mu transposition, are the Mu A protein, Mu B protein and protein HU. Experiments are in progress to determine which of these proteins are involved in recognition of the target site consensus sequence.

1212 Tn10 TRANSPOSITION IN VITRO, Donald Morisato and Nancy Kleckner, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

Mechanistic aspects of Tn10 transposition have previously been analyzed by experiments in vivo. We are now able to observe both intermolecular transposition (from a plasmid onto phage lambda DNA) and intramolecular transposon circle formation (Morisato and Kleckner, Cell 39, 181-190) in cell-free extracts. We have initially characterized the requirements for the intramolecular reaction, which occurs at substantially higher levels than the complete transposition reaction. Extracts are prepared from cells overproducing transposase. In optimized reactions, supercoiled plasmid substrate is incubated with the extract at 30°C, in a reaction mix containing 25mM Hepes (pH 7.6), 50mM KCl, 5mM $MgCl_2$, and 1mM DTT. When the substrate plasmid, which contains a pair of suitably oriented Tn10 termini, is relaxed with topoisomerase I prior to incubation with the extract, circle formation is reduced substantially. There is no ATP requirement in the reaction.

Within the extract, transposase is tightly associated with chromosomal DNA. This property allows the separation of transposase from host proteins by simple size fractionation on a sucrose gradient. Fractions containing transposase do not promote transposon circle formation alone, but do so when supplemented with an extract made from cells producing no transposase. This host component is trypsin-sensitive and heat-stable. This raises the possibility that Tn10 transposition requires one of the family of small heat-stable proteins necessary for many site-specific recombination reactions and Mu transposition, although these results are still formally consistent with the inactivation of an inhibitor present in the transposase fraction.

1213 INTERACTION OF SSB WITH RECA-SSDNA COMPLEXES, Scott Morrical, Jongwon Lee, and Michael M. Cox, Department of Biochemistry, School of Agriculture and Life Sciences, University of Wisconsin-Madison, Madison, WI 53706.

The single-stranded DNA binding protein of Escherichia coli (SSB) stimulates recA protein-promoted DNA strand exchange reactions by promoting and stabilizing the interaction between recA protein and ssDNA. Previous models have proposed that SSB acts transiently in this system, melting out regions of secondary structure in ssDNA before being displaced by recA protein. Utilizing the intrinsic tryptophan fluorescence of SSB, we have detected an ATP-dependent interaction between SSB and recA-ssDNA complexes. This interaction is continuous for periods exceeding one hour under conditions that are optimal for DNA strand exchange. In the absence of ATP, SSB and recA binding are mutually exclusive; SSB completely displaces recA protein from ssDNA. In contrast, binding of recA protein and SSB in the complex in the presence of ATP is not mutually exclusive. Instead, both proteins appear to be present in defined stoichiometries.

The properties of the joint recA-SSB-ssDNA complex formed in the presence of ATP are consistent with the properties of SSB-stabilized recA-ssDNA complexes determined by other methods. Complex formation is kinetically competent to be the first step of recA protein-promoted DNA strand exchange.

Our data is incompatible with models in which SSB is displaced after acting transiently in the formation of recA-ssDNA complexes. A continuous association of SSB with recA-ssDNA complexes may therefore be an important feature of the mechanism by which SSB stimulates recA protein-promoted reactions.

1214 PHYSICAL ANALYSIS OF THE ESCHERICHIA COLI RECO GENE: A NEW RECF RECOMBINATION PATHWAY GENE. Paul T. Morrison and Richard Kolodner. Laboratory of Molecular Genetics, Dana-Farber Cancer Institute and Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

Mutations in the Escherichia coli recO gene decrease the frequency of conjugation-mediated recombination events and increase the sensitivity to killing by UV-irradiation and mitomycin-C when present in a recBrecCsbcB genetic background. When present in an otherwise wild type genetic background, recO mutations decrease the frequency of plasmid recombination events and cause a UV-sensitive phenotype but have no effect on conjugation-meidated recombination or killing by mitomycin-C. The recO gene was cloned by identifying a plasmid from the Clarke and Carbon collection that complemented the UV-sensitive pheontype caused by a recO::Tn5 insertion mutation. Restriction mapping, deletion mapping and subcloning studies were carried out to locate the recO complementing region to a 3.3 Kb region of DNA. Southern blotting experiments demonstrated that this 3.3 Kb region was homologous to a region of E. coli chromosomal DNA containing the recO::Tn5 insertion mutation and indicated that the 3.3 Kb region contained the recO structural gene. DNA sequencing studies, in vivo expression studies and protein purification studies are being carried out in order to characterize the recO protein.

1215 SITES AND FUNCTIONS REQUIRED FOR TRANSPOSITION OF Tn554, E. Murphy, M. Bastos, L. Huwyler and E. Reinheimer, The Public Health Research Institute of the City of New York, Inc., New York, NY 10016

The Staphylococcus aureus transposon Tn554 is 6691 bp in length and contains 6 major open reading frames, of which 2 correspond to the known antibiotic resistance genes ermA and spc, and 3 encode functions required for transposition of Tn554. The transposition genes tnpA, tnpB and tnpC, encompassing 3.7 kb of Tn554, potentially encode basic proteins of 43, 74 and 14 Kdal, respectively. Small insertion and deletion mutants within these genes abolish the ability of Tn554 to transpose. All mutants can be complemented in trans. Tn554 is extremely site-specific, inserting at a single location in the S. aureus chromosome. Its ends are asymmetric, lacking either inverted or direct terminal repeats, and it does not generate a duplication of any target sequences. Insertions into the cloned attachment site occur in both orientations, in contrast to insertions into the chromosome, which occur in only one orientation. Transposition occurs specifically on one or the other side of a 6 bp "core" sequence GATGTA, that is identical to the terminal 6 bp at the right end of Tn554. Deletion analysis and subcloning of the chromosomal attachment site has shown that the functional target site is greater than 18 nt but smaller than 36 nt in length. On the right side, deletions that leave the core sequence and the adjacent 5 bp intact retain target function; those that remove one additional base are non-functional. On the left, at least 8 nt, but fewer than 23, are required. In both size and organization (but not primary sequence) the Tn554 attachment site thus appears to be similar to attB.

1216 FACTORS INVOLVED IN TRANSPOSITION OF THE INSERTION SEQUENCE IS2. Richard E. Musso, Susan C. Barbieri, Todd Black and Lee Wilson. Univ. of So. Car., Columbia, SC 29208.

The bacterial insertion sequence IS2 is a 1330 bp transposable DNA element which occurs normally in several copies within the chromosome of Escherichia coli K-12 strains but not in the genome of E. coli C. Although virtually the entire IS2 DNA can be transcribed from two overlapping promoters located near the right end of IS2, maxi-cell experiments reveal only a single 14 kd protein encoded in the right one-third of IS2. The remaining two-thirds of IS2, downstream of the 14K gene, has one long and other short open reading frames.

To assess the role of the 14 kd protein and the possibility of other IS2 encoded factors, we have developed genetic assays for IS2 transposition. A fragment carrying a gene, kan, for kanamycin-resistance has been inserted at different sites within the interior of IS2. Using E. coli C (lacks chromosomal IS2) infected by appropriate λIS2-kan phage that neither grow lytically nor integrate, we can detect IS2-kan transposition to the chromosome as KAN^r cells. Depending on the location of the kan insert within IS2, different results are obtained. Transposition occurs at a low frequency ($7x10^{-8}$) when kan is within the 14K gene and only cointegrate products are obtained; whereas if kan is located just downstream of the 14K gene, a much lower transposition frequency is observed ($2x10^{-9}$) and gives simple insertion products. If an intact IS2 is also provided by coinfection with either marked IS2-kan, the transposition frequency increases ($2\text{-}3x10^{-7}$) and the vast majority of products are simple insertions. Thus IS2 encodes a diffusable factor (besides the 14 kd protein) which is required for transposition and the 14 kd protein may resolve cointegrates or modulate the relative amounts of cointegrate vs. simple insertion products.

1217 **REPLICATION OF YEAST CHROMOSOME III,** Carol S. Newlon, Timothy G. Palzkill and Ann Dershowitz, Department of Microbiology, U.M.D.N.J.-New Jersey Medical School, 100 Bergen Street, Newark, N.J. 07103.

A 200 kb circular derivative of yeast chromosome III, containing sequences between the HML and MAT loci has been cloned and restriction mapped. This ring chromosome contains at least twelve autonomously replicating sequences (ARS's). Four of these ARS's have been analyzed in more detail. They vary at least ten-fold in efficiency as measured by the rate of 1:0 segregation, assayed by a simple colony color test (1). Comparison of the DNA sequences of fragments containing these ARS's with other published ARS sequences has revealed highly conserved nucleotides in addition to the previously reported 11 bp consensus (2).

To determine whether ARS's are essential for mitotic and meiotic chromosome stability, we are systematically deleting ARS's from the wild type, linear chromosome III and two circular derivatives. Deletion of either ARS from a 66 kb circular chromosome which contains only two strong ARS's destabilizes it significantly while deletion of either of the same two ARS's from the wild type chromosome has no measurable effect on mitotic stability.

1. Hieter, P.W., C. Mann, M. Snyder and R.W. Davis (1985) Cell 40:381.
2. Broach, J.R., Y.-Y Li, J. Feldman, M. Jayaram, J. Abraham, K. Nasmyth and J.B. Hicks (1983) Cold Spring Harbor Symp. Quant Biol. 47:1165

1218 GENETIC REARRANGEMENTS FOLLOWING THE INSERTION OF AMPICILLIN TRANSPOSONS INTO THE PLASMID DNA OF NEISSERIA GONORRHOEAE. Jean-Claude Piffaretti and Achille Arini. Inst. Cantonale Batteriologico, 6904 Lugano, Switzerland.

Penicillinase producing N. gonorrhoeae (PPNG) isolated throughout the world harbour a 7.3 kb resistance plasmid or its 5.1 kb deletion derivative: both elements contain 40% of the transposon TnA. In order to better understand the emergence of such plasmids and to establish whether these can originate from multiple events of TnA insertions into a specific site of a core plasmid, we constructed bla derivatives of the PPNG resistance elements. Transposition experiments performed in E. coli showed indeed that these recombinant plasmids have a hot-spot insertion site for the TnA transposons. Furthermore, when introduced into N. gonorhoeae by transformation or conjugation, these plasmids originated undistinguishable derivatives, characterized by specific genetic rearrangements and deletions in the TnA element and the gonococcal DNA. These results can be related to the observation that no complete functional Tn3 transposons could ever be found in gonococci and support the hypothesis that the spread of the gonococcal resistance plasmids might be due to multiple but specific TnA insertional events in a unique core plasmid.

1219 ROLE OF oriT IN THE ENHANCED RECOMBINATION OF F42lac, Ronald D. Porter, Anne V. Thomas, Jeffrey C. Carter and H. Steven Seifert, The Pennsylvania State University, University Park, PA 16802

The F42lac plasmid of E. coli recombines with a λplac5 transducing phage about thirty times more efficiently than does a chromosomal lac gene. This enhancement of recombination depends upon the RecBC enzyme of the recipient cell and the constitutive expression of the tra regulon of F42lac. The tra dependence of enhancement can be broken down into cis-and trans-acting components. We are continuing our investigations to determine which tra gene products are required to act in-trans for the enhancement. The current list of candidates is traY, A, L, I, and Z.

The cis-acting component is located within a 2.5kb restriction fragment that contains the oriT site of the F factor and no other known tra functions. Further analysis has shown, however, that the presence of a conjugationally active oriT site in-cis to the recombing lac gene is not sufficient for recombination enhancement. Our working hypothesis is that the in-trans action of the tra gene proteins at the oriT site begins a series of events that allow the RecBC enzyme to enter the F42lac molecule. We are currently investigating the possibility that a double-stranded break is made in the oriT region to allow the entry of the RecBC enzyme.

1220 CONSTRUCTION AND TRANSPOSITION OF ARTIFICIAL IS1 ELEMENTS. P. Prentki*, D. J. Galas*, P. Gamas+, and M. Chandler+, Molecular Biology, U.S.C., Los Angeles, CA 90089-1481, U.S.A.* and C.R.B.G.C. 31062 Toulouse, France+.

Our previous studies have shown that the 25 bp-long inverted repeats at the ends of IS1 contain the sequences required for transposition, as well as binding sites for the E. coli protein integration host factor (IHF). To determine more precisely the DNA sequence requirements for function of the ends in transposition we have synthesized a family of oligonucleotides carrying the ends of IS1 (wild-type or with specific mutations) and cloned each of them in an inverted repeat configuration at the extremities of the selectable marker omega. The activity of these artificial segments as transposition end-points was then assayed in vivo. We can conclude the following from preliminary experiments: i) Oligonucleotides carrying sequences with either the right (IRR) or left (IRL) end of IS1 are fully active; ii) A complementing copy of IS1 is required in cis; iii) Mutations at the third, but not at the first, base reduce activity; iv) Not all mutations in the IHF recognition sequence abolish transposition. A clearer picture of the exact sequences involved should emerge from the analysis of the much larger set of mutants which are currently under analysis.

1221 DNA SUPERCOILING IN TOPOISOMERASE I MUTANTS: NUCLEOTIDE SEQUENCES IN PLASMID pBR322 RESPONSIBLE FOR HIGH LEVELS OF SUPERCOILING. Gail J. Pruss* and Karl Drlica**, *University of Rochester, Rochester, N.Y. 14627 and **The Public Health Research Institute of the City of New York, Inc., New York, N.Y. 10016

Plasmid pBR322 DNA isolated from topoisomerase I mutants of E. coli and S. typhimurium exhibits a novel supercoiling distribution that is characterized by the presence of highly negatively supercoiled topoisomers (Pruss, J. Mol. Biol. 185, 51-63, 1985). This distribution is obtained even when pBR322 DNA is isolated from a topoisomerase I mutant carrying a compensatory mutation in DNA gyrase, although bacterial chromosomes isolated from the same strain are less negatively supercoiled than normal (Pruss et al., Cell 31, 35-42, 1982). Analysis of the supercoiling distributions of deletion and insertion derivatives of pBR322 has shown that the presence of the gene encoding tetracycline resistance on pBR322 is responsible for the unusual supercoiling distribution. Both an intact promoter and a portion of the remainder of the gene are required. However, no particular section of the gene outside of the promoter appears to be necessary; only the size of the section remaining (about 600 base pairs) appears to be important. These observations suggest that expression of the gene, rather than the presence of preferred sites of gyrase activity, may be responsible for the gene's effect on supercoiling.

1222 ANALYSIS OF TN7 TRANSPOSITION, Mark Rogers, Elaine Nimmo, Nelly Ekaterinaki and David Sherratt, Inst. of Genetics, University of Glasgow, Glasgow, Scotland

Tn7 is a 14Kb transposon that exhibits a high specificity for an "attachment" site in the chromosome of E. coli and other genera and inserts into plasmids in a specific relative orientation. Transposition assays have revealed differences in efficiency of transposition to the cloned attachment site that are determined by the context of the donor transposon. Transposition from the attachment site is less effecient than transposition from a plasmid. There is no effect of donor context on transpositions to plasmids. This, and the analysis of deletion mutants which are affected only in transposition to plasmids suggests that more than one transposition mechanism is employed.

Sequencing, translational and transcriptional fusions and complementation studies using a chloramphenicol resistance gene bounded only by the ends of Tn7 have helped define the regions important for transposition. These have been cloned into expression vectors for further analysis.

1223 MECHANISM OF INHIBITION OF DNA GYRASE BY QUINOLONE ANTIBACTERIALS: A COOPERATIVE DRUG-DNA BINDING MODEL. Linus L. Shen, John Baranowski and Thanda Wai, Abbott Laboratories, Abbott Park, Illinois 60064

Quinolones, a series of nalidixic acid analogues, are a new class of synthetic antibacterial agents with extremely high potencies and enhanced bactericidal activity. The drugs have been shown to inhibit DNA gyrase. Using radiolabeled norfloxacin, we have demonstrated that the drug receptor site is not located on gyrase, but on the substrate DNA (PNAS 82, 807, 1985). The drug binding specificity was further studied using RNA, oligo- and poly-deoxynucleotides as model systems. Results show that the drugs, in general, are poor DNA binding agents, since they do not bind to double-stranded DNA and bind very poorly to RNA or to single-stranded DNA. They bind selectively and in a highly cooperative manner, at low inhibitory drug concentrations, to certain specific denatured regions of the DNA. A binding model will be proposed.

1224 ANALYSIS OF THE ACTIVITY OF RETROVIRAL pol GENE PROTEINS EXPRESSED IN BACTERIA. D. Soltis, F. Alexander, R. Terry, J. Leis* and A.M. Skalka, Department of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110. *Case Western Reserve University, Cleveland, OH 44106.

Reverse transcription and integration of retroviral DNA into the host genome requires the function of the retroviral pol gene. The pol gene in the avian sarcoma/leukosis viruses encodes several enzymatic activities and its protein domains are organized into at least three, differentially processed molecular forms. One of these, the $\alpha\beta$ form, includes a 90 kd polypeptide chain (β) containing most of the coding capacity of pol. The β chain is complexed to a 60 kd α chain which contains a proportionately smaller amount of the pol information. This $\alpha\beta$ complex has a number of distinct activities including reverse transcriptase and DNA endonuclease. pp32 protein, another molecular form encoded in pol, also exhibits endonuclease activity. It is the presence of the pp32 domain in β (but not α) that accounts for the endonuclease activity of $\alpha\beta$.

We have constructed bacterial expression clones which produce the different molecular forms of the pol gene products. These proteins are being used to study the mechanisms of reverse transcription and retroviral DNA integration. Recombinant proteins containing the β and pp32 domains have been partially purified from bacterial extracts and, as will be shown, exhibit the expected reverse transcriptase and endonuclease activities. Our initial studies revealed a short segment at the C-terminus of pol which is present in primary translation products of the β and pp32 domain, but appears to be removed from the majority of the "mature" proteins isolated from virus particles by viral-specific proteolytic processing. Results from our comparison of the properties and activities of recombinant protein with and without these additional sequences will also be discussed.

1225 HOMOLOGOUS RECOMBINATION IN MAMMALIAN CELLS, N. Sternberg, S. Brenner, K. Sperle, M. Lin, E. I. Du Pont de Nemours and Company, Inc., CR&D, Experimental Station, Wilmington, DE 19898

We continue to study the process of homologous recombination in cultured mammalian cells using gene transfer techniques. Previously, we, and others, showed that when DNA is added to mammalian cells it can recombine efficiently. We proposed a four step model to account for the properties of that recombination (Lin et.al., Mol. Cell. Biol. 4, 1984). The model evokes a 5' exonuclease that degrades DNA strands in opposite directions from a DNA end exposing homologous complementary single-stranded regions. After those regions have paired, frayed 3' single-stranded ends are degraded generating a gap whose repair produces the recombinant. An alternative model evokes a 3' exonuclease that also degrades the DNA strands from a DNA end. In this case, the recombinant is generated, following strand pairing, by a resynthesis or repair of the degraded strand and a subsequent ligation of the newly synthesized DNA to the paired homologous region. Recent results with double-stranded DNA substrates are consistant with either of the two model. However, results obtained by transforming cells with recombination intermediates are more consistant with the second model. We also have been studying the process of targeting DNA introduced into mammalian cells into homologous DNA sequences placed in chromosomes in a previous transformation step (Lin et.al., Proc. Natl. Acad. Sci. 82, 1985). The targeting event is recognized by virtue of its reconstruction of a selectable gene (tk or neo). Those studies indicated that targeting can occur, but that it is very much dependent on the target transformant cell line used; some cell lines can be targeted while others can not. We will describe studies designed to study the factors that effect that variability. To analyze in greater details the mechanism of homologous recombination we have established an in vitro recombination system, using mammalian cell extracts, that is capable of promoting recombination between two defective neo genes on the same DNA molecule. Our results indicate that recombination can be as efficient as 2% and is accompanied by the appearance of recombined DNA in the extracts.

1226 PURIFICATION, CHARACTERIZATION, GENE CLONING, NUCLEOTIDE SEQUENCE AND GENETICS OF YEAST SINGLE-STRANDED DNA BINDING PROTEINS. Robert K. Hamatake, P. Sue Alexander, Akira Sakai, Rupa Desai and Akio Sugino, NIEHS, NIH, RTP, NC 27709.

By analogy to prokaryotes, single-stranded DNA binding proteins (SSBs) are believed to be required for DNA replication, recombination and repair in eukaryotes. Several eukaryotic SSBs have been purified and characterized, but none of their functions are yet known with the exception of the adenovirus SSB. To study the functions of yeast SSBs, three SSBs (which specifically stimulate yeast DNA polymerase I reactions by several-fold), have been purified to homogeneity from S. cerevisiae (Mr = 38,000, 20,000 and 14,000 daltons). They are located mainly in the nuclei. Antibodies against each have been raised in rabbits and are very specific, not cross-reacting with other proteins. Using the antibodies, the gene for each SSB has been cloned, sequence, and mapped both physically and genetically on the chromosome. The cloned genes have been modified by deletion and/or disruption and substituted for the chromosomal genes in order to determine the roles of each SSB in cell viability, UV and X-ray sensitivity and recombination proficiency.

1227 STUDIES OF AN ACTIVE COVALENT COMPLEX BETWEEN E. coli DNA TOPOISOMERASE I AND OLIGONUCLEOTIDES. Yuk-Ching Tse Dinh, E. I. du Pont de Nemours & Company, Central Research & Development Department, Wilmington, DE 19898.

DNA topoisomerases have been shown previously to cleave DNA phosphodiester bond and simultaneously become linked to the DNA at the cleavage site via a phosphotyrosine linkage. For prokaryotic DNA topoisomerases, this is observable only when denaturant or protease is added to the topoisomerase incubation mixture. Previous attempts to reform phosphodiester bonds from the covalent complex have been unsuccessful. Using oligonucleotides as substrates, the cleavage reaction of *E. coli* DNA topoisomerase I is found to occur spontaneously. Upon reaction with oligo(dA) labeled at the 3' end using terminal transferase and α-^{32}P dATP, the enzyme becomes covalently linked to the ^{32}P-labeled oligonucleotide. This ^{32}P-label can then be transferred to the 3'-OH end of a linear or nicked duplex DNA molecule freshly added to the reaction mixture. This phosphodiester bond rejoining reaction can occur at a recessed, blunt or protruding 3'-end. It requires magnesium ions. Implications on the structure of prokaryotic type I DNA topoisomerases as compared to their eukaryotic counterparts are discussed.

1228 PRODUCTS OF THE GIN MEDIATED DNA-INVERSION REACTION. R. Kanaar[1], P. van de Putte[1] and N. Cozzarelli[2]. [1]Laboratory of Molecular Genetics, State University of Leiden, Leiden, The Netherlands. [2]Department of Molecular Biology, University of California, Berkeley, USA.

The site-specific DNA-inversion reaction of phage Mu was studied *in vitro*. Components of the system are the phage encoded Gin protein, a host factor protein called GHF for Gin Host Factor, and a supercoiled substrate DNA carrying two 34 bp recombination sites (IRs) in inverted orientation relative to each other. Also an additional sequence on the DNA is required to enhance the recombination.

Like the recombination activity the relaxation activity of the system was shown to be site-specific. Relaxation appeared to be much lower than reported for other site-specific recombinases such as resolvase and Hin and suggests that breakage and reunion might be much more tightly coupled to recombination.

The topology of the reaction products was found to be quite simple. Over 99% of the molecules were open circles. Using DNA which was purified from *in vivo* knots small amounts of a trefoil product could be detected. This knot is a product of an iterative reaction which shows that the synaptic complex contains two binding nodes.

1229 EFFECT OF recF::Tn5 ON THE INDUCTION OF SOS GENES IN Escherichia coli K12. Wilfried Wackernagel and Brigitte Thoms, Fachbereich Biologie, AG Genetik, Universität Oldenburg, 2900 Oldenburg, West Germany

We have determined the influence of a newly isolated recF mutation (obtained by insertion of Tn5 into recF) on the induction by UV or nalidixic acid of nine SOS genes. Induction was measured by ß-galactosidase assays of appropriate Mud(Ap lac) fusion strains which were recA$^+$. The results show that the absence of recF function (i) reduces significantly the induction of dinD, uvrA, recA and recN, (ii) reduces slightly the induction of dinA, dinB and sfiA, (iii) does not affect induction of umuC and (iiii) abolishes UV-induction of dinF. Reduced induction of dinD was not alleviated by a recAoc mutation. A recB mutation had no significant effect on the induction of dinA and uvrA. It is concluded that recF plays an important role in the derepression of most SOS functions. However, this finding is not sufficient to explain the UV-sensitivity of recF strains (having either a recBCsbcB or a recB$^+$C$^+$ genetic background). Rather, complementary results (e.g. the extreme UV-sensitivity of a uvrA lexA102 recF mutant) suggest that recF participates also in a more direct way in the repair of UV-damaged DNA. We also present a hypothesis on the function of recF in the recF pathway of genetic recombination.

1230 RECOMBINATION BETWEEN REPEATING UNITS WITHIN A SINGLE GENE, Gareth Warren, Robert Green and Loren Corotto, Advanced Genetic Sciences, Inc., 6701 San Pablo Avenue, Oakland, CA 94608

The ice nucleation genes of *Pseudomonas syringae* and *Pseudomonas fluorescens* are internally repetitive. Comparisons between the two genes, and of the codon usage between repeats within each gene, indicate that intramolecular recombination is responsible for the maintenance of the repeats' fidelity. Such intramolecular recombination in these genes is experimentally detectable by the pseudo-reversion of an insertion mutation, which results from deletion of the insert and of some of the repeats which flank it. (The ice nucleation phenotype provides an exceptionally sensitive assay for the detection of revertants, and the ice nucleation protein can tolerate the loss of some repeats.) Although such reversion must occur by precise recombination between homologous sequences, its frequency is independent of of recA. We are studying the influence of other rec genotypes.

1231 TOPOLOGICAL STUDIES OF SITE-SPECIFIC RECOMBINATION BY CRE PROTEIN, Robert L. Weinberg, Steven A. Wasserman, Nicholas R. Cozzarelli, and Kenneth Abremski. University of California, Berkeley, Berkeley CA 94720; E. I. du Pont de Nemours and Co., Inc., Wilmington DE 19898.

The Cre-loxP site-specific recombination system from phage P1 requires only the Cre protein and DNA containing the 36 bp loxP site. We have employed a topological approach to study the mechanisms of site alignment and strand exchange. The substrate DNA may be supercoiled, nicked, or linear. We find that recombination of a supercoiled substrate with two directly repeated (head-to-tail) loxP sites produces knots and catenanes containing up to twenty crossings or nodes. The products appear as interdigitating ladders when examined by gel electrophoresis. Our data suggest that this array of products is the result of two simple events rather than that of a complex or variable mechanism. Singly-linked catenanes (a first round product) react to form trefoils. We suggest that more complex catenanes also undergo a second round to form the other knots we observe. We also find that nicked substrates form different products than supercoiled ones. We have designed an experiment to test the change in linkage induced by Cre. Using a substrate containing alternating and directly repeated res sites and loxP sites, we can make singly-linked (-) catenanes by recombining with the resolvase of Tn3. These will then be reacted with Cre. If the product formed is a trefoil knot we can deduce that Cre introduces a negative node in the process of strand exchange. If we observe an unknotted dimeric product, this would indicate a +1 mechanism for Cre recombination.

1232 INITIATION OF POLYOMA DNA REPLICATION, M. DePamphilis, D. Wirak, L. Chalifour, R. Perona, S. Fields-Berry, P. Wassarman[1], J. Hassell[2], E. Hendrickson, C. Fritze, U. Heine, and W. Folk[3]. Harvard Medical School, Boston, MA; [1]Roche Institute, Nutley, NJ; [2]McGill Univ., Montreal, Canada; [3]Univ. Texas, Austin, TX.

What constitutes the polyoma (PyV) origin of replication (ori), how does it differ from that of SV40, and how does initiation of DNA replication in differentiated mammalian cells compare with embryonic mammalian cells? We have provided some insight to these questions by measuring DNA replication and gene expression following microinjection of various plasmids into the nuclei of preimplantation mouse embryos, and by mapping the sites of DNA synthesis in wild-type and mutant PyV(RI) DNA. The principle conclusions at this time are: (i) DNA replication in mouse embryos requires specific cis-acting sequences such as the PyV ori, in contrast to sequence-independent replication of DNA injected into Xenopus eggs. (ii) Mouse embryos are permissive for PyV DNA replication, in contrast to mouse embryonal carcinoma cells. (iii) The PyV ori requires an enhancer element, in contrast to the SV40 ori, and some of these elements can activate ori-core in differentiated cells but not in embryos. (iv) The role of enhancer elements in activating ori-core can be distinguished from their role in activating gene expression. (v) The arrangement of DNA synthesis initiation sites that define the origin of bidirectional replication (OBR) is distinctly different in PyV compared with SV40, although both OBRs lie within the ori-core sequence.

1233 LINKER INSERTION MUTAGENESIS OF E. COLI TOPOISOMERASE I, Louis Zumstein and James C. Wang, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

We have constructed twenty in-frame linker insertion mutations of a cloned E. coli topoisomerase I gene. These twenty mutant clones sort into two groups; seven abolish all activity as determined by their ability to a) relax supercoiled DNA, b) cleave and covalently attach to single-stranded DNA, and c) complement a conditional lethal topA mutation in vivo. The other thirteen mutants have in vitro activities ranging from several percent to nearly 100% of wild-type levels, and all thirteen complement the topA conditional lethal mutation. Compared to other proteins, topoisomerase I seems to be very tolerant of the insertion of four amino acids in many different positions throughout the protein. The carboxyl terminus of the protein is especially tolerant of such insertions, with the seven insertions in the last third of the protein retaining at least 40% of wild-type activity. We have also constructed mutants which have premature termination codons and which therefore produce truncated topoisomerase I proteins. Removal of 6% or 11% of the protein from the carboxyl terminus has little effect on the in vitro topoisomerase I activities or on the in vivo complementation activity. Mutant proteins with 22% or 33% of the protein removed from the carboxyl terminus are completely inactive by all three assays.

DNA Recombination II: Eukaryotes

1234 MODEL SYSTEMS FOR SOMATIC RECOMBINATION IN MAMMALIAN CELLS, Richard A. Anderson, Vet. Admin. Med. Ctr. & University of Iowa, Iowa City, IA 52242.

The co-transfection of complementary, overlapping partial fragments of selectable genes into eukaryotic tissue culture cells and the monitoring of the joining of similar substrates in cell-free extracts have both been proposed as models for genetic recombination in mitotic cells. Experiments have been done to evaluate whether the same process is being measured in both approaches. Potentially complementing pairs of deletion mutants of the neo gene in pSV2 vectors were co-transfected into L-cells using calcium phosphate. Cells that had recombined and integrated intact neo genes were selected in medium containing G418. Relative to the recombination frequency measured when both transfected molecules were super-coiled there was a large stimulation of homologous joining seen when double-stranded cleavages in specific locations were made on one (2-20x) or both parental molecules (30-100x). Pairs of cleavages that were equidistant from the overlap produced the greatest stimulation. Specific single-strand nicks in the circular molecules had relatively little effect. Gene conversion-like events without exchange of flanking markers were not seen. When the same series of constructions were added to nuclear extracts of L-cells and homologous joining measured by Southern blotting the same pattern of stimulation was evident. When recombination was measured by the number of kanamyin resistant colonies produced upon transforming bacteria with the reaction products, a linear + a circular substrate produced the best signal relative to background. These results are consistent with a joining of the DNAs at homologous overlapping ends. This may represent a DNA-repair activity that is stimulated by free DNA ends.

1235 CHARACTERIZATION OF SPECIFIC MEVALONATE-DERIVATIZED PROTEINS IN THE CHO CELL LINE MEV-1. Lawrence A. Beck and Michael Sinensky, Eleanor Roosevelt Institute for Cancer Research, University of Colorado Health Sciences Center, Denver, CO 80262.

Recent experimental evidence indicates that mevalonate, in addition to its well characterized role as an intermediate in sterol biosynthesis, is a precursor for several non-sterol metabolic products that regulate 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) via a translational control mechanism and appear to be involved in the control of cell cycle progression.

We have presented evidence that mevalonate-derivatized proteins function as trigger proteins in controlling the progression of cells past a restriction point of the cell cycle and are continuing studies designed to elucidate the biological functions of these proteins. Growth of a CHO cell line auxotrophic for mevalonate (Mev-1) in the presence of ^{3}H-mevalonate results in the incorporation of approximately 45% of the label into specific proteins having molecular weights of 22,24,26, and 43 kd as determined by SDS-PAGE and radioautography. Proteins of identical molecular weights have been identified in other cell lines indicating the highly conserved nature of these mevalonated proteins. We have begun purification of these mevalonate-derivatized proteins by conventional and HPLC methods and are examining the subcellular localization of these proteins in order to determine the site and mechanism of action of these proteins.

1236 MISMATCH REPAIR FOLLOWING TRANSFORMATION OF PMS$^+$ AND pms1 YEAST WITH PURIFIED HETERODUPLEX PLASMIDS
D. K. Bishop[1], M. S. Williamson[2], S. Fogel[2], and R. D. Kolodner[1], [1]Dana Farber Cancer Institute, Boston, Ma 02115; [2]Department of Genetics, University of California, Berkeley, Ca 94720

We have developed a system which allows us to examine the repair of mismatched DNA in yeast. Yeast shuttle vectors containing mismatches were prepared and purified. The closed circular heteroduplex plasmid was introduced into mitotic yeast cells by transformation. Heteroduplex plasmid, having two small insertion mismatches separated by 943 bp, was constructed from plasmids which differ at two restriction sites. The identity of repair products was determined by physical analysis of plasmid DNA derived from transformed clones. In addition to wild type strains, we examined repair of the same heteroduplex DNA following transformation into a strain carrying a pms1 mutation. Strains carrying pms1 show increased frequencies of post-meiotic segregation. Most PMS1$^+$ transformants carried plasmids which suggested that repair had occurred at both mismatched sites; both double independent repair and co-repair events could be detected, although their relative numbers varied 6 fold from one transformation to the next. In wild type cells, one site was repaired about 85% of the time while the second site was repaired at least 97% of the time. When the pms1 strain was transformed the frequency of repair was 55 and 79% at the two sites respectively. Wild type cells showed a slight preference for repair in favor of the insertion strand in some experiments, while pms1 cells showed a strong preference for repair in favor of the deletion (>90%). The data presented support the hypothesis that the increase in post-meiotic segregation observed in pms1 strains is the result of a decrease in mismatch repair efficiency. One consequence of this hypothesis is that at least some meiotic gene conversion results from the repair of heteroduplex DNA. We have recently developed a system for identifying repair products genetically, and we hope to use this system to examine the repair of substitution and frameshift mismatches in a variety of genetic backgrounds.

1237 MEIOTIC RECOMBINATION BETWEEN REPEATED SEQUENCES IN YEAST. Rhona H. Borts, Michael Lichten, and James E. Haber, Brandeis University, Waltham, MA 02254

We have examined homologous meiotic exchange involving copies of the LEU2 gene inserted at several places in the Saccharomyces cerevisiae genome (at LEU2, HIS4, MAT, or URA3). In crosses where a pair of defined leu2 two heteroalleles were inserted at the same position in both homologs, the frequency of LEU$^+$ recombinants exhibited a strong locus dependence, and ranged from 0.09% to 1.7% of random spores. These results suggest that recombination within an interval can be modulated by sequences present in flanking regions.

We have also examined unequal gene conversion and crossing-over in diploids where one leu2 allele was located at its normal chromosomal location and the other mutant allele was present in a 2.2 kb leu2 fragment inserted elsewhere in the genome. LEU$^+$ meiotic recombinants occurred frequently (0.02% to 0.05% of random spores), often at frequencies similar to or higher than those observed in crosses when both alleles were inserted as 2.2 kb fragments at the same position on homologous chromosomes. Preliminary experiments indicate that these unequal exchange events are frequently associated with exchange of flanking sequences. Therefore, the presence of large regions of contiguous flanking homology is not a requirement for efficient meiotic recombination.

When exchange is between one allele at leu2 and an allele inserted at MAT, on the opposite arm of the same chromosome, Southern blot analysis of the recombination products shows that there is no constraint on intra-chromosomal (or unequal sister chromosomal) versus inter-chromosomal crossing-over.

1238 PROTEINS AND DNA SEQUENCES NEEDED FOR HIN RECOMBINATION, M. F. Bruist, R. C. Johnson, C. Lee, M. B. Glaccum, and M. I. Simon, California Institute of Technology, Pasadena, CA 91125

The Hin site-specific recombination system inverts a 995 bp segment of DNA in order to control the expression of flagellin genes in Salmonella. We have developed an in vitro system with E. coli extracts for studying this reaction. At least three different proteins are necessary for efficient recombination: Hin, HU (a histone-like protein), and an unidentified protein factor. A 26-bp sequence (hix site) is needed at each crossover point and a separate site, the recombinational enhancer, makes recombination efficient.

Nuclease and dimethyl sulfate (DMS) protection experiments have shown that Hin binds to the hix sites. A synthetic peptide composed of the last 52 amino acids of Hin also protects the hix site; thus, the C-terminal domain of Hin recognizes these sites. Portions of the enhancer sequence are protected by crude Hin extracts, but not by a highly purified Hin extract which requires the factor for activity. The factor is presumed to bind the enhancer sequence. This is being tested by purifying the factor. HU protein does not alter the Hin nuclease protection patterns. DMS cleavage patterns have been compared for supercoiled and linear substrates. No differences were detected even though supercoiling is necessary for recombination.

1239 THE EFFECT OF BLOOM'S SYNDROME FIBROBLASTS ON GENETIC RECOMBINATION AND MUTATION, Glenn Bubley, Lowell Schnipper, Charles A Dana Research Institute & the Department of Medicine, Beth Israel Hospital & Harvard Medical School, Boston, MA 02215.

The effect of Bloom's Syndrome (BS) and normal fibroblasts (NF) on homologous recombination of temperature sensitive (ts) mutants of herpes simplex virus type 1 (HSV-1) was determined. The viruses employed were HSV-1 ts K-13, ts J-12 and ts N-20 which map at .095 - .108, .345-.370, and .445 - .448, respectively, in the unique long (U_L) segment of the genome. Following infection of Vero cells with 5 pfu/cell of each member of a cross, or 10 pfu/cell of a self cross, the recombination frequency (RF) was quantitated as the virus titer at the non-permissive temperature (39°C) divided by the titer at the permissive temperature (33°C) x 200. Reversion rates for the self cross were insignificant (<0.1%). BS supported a 3.4 - 4 fold increase in RF with all crosses studied, as demonstrated by the J x K cross (3.7±1.8% for BS, 1.1±0.4% for NF). To determine the effect of low fluence UV light, NF were irradiated with 4 J/m^2. Little or no increase in the RF was observed immediately, or 24 hrs following UV irradiation. At 48 hrs, the RF increased to 5.7±2.1% from a 1.1% baseline in the J x K cross, suggesting that UV irradiation enhances the RF of HSV-1 in NF.

To determine the expression of a mutator phenotype in BS or NF cells, the reversion frequency of ts mutants was studied. Following infection at 0.01 - 0.1 pfu/cell to reduce the probability of multiplicity reactivation, the reversion frequency to the ts^+ (39°C) phenotype was 5-7 fold higher in BS vs NF ($2.4\pm1.1 \times 10^{-3}$ vs. $4.1\pm1.8 \times 10^{-4}$ for ts J-12, and $3.8\pm1.2 \times 10^{-3}$ vs. $5.2\pm2.1 \times 10^{-4}$ for ts K-13, respectively on the two different cell types). The biochemical basis for the increased reversion frequency in BS is under study.

1240 SV40 EXCISION CROSS-OVER POINTS ARE ASSOCIATED WITH TOPOISOMERASE I CLEAVAGE SITES. P. Bullock,[1] J. J. Champoux[2] and M. Botchan.[3] Imperial Cancer Research Fund, London, WC2A 3PX, England; [2]Dept. of Microbiology and Immunology, U. of Washington, Seattle, Washington, 98195; Dept. of Molecular Biology, U. of California, Berkeley, California 94720, USA.

Non-homologus DNA recombination occurs during numerous types of chromosomal rearrangements. In an effort to determine the mechanism(s) for non-homologous recombination, we have been analysing the sequences involved in SV40 excision from the chromosome of cell line 14B. Previous studies of the cross-over points used during SV40 excision indicated that these recombination events were dependent on sequences either at, or very close to the cross-over points. The cross-over point sequence features previously identified included small homologies at the point of strand exchange (2-3 bp), and sequences similar to those reported to be preferential binding sites for eukaryotic topoisomerase I. Furthermore, it was noted that the correlation between sites for topoisomerase I interaction, and sequences at cross-over points, could be extended to many other non-homologous recombination events.

Results from in vitro studies will be presented showing that the DNA sequence at the cross-over points used during SV40 excision are associated with topoisomerase I cleavage sites. The arrangement of the topoisomerase I cleavage sites has led to the proposal of a model for topoisomerase I mediated non-homologus recombination.

1241 PURIFICATION AND CHARACTERIZATION OF A MAMMALIAN REC PROTEIN, Era Cassuto, Yale University, New Haven, CT 06511

We have purified from crude extracts of HeLa cells and human foreskin fibroblasts a protein fraction which displays a number of activities that are thought to be part of the recombination process. These activities include binding to single-stranded DNA, annealing of complementary DNA strands, single-stranded DNA dependent ATPase, formation of joint molecules and strand exchange between two homologous duplexes and between duplex and single-stranded DNA molecules. The inducibility of these activities by mitomycin C and UV irradiation has also been studied.

1242 INTERMOLECULAR RECOMBINATION IN MAMMALIAN CELLS WITHOUT SELECTION. Pierre Chartrand and Suzanne Brouillette, Université de Sherbrooke, Sherbrooke, Canada, J1H 5N4.

In order to study intermolecular recombination in mammalian somatic cells we have devised an assay which permits recovery of the recombination products without selection. In our assay we co-transfect mouse 3T6 cells with a eucaryotic and a procaryotic vector, Hirt extract after 48 hrs and use the supernatant to transform E. coli. The eucaryotic vector is a polyoma variant that consists of a complete polyoma genome with an insertion of mouse cellular DNA. This cellular DNA contains both unique and highly repetitive sequences. This polyoma variant is thermosensitive for replication. The procaryotic vector is pAT153 with insertions of various lengths of mouse cellular DNA homologous to the sequences present in the polyoma vector. The recombinant molecules rescued in E. coli are identified by their positive in situ hybridization with polyoma sequences.

This assay makes it possible to control various parameters such as 1) the length and the degree of shared homology between the two vectors, 2) the structure of the vectors (circular or linear), with the double strand break in homologous or non-homologous sequences and 3) the replicative state of one of the vectors.

In our first assay we have co-transfected two vectors as circular molecules with 191 bp of shared homology. Analyses of 20 recombinants reveals no evidence of homologous recombination between the two molecules. Furthermore the homologous segments can be found close to each other on recombinant molecules. This would seem to indicate a lack of homology seeking mechanism in this type of intermolecular recombination. Other assays involving longer segments of homology are presently being conducted.

1243 FUNCTIONS OF PHAGE P1 RepA PROTEIN AT ITS MULTIPLE BINDING SITES IN THE ORIGIN OF PLASMID REPLICATION, Dhruba K. Chattoraj and Subrata K. Pal, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Phage P1 lysogenizes E. coli as a unit copy plasmid. For the plasmid mode of replication, an origin which depends on a 32 Kd P1 protein, RepA, for function has been identified within a 245 bp region. A salient feature of the origin is the presence of five tandem 19 bp repeat sequences to which RepA binds (1). Other than its role in initiating DNA replication RepA can also mediate two other functions: turning off its own promoter which maps within the repeat sequences and, at higher than physiological concentration, turning off plasmid replication itself. Controlling the amount of RepA protein is thus required for plasmid maintenance. In order to establish whether the low copy number of P1 plasmids is due to limiting amounts of RepA in the cell, we have cloned the repA gene under the control of foreign promoters so that its transcription can be varied systematically. Our preliminary results are consistent with the notion that RepA is normally rate-limiting for replication.

The 245 bp origin apparently interacts with at least two other host proteins: DnaA (2) and Dam (1). The study of these interactions is expected to reveal the role of multiple binding sites and multiprotein interactions in the control of DNA replication.

1. Abeles: These abstracts; Austin et al. JMB (1985) 183, 195.
2. Hansen and Yarmolinsky: These abstracts.

1244 Mutants of Saccharomyces cerevisiae with altered levels of sister chromatid recombination. A. Chaudhury and M.A. Resnick, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

A genetic analysis of sister chromatid recombination would not only provide a means for examining the process, but it would also be expected to lead to the identification of mutants altered in other types of recombination. Since sister chromatid recombination may occur during replication, some of the mutants might prove useful in identifying genes coding for important proteins involved in DNA synthesis. We initiated studies to identify and characterize mutations that alter wild type levels of recombination between sister chromatids. The mutants were obtained using a haploid yeast strain that is deleted for the original HIS3 gene but instead contains this gene split into two fragments with a region of homology on each fragment. The fragments are arranged so that only recombination between sister chromatids can generate HIS^+ colonies (M. Fasullo, Stanford University).

Following EMS mutagenesis, we obtained two classes of mutants: esr mutants exhibiting enhanced frequencies (>10X over the wild type) and dsr mutants having low levels of sister chromatid recombination. Among the mutants isolated, three esr and two dsr mutants have been examined further. The phenotypes segregate in a Mendelian fashion. One of the esr mutants (esr1) is sensitive to DNA damaging agents and shows increased mutability; the esr1/esr1 homozygous diploids also display poor sporulation and spore viability. The two dsr mutants are sensitive to γ-radiation but not UV. Experiments are in progress to determine the effect of these mutations on other genetic endpoints such as gene conversion and reciprocal recombination during mitotic growth and meiosis.

1245 Repair and recombination defective mei-41 mutants of Drosophila lack a DNase which is related to nucleases from fungi. Chow, T. Y.-K., Yamamoto, A.H., Mason, J.M. and Resnick, M.A. National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

In Drosophila melanogaster, the mei-41 gene controls the repair of DNA damage, meiotic recombination and fertility. The phenotype of mei-41 mutants is similar to that of the rad52 mutants of Saccharomyces cerevisiae, and of many recombination/repair deficient mutants of Neurospora crassa. Several of the N. crassa mutants have been shown to exhibit reduced levels of a single-strand DNA binding endo-exonuclease. We recently identified and purified a yeast deoxyribonuclease of 72 Kd that is precipitated by antiserum raised against the endo-exonuclease from N. crassa. The yeast deoxyribonuclease was shown to be under the control of the RAD52 gene.

Utilizing this antiserum, we have found a similar cross-reacting deoxyribonuclease in D. melanogaster; its activity is not detected in mei-41 mutants. In extracts of wild-type Oregon-R embryos a cross-reacting protein of approximately 70 kd was identified.

We used a λgt11 yeast genomic expression library to identify clones expressing an antibody cross-reacting protein. A yeast DNA segment that expressed a cross-reacting protein was obtained. It hybridizes in situ to a band in region 56D of the right arm of chromosome II of D. melanogaster. This is a different site than that of the mei-41 gene.

1246 MAPPING OF BABOON ENDOGENOUS VIRUS SITE SPECIFIC INTEGRATION ON HUMAN CHROMOSOME 6, J. C. Cohen, L-H Huang, J. Silberman, D. Pious, and M. Murphey-Corb. Louisiana State University Medical Center, New Orleans, LA 70112, University of Washington, Seattle, WA 98195 and Tulane University Delta Regional Primate Center, Covington, LA.

Baboon endogenous virus (BEV) infection of human cells was shown previously by somatic cell genetics and restriction endonuclease digestion to result in the sequence specific integration of viral DNA into human chromosome 6. In this study DNA from BEV-infected human VA2 cells was cloned into lambda bacteriophage and BEV positive clones were isolated. Restriction endonuclease maps indicated the presence of previously predicted clusters of PstI sites adjacent to integrated BEV proviruses. Three proviruses obtained from two independently derived lambda clones were shown to represent an identical region of the human genome by hybridization of flanking cellular sequences to digestions of normal human DNA. Hybridization of this flanking DNA to VA2 cells infected with BEV revealed that this region was a preferred site of integration

To genetically map the site of BEV specific integration a series of human lymphoid cell clones with various deletions in the short arm of chromosome 6 were used. DNA was digested and the flanking junction DNA was used to demonstrate the presence or absence of the BEV preferred integration site in each of the clonal human deletion cell lines. It was found that the preferred integration site mapped between the Glo and HPCK loci in the region defined by the insulin gene-like locus.

1247 THE ROLE OF RECOMBINATION IN MEIOSIS I HOMOLOG SEGREGATION, Dean s. Dawson Andrew W. Murray and Jack W. Szostak, Mass. Gen. Hosp., Boston, MA 02114

Cytological and genetic studies in a number of organisms have shown that the physical linkage of homologs in meiosis I is necessary to insure their segregation. These linkages, chiasmata are probably the cytologically visible consequence of reciprocal crossovers between homologs. We have undertaken experiments in yeast to examine the requirements for meiotic reciprocal crossing-over between homologs and the effect of reciprocal crossovers on the segregation of homologous model chromosomes. Artificial chromosomes composed of a bacteriophage lambda DNA backbone and yeast centromeres, replicators, telomeres and selectable markers, were shown to recombine ten to twenty times less frequently than similarly sized truncated versilns of chromosome III. We are currently in the process of determining the basis for this difference. Reciprocal crossing-over between the artificial chromosomes did not measurably increase their levels of proper disjunction. We are investigating the structural requirements which are prerequisites for the mediation of proper disjunction by reciprocal crossing-over.

1248 *IN VIVO* RESOLUTION OF CLONED POXVIRUS TELOMERES, A.M. Delange and G. McFadden
Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

The poxvirus genome consists of a single double-stranded DNA molecule which is covalently crosslinked to form terminal hairpin structures. The terminal hairpin sequences of two poxviruses, Vaccinia and Shope Fibroma Virus (SFV), have been cloned in yeast/*E. coli* shuttle vectors and DNA sequencing studies have shown them to be maintained in yeast as inverted repeats, with the axis of symmetry at the original hairpin. Similar inverted repeats have been detected during poxvirus replication, and are presumed to be telomeric replicative intermediates. Efficient resolution of the cloned inverted repeat sequences to hairpin termini was achieved following their transfection into poxvirus-infected cells. Although the terminal DNA sequences of Vaccinia and SFV are nonhomologous, except for two 10-11bp regions of identity, cloned telomeres from either virus were efficiently and accurately resolved in heterologous transfections, suggesting a common mechanism. The 320bp AluI fragment of the SFV-yeast clone and the 260bp ClaI fragment of the Vaccinia-yeast clone, both of which are imperfect palindromes, were subcloned into pUC vectors, using a recombination defective strain of *E. coli* as host. Unidirectional deletion derivatives of these inverted repeats were constructed and tested for their resolution to hairpin termini. A minimum DNA sequence of 150-200 base pairs long was found to be necessary for the resolution of each viral telomere.
(Supported by AHFMR and MRC.)

1249 TELOMERE REPLICATION AND CRUCIFORMS, Peter Dickie, Grant McFadden and A. Richard Morgan, Dept. of Biochemistry, Univ. of Alberta, Edmonton, Alberta, Canada T6G 2H7.

The DNAs of pox viruses have hairpin termini, and appear linear morphologically. Replication through the hairpin termini (telomeres) generates a palindrome, which potentially can extrude into a cruciform. Holliday cross-overs and cruciforms are identical structurally and endonucleases from T4 and T7 phages can cut the DNA at the crossing phosphodiesters resolving the linked duplexes. If the "opened-out" (replicated) telomeric palindromes undergo cruciformation and resolution, the original telomeres will be regenerated. The "opened-out" telomeres of vaccinia and Shope fibroma viruses have been cloned into pUC13 and, when transfected into pox virus infected cells, the plasmids are resolved into linear hairpin DNA. To date a cruciform can only be extruded from a palindrome when the DNA is stressed by negative superhelical tension. Extrusion of a cruciform might be expected to follow DNA melting in the region of diad symmetry and branch migration. However this simple mechanism is in conflict with several experimental findings. In order to study the recombinationally relevant processes of intermediate formation, branch-migration and resolution as they occur during cruciformation, we have constructed circular DNAs which are entirely palindromic in vitro. We have some evidence from electronmicroscopy of possible intermediates, which we tentatively believe may be tetrastranded DNA. The latter, if formed intramolecularly from palindromic DNA, on strand exchange, will give cruciforms. Using a novel fluorescence assay we can detect the activities of T7 gene 3 protein, a resolvase of cruciforms, and hence we are proceeding to attempt to isolate the protein(s) in Shope fibroma infected cells which resolve the "opened-out" telomeres in pUC13. (Supported by AHFMR and MRC Canada.)

1250 SPECIALIZED NUCLEOPROTEIN STRUCTURES AT THE REPLICATION ORIGIN OF BACTERIOPHAGE λ. M. Dodson[1], H. Echols[1], S. Wickner[2], J. D. Roberts[3], B. Gomes[3], C. Alfano[3], J. Lebowitz[3], and R. McMacken[3]. [1]Dept. of Molecular Biology, University of California, Berkeley, CA; [2]Laboratory of Molecular Biology, Natl. Cancer Institute, Bethesda, MD; [3]Dept. of Biochemistry, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

Bacteriophage λ initiates replication at a unique origin (oriλ). The oriλ region contains four direct repeats of 18 bp. Two phage-encoded proteins, O and P, are required for replication. Electron microscopy demonstrates that O protein interacts with the ori region to generate a specific nucleoprotein structure in which the DNA is inferred to be folded or wound. A similar structure is observed on substrates deleted for the two leftward repeats, but not on substrates with deletions extending into the third repeat. We surmise that the nucleoprotein structure is formed by an initial interaction of O with at least two of the direct repeats, which then serves as the nucleation event for a protein-protein association that generates the structure seen in the electron microscope. The O, P, and host dnaB proteins interact at oriλ to generate a structure larger than that formed by O alone. When dnaJ, dnaK, and ssb proteins are included in the reaction mixtures, ring-like structures whose formation is ATP-dependent are observed; we interpret these as an early event in initiation. The formation of ring-like structures requires DNA sequences both within and outside of the directly repeated region of oriλ. We conclude that the specialized nucleoprotein structure formed by O and oriλ provides for localized initiation of DNA replication by serving as the foundation for the assembly of the initial priming structure.

1251 SPECIALIZED NUCLEOPROTEIN STRUCTURES IN INITIATION OF DNA REPLICATION AND SITE-SPECIFIC RECOMBINATION. H. Echols, Department of Molecular Biology, University of California, Berkeley, California.

The initiation of DNA replication and site-specific recombination frequently involve multiple binding interactions by the proteins that localize the reaction. In the case of λ O, λ Int and E. coli DnaA, electron microscopic and other evidence indicates that these proteins generate organized nucleoprotein structures at their target sites. The association of proteins bound at multiple sites on DNA presumably folds or winds the DNA to produce a specific three-dimensional conformation. This specialized nucleoprotein structure may be a general mechanism for achieving exceptional accuracy in DNA transactions in which even a rare mistake must be avoided. To convert binding precision into specific reactivity, the initiating nucleoprotein structure can direct additional protein-protein associations and structural changes in the B-type DNA duplex (transition state complexes). For site-specific recombination, we presume that the initial pairing of substrate sites occurs by protein-protein interactions that generate the appropriate augmented nucleoprotein complex; in addition, the B-DNA duplex in the nucleoprotein structure might be distorted into a reactive configuration favoring a strand-switched, base-paired intermediate. For initiation of replication, the site-localization by the sequence-specific initiator probably targets the sequential addition of other critical proteins; a reactive configuration of DNA may also be generated (e.g. partial unwinding for the start of helicase activity of DnaB). The overall interactions noted above are more complex than found for typical prokaryotic transcription regulators (some may, however, have separated binding sites). Eukaryotic transcription regulators utilize multiple binding interactions extending over large regions of DNA and thus may also generate specialized nucleoprotein structures.

1252 CHARACTERISATION OF E. coli RecB AND RecC PROTEINS, Peter T. Emmerson, Paul W. Finch, Ian D. Hickson, Alan Storey and Rosemary E. Wilson, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU, UK.

The RecB and RecC proteins have been purified to homogeneity from cells harbouring plasmids in which the recB and recC genes were harnessed to strong promoters. When combined, these proteins exhibit the ATP-dependent ds DNA exonuclease activity characteristic of the RecBC enzyme. The reconstituted enzyme also cleaves ds DNA adjacent to Chi sequences. Partial properties of the individual subunits have been studied with a view to assigning, where possible, the wide range of properties of the holoenzyme to either subunit. The DNA-dependent ATPase activity resides in the RecB subunit. This correlates well with nucleotide sequence analysis of the thyA-recC-ptr-recB region which reveals a consensus adenine nucleotide binding site in the recB gene. In filter-binding studies, RecB but not RecC protein binds to ss DNA. Both proteins possess endonuclease activity, although their substrate specificities differ. RecB protein appears to cut linear ss DNA, while RecC protein cuts linear ds DNA in a site-specific manner. Experiments are in progress to define the cleavage sites in model substrates.

1253 PURIFICATION OF A YEAST ENZYME THAT CLEAVES HOLIDAY JUNCTIONS AND CHARACTERIZATION OF THE ENZYME USING AN OLIGONUCLEOTIDE SUBSTRATE ANALOG, David H. Evans and Richard Kolodner. Laboratory of Molecular Genetics, Dana-Farber Cancer Institute and Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

We have partially purified an endonuclease from Saccharomyces cerevisiae that cleaves Holliday junctions. The enzyme has been purified greater than 300 fold and contains 4-5 peptides as determined by electrophoresis on SDS-acrylamide gels. Based on gel filtration experiments the activity eluted as a 94,000 M_r protein but may be composed of smaller subunits.

In order to further characterize this enzyme we have constructed an artificial Holliday junction from synthetic oligonucleotides. Mixing curves showed that these oligonucleotides annealed to form a four-armed analog of the crossover region of a Holliday junction. Analysis of the equilibrium between the junction and component single strands demonstrated that these structures were stable (ΔH° = ~8.0 Kcal, mol bp^{-1}, ΔS° = ~25 cal, mol bp^{-1}, deg^{-1}; in 0.1M NaCl) in agreement with the model building studies of Sigal and Alberts. The synthetic Holliday junction was found to be a specific inhibitor of the enzyme in assays that measured the ability of the enzyme to cleave within extruded palindromic sequences of supercoiled plasmids. Fifty percent inhibition of this reaction was observed at an inhibitor/substrate ratio of 7:1 which supports the contention that cruciform junctions are the structures recognized by the enzyme. Finally, unlike plasmid borne cruciform structures, the sequences of the arms of this synthetic Holliday junction are unique and therefore the junction cannot branch migrate. We are currently using this property to determine precisely where the enzyme cleaves relative to the Holliday junction.

1254 SITE-SPECIFIC RECOMBINATION IN THE PARVOVIRUS CHROMOSOME: ROLE FOR A $CT^{A}_{T}TT^{C}_{T}$ MOTIF. Emanuel A. Faust, Jim Lickers and Aileen Hogan, University of Western Ontario, London, Ontario, Canada, N6A 5B7.

Minute virus of mice (MVM), a helper-independent parvovirus, exhibits a high frequency of spontaneous deletions that are mediated by 4-10 base pair direct repeats (Hogan and Faust (1984) Mol. Cell. Biol. 4; 2239-2242). We have examined the DNA sequences at deletion junctions in eight deleted variants of MVM and found a hexanucleotide motif of the type $CT^{A}_{T}TT^{C}_{T}$ in the vicinity of these junctions. This motif closely resembles sequences that are required for FLP-mediated recombination in yeast, Tn10 transposition and conservative integration of λ DNA into the attB site of the E. coli chromosome.

In addition, we have detected a site-specific endonuclease activity in purified mutant virus particles that cleaves the encapsidated DNA at either 3'-CTATT|C-5' or 3'-CTTAT|C-5' sequences. The results suggest that a $CT^{A}_{T}TT^{C}_{T}$ motif is involved in the formation of spontaneous deletions in the parvovirus chromosome and that the deletion mechanism involves cleavage at $CT^{A}_{T}TT^{C}_{T}$ sequences by a site-specific endonuclease.

1255 THE FATE OF THE VIRAL GENOME IN INFECTIONS OF NON PERMISSIVE CELLS BY POLYOMA VIRUS. Michele M. Fluck, David L. Hacker, Susan Kalvonjian and Claudette Priehs, Michigan State University, East Lansing, Mi. 48824-1101

We are following the fate of the polyoma viral genome from the time of infection of non permissive rat cells to the time of integration into the host genome. Extensive amounts of replication are detected under some circumstances. We are in the process of characterizing replicative intermediates. Examination with different strains of virus reveals extensive strain specific differences which are not observed in permissive mouse cells.

We have carried out some of our analyses in mixed infections with two strains which differ in the presence of restriction endonuclease sites. This allows us to examine the role of recombination in the transformation pathway. Whereas no recombination can be detected in the pool of viral DNA molecules present in the whole cell population up to 10 days after infection, transformants which arise around that time show a high level of recombination (33% in a 1,000 basepair interval). Since a single parent is able to transform on its own, the experiments strongly suggest that transformants are derived from a special pool of cells, selected aside from or among cells in which viral DNA synthesis is occuring. The observations also suggest that the bulk of tandem viral integrations are due to recombination.

1256 A POSSIBLE ROLE FOR NEUROSPORA ENDO-EXONUCLEASE IN RECOMBINATIONAL REPAIR OF NUCLEAR DNA, Murray J. Fraser, Dindial Ramotar and Zafer Hatahet, McGill University, Montreal, Quebec, Canada H3G 1Y6.

The recombination-defective, mutagen-sensitive uvs-3 mutant of Neurospora crassa is deficient in endo-exonuclease in both nuclei and mitochondria and yet these organelles have high levels of trypsin-activatable endo-exonuclease, a 90 kDa protein which is apparently a precursor of the active enzyme. Two approaches are being used to ask how endo-exonuclease might be involved in repair of nuclear DNA: (i) to determine whether pre-treatment of mycelia with the DNA-damaging agent, 4-nitroquinoline-1-oxide (4-NQO) has any effect on nuclear endo-exonuclease levels and (ii) to study the mechanism of action of pure endo-exonuclease in vitro. In response to pre-treatment of wild-type mycelia with 4 μg/ml 4-NQO, nuclear precursor was converted to active endo-exonuclease. At higher doses (8-16 μg/ml), the precursor was lost. Neither of these response appeared to be inducible phenomena since they were not blocked by 5 μg/ml cycloheximide. A salt- and polyamine- activated protease activity has been identified in nuclear extracts which destroys both precursor and active enzyme. Pure endo-exonuclease, under appropriate conditions, was found to make double-strand breaks in native DNA with some site specificity. Electron microscopic examination of the treated DNA indicates that the enzyme remains attached to the broken ends of the DNA. A very potent heat-stable protein inhibitor has been purified which preferentially inhibits the exonuclease activity of endo-exonuclease. A possible role for endo-exonuclease in double-strand break repair is suggested. (Supported by the Medical Research Council of Canada).

1257 Molecular Cloning and Analysis of Genes Required for Meiotic Recombination and DNA Metabolism in Yeast; C.N. Giroux[1], H.F. Tiano[1], M.E. Dresser[2], and M. Moses[2]; [1]NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709; [2]Dept. of Anatomy, Duke University Medical School, Durham, NC 27710

The goal of this project is to identify and analyze the proteins required for chromosome behavior and DNA metabolism during yeast meiosis. We are focusing on the analysis of two meiosis specific genes: SPO11 is required for the initiation of meiotic recombination while PAC1 is required for its resolution. A general system has been developed to isolate meiosis specific genes for which mutants are available. Using this system, the SPO11+ gene has been cloned and its structure and DNA sequence determined. Both insertion and deletion mutants of the SPO11 gene have been constructed in vitro. These results, combined with genetic complementation of a spo11- mutant in vivo, identify a candidate coding sequence for the SPO11 gene product. We are attempting to directly identify this gene product by expression of the cloned SPO11 gene in an E. coli system. Similar studies are in progress with the PAC1 gene. As a structural approach to meiotic recombination and chromosome pairing, we have initiated an immunocytological analysis to identify protein components of the synaptonemal complex. Monoclonal antibodies which react with the SCs of several species during pachytene have been isolated; their characterization will be presented.

1258 NUCLEASE MECHANISM OF THE AVIAN RETROVIRUS pp32 INTEGRASE, Duane P. Grandgenett, St. Louis University, St. Louis, MO 63110

The integration of retroviruses requires a virus-coded DNA endonuclease function. The avian retrovirus pp32 integrase is a partially phosphorylated 32,000 dalton protein which possesses a DNA nicking activity in the presence of Mg^{2+} or Mn^{2+}. The pp32 protein forms a nucleosome-like structure on viral LTR DNA including the circle junction region of two tandem LTR copies. The circular viral molecule containing two LTRs in tandem is the presumed precursor molecule to the integrated provirus. In the presence of Mg^{2+}, the integrase prefers to nick supercoiled DNA containing LTR circle junction sequences at one or the other of two sites, each which mapped two nucleotides back from the circle junction. The sequence at the sites of nicking is

CA↓TT|AA TG
GT AA|TT↑AC

where ↑ indicates the position of the two alternative sites. These in vitro data support other biochemical and genetic data demonstrating the role of the pp32 protein in viral integration and retrovirus transposition. In addition, other laboratories have demonstrated using amino acid sequence comparisons that the viral pp32 sequences are conserved in various systems employing transposition including Drosophilia copia and Saccharomyces cerevisiae Ty.

1259 IMMUNOGLOBULIN GENE EXPRESSION IN DIFFERENTIATIVE PRE-B LYMPHOCYTES, Johanna A. Griffin and David Spalding, University of Alabama at Birmingham, Birmingham, AL 35294

The generation of mature genetic codes for an immunoglobulin molecule requires a number of precise DNA recombination events. Since it has previously been possible to study only transformed cells that have already completed the recombination process, it has not been possible to study the molecular mechanism by which the processes occur. We have established a means of inducing non-transformed cells to undergo differentiation from one of the earliest stages of B lymphocyte differentiation, pre-B cells, to the latest plasma cell stage of immunoglobulin secretion. These cells must undergo all of the DNA recombination events necessary to form the light chain variable exon and the switch recombination that translocates the heavy chain variable exon from a position 5' to the code for the IgM to a new location 5' to the IgA heavy chain code in vitro. Our data indicate that interation of the pre-B cells with accessory cells from different lymphoid tissues results in very specific genetic modifications. Pre-B cells induced by spleen accessory cells to secrete IgM undergo deletions in the DNA flanking C_μ that could include the switch recombination sequences and a potential z-DNA region that might result in the loss of the code for the trans-membrane portion of the IgM heavy chain. Therefore, the commitment to the secretion of IgM may be due to the specific deletions of sequences that would allow the cell to pursue other pathways of differentiation.

1260 SEQUENCES ADJACENT TO THE YEAST MATING-TYPE LOCUS AFFECT PAIRING WITH HML OR HMR DONORS DURING HOMOTHALLIC SWITCHING. James E. Haber, Norah Rudin, Sue Stewart and Bernadette Connolly. Brandeis University, Waltham MA 02254

Homothallic switching in Saccharomyces cerevisiae is normally an intra-chromosomal gene conversion event in which unexpressed a or α sequences at the donor loci, HML and HMR, are transposed to replace sequences at the mating-type locus, MAT. Normally, MATa cells interact preferentially with HML, while MATα cells usually use HMR. When the normal MAT locus is deleted and a cloned fragment containing MAT is inserted either on the same chromosome or on a different chromosome, switching is much less efficient. In many cases, the MAT locus fails to pair with either donor and the MAT locus (cut by the HO-endonuclease) is degraded. In the most extreme case (inter-chromosomal) 90% of MATα cells fail to switch to the opposite mating type, while MATa fails 50% of the time. It appears that pairing of MATα with HMR requires additional MAT-adjacent sequences not included in the transformed segment and beyond those required by MATa to interact with HML. When all of the region distal to MAT is deleted from the cloned segment, MATa as well as MATα switching nearly always fails. We are currently constructing deletions at the normal MAT locus to analyze the role these regions play in normal donor selection.

We have also begun to characterize a trans-acting mutation that appears to abolish normal directionality of switching and results in nearly equivalent use of either HML or HMR in switching cells of either mating type.

1261 DEFECTIVE DNA TOPOISOMERASE I ACTIVITY IN A DNA^{ts} MUTANT, Roger Hand, Gui-Chao Zeng and Harvey L. Ozer, Dept. of Medicine, University of Illinois at Chicago and Dept. of Biology, Hunter College of CUNY, New York.

Cell and polyomavirus DNA synthesis in ts20, a temperature-sensitive mutant derived from Balb/3T3 cells, is inhibited at an early step in chain elongation in vivo and in vitro. Virus DNA synthesized under restrictive conditions contained a series of equally spaced bands migrating between form I and form II on electrophoresis. If restrictive conditions were prolonged, the relative amount of these topoisomers increased while the overall amount of virus DNA decreased. DNA topoisomerase I activity was lower and more heat-labile in mutant cells compared to wild-type (wt) and revertant cells. An assay in which extracts from wt cells corrected defective cell DNA synthesis in lysed mutant cells was applied to purification of the active factor from such extracts. Salt fractionation and three cycles of column chromatography resulted in the isolation of the activity in a fraction containing 10 major polypeptides. The specific activity in the final preparation was increased 5-fold and was accompanied by the activity of DNA topoisomerase I. Our results provide evidence that DNA topoisomerase I functions at an early step in chain elongation of cell and polyomavirus DNA synthesis and that the enzyme activity may be decreased as a result of the mutation in ts20.

1262 PROTEIN-DNA RECOGNITION IN THE REGULATION OF TETRACYCLINE RESISTANCE GENES, W. Hillen, Institut fur Physikalische Biologie, Universitat Dusseldorf.

The expression of Tetracycline resistance genes in gram negative bacteria is regulated at the level of transcription. Their regulatory mechanism is complex and consists of two overlapping promotors with opposite polarity and two operators of nearly identical palindromic sequences (1). Each operator is occupied by a Tet repressor dimer when transcription is turned off. Induction of expression is mediated by tetracylcline which binds to the Tet repressor and reduces its affinity for the operator DNA (2). Resistance genes isolated from different species contain natural repressor operator recognition mutants with varying affinities for the heterologous interactions (3). These as well as mutants constructed in vitro provide the basis for oligonucleotide directed mutagenesis in the operator and repressor sequences. The mutants are used to define the protein DNA interaction at the single amino acid resolution and to identify the function of the tandem arrangement of the operators. The binding of the inducer tetracycline to the Tet repressor is studied by fluorescence spectroscopy. The results indicate energy transfer from tryptophan of the repressor to bound tetracycline. The primary structure of the repressor is engineered by site directed mutagenesis to allow identification of the energy transferring tryptophan and to study the behaviour of the DNA binding domain in the presence and absence of inducer.

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3. G. Klock, B. Unger, C. Gatz, W. Hillen, J. Altenbuchner, K. Schmid and R. Schmitt. 1985. J. Bacteriol. 161:326.

1263 **An endonucleolytic activity that cleaves immunoglobulin recombination sequences.**
Thomas J. Hope, Renato J. Aguilera, Mark E. Minie and Hitoshi Sakano, Department of Microbiology and Immunology, University of California, Berkeley, CA 94720.

In order to better understand the molecular mechanism of Ig gene recombination, we have attempted to identify a recombination enzyme which mediates V-(D)-J joining (1,2). Our studies are based on the assumption that three main activities should be involved in Ig gene recombination: a DNA binding activity, an endonucleolytic activity, and a ligase activity. An endonucleolytic activity which cleaves in the Ig joining segment region was identified using a Southern blot hybridization assay and a sequencing gel assay. The activity introduces a double-strand cut in the vicinity of the recombination site of immunoglobulin joining gene segments. The cleavage occurs at the dinucleotide pair $\frac{TG}{AC}$ generating a product consisting of a 3' overhanging guanidine nucleotide. The endonucleolytic activity is found in mouse fetal and adult liver, and chick embryo bursa nuclei and is not present in any other tissues and cell lines analysed. These results indicate that this activity is a good candidate for the putative endonuclease involved in immunoglobulin V-D-J recombination. This activity is distinct from the endonuclease activities identified in two similar studies. We are currently purifying this activity for further characterization, as we try to identify the associated DNA binding activity.

1. **Identification of an endonucleolytic activity which cleaves immunoglobulin recombination sequences.** [T.J. Hope, R.J. Aguilera, M.E. Minie, and H. Sakano (1985) Science in press.]
2. **The immunoglobulin heavy-chain enhancer is deleted is some murine plasmacytomas.** [R.J. Aguilera, T.J. Hope, H. Sakano (1985) EMBO in press.]

1264 BINDING OF GENE II PROTEIN TO THE REPLICATION ORIGIN OF BACTERIOPHAGE f1, Kensuke Horiuchi and David Greenstein, Rockefeller University, New York, NY 10021

The replication of plus strand of the filamentous bacteriophage f1, M13 and fd is initiated by introduction of a site-specific nick in the plus strand of RFI by the action of phage-coded gene II protein. Gene II protein binds the origin in vitro to form a complex which can be trapped on nitrocellulose filters. The binding occurs with both superhelical form DNA and linear DNA fragments. Screening of a number of defective mutants of the origin for the ability to bind gene II protein indicates that the region of DNA required for the binding overlaps, but is not identical to, the region required for the nicking reaction. The nicking site itself is dispensable for the binding. The binding of gene II protein to the origin results in protection of a 43 bp segment from DNase I digestion. The protected region covers whole domains which are required for the nicking and for the binding. In vivo, a number of defective mutants of the origin, when in a plasmid, inhibit growth of superinfecting phage if the intracellular level of gene II protein is low. The domain of DNA sequence required for the in vivo inhibition is consistent with that for the in vitro binding.

1265 RECONSTRUCTING HUMAN α-THALASSEMIA 2 GENOTYPE IN PRIMATE CELLS: UNUSUALLY HIGH FREQUENCY OF HOMOLOGOUS DNA RECOMBINATION AND MODULATION BY SPECIFIC DNA SEQUENCES IN CIS, W.-S. Hu and C.-K. James Shen, University of California, Davis, California 95616

The duplicated human adult α globin genes, α2 and α1, provide an excellent system for exploring the molecular mechanism of both general and site-specific DNA recombination processes in mammalian cells. These loci are contained within tandemly arranged units; each unit spans approximately 4 kb of DNA containing three homology blocks (X, Y, and Z) separated by non-homologous regions. This high degree of homology is presumably maintained by gene conversion and unequal crossing-over events. These unequal crossing events have resulted in high frequency of two types of deletion of one α gene (α-thalassemia 2) in the human populations: the leftward resulting from recombination between the X blocks, and the rightward resulting from recombination between the Z blocks. Our sequencing study of the X blocks further suggests the existence of hot spot(s) of initiation of DNA recombination near their 5' ends.

We have now successfully reconstructed these α-thalassemia 2 genotypes in monkey cells. The duplication units have been cloned in SV40 ori-containing vector, and transfected into COS-7 cells. Newly replicated, supercoiled DNA were analyzed by blotting-hybridization, and by rescuing from bacterial strain HB101. Unusually high frequency of intrachromosomal homologous DNA recombination (> 20% per kb of homology) has resulted in both the rightward and leftward α-thalassemia 2 genotypes. Deletion of the 5' end of one X block greatly affects the relative frequencies of the leftward and rightward deletions. These results suggest that the homologous recombination of the α globin locus in primate cells is modulated both by specific sequences and by the spatial arrangement of the recombination units.

1266 GENE-A-PROTEIN OF BACTERIOPHAGE PHI-X174; INTERACTION WITH THE ORIGIN OF DNA REPLICATION, H.S. Jansz, A.D.M. van Mansfeld, A.C. Fluit and P.D. Baas, State University of Utrecht, Vondellaan 24 a, 3521 GG Utrecht, The Netherlands.

The protein encoded by gene-A of bacteriophage ϕX174, the ϕX A-protein, cleaves the viral strand of supercoiled ϕX RF DNA at a unique site, the origin of DNA replication. The origin is located in a specific sequence of 30 bp, ĊAACTTGATȦTTAATAACACĊTATAGACCAĊ, which is recognition spacer binding highly conserved among isometric single-stranded DNA phages. ϕX A-protein cleaves the phosphodiester bond between G (position 7) and A (position 8) in this origin region yielding a free 3' OH at the G residue and a covalent bond between tyrosine-OH of the A protein and 5' phosphate of the A residue. Therefore, this type of phosphodiester bond cleavage is called tyrosinolysis. We have studied the mechanism of tyrosinolysis by varying the DNA substrate and by further analysis of the covalent complex between A-protein and the DNA. The results indicate that: 1. tyrosinolysis of single-stranded DNA requires the presence of only the first 9 nucleotides of the origin region whereas for the cleavage of supercoiled DNA almost the complete origin region is both required as well as sufficient. 2. two different tyrosine residues, whose positions in the A protein have been determined, are involved in DNA cleavage. Either one of these tyrosine residues can function as the acceptor of the DNA chain during tyrosinolysis. These results suggest underlying molecular mechanisms of the ϕX A-protein catalysed initiation and termination reactions of the looped rolling circle model for DNA replication as postulated by Kornberg and co-workers.

1267 THE 2 MICRON CIRCLE: A MODEL FOR SITE-SPECIFIC AND GENERALIZED RECOMBINATION, Makkuni Jayaram, Scripps Clinic and Research Foundation, La Jolla, CA 92037

The FLP-protein of 2 micron circle is a site-specific recombinase that acts on the 599 base-pair inverted repeats of the molecule. The minimal FLP substrate has been localized to a 13 base-pair dyad symmetry with an 8 base-pair core. The terminal 3 base-pairs of the symmetry elements can be deleted without abolishing the reaction. The interactions between a random set of mutant FLP proteins and the minimal substrate are under investigation.

I have used a flp$^-$ 2 micron circle as a model substrate for studying the mechanistic consequences of gene conversion. The conversion event is initiated by making a double-stranded gap within one of the repeats and allowing the gap to be repaired in yeast. The intact repeat serves as the template for gap-repair. The association of flanking-marker recombination with the conversion event is assessed by determining whether the plasmid has retained its parental configuration or undergone intramolecular inversion.

The FLP protein can promote inversion in yeast of the bacterial transposon Tn5 contained within a 2 micron circle plasmid. I have shown that this inversion reaction is the mechanistic consequence of a gene conversion event initiated, presumably, by a FLP-mediated cut made within the 2 micron circle repeat. The sequence of events involved in the reaction are reminiscent of the steps leading to mating type interconversion in yeast.

1268 **SEGREGATION OF CEN CONTAINING PLASMIDS AWAY FROM INTACT CHROMOSOMES DURING MEIOSIS I IN SACCHAROMYCES CEREVISIAE.** David B. Kaback and Vincent Guacci, Department of Microbiology, UMDNJ-Graduate School of Biomedical Sciences - New Jersey Medical School, Newark, N.J. 07103.

Meiosis I is characterized by pairing, recombination and segregation of homologous chromosomes. To determine the chromosomal DNA elements required for these processes, we have examined the meiotic segregation of centromere containing plasmids with respect to unpaired intact chromosomes. Whereas centromeric DNA appears essential for proper segregation of homologs, it does not appear to provide the specificity that causes the homologs to interact. To determine if DNA homology provides this specificity, plasmids containing inserts from different parts of chromosome I were transformed into strains of S. cerevisiae that were either monosomic or trisomic for this chromosome. Transformed strains were sporulated and the meiotic behavior of plasmids and the intact copies of chromosome I were examined. Three plasmids, each containing a 12-14 kb insert from a different part of chromosome I were tested. In all three cases the plasmids segregated away from the unpaired copy of this chromosome in approximately 80% of the asci examined. In contrast, when the plasmid vector contained no chromosome I insert or a 16.8 kb DNA insert from bacteriophage λ , it segregated randomly with respect to the unpaired copy of chromosome I. These results suggest that a small amount of homologous DNA is sufficient to direct meiotic segregation of a plasmid away from its chromosomal homolog.

1269 THE ICP8 DNA-BINDING PROTEIN OF HERPES SIMPLEX VIRUS BINDS PREFERENTIALLY TO THE JOINT REGION OF THE VIRAL GENOME IN VIVO, Susan S. Leinbach, Univ. of South Alabama, College of Medicine, Mobile, AL 36688

Herpes simplex virus type 1 (HSV-1) encodes a 130,000 molecular weight DNA-binding protein, ICP8. This protein is required for viral DNA replication as determined by analysis of temperature-sensitive mutants. ICP8-DNA complexes can be immunoprecipitated from nuclear extracts prepared from wild-type HSV-1 infected cells using monoclonal antibody directed against ICP8. Studies were performed to identify the regions of the viral genome present in these complexes. DNA was extracted from immunoprecipitated ICP8-DNA complexes, nick-translated and used as a hybridization probe (the ICP8 DNA probe). This probe was hybridized to restriction endonuclease-generated HSV-1 DNA fragments which had been fractionated by agarose gel electrophoresis and transferred to nitrocellulose paper. DNA fragments representing the entire HSV-1 genome were identified by the ICP8 DNA probe. However, fragments from the joint region, that region where the long and short components of the viral genome join, were over-represented. Dot-blot hybridizations were used to confirm these results. ICP8 DNA contained about 8 times more of the DNA sequences present in a 1500 base pair (bp) HinfI joint fragment than in an adjacent 1200 bp HinfI fragment. These results suggest that ICP8 binds preferentially in vivo to the joint region of the viral genome. Experiments are in progress to determine if such preferential binding occurs in vitro. In addition, since ICP8 binds preferentially to single-stranded DNA in vitro, in vivo ICP8-DNA complexes are being examined for single-stranded DNA.

1270 DNA JOINING ACTIVITIES IN MAMMALIAN CELL EXTRACTS OF DIFFERENT ORIGIN Kerstin Kenne and Siv Ljungquist*, Department of Medical Cell Genetics, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden. * National Institute of Environmental Medicine, Box 60208, S-104 01 Stockholm, Sweden.

Various mammalian cell lines and organs have been investigated with respect to the DNA joining activity, with similarities to the E. coli RecA activity, detected in fibroblasts from patients suffering from the inherited disease Bloom's syndrome (BS).

The recovery of joint molecules was analyzed by a filterbinding assay where D-loop containing joint molecules, formed from ØX174 RFI DNA and fragments of ØX174 ssDNA by partially purified cell extracts, was separated from the substrates. In addition to BS(GM1492), CHO cell extracts were positive. No activity, or very little, was present in normal human fibroblasts, Fanconi's anemia fibroblasts, SV40 and EBV transformed human cells. No significant increase in the DNA joining activity was noticed in the filterbinding assay on treatment with mitomycin C, an inducer of mitotic recombination. Cell extracts from human placenta, calf thymus, rat spleen and rat testis were inactive.

The cell extracts positive in the filterbinding assay (GM1492 and CHO) were also positive in another DNA joining assay utilizing ØX174 RFIII DNA as substrate and agarose gelelectrophoresis for analysis. The formation of joint molecules was dependent on ATP and Mg^{2+} as in the filterbinding assay. All other cell extracts tested were negative. These results indicate specific DNA joining activities in Bloom's syndrome (GM1492) and CHO cells.

1271 ISOLATION OF A LOW COPY NUMBER DNA SEQUENCE THAT NEIGHBORS CENTROMERIC SATELLITE DNA IN MONKEY, HUMAN AND MOUSE GENOMES. Antonella Maresca*, Ronald E. Thayer[1], Chantal Guenet[1] and Maxine F. Singer[1], [1]Lab. of Biochemistry, N.I.H., Bethesda, MD 20892 and *Lab. of Molecular Endocrinology, CHUL, Quebec, G1V 4G2, Canada.

Long arrays of tandemly repeated DNA sequences known as satellite DNA occur at many mammalian centromeres. Structural analysis has suggested that the organization of centromeric satellite changes continually, presumably through alternating cycles of mutations, amplifications and deletions. It is possible that these events and the localized maintenance of the satellites are by-products of genomic processes involving recombination and replication that are characteristic of centromeres. This model suggests that special (non-satellite) sequences in the vicinity of satellite DNAs may be involved in satellite DNA maintenance and evolution. To begin to test this hypothesis we cloned, from an African green monkey (C. aethiops) genomic library, 3 segments containing the monkey deca-satellite joined to 3 different low copy number sequences (LCNS). The LCNS of one of the clones (λCJA1) has been studied in detail. This LCNS is conserved in human and rodent genomes. In clones selected from human and mouse genomic libraries with the monkey LCNS, the LCNS-homologous segments are joined to centromeric satellite sequences specific to those species. The conservation of the LCNS sequence and location indicates that the segment may participate in centromeric processes or structures. Moreover certain unusual properties of the LCNS-containing clones including poor growth, sequence rearrangements and the presence of poly d(GT·CA) stretches suggest that the segment may recombinogenic.

1272 RECOMBINATION OF DNA MOLECULES INJECTED INTO XENOPUS OOCYTES, Ed Maryon and Carroll, University of Utah, Salt Lake City, Utah 84132

DNA molecules injected into Xenopus oocyte nuclei undergo genetic recombination. Using both bacteriophage lambda and plasmid DNAs, it has been found that this process occurs only with linear substrates, is dependent on sequence homology, and is blocked by terminal non-homologies. Because molecular ends are important for recombination, we examined the structure of the ends of non-recombining linear DNAs after injection. These DNAs are not assembled into normal chromatin, and are degraded exonucleolytically. At early stages of degradation, some fraction of molecules have single-stranded tails, and at least a portion of these have protruding 5' ends. A model is suggested in which this single stranded tail, in conjunction with activities provided by the oocyte, invades a homologous duplex to initiate recombination. Because nanogram quantities of DNA are processed by each oocyte, this system offers exceptional opportunities for detailed analysis of the process of recombination and of the components which catalyse individual steps.

1273 TOPOISOMERASE I IN CRITHIDIA FASCICULATA, Thomas Melendy and Dan S. Ray, Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA 90024

An enzymatic activity capable of relaxing negatively supercoiled DNA substrates has been identified in the trypanosomatid, Crithidia fasciculata. The enzyme is appears to be a type I topoisomerase in that it functions in an ATP-independent manner and is incapable of catenating covalently closed circular DNA molecules under normal catenation assay conditions. A several hundred-fold purification has been achieved using several chromatographic steps including: Heparin Sepharose, Hydroxyapatite and Mono-Q anion exchange chromatography.

1274

Molecular basis of spontaneous gene rearrangements at the hamster aprt locus
Mark Meuth, Josephine Nalbantoglu and Gerry Phear, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, U.K.

To examine the role of gene rearrangements in producing genetic variation in cultured mammalian somatic cells, DNA from a large collection of adenine phosphoribosyl deficient mutants was analyzed for gene structural alterations by probing Southern blots with a unique aprt DNA fragment. We previously reported a number of mutations which resulted in the loss or gain of restriction sites, deletion of sequences, or insertion of a new DNA fragment. We have now cloned aprt fragments containing these alterations from genomic libraries of these mutant DNAs prepared in λ vectors and characterized the changes occurring at the nucleotide level by DNA sequencing. Here we report data concerning rearrangements of aprt sequences. More specifically we show:

(1) Most deletion termini have short, 3 to 5 base pair homologies.
(2) Several deletion termini map to a 40 base-pair region of aprt indicating the presence of a "hot spot" for deletion formation.
(3) The only insertion mutation detected involves the transposition of a unique DNA fragment from elsewhere in the genome into the aprt locus. No structures characteristic of transposable elements (terminal repeats or duplication of flanking cellular-sequences) were found in the mutant gene.

1275 INDUCIBLE GENE EXPRESSION BY DNA REARRANGEMENTS IN HUMAN CELLS. John P. Murnane, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143

A permanent human cell line, LM205, was established by transformation of primary human fibroblasts with a plasmid containing both SV40 sequences with a defective origin of replication and a G418-resistance gene (neo) that lacked a eucaryotic transcriptional promoter. G418-resistant cells appeared spontaneously in clonal populations of LM205 with a frequency of approximately 10^{-5} per cell plated in 400 µg/ml G418. G418 resistance was stable and correlated with the appearance of neo-specific RNA. Characterization of the neo gene in the G418-sensitive parental cell line by both Southern blot analysis and restriction map analysis of cloned sequences demonstrated a stable integration site containing a single neo coding sequence. Similar analysis of fragments containing the neo gene isolated from G418-resistant subclones demonstrated tandem duplications that resulted in the relocation of the SV40 bidirectional transcriptional promoter 5' to the neo gene. The newly acquired SV40 promoter appears to be responsible for G418-resistance, because the small increase in neo gene copy number shows no correlation quantitatively with the large increase in neo-specific RNA. Heterogeneous sites of recombination indicate nonhomologous recombination, and therefore these tandem duplications appear to represent the first steps in amplification of a nonrepetitive sequence. One G418-resistant subclone had undergone further amplification of the neo gene during G418 selection (2 or more copies), and various subpopulations containing sequential numbers of neo gene copies are being studied to determine the series of recombination events involved in the development of a multi-copy tandem array.

1276 DNA RECOMBINATION AND REPLICATION IN PLANTS: OBSERVATIONS FROM EXPERIMENTS ON PROTOPLAST TRANSFORMATION AND FUSION IN NICOTIANA PLUMBAGINIFOLIA. I.NEGRUTIU, M.HORTH, A. MOURAS, R.D.SHILLITO, I.POTRYKUS. F.M.I. BASEL (Switzerland) and I.M.B. Brussels(Belgium)

(1) Autonomous replicating systems? Cloned sequences from total plant DNA exhibiting ARS function in yeast were transferred to plant protoplasts via transformation with a pLGVneo expression vector. Those sequences showing highest transformation rates and prolonged overexpression of NPT in transient expression experiments were further studied for mitotic and meiotic stability, and used to retransform E. coli. In a similar approach, plant mitochondrial Plasmids were employed.

(2) Factors enhancing recombination events. Ultraviolet light(UV) and 3-aminobenzamide were tested, alone or in combination, in experiments of the direct gene transfer type and making use of the NPT gene as a selectable marker(pABDI and pLGVneo constructs). Transformation rates were increased by a factor 10 to 30. Molecular and genetic data will be presented.

The system was transposed to chromosome transfer experiments by means of protoplast fusion following inactivation by lethal irradiation of the donor (selectable) fusion partner.

1277 EUKARYOTIC TOPOISOMERASE II: CHARACTERIZATION OF ENZYME TURNOVER, Neil Osheroff, Vanderbilt University School of Medicine, Nashville, TN 37232

While the binding of adenyl 5'-yl-imidodiphosphate (APP(NH)P) to Drosophila melanogaster topoisomerase II induces a double-strand DNA passage event, the presence of its non-hydrolyzable β-γ imidodiphosphate bond prevents enzyme turnover. Therefore, this ATP analog was used to characterize the interactions between Drosophila topoisomerase II and DNA which occur after DNA strand passage, but before enzyme turnover. In the presence of APP(NH)P, a stable post-strand passage topoisomerase II·nucleic acid complex is formed with supercoiled, relaxed, or nicked circular DNA substrates. Although non-covalent in nature, these complexes are resistant to increases in ionic strength and show less than 5% dissociation under salt concentrations (>0.5 M) that disrupt 95% of the enzyme-DNA interactions formed in the absence of APP(NH)P or under a variety of other conditions that do not allow DNA strand passage to take place. These results strongly suggest that the process of enzyme turnover not only regenerates the active conformation of topoisomerase II, but also confers upon the enzyme the ability to dissociate from its nucleic acid product. Further experiments with linear DNA substrates indicate that after strand passage has taken place, topoisomerase II may be able to translocate along the DNA molecule by a process that is independent of enzyme turnover. Finally, topoisomerase II is able to cleave double-stranded DNA substrates, even after strand passage has occurred. Therefore, enzyme turnover may not be necessary to regenerate the catalytic center of topoisomerase II. Supported by NIH Research Grant GM-33944 and Biomedical Research Support Grant RR-05424.

1278 IDENTIFICATION AND CHARACTERIZATION OF A 65 K DNA BINDING PROTEIN OF HSV-1 ASSOCIATED WITH TOPOISOMERASE ACTIVITY, D.S. Parris, M.T. Muller, M.L. Gallo, H.S. Marsden, and L.D. Goodrich, The Ohio State University, Columbus, OH, and The MRC Virology Unit, Glasgow, U.K.

A protein of 65,000 daltons (p65) was purified from herpes simplex virus type 1 (HSV-1) infected cells to near homogeneity using a double stranded DNA cellulose column. This fraction contained a type I DNA topoisomerase (topo) activity as evident from titration of its ability to relax supercoiled DNA, titration of its ability to bind covalently to DNA, and by its ability to alter the linking number of an isolated topoisomer in steps of one. Antibody prepared in rabbits by inoculation of this fraction reacted only to p65 on Western blots and inhibited the topo activity found in purified virions. Using the antibody to follow the separation of p65 by other chromatographic means, we found that p65 consistently co-fractioned with topo activity through heparin agarose, PEI cellulose, and Mono S. The fractionated activity and that present in virions appeared to be Mg^{++} but not ATP dependent, distinguishing it from most other type I topos. The synthesis of p65 appears to occur relatively late in the viral replicative cycle at 12 hr. p.i., and fails to accumulate in DNA- ts mutants at the nonpermissive temperature. Because p65 is synthesized at low levels in wild-type virus infected cells when viral DNA synthesis is completely blocked with phosphonoacetic acid, synthesis of p65 does not have an absolute requirement for viral DNA replication.

1279 Studies of homologous recombination in the plant genome
J.Paszkowski, M.Baur, M.Saul, R.D.Shillito and I.Potrykus
Friedrich Miescher-Institut, P.O.Box 2543, CH-4002 Basel, Switzerland

There is still a virtually complete lack of studies on homologous DNA recombination in plants except for the specific extra chromosomal case of the DNA virus Cauliflower Mosaic virus. Direct, vectorless transformation of plant protoplasts has opened new possibilities of studying the DNA recombination process in plants. Using this method we have constructed two lines of tobacco plants, one containing bacterial plasmid sequences and partial non-functional copies of a plant selectable marker gene [hybrid APH(3')II gene] and the other containing a functional nopaline synthase gene derived from the Agrobacterium tumefaciens Ti plasmid. The foreign DNA in these strains is now being used as a target for homologous recombination of DNA into the genome after DNA transformation.

1280 TOPOISOMERASE II-INDUCED DNA STRAND BREAKS AND DNA-PROTEIN LINKS IN RELATION TO THE CELL CYCLE, Yves Pommier, Judith Markovits, Donna Kerrigan and Kurt W. Kohn, National Cancer Institute, Bethesda, MD 20892

m-AMSA and VP-16 produce topoisomerase II-mediated DNA strand breaks and DNA-protein links in mammalian cells. NIH 3T3 cells stop proliferation when they reach a certain density on the plate. m-AMSA and VP-16-dependent DNA breakage was greater in proliferating cells than in arrested cells. The cells can be initiated to proliferate by replating at a lower density and feeding with fresh medium containing calf serum. Two peaks of [^{3}H]-thymidine incorporation were observed at 4-6 h and 16-28 hr after replating-splitting the cells. 30 min treatments of the cells with m-AMSA and VP-16 produced DNA double-strand breaks whose frequency followed the DNA synthesis peaks. Similar experiments were performed in isolated nuclei. The yield of drug-induced DNA-protein links measured by alkaline elution was greater in nuclei from exponentially growing cells than in those from growth arrested cells and followed the peaks of [^{3}H]-thymidine incorporation in cells initiated to proliferate. These observations suggest that the association of topoisomerase II with DNA is increased during DNA replication.

1281 DNA-PROTEIN INTERACTIONS IN 2 MICRON CIRCLE SITE-SPECIFIC RECOMBINATION, Paruchuri V. Prasad, Deborah Horensky and Makkuni Jayaram, Scripps Clinic and Research Foundation, La Jolla, CA 92037

We have started a detailed analysis of the DNA-protein interactions involved in the FLP-catalyzed site-specific recombination in the yeast plasmid, 2 micron circle. We have determined that the minimal FLP-substrate is a 24 base-pair DNA segment, which consists of a nearly perfect 8 base-pair dyad symmetry and an 8 base-pair core region. We have chemically synthesized a set of FLP-substrates which contain symmetric or asymmetric mutations within the symmetry elements of the minimal substrate. We have also synthesized substrates with point mutations within the spacer region. The nature of the interactions of these modified substrates with FLP, both in vivo and in vitro is being investigated. Further, we have created a library of mutant FLP proteins by using in vitro mutagenesis. The interactions of mutant FLP with the wild type or altered FLP substrates are also under investigation.

1282 INTERSPECIES RECOMBINATION BETWEEN AGROBACTERIUM DNA AND PLANT DNA, Dennis Prosen, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568

Agrobacterium tumefaciens causes crown gall tumors on many dicotyledonous plants by transfer and integration of a specific segment of bacterial DNA into the nuclear genome or in some cases (1), the chloroplast genomes of plants. The transferred DNA (T-DNA) is flanked by 25 bp imperfect direct repeats in the bacterium. It has been proposed (2) that recombination between the direct repeats occurs in the bacterium, resulting in a circular intermediate. The only portions of the T-DNA which are required for faithful transfer are the 25 bp flanking direct repeats. The Vir genes are also required for the transfer and integration of the T-DNA into plants but the Vir genes are not transferred or integrated. The mechanism of integration of T-DNA into the plant genome is being investigated. Three aspects of our investigation are as follows: a.) How many copies of T-DNA are transferred to each plant cell? b.) How many bacterial cells can transfer T-DNA to one plant cell? c.) Does the size of the T-DNA effect the transfer efficiency?

1. M. De Block et al., EMBO Journal vol. 4, pp. 1367-1372, 1985.
2. Z. Koukolikova-Nicola et al., Nature, vol. 313, pp. 191-196, 1985.

1283 MODULATION OF THE DNA-BINDING ACTIVITY OF SV40 LARGE T ANTIGEN BY REVERSIBLE PHOSPHORYLATION, Karl H. Scheidtmann* and Ernst Baumann§ *Institute of Immunobiology, University of Freiburg; §Faculty of Biology, University of Konstanz, FRG.

SV40 large T specifically interacts with regulatory sequences at the origin of replication on the viral genome, thereby mediating initiation of replication and regulation of transcription. The DNA-binding activity depends on the state of phosphorylation, molecules in a low phosphorylation state being more active than highly phosphorylated ones. Two residues seem to be directly involved in DNA-binding, Thr124 and Ser123. Phosphorylation of Thr124 appears to be a prerequisit for initial binding, since dephosphorylation of this residue results in loss of Ori binding. Phosphorylation of Thr124 occurs in the cytoplasm and is metabolically stable. Subsequent phosphorylation of Ser123, which takes place in the nucleus, reduces the binding activity. However, the phosphate at Ser123 is rapidly turned over, thereby regenerating the original phosphorylation, and perhaps functional, state.

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1284 GENE TRANSFER FROM BACTERIA TO PLANT CELLS: ENZYMATIC EXCISSION OF THE T-REGION FROM TI PLASMIDS OF AGROBACTERIA, Joachim Schröder and Juliane Alt-Moerbe, Institut für Biologie II, Universität, Schänzlestr. 1, D-7800 Freiburg, FRG

Tumor induction in plants by Agrobacteria is the result of DNA transfer from the procaryotic to the eucaryotic cells. Ti plasmids play a central role in this process; they contain two physically separated regions which cooperate in tumor induction: The Vir-region (genes responsible for DNA transfer) and the T-region (defined by specific border sequences and transferred to the plant cells). It seems likely that the genes of the Vir-region function in the Agrobacteria, and that excission of the T-region is one of the first steps in DNA transfer.
We have developed a simplified approach to analyze the excission event. The system uses as first component a small plasmid containing a selectable marker gene cloned between T-region border sequences, and as second component a set of compatible plasmids containing fragments of the Vir-region. The two components are brought together into the same cell, and it is then analyzed whether specific Vir-region fragments code for functions excising the selectable marker gene at the border sequences. The results indicate that a specific part of the Vir-region is responsible for DNA excission. Its size is about 2 Kb, and it expresses defined proteins in E. coli minicells.

1285
L1Md SHARES FEATURES WITH RETROTRANSPOSONS Shehee, W.R., S.F. Chao, C.A.Hutchison III and M.H. Edgell

Two full length members of the long interspersed repetitive family L1 from Mus domesticus, L1Md-A2 and L1Md-A13, have been completely sequenced. 4.3kb from the 5' end of a third long member, L1Md-9, has also been sequenced. Analysis of these sequences reveals several features shared with protein coding transposable elements. The size and relationship of two open reading frames (ORFs) are analagous to retroviruses and the Ty element of yeast. The ORFs are 1137bp and 3900bp, and they overlap by 14bp. Also, the 3900bp ORF has homology to several reverse transcriptases. One difference L1Md has with retrotransposons is the absence of LTRs, indicating a different mode of replication. The comparison of three long elements also defines one 5' end of L1Md. All three elements contain a 208bp repeating motif at the 5' end. They have different numbers of the repeating unit (L1Md-A2, 4 2/3; L1Md-A13, 2 2/3; L1Md-9, 1 2/3). However, they all end at approximately the same position in a repeating unit. The functional significance of this end point is not clear. One possibility is that the 208bp unit can act as a promoter with transcription beginning at the 2/3 point. Comparison of these three sequences with that of a truncated element, L1Md-4, allows construction of the consensus sequences for the proteins encoded by the ORFs.

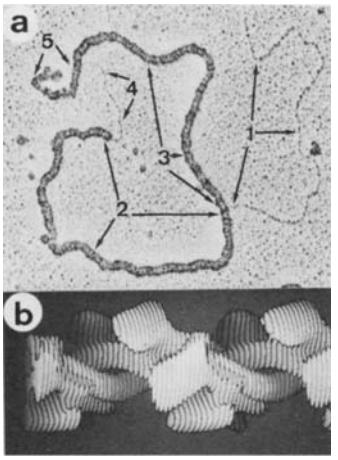
1286 **A TOPOISOMERASE INHIBITOR, NOVOBIOCIN, INHIBITS THE REACTIVATION OF LATENT HERPES SIMPLEX VIRUS TYPE 1. Jordan G. Spivack, Donald R. O'Boyle II, and Nigel W. Fraser, The Wistar Institute, Philadelphia, PA 19104**

After an initial infection herpes simplex virus type 1 (HSV-1) often persists, for the life of the individual, within neurons of the sensory ganglia that innervate the site of infection. Periodic reactivation of the latent virus to cause recrudescent lesions is a serious health problem. None of the antiviral agents currently in use affect the reactivation process. In this poster evidence is presented that novobiocin, a topoisomerase inhibitor, inhibits the reactivation of latent HSV-1 in a mouse model system. The effect of other topoisomerase inhibitors has also been investigated. It is hypothesized that this novobicin-sensitive event may be mediated by a DNA topoisomerase.

Balb/c mice were infected in the eye with HSV-1 after corneal scarification. During the acute phase of the infection virus replicates in the eye, trigeminal ganglion and the CNS. Four weeks later infectious virus was no longer detectable but could be reactivated following explant cultivation of the trigeminal ganglia. No infectious virus was detected until two days post-explant. This model system serves as the basis for studying the reactivation process. When 80uM novobiocin was added at the time of explant the reactivation of latent HSV-1 was significantly inhibited. A time course of drug addition indicates that novbiocin inhibits an event(s) that occurs early in the reactivation process. The effect of novobiocin on the reactivation of latent HSV-1 was not the result of antiviral activity. The implications concerning the reactivation process, and the potential use of topoisomerase inhibitors in the treatment of latent herpes infections are discussed.

1287 RECA PROTEIN-DNA INTERACTIONS IN RECOMBINATION, Andrzej Stasiak, Edward H. Egelman* and Theo Koller, Institute for Cell Biology, ETH, Zürich, and *Yale University, New Haven, CT 06511

Using electron microscopy and three-dimensional image reconstruction we were able to visualize sequential stages of the recA-mediated strand exchange reaction and to determine the molecular structure of recA-DNA complexes involved in the recombination process. Picture (a) shows how a naked linear double-stranded DNA molecule (1) invades the helical recA complex (2) formed on a circular single-stranded DNA molecule, leading to the formation of a recA covered "triple- stranded" DNA region (3). The newly formed heteroduplex region is visible as naked DNA (4) on the circumference of the circle. The displaced strand (5) is covered by recA protein. Image reconstruction (b) shows molecular details of the helical recA-DNA complex. The combined results offer a precise molecular model of recA-promoted recombination.



1288 MUTATION RATES AND dNTP POOL SIZES IN HYDROXYUREA RESISTANT CELL LINES, Aaron Y. Tagger and Jim A. Wright, Manitoba Institute of Cell Biology and Department of Biochemistry, University of Manitoba, Winnipeg, Manitoba.

Among factors which are believed to affect the fidelity of DNA replication are the relative sizes of the precursor dNTP pools. Several studies have suggested that pertubations of these pools can lead to increased rates of spontaneous mutation at independent loci. It has not, however, been established whether or not pool imbalances act directly by biasing their incorporation into DNA or if they function by some other mechanism. In this study, the rate of spontaneous mutation to ouabain resistance and 6-thioguanine resistance was examined in two CHO mutant cell lines HU^R-2 and NC^R30A2 selected for resistance to hydroxyurea and N-carbamyloxyurea respectively. Both cell lines were shown to exhibit similar rates of mutation to these markers compared to the wild type parental line. In addition, the HU^R-2 cell line, although having alterations in ribonucleotide reductase properties, did not show any significant changes in it's dNTP pool size. On the other hand, the NC^R-30A2 cell line had a decrease of approximately five fold in the dCTP pool while the dGTP pool was elevated by a factor of five. Taken together, these observations suggest that alterations in ribonucleotide reductase or significant changes in dNTP pool sizes do not necessarily contribute to increases in mutation rates in mammalian cells. (Supported by the N.C.I. of Canada and N.S.E.R.C.).

1289 RECOMBINATION OF PLASMID DNA IN IMMORTALIZED XERODERMA PIGMENTOSUM FIBROBLASTS, Terry L. Timme and Robb E. Moses, Baylor College of Medicine, Houston, TX 77030

The recombination frequency of co-transfected plasmid DNA has been compared in SV40-transformed fibroblasts from humans with DNA repair-deficient diseases and from repair-proficient human fibroblasts. We have constructed pSV2neo plasmids with non-overlapping deletions or linker insertions. Recombination has been detected by either growth of colonies of the human cells in the presence of G418, or Hirt extraction of plasmid DNA from the human cells followed by transformation into recA$^-$ bacteria and selection of recombinant plasmids on kanamycin-containing media. The colony forming ability in G418 after co-transfection is about 10% of that of pSV2neo in cell lines from xeroderma pigmentosum (XP) complementation groups A, C, D, E and G. This is comparable to the level in Bloom syndrome or ataxia telangiectasia (AT) fibroblasts, but greater than Fanconi anemia (FA) fibroblasts. The level of recombination detected in Hirt extracts from human cells is less than determined by G418-resistant colonies. Repair-proficient cells have the highest level of recoverable plasmids, and less than 1% of these are recombined in the neo gene. The XP cell lines have less recoverable plasmid and a 10-fold decrease in recombination frequency. AT and FA fibroblasts have intermediate levels of recovery and recombination. Transfected plasmid DNA appears to be replicated less in the XP cells than in normal or FA cells. Although the level of recombination in DNA repair-defective fibroblasts is normal when recombinant molecules are selected by colony formation, the XP, but not AT or FA, cells appear to be less efficient at recombination prior to integration. Supported by USPHS CA37860.

1290 VACCINIA VIRUS: ANALYSIS OF VIRAL REPLICATION MUTANTS AND CHARACTERIZATION OF THE VIRAL TOPOISOMERASE. Paula Traktman, Rose Shaffer-Puelle and Elizabeth Evans. Cornell University Medical College, New York, N.Y. 10021.

Vaccinia virus is a complex DNA virus which replicates lytically in the cytoplasm of infected cells. It appears to encode virtually all of the enzymes involved in DNA replication and the regulation of DNA topology. Because of this physical and genetic autonomy from the host cell, it is an ideal system in which to couple biochemical and molecular genetic analyses of DNA metabolism. We are studying various viral enzymes involved in DNA metabolism, especially the viral topoisomerase. The purified enzyme relaxes both negatively and positively supercoiled DNA in the absence of an energy cofactor, and is sensitive to coumermycin and novobiocin. Label-transfer experiments have revealed that the topoisomerase species which covalently binds to the DNA substrate has a molecular weight of 37,000. Attempts to identify and analyze the gene encoding the topoisomerase are underway. We have molecularly cloned and extensively characterized the gene for the viral DNA polymerase. DNA sequence studies are in progress to define the exact lesion in several mutant alleles. Two additional complementation groups of replication mutants are being studied. Marker rescue and hybrid-selection techniques are being used to define the lesions in these mutants. To date, the genomic position of the lesions, and the identity of the gene and polypeptide affected in each group, has been established. In addition to these structural analyses, various approaches are underway to determine the functional defect in these mutants.

1291 SPECIFIC CLEAVAGE OF CRUCIFORM DNA STRUCTURES BY AN ENZYME PURIFIED FROM SACCHAROMYCES CEREVISEAE. Stephen C. West, Imperial Cancer Research Fund, Clare Hall Labs, South Mimms, Herts, England.

Supercoiled plasmids that contain inverted repeat sequences are known to extrude the repeats into cruciform structures to reduce the free energy of supercoiling. Extruded cruciform structures are cleaved in vitro by two classes of enzymes: (a) Single strand nucleases such as S1 nuclease which non-specifically cleave the single stranded hairpin loops of the cruciform, and (b) Holliday junction resolving enzymes, such as T4 endo VII, which show a more specific interaction and cleave diagonally across the cruciform junction.

We have now purified an activity from yeast that also cuts plasmid DNA at extruded cruciform structures. The native molecular weight of the enzyme is 200-300 kd.

Using the plasmid pColIR215, which contains a cloned inverted repeat from ColE1, we have investigated the sites of cleavage relative to the cruciform structure. We find that cuts are introduced within the inverted repeat sequences at positions that are symmetrically opposed across the cruciform junction.

The purified nuclease is presently being tested on a variety of DNA substrates, including other plasmids that contain inverted repeats, stable Holliday junctions constructed from phage attachment sites, and figure-8 DNA molecules.

Ref: West, S.C. and Korner, A. Proc. Natl. Acad. Sci. USA 82, 6445-6449 (1985).

Proteins in DNA Replication

1292 P1 PLASMID REPLICATION: SPECIFIC INTERACTION OF RepA WITH P1 REPLICON DNA, Ann L. Abeles, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

The P1 genes, which control replication of the P1 plasmid, occupy about 1250 bp of contiguous DNA containing an origin including a control element (incC), a gene for an essential replication protein (repA), and another control element (incA). Both incC and incA consist of groups of 19-bp repeated sequences. The 5 incC repeats are required for origin function and also overlap the autoregulated repA promoter. The 9 incA repeats are located at the 3' end of repA and apparently regulate copy number by titrating the RepA.

The interaction of RepA with the origin, incC, and incA DNA was studied in order to investigate the role of RepA in the control and initiation of P1 replication. The interactions were assayed in vitro by allowing RepA to bind to specific fragments of DNA and by following the mobility of the DNA-protein complexes in polyacryamide gels. These complexes were further examined by DNA footprinting and Western blot analyses to determine the exact regions of protein-DNA interaction and to quantitate the amount of protein in each complex. These data provide an explanation for some important features of P1 plasmid replication. There are several analogies to replication of similar unit-copy plasmids.

Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-23909 with Litton Bionetics, Inc.

1293 BOWLS ON THE NUCLEOSOME, Gerard Andlauer, Mundelheim, France.

The nucleosome is the elemental unit of the nucleoprotein chain. It results in a core particle and a linker that include histone and DNA molecules.

The Kornberg model refers to the core particle as a DNA strand coiled around a mean histone octamer.

The proposed 'abacus model' defines the particle core as a double helix covered on the outside with the globular zone of the histones.

1294 FUNCTIONAL EFFECTS OF THE araC LESION IN DNA. G. Peter Beardsley and Thomas Mikita. Departments of Pediatrics and Pharmacology, Yale University School of Medicine New Haven, Connecticut 06510

Cytosine arabinoside (araC) is an important anti-leukemic drug whose action involves misincorporation into DNA. The nature of the putative functional deficits resulting from this DNA structural lesion have not heretofore been defined. We developed a chemical synthetic method for the introduction of araC into defined sequence oligomers and utilized these for study of site specific effects on processes of DNA replication. AraC at 3' primer terminimarkedly inhibited the rate of addition of the suceeding nucleotide by E. coli Pol I (Kf) and T4 DNA polymerase. These polymerases, with associated 3' 5' exonuclease activity showed an overwhelming preference for excision of 3' terminal araC rather than addition. AraC at 3' primer termini slowed chain elongation by 50 fold, and with HeLa cell pol α_2 holoenzyme, no chain elongation whatever was detected. In contrast to the profound inhibition of polymerases, 3' terminal araC did not affect the rate at which T4 DNA ligase joined oligomers annealed adjacently on M13 single strand DNA, although the ligase enzyme catalyzes formation of the same bond as the polymerases. When an araC lesion was placed in the template strand, interference with primer elongation by Pol I (Kf) and Pol α_2 was observed. The rate of nucleotide insertion prior to the lesion site was normal, but the rate of insertion opposite the lesion was markedly decreased. Structural models of the araC lesion in duplex B-DNA show the additional hydroxyl group of the arabinose sugar projecting into the major groove. We propose that this interferes with the relative motion between DNA and protein necessary for the action of processive, but not distributive, enzymes.

1295 ROLE OF THE Ø29 DNA POLYMERASE IN THE VIRAL DNA REPLICATION, Luis Blanco, José M. Lázaro, Ignacio Prieto, Antonio Bernad, José M.Hermoso and Margarita Salas, Centro de Biología Molecular (CSIC-UAM) Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

The product of the Ø29 gene 2, needed for the viral DNA replication in vivo, was shown to be a DNA polymerase. In vitro studies indicated that protein p2 is required for the protein-primed initiation of replication as well as for elongation of the Ø29 DNA chain. This viral DNA polymerase has a 3'→ 5' exonuclease activity that is likely to act as a proof-reading mechanism. Ts2 mutants are available and they are being tested to look for different active sites in protein p2. In addition to the Ø29 DNA polymerase, the initiation reaction requires the presence of free terminal protein p3 and the p3-DNA complex as template and is stimulated by NH_4^+ ions. In these conditions, the p3-dAMP initiation complex formed at each end of the DNA molecule is elongated by the DNA polymerase activity of protein p2 giving rise to full length Ø29 DNA. However, the slow rate of elongation obtained in vitro (10 nt/sec) suggests the need of accessory proteins. Protein p6, involved in DNA replication in vivo, stimulates the formation of the initiation complex decreasing the Km value for dATP. The effect of protein p6 in elongation, as well as the binding of protein p6 to Ø29 DNA will be also presented.

1296 REGULATION OF ColE1 SYNTHESIS BY ROM PROTEIN, Michael Brenner and Jun-ichi Tomizawa, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, MD 20892. Replication of ColE1 requires an RNA primer for the initiation of DNA synthesis. The RNA primer is formed from a transcript which initiates 555 base pairs upstream from the origin, and whose synthesis proceeds through the origin. In the origin region the transcript can form a persistent hybrid with its DNA template. Cleavage of the hybridized RNA by RNase H yields the functional primer.

Formation of the RNA/DNA hybrid, and hence primer formation, is inhibited by binding of a second RNA to the 5' end of the elongating primer transcript. The binding of these two RNAs is in turn catalyzed by the Rom protein, a 7,000 MW polypeptide encoded by the ColE1 plasmid. This pivotal role of the Rom protein suggests it may be a physiological control point for regulating plasmid copy number.

We have investigated the possibility that transcription of the Rom protein is under physiological control. The in vivo activity of the Rom promoter has been determined under a variety of physiological conditions. In vitro transcription studies have determined the precise location of the Rom promoter, and examined the effects of several possible regulators. The results obtained will be discussed in reference to in vivo measurements of Rom protein levels and plasmid copy number.

1297 PURIFICATION AND CHARACTERIZATION OF A SUPPRESSION PRODUCT OF THE polA1 GENE IN ESCHERICHIA COLI, Sharon K. Bryan and Robb E. Moses, Baylor College of Medicine, Houston, TX 77030

We found that 95% of temperature-resistant revertants from HS432 (polA1, polB$^-$, polC$_{ts}$, pcbA$^-$, su$^+$), were methylmethane sulfonate-resistant (MMS^R) and UV^R, indicating the presence of DNA polymerase I activity. These revertants fell into 2 categories: 1) a mutation in the polA1 gene made some polA$^+$; and 2) a suppressor acting on the polA1 gene gave a low (10%) level of DNA polymerase activity. Since 70% of the revertants fell into the second category, we characterized the suppression product of the polA1 (am) gene. Using CSM61, a revertant of the second category, we purified this enzyme several thousand-fold. During purification of the polA1 suppression product, we found that it differed from authentic DNA polymerase I and is unstable without glycerol or in KPO_4 less than 0.1 M. The purified enzyme is slightly more heat-sensitive than native DNA polymerase I, but shows the same inactivation by anti-DNA polymerase I antibody. The apparent molecular weight of the purified enzyme is about 10% lower than native DNA polymerase I as indicated by sucrose gradient sedimentation, gel filtration (size exclusion) chromotography, HPLC, and polyacrylamide gel electrophoresis. However, the low (10%) level of polymerase activity is enough to make the cell normal with respect to cell survival after UV or MMS treatment, and adequate to drive replication with pcbA$^-$ in the absence of DNA polymerase III. Supported by NIH GM19122.

1298 MULTIPROTEIN INTERACTIONS IN THE REPAIR OF DAMAGED DNA BY THE E. COLI UVRABC SYSTEM, Paul R. Caron and Lawrence Grossman, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, MD 21205

The bimodal nature of the E. coli UvrABC catalyzed incision reaction of DNA damaged by a UV irradiation or containing various "bulky" adducts (AAF, benzo(a)pyrene, psoralens, cis-Pt,...) leads to potential excision of a 12-13 base long damaged fragment. However, the oligonucleotide fragment containing the damaged site is not released under non-denaturing in vitro reaction conditions. The UvrABC proteins, also, are stably bound to the incised DNA and do not turnover following the incision event. Damaged fragment release from the parental UvrABC incised DNA is dependent on either chelating conditions or upon the simultaneous addition of the uvrD gene product (helicase II) and the polA gene product (DNA polymerase I) when catalyzing concommitant polymerization of deoxynucleoside triphosphate substrates. The product of this multiprotein catalyzed series of reactions serves as a substrate for polynucleotide ligase which results in the restoration of the integrity of the strands of DNA. In some cases an intermediate in the incision reaction can be identified in which UvrABC generates a 5' incision before the 3' incision. In these cases the 5'-3' exonuclease activity of DNA polymerase I or exonuclease VII (xse) can lead to a 3' incision allowing the excision of the damage. The rate of incision may be correlated with the extent of distortion that various "bulky" adducts produce. The addition of the UvrD protein to the incised DNA-UvrABC complex also results in turnover of the UvrC protein. The addition of SSB leads to stimulation of the incision reaction and also appears to compete for UvrA binding at nonspecific sites. It is suggested that the repair processes of incision, excision, resynthesis and ligation are coordinately catalyzed by a complex of proteins in a 'repairosome' type of configuration.

1299 STUDIES OF THE DNA HELICASE-RNA PRIMASE UNIT FROM BACTERIOPHAGE T4: A TRINUCLEOTIDE SEQUENCE ON THE DNA TEMPLATE STARTS RNA PRIMER SYNTHESIS, Tai-An Cha and Bruce M. Alberts, Dept. of Biochemistry and Biophysics, Univ. of California, San Francisco, CA 94143

The purified DNA replication proteins encoded by genes 41 and 61 of bacteriophage T4 catalyze efficient RNA primer synthesis on a single-stranded DNA template. In the presence of additional T4 replication proteins, we demonstrate that the template sequences GTT and GCT serve as necessary and sufficient signals for RNA primer-dependent initiation of new DNA chains. These chains start with primers that have the sequences pppApCpNpNpN and pppGpCpNpNpN, where N can be any one of the four ribonucleotides. Each primer is initiated from the T (A-start primers) or C (G-start primers) in the center of the recognized template sequence. A subset of the DNA chain starts is observed when one of the four ribonucleoside triphosphates used as the substrates for primer synthesis is omitted; the starts observed reveal that both pentaribonucleotide and tetraribonucleotide primers can be used for efficient initiation of new DNA chains, whereas primers that are only three nucleotides long are inactive.

It was known previously that, when 61 protein is present in catalytic amounts, the 41 and 61 proteins are both required for observing RNA primer synthesis. However, by raising the concentration of the 61 protein to a much higher level, a substantial amount of RNA-primed DNA synthesis is obtained in the absence of 41 protein. The DNA chains made are initiated by primers that seem to be identical to those made when both 41 and 61 proteins are present; however, only those template sites containing the GCT sequence are utilized. The 61 protein is therefore the RNA primase, whereas the 41 protein should be viewed as a DNA helicase that is required (presumably via a 41/61 complex) for efficient primase recognition of both the GCT and GTT DNA template sequences.

1300 REPLICATION OF COLE1 PLASMID UNDER RNase H⁻ CONDITION, Santanu Dasgupta, Hisao Masukata and Jun-ichi Tomizawa, NIADDKD, NIH, Bethesda, MD, 20892

Replication of ColE1 DNA can be initiated in vitro from the unique origin with three enzymes, RNA polymerase, RNase H and DNA polymerase I. RNase H cleaves the primer transcript that hybridizes to the template DNA, to produce the primer for elongation by DNA polymerase I.

However, it has been shown that ColE1 DNA can replicate in vivo in bacteria that are deficient in the RNase H activity. We therefore analyzed replication of the plasmid DNA in vitro as well as in vivo in the absence of RNase H. To create RNase H⁻ condition, we found it necessary to use a bacterial strain where defect in RNase H action is severer than those that were available. Such a strain was constructed by inserting the gene for Kanamycin resistance at a site very close to the 5'-end of the sequence that encodes the enzyme. In the Km^R rnh⁻ strain, the wild type plasmid can still replicate fairly well. Experiments with various mutant plasmids showed that, at least, hybridization of the primer transcript to the template DNA is necessary for replication even in the rnh⁻ host. Results of in vitro experiments with cell extracts from the wild type and rnh⁻ mutants showed that replication initiates at new sites that include approximately 10 bp and 25 bp downstream from the unique replication origin seen in the wild-type extract.

1301 **CHARACTERIZATION OF PROTEIN WHICH BINDS SPECIFICALLY TO A PUTATIVE ORIGIN OF CELLULAR DNA REPLICATION.** John F.X. Diffley and Bruce Stillman. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

We have developed a rapid and sensitive nitrocellulose filter binding assay for the detection of sequence specific DNA binding proteins in crude cellular extracts and have applied this assay to the detection of factors in whole cell extracts of *Saccharomyces cerevisiae* which bind specifically to a putative origin of replication, ARS1. One such protein, ARS1 binding factor I (ABFI) has been detected and partially purified. This partially purified protein protects approximately 20*bp* from DNaseI digestion in footprinting experiments. This region of DNaseI protection lies entirely within domain B, a region shown to be important in ARS function (1) and we suggest that it is this binding site that defines the functional domain B. We are currently investigating the effects on ARS activity of point mutations within domain B which effect ABFI binding and furthermore, we are attempting to purify ABFI to homogeneity. Our results suggest that this putative cellular origin of replication may have an organization analogous to the organization of the adenovirus and SV40 origins of replication in which a site for a sequence specific DNA binding protein is located adjacent to a critical A+T rich region.

(1) Celniker, S.E., K. Sweder, F. Srienc, J.E. Bailey and J.L. Campbell. 1984. Mol. Cell. Biol. 4:2455-2466

1302 PURIFICATION AND CHARACTERIZATION OF THE YEAST DNA POLYMERASE - PRIMASE COMPLEX: Russell J. DiGate and David C. Hinkle; Department of Biology, University of Rochester, Rochester, New York 14627

We have purified yeast DNA polymerase I to near homogeneity using procedures designed to minimize proteolysis. A DNA primase activity is associated with the DNA polymerase and the ratio of primase to polymerase activity remains constant until the last step of the purification. Sedimentation on a sucrose gradient reveals two species of DNA polymerase (9.6s and 7.9s). The primase activity is associated with the 9.6s form which comprises only 20-30% of the total DNA polymerase activity. Both forms of the enzyme have a Stokes radius of about 72 Å (measured by gel filtration), which indicates a molecular weight of approximately 290,000 for the 9.6s form and 240,000 for the 7.9s form.

Analysis of DNA polymerase fractions by SDS-PAGE shows two major polypeptides (175 kDa and 70 kDa) associated with both forms of the DNA polymerase. Both of these polypeptides copurify with polymerase activity and they probably comprise the 240 kDa form of the enzyme. Two additional major polypeptides (52 kDa and 48 kDa) sediment with the 9.6s form of the enzyme. The primase has been separated from polymerase by sedimentation in the presence of 2.8M urea. The recovery of primase activity is very low but only the 48 kDa and 52 kDa polypeptides are present in the fractions containing primase activity. One or both of these peptides may be required for primase activity.

We have characterized the RNA primers synthesized by the polymerase-primase complex on a variety of synthetic and natural single-stranded DNA templates. On natural DNAs there are preferred sites for initiation but no obvious consensus sequence for these sites has been identified.

1303 MAPPING THE DNA TEMPLATE CONTACTS OF THE T4 DNA POLYMERASE. Mary K. Dolejsi, Mary C. Dasso, Joel W. Hockensmith and Peter H. von Hippel. Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403.
We are studying the enzymatic properties of the T4 DNA polymerase (gene 43 protein) as a probe of its structural interactions with DNA primer-templates. The gene 43 protein has both DNA synthesis and single-stranded exonuclease activities. When the gene 43 protein is incubated with an excess of single-stranded oligonucleotides, exonuclease products of 9-10 nucleotides accumulate (independent of the length of starting oligomer). By measuring the rate of hydrolysis as a function of the length of starting oligomer, we can determine apparent K_m's. There is an increase in K_m as the template drops below 12 nucleotides. We conclude that the gene 43 protein needs a DNA oligomer of length 10-11 for fully functional interactions. A similar analysis is being conducted with double-stranded primer-template models, and will be expanded with template competition studies. Supported by USPHS Training Grant GM07759 and Research Grant GM-29158.

1304 THE dnaA PROTEIN AND THE REGULATION OF TRANSCRIPTION IN E. coli, H. Eberle, G. Kampo and D. Sporn, University of Rochester School of Med., Rochester, N.Y. 14642
The dnaA protein of E.coli is necessary for the initiation of DNA synthesis and has been demonstrated by others to bind to a nine base pair sequence between P1 and P2 of the dnaA gene and to regulate its own transcription. This nine base pair sequence (TTATCCACA) occurs with slight variations near the origin of chromosomal replication and in front of the coding region of other genes. Some of these genes appear to have functions related to DNA replication, repair and cell division; other genes appear to have unrelated functions. Two examples of the later type are the ompF gene with the sequence TTATCATA in the promoter and the rrn ribosomal RNA operons, where the sequence TTATCAGA occurs. In order to determine whether the dnaA protein might influence the transcription of the ompF gene, we examined the transcription of lacZ from the plasmid, ORF 2, (which has the ompF promoter cloned in front of the lacZ gene) when this plasmid was resident in various dnaA temperature sensitive mutants. The results indicate that in some dnaA mutants the level of lacZ transcripts is much higher at permissive temperature as compared with nonpermissive temperature. This would suggest that active dnaA protein increases the level of transcription from the ompF promoter. Other results suggest that under special conditions active dnaA protein also increases the level of 16s rRNA transcripts. The essential sequences in the ompF promoter necessary for this regulation is currently under investigation.

1305 ISOLATION OF A DNA-BINDING PROTEIN SPECIFIC FOR THE REPLICATION ORIGIN OF HERPES SIMPLEX VIRUS I, Per Elias, Michael E. O'Donnell and I. Robert Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

As a part of a study of the mechanism of HSV-I DNA replication we are seeking proteins that interact with the origin of replication. Using a nitrocellulose filter binding assay we have identified a protein that specifically recognizes the ORIs sequence of HSV-I. This protein has been partially purified and its binding site in the ORIs region has been mapped using deletion mutants obtained by site-directed mutagenesis. The influence of this protein on in vitro DNA replication assays will be discussed.

1306 THE GENETIC AND BIOLOGICAL CHARACTERIZATION OF Ø105 INDUCTION IN BACILLUS SUBTILIS. Bradford M. Friedman and Ronald E. Yasbin, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642
Ø105 is a temperate bacteriophage of B. subtilis containing double-stranded DNA with a molecular weight of approximately 25Kda. Bacteria lysogenic for Ø105 are significantly reduced in their ability to be transformed by exogenous DNA due to the induction of the prophage in competent cells. Four recombinant plasmids have been generated which render B. subtilis resistant to superinfection by Ø105cl$_2$, a clear plaque mutant of Ø105. The parental plasmid is capable of replicating both in E. coli and in B. subtilis. The cloned DNA inserts consist of an EcoRI "F" fragment from whole vegative Ø105 DNA ca. 3.2Kbp and three smaller pieces from the "F" fragment (each ca. 700bp). Although these recombinant plasmids confer resistance to superinfection by Ø105cl$_2$, they differ in their biological activitives with regard to prophage induction and transformability of lysogenic strains. Specifically, one of the fragments (a Sau3A digest) caused homoimmune prophage to be lost from the chromosome, while another fragment (an EcoRI digest) decreased, beyond the level of detection, the abilitiy of lysogenic strains carrying either heteroimmune or homoimmune prophage to be transformed. In addition, there are unique proteins generated from each DNA fragment detected in B. subtilis minicells and E. coli maxicells. The elucidation of the activities associated with these cloned DNA fragments are aiding in the understanding of the mechanisms that control lysogeny and replication of the B. subtilis bacteriophage Ø105.

1307 ØX GENE A PROTEIN CATALYZED REPLICATION: SEQUENCE REQUIREMENTS AND THE ØX A PROTEIN - DNA COMPLEX, George S. Goetz, Thomas Schmidt-Glenewinkel, Nilda Belgado, and Jerard Hurwitz, Memorial Sloan-Kettering Cancer Center, New York, NY.

We have examined the mode of action of the ØX 174 gene A protein, the site-specific nuclease-ligase required for synthesis of single strand circular (SS(c)) ØX 174 DNA from supercoiled ØX 174 RFI DNA. The A protein initiates this pathway by nicking ØX 174 RFI between nucleotide residues 4305 and 4306 and is linked to the dAMP residue at position 4306 through a tyrosine-dAMP bond. Protease treatment of an oligonucleotide-A protein covalent complex suggests that the protein contains two distinct active sites. The sequence requirements supporting the various activites of the ØX A protein were examined by in vitro analysis of a series of mutations in the 30 nucleotide ØX 174 origin. Mutations or deletions within the first three nucleotides at the 5' end of the 30 nucleotide origin sequence do not alter the nicking reaction by the A protein, but result in reduced RF→SS(c) synthesis due to poor reinitiation. A G→A transition at nucleotide residue 4307 prevents cutting, while an A→G transition at residue 4306 permits efficient cutting and a reduced level of replication. 3' deletions truncating the origin region by 4 to 7 nucleotides support replication but are deficient in termination, producing long linear products. Through analysis of plasmids containing two origins of replication, one wild type and one mutant, on the same strand, it appears that a mutant origin unable to support initial cutting is also unable to support termination and reinitiation. No evidence for uncoupling of the termination and reinitiation reactions has been obtained.

1308 ASSEMBLY OF PRIMOSOME COMPLEXES MEDIATED BY THE BACTERIOPHAGE λ O AND P REPLICATION INITIATORS. Bruce Gomes, Jonathan H. LeBowitz and Roger McMacken, Johns Hopkins University, Baltimore, MD 21205

The λ O and P replication proteins, in conjunction with several E. coli proteins, promote the replication of single-stranded (ss) DNA templates that are coated with the ss DNA binding protein (SSB). This strand initiation system has recently been established with 8 purified proteins (LeBowitz et al., PNAS 82, 3988-3992, 1985). Since each of the 8 required proteins functions in λ DNA replication in vivo, we anticipate that that analysis of the 'λ ss replication reaction' will yield clues about the protein-protein interactions that occur prior to the initiation of DNA chains at the λ replication origin. The rate-limiting step in the λ ss replication reaction is the ATP-dependent transfer of the E. coli dnaB protein helicase onto the template DNA strand. Our data suggests (i) that the λ P protein mediates the interaction of a P-dnaB protein complex with a ternary complex of λ O protein, SSB and ss DNA and (ii) that action by the E. coli dnaJ and dnaK heat shock proteins frees the dnaB protein to actively migrate away from the initial assembly point. The activated dnaB protein apparently migrates processively along the SSB-coated template strand, serving as a locus for the synthesis of multiple primers by the E. coli primase. In related studies we have discovered that a functionally similar primosome-like complex can be formed with just dnaB protein, primase and SSB, but only if the dnaB protein is permitted to bind to the ss DNA prior to the addition of SSB. Using poly dT_{14} as a trap for free dnaB protein, we have found in this minimal system that the dnaB protein acts processively and does not dissociate during the synthesis of multiple RNA primers.

1309 CONFORMATIONAL BEHAVIOR OF NUCLEOTIDES RELEVANT TO DNA REPLICATION FIDELITY. Myron F. Goodman, John Petruska, Lawrence C. Sowers, Michael Boosalis, Sandra K. Randall, Larry Dalton, Dhananjaya N. Mhaskar, Tasneem Khwaja, G. Victor Fazakerley+, Ramon Eritja*, and Bruce Kaplan*, University of Southern California, Los Angeles, CA 90089-1481, +CEN Saclay, Gif sur Yvette, France, *Beckman Research Institute, City of Hope, Duarte, CA 91010

The susceptibility of nucleotides to conformational changes profoundly influences the fidelity of DNA replication. Disfavored tautomers of common bases and base analogues are generally assumed to explain base substitution mutagenesis. For 2-aminopurine (AP), a mutagenic analogue of adenine (A), we provide evidence from NMR and UV spectra that the AP:C mispair exists in DNA and forms two hydrogen bonds. We propose that protonation rather than tautomerization is the most likely cause of AP:C mispairs. We also provide evidence that AP:A mispairs exist in DNA and that DNA polymerases insert AP opposite template A sites. We suggest that AP:A is a wobble base pair with two hydrogen bonds. Kinetic data (K_M and V_{max}) are provided for the formation of single phosphodiester bonds for matched and mismatched base pairs, including insertions opposite apurinic sites on DNA. We present a model, based on water exclusion in the enzyme cleft, to explain how DNA polymerase amplifies (2- to 5-fold) the free energy differences between correct and incorrect base pairs measured in aqueous solution, without invoking enzyme conformational changes or kinetic proofreading.

I310 IN VIVO POINTS OF DNA-PROTEIN CONTACT WITHIN THE PROMOTER REGION OF THE YEAST HSP82 GENE. David S. Gross and William T. Garrard, Dept. Biochemistry, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Saccharomyces cerevisiae is an attractive biological system to dissect the cis-acting DNA sequence determinants of gene expression and to study the DNA contact points of trans-acting regulatory proteins. Genetic manipulation of this organism readily permits positive selection of site-directed integration, making it possible to change any base sequence within the normal chromosomal environment of a given gene. We have chosen to study the locus encoding an 82 kd heat shock protein, HSP82, and report here identification of regulatory protein contacts within the promoter of this gene. We have exposed living haploid yeast cells to dimethyl sulfate (DMS) and have assessed the extent to which the N-7 moiety of guanine, which resides in the major groove of DNA, is accessible to methylation. Mapping of piperidine-induced cleavage sites along an ~500 bp segment of the upstream region was by indirect end-labeling, using a modification of the genomic sequencing technique of Church and Gilbert (1984). Strikingly, this method reveals substantial reactivity of an A residue at -79 on the template strand, a position opposite the first T of the TATA box, as well as unusual reactivities of adenine clusters of 3-4 residues which flank the TATA box on the upper strand. These adenines are presumably methylated by DMS at the N-7 position, since they are unusually labile to treatment that normally results in chain cleavage exclusively at 7-methylguanine. We are currently constructing M13 clones that contain single base mutations at these sites.

I311 ENZYMOLOGICAL ASPECTS OF DNA REPLICATION IN MAMMALIAN CELLS, Frank Grosse, Ulrich Schomburg, Stephan Scholtissek and Heinz-Peter Nasheuer, Max-Planck-Institut f. Exp. Med., D-3400 Göttingen, FRG

Several proteins that are involved in DNA replication were purified to homogeneity. These proteins include the DNA polymerase α- primase complex, a stimulatory protein of the α-polymerase that most probably is a further subunit (SF-35), the single-stranded DNA binding proteins UP1 and its undegraded form (SSB-38), and the type I and type II topoisomerases from calf thymus. By employing in vitro DNA synthesis on single-stranded M13-DNA as a model system, the influence of these proteins on the reaction rate, the processivity and the accuracy of DNA polymerase α was studied. We found that the stimulatory protein diminishes the time demand for the complete replication of M13-DNA from 90 min to 40 min. The accuracy of DNA polymerase α increases at least twofold in the presence of SF-35. UP1 and its undegraded form SSB-38 increases the processivity of pol-α from 18 to about 30. Both SSB-proteins allow a faster replication through hairpin structures. By contrast, neither topoisomerase I nor topoisomerase II seem to have any influence on the in vitro replication of single-stranded M13-DNA by the homogeneous DNA polymerase α- primase complex.

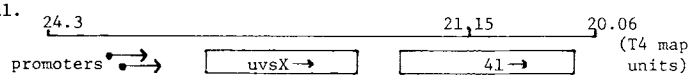
I312 TERMINAL PROTEIN OF PHAGE Ø29. SITE OF LINKAGE TO THE DNA AND INTERACTION WITH THE VIRAL DNA POLYMERASE AND DNA, José M. Hermoso, Ignacio Prieto, Cristina Garmendia, Luis Blanco, Enrique Méndez* and Margarita Salas, Centro de Biología Molecular (CSIC-UAM) Universidad Autónoma, 28049 Madrid, and *Centro Ramón y Cajal, Carretera Colmenar Viejo, 28034 Madrid, Spain.

Phage Ø29 has a protein, p3, covalently linked to the ends of the DNA through a phosphoester bond between a serine residue and the 5' terminal nucleotide dAMP. We have identified the site of linkage to the DNA as the serine residue 232, by amino acid analysis of a proteinase K resistant peptide linked to the DNA, that contains only one serine residue. This result has been confirmed by sequence determination of a tryptic peptide, covalently attached to the DNA, that includes serine residue 232.

Purified free terminal protein forms a complex with the Ø29 DNA polymerase. The formation of this complex requires NH_4^+ ions. Protein p3 also binds to DNA, though this interaction is not sequence specific. Purified parental terminal protein, obtained by nuclease digestion of the Ø29 DNA-p3 complex, does not interact either with the viral DNA polymerase or with DNA and, in contrast to free protein p3, forms dimers. The presence in the parental terminal protein of a short oligonucleotide covalently linked may change its structure and explain the different behaviour of the two proteins, reflecting their different roles in the initiation of replication of Ø29 DNA.

I313 EXPRESSION OF THE BACTERIOPHAGE T4 GENES uvsX AND 41, GENES REQUIRED FOR NORMAL DNA RECOMBINATION AND REPLICATION. Deborah M. Hinton, National Institutes of Health, Bethesda, MD 20892

The prereplicative T4 genes uvsX (recombination protein) and 41 (replication primase component and helicase) lie in a region of T4 DNA that is expressed early after T4 infection. Such early expression is thought to use transcription signals similar to those of uninfected E. coli, although detailed analyses of early T4 operons has only recently begun. My construction of plasmids encompassing the uvsX-41 region has positioned these genes more precisely on the T4 genome (see below). uvsX$^+$ plasmids express uvsX protein at a high level both in vivo and in vitro regardless of the orientation of the T4 DNA within the vector. Transcription of T4 DNA restriction fragments in vitro using E. coli RNA polymerase yields two major transcripts initiating approximately 700 and 800 bases upstream of the uvsX gene. In a plasmid containing both uvsX and 41, the level of the protein from the downstream gene 41 is much lower than that from uvsX both in vivo and in an S-30 extract. These results indicate that, on the plasmid, transcription begins within the T4 DNA upstream of uvsX but some type of regulation diminishes the level of gene 41 expression. It is hoped that the study of the expression of this region will provide an understanding of the expression of early T4 genes in general.



I314 LASER CROSSLINKING OF BACTERIOPHAGE T4 DNA REPLICATION PROTEINS TO NUCLEIC ACIDS. Joel W. Hockensmith, Thale A. Cross, William L. Kubasek, William R. Vorachek and Peter H. von Hippel. Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403.

Single-pulse (~8 nanosecond) ultraviolet laser excitation of protein-nucleic acid complexes can result in efficient and rapid covalent crosslinking of proteins to nucleic acids. The reaction produces no nucleic acid-nucleic acid or protein-protein crosslinks, and no nucleic acid degradation. Laser lines ranging from 204 nm to 309 nm have been examined to determine their ability to induce protein-DNA crosslinks. The efficiency of crosslinking is dependent on the wavelength of exciting radiation, the nucleotide composition of the nucleic acid, and the total photon flux. The method was calibrated using the T4 gene 32 protein interactions with oligonucleotides, for which binding constants had been previously measured by standard techniques. The method appears to be applicable to all proteins that bind to nucleic acids. Our primary application has been examination of the five gene products of T4 phage that are involved in leading-strand DNA synthesis. Our results include apparent binding constants for individual proteins to various substrates, as well as the way in which binding is altered by addition of cofactors necessary for DNA replication (dNTPs, ATP and other proteins). Additionally, laser crosslinking yields information on specificity of protein-nucleic acid interactions, including points of contact and site sizes. This information allows us to define the protein-protein and protein-nucleic acid interactions that take place during DNA replication. (Supported by NIH grants GM-29158, GM-07759 (to PHvH) and GM-09353 (to JWH).)

I315 REPLICATION AND TRANSCRIPTION OF BACTERIOPHAGE MU IN ESCHERICHIA COLI DNA GYRASE MUTANT STRAINS, Martha M. Howe, Scott H. Shore, and Wilma Ross, University of Wisconsin, Madison, WI 53706.

Mutations in E. coli DNA gyrase genes, gyrA and gyrB, which inhibit the growth of bacteriophage Mu but have little or no effect on cell growth or colony morphology were isolated. The Mu growth inhibition was reflected by reductions in plaque size, efficiency of plating, and burst size and by a delay in the time of lysis. To determine the mechanism responsible for Mu growth inhibition, Mu DNA replication and transcription were assayed. Analysis of Mu-specific DNA synthesis demonstrated a delay in Mu replication comparable to the delay in lysis time. Quantitative studies of Mu transcription indicated that this delay in Mu DNA replication could not be attributed to reduced transcription of the Mu replication and transposition genes A and B. Southern blot analysis of Mu RNAs isolated at various times after induction showed no difference in Mu transcription pattern between the wild-type and gyrase mutant strains other than those attributable to the delays observed in the other assays. It is not clear whether the effects of the gyrase mutations are indirect, for example by causing an alteration in the level of DNA supercoiling, or whether they reflect a direct role for DNA gyrase as an active component of the transposition process.

I316 INTERACTION OF MUTANT THIOREDOXIN WITH T7 DNA POLYMERASE, Hans Huber and Charles C. Richardson, Harvard Medical School, Boston, MA 02115

DNA polymerase activity in Escherichia coli cells infected with bacteriophage T7 resides in a protein complex consisting of the T7 gene 5 protein and E. coli thioredoxin in a one-to-one stochiometry. We have analyzed nine mutationally altered thioredoxins, both in vivo and in vitro, for their ability to interact with the T7 gene 5 protein and stimulate the DNA polymerase and exonuclease activities inherent in gene 5 protein. The efficiency of plating of T7 on E. coli thioredoxin mutants correlates well with the affinity of the purified mutant proteins for T7 gene 5 protein. The observed dissociation constant K_{obs} is increased between 5 and several hundred fold compared to wild-type thioredoxin. The maximum polymerase and exonuclease activities of the gene 5 protein/thioredoxin complex at saturating concentrations of mutant thioredoxins, however, are reduced by less than 20%. Consequently, none of the mutant thioredoxins acts as a competitive inhibitor of wild-type thioredoxin. The active-site disulfide of thioredoxin is not essential for the activities of the gene 5 protein/thioredoxin complex. Both cysteines can be replaced without significantly affecting the maximum polymerase or exonuclease activities. Substitution or alkylation of either cysteine, however, reduces the affinity for gene 5 protein drastically, indicating that the active-site is part of the thioredoxin surface involved in the protein-protein interaction.

I317 **A mutation in the 3′-5′ exonuclease active site of DNA polymerase I of E. coli.**
Catherine M. Joyce, Vicky Derbyshire and Nigel D.F. Grindley. Yale University, New Haven, CT 06510.

The Klenow fragment of DNA polymerase I is the only DNA polymerizing enzyme for which high resolution structural data are available. The 3A structure determined by Steitz and co-workers shows the molecule to be folded into two domains (1). A variety of biochemical and model-building experiments suggest that the two domains correspond to the two enzymatic activities of Klenow fragment: polymerase and 3′-5′ (editing) exonuclease (2). Since the 3′-5′ exonuclease reaction is inhibited by deoxynucleoside monophosphates (3), the dNMP binding site observed crystallographically probably marks the 3′-5′ exonuclease active site. In order to identify amino acid side chains involved in the exonuclease reaction, we are using site-directed mutagenesis to change residues that interact with the dNMP molecule. Our preliminary experiments suggest that substitution of Ala for Asp 424 affects the product dissociation step of the reaction.

(1) Ollis, D.L. et al. (1985) Nature 313:762-766.
(2) Joyce, C.M. et al. UCLA Symposium: Protein Structure, Folding and Design, (D. Oxender, editor), in press.
(3) Que, B.G. et al. (1978) Biochemistry 17:1603-1607.

I318 AN ALPHA-LIKE DNA POLYMERASE AND REVERSE TRANSCRIPTASE-PRIMASE FROM H. HALOBIUM. M. Nakayama, K.B. Mahrez and M. Kohiyama, Institut J. Monod, Universite Paris 7, France.

The inhibition of H. halobium DNA replication by aphidicolin observed in our laboratory led us to isolate from this bacterium a DNA polymerase sensitive to the drug and similar to the alpha DNA polymerase of eucaryotes. The cell free extracts were first treated with a phase partition technique and the resulting DNA free extracts were chromatographed on a column of DEA Sephacel. Two peaks of polymerase activity were found; one in the non adsorbed fraction and the other eluted by 0.15 M NaCl. The latter activity was further purified by successive column chromatographies and by sucrose gradient centrifugation. Two DNA polymerases were separated at the last step; one sensitive to aphidicolin and the other resistant. The drug-sensitive DNA polymerase having a native molecular weight of 250 KD was found to be very similar to alpha type of eucaryotic origin; its resistance against ddXTP, and sensitivity to NEM. The polymerase reaction was high salt dependent (5 M NaCl) in the absence of which the enzyme dissociated into subunits. The antiserum prepared against this polymerase cross-reacted strongly with the 70 KD subunit of alpha DNA polymerase obtained from rat regenerating liver. The other DNA polymerase was sensitive to ddXTP but resistant to NEM. The polymerase found in the non adsorbed fraction from DEAE Sephacel was further purified by hydroxyapatite and carboxymethyl cellulose column chromatography. It can incorporate either rXTP or dXTP using a single-stranded DNA or RNA as template. In the presence of DNA polymerase alpha of H. halobium, DNA synthesis resumed on the template poly(d(A) when this small enzyme was added. The enzyme has both the properties of reverse transcriptase and primase.

1319 INTERACTION OF THE β SUBUNIT OF E. COLI DNA POLYMERASE III HOLOENZYME WITH THE CATALYTIC CORE. R. LaDuca[‡], J. Crute*, C. McHenry[+], and R. Bambara*, [‡]Eastman Kodak Company and *University of Rochester, Rochester, New York 14642, and [+]University of Colorado, Denver, Colorado 80262.

A direct interaction between the β subunit of DNA polymerase III holoenzyme with the catalytic core of the holoenzyme was demonstrated, resulting in a new form of the enzyme with enhanced catalytic and processive capabilities. The addition of saturating levels of the β subunit to the core DNA polymerase III enzyme results in as much as a 7-fold stimulation of synthetic activity. Two populations of DNA products were generated by DNA polymerase III-β enzyme complex. Short products resulting from the addition of 5 to 10 nucleotides per primer fragment were generated by DNA polymerase III in the presence and absence of added β. A second population of much longer products was generated only in β-supplemented DNA polymerase III reactions. The DNA polymerase III-β reaction was inhibited by single-stranded DNA binding protein and unaffected by ATP, distinguishing it from the holoenzyme reaction. Complex formation of DNA polymerase III with β increased the residence time of the enzyme on synthetic DNA templates. Our results demonstrate that the β-stimulation of DNA polymerase III can be attributed to a more efficient and highly processive elongation capability of the DNA polymerase III-β complex. (This work was supported by NIH grants CA11198, CA09363, GM24441 and ACS grant NP 232.)

1320 DISSOCIATION OF THE β SUBUNIT FROM THE E. COLI DNA POLYMERASE III HOLOENZYME, Roger S. Lasken and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

E coli DNA polymerase III holoenzyme (HE) is separable into the nonprocessive polymerase subassembly, pol III*, and the β subunit. Mixing of pol III* and β reconstitutes processive HE synthesis. Purified HE fractions contain pol III* and free β subunit, presumably in an equilibrium state. HE forms an isolable initiation complex with a primer-template in which β remains associated. However, it appears that HE, when not complexed with DNA, dissociates into pol III* + β as diagramed:

$$\text{pol III*} + \beta \rightarrow \text{HE} \xrightarrow{\text{primer-template}} \underset{\text{complex}}{\text{initiation}} \rightarrow \text{pol III*} + \beta + \text{DNA}$$

Consistent with this model, recycling of HE from a replicated template to a second primer-template was dependent upon the concentration of free β subunit. This was true for purified HE and also for HE initiation complexes isolated from crude cellular extracts. Also, radioactively labeled β subunit combined with purified HE in initiation complexes indicating that β is freely exchanged. The results suggest that the β subunit concentration in a cell could be a limiting factor in HE formation.

1321 ISOLATION AND CHARACTERIZATION OF HUMAN PLACENTAL DNA POLYMERASE δ. Marietta Y.W.T. Lee, N. Lan Toomey, Mary J. Fenna, University of Miami, Miami, FL 33101.

DNA polymerase δ was isolated from human placenta and characterized. It was characterized as DNA polymerase δ on the basis of its association with a 3' to 5' exonuclease activity. This association was maintained throughout purification and attempted separations by physical or electrophoretic methods. Moreover, ratios of the two activities remained constant during the purification steps. The purified enzyme had an estimated molecular weight of 172,000, based on a Stokes radius of 53.6 Å and a sedimentation coefficient of 7.8 S. Like polymerase α, polymerase δ is inhibited by aphidicolin, but it can be distinguished from DNA polymerase α by a number of properties. DMSO was found to stimulate polymerase δ but to inhibit polymerase α activity. BuPdGTP [N^2-(p-n-butylphenyl)-9-(2-deoxy-β-D-ribofuranosyl)guanine 5'-triphosphate] and BuAdATP [2-(p-n-butylanilino)-9-(2-deoxy-β-D-ribofuranosyl)adenine 5'-triphosphate] were found to be potent inhibitors of polymerase α, whereas polymerase δ was relatively insensitive. In addition, preliminary studies using both polyclonal and monoclonal antibodies to polymerase δ suggest that it is immunochemically distinct from polymerase α. (Supported by GM 31973; M.Y.W.T. is an Established Investigator of the American Heart Association.)

1322 REPLICATION OF UV-IRRADIATED SINGLE-STRANDED DNA WITH DNA POLYMERASE III HOLOENZYME: IMPLICATIONS FOR S.O.S.-MUTAGENESIS, Zvi Livneh, Department of Biochemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel

In vitro replication of UV-irradiated single-stranded DNA with DNA polymerase III holoenzyme in the presence of SSB results in a significant fraction of fully replicated DNA molecules in addition to termination products. This is the result of bypass of pyrimidine-photodimer by the polymerase. Under similar conditions, T4 DNA polymerase, in the presence of gene 32 protein, is at least 10-fold less efficient in fully replicating UV-irradiated single-stranded DNA. Inhibition of the 3'→5' exonucleolytic activity of DNA polymerase III holoenzyme or the addition of purified recA protein do not increase bypass, suggesting that inhibition of proofreading is unlikely to be a rate limiting factor in bypass of pyrimidine-photodimers. Based on these results and others, a model for S.O.S. mutagenesis is proposed.

1323 SALT DEPENDENT CHANGES IN THE DNA BINDING COOPERATIVITY OF E. coli SSB PROTEIN, Timothy M. Lohman, Leslie B. Overman & Santanu Datta, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843

The cooperative binding of E. coli SSB protein to s.s. nucleic acids has been examined to determine if different degrees of cooperativity are associated with the two SSB binding modes recently identified [Lohman and Overman (1985) J. Biol. Chem., 260, 3594]. Quantitative estimates of the binding constant and cooperativity parameter, ω, to s.s. DNA and RNA homopolynucleotides have been obtained from equilibrium binding isotherms at high salt ([NaCl]>0.2M), using fluorescence. Under these conditions, where the site size for SSB binding is 65±5 nucleotides/tetramer, we find only moderate cooperativity for SSB binding to both DNA and RNA, (ω=50±10), independent of [NaCl] and [$MgCl_2$]. This value for ω is much lower than most previous estimates. At lower salt concentrations, where the low site size SSB binding mode (33±3 nucleotides/tetramer) exists, we have used agarose gel electrophoresis to qualitatively examine SSB cooperativity with s.s. M13 DNA. The apparent cooperativity increases dramatically below 0.20M NaCl as judged by the nonrandom distribution of SSB among the s.s. DNA population at low SSB/DNA ratios. However, the highly cooperative complexes are not at equilibrium at low SSB/DNA binding densitites, but are only formed transiently when SSB and s.s.DNA are directly mixed at low [NaCl]. At equilibrium, the SSB-s.s.DNA complexes seem to possess the same low degree of cooperativity (ω=50±10) under all conditions tested. The highly cooperative SSB binding mode, although metastable, may be important during nonequilibrium processes such as replication. The two SSB binding modes may be related to the high and low cooperativity complexes reported here and each may function selectively in replication, recombination and repair processes, in vivo. (Supported by NIH grant GM30498 and Welch grant A-898.)

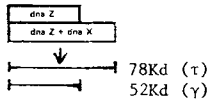
1324 Base Pair Specific Interaction of dNTP Substrates and the T4 DNA Polymerase Active Site, David C. Mace, Waksman Institute, Rutgers University, Piscataway, NJ 08854

Recently, studies have been undertaken using T4 DNA polymerase and base analog dNTPs such as dITP and dDAPTP (2,6-diaminopurine deoxynucleoside-5'-triphosphate). Kinetic analysis of the insertion step during DNA synthesis or idling turnover has shown that dGTP is used no better than dITP as a substrate, and dDAPTP is used considerably more poorly than dATP as a substrate. Yet the bases of both dGTP and dDAPTP make more stable base pairs with C and T respectively than do the base of dITP and dATP.

A model for the structure of the active site that accounts for this is presented. It proposes a specific, unfavorable interaction of the enzyme active site with the 2-amino group (if present) of purines in the substrate-template base pair. This model also offers an explanation of extant data (Clayton, et al., (1979) J.Biol.Chem. 254, 1902-1912) for utilization of dAPTP (2-aminopurine deoxynucleoside-5'-triphosphate) as compared to dATP. It also accounts for the general observation for DNA polymerases that the Kms for dGTP and dCTP are no greater than for dATP and dTTP despite the much greater stability of the G:C base pair.

1325 RECONSTITUTION OF DNA POLYMERASE III HOLOENZYME FROM PURIFIED SUBUNITS, Satoko Maki and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

DNA polymerase III holoenzyme of E. coli, a multi-polypeptide complex, catalyzes DNA synthesis with high processivity. Subunit composition and function in processivity have been approached by reconstitution of the holoenzyme from highly purified subunits. The dnaX and dnaZ genes, known to serve in chain elongation, are contiguous at 10.4 min and produce two polypeptides (78 kd, 52 kd):



These genes were cloned in a P_L-promoter mediated expression vector and the products overproduced. The 52 kd protein was purified to homogeneity using an in vitro dnaZ complementing assay. Western-blot analyses with antisera against this protein revealed that the 78 kd and 52 kd proteins are related immunologically and correspond to the τ and γ subunits of the holoenzyme, respectively. The holoenzyme activity could not be reconstituted by combining purified Pol III core (α, ε and θ), β and γ proteins. Based on a reconstitution assay with these subunits, a new component, overproduced in the dnaX-Z overproducing strain, is being purified and will be characterized.

1326 OVERPRODUCTION AND PURIFICATION OF AN F PLASMID REPLICATION PROTEIN, PIFC. L. Masson and D.S. Ray, U.C.L.A., Los Angeles, Ca.

In addition to possessing at least two known origins of replication, the 94.5 kilobase F plasmid is maintained in vivo under stringent control (approx. 1-2 copies per cell). In order to develop an in vitro DNA replication system to further identify both the cis and trans acting elements governing the initiation of DNA replication, our efforts were directed towards the purification of the F encoded, 40 kilodalton (kd) replication protein pifC.

Through the use of oligonucleotide-directed mutagenesis, an Aha III restriction endonuclease site was created in order to separate the pifC coding sequence from its promoter. The pifC coding sequence was subsequently cloned into the expression vector pING 1 behind the strong, inducible araB promoter and a truncated araB gene. Due to an intergenic translational stop codon, induction by l-arabinose resulted in the high level expression of a non-fused 40 kd protein as detected by SDS-PAGE. This overproduced protein was subsequently purified to near homogeneity by Sephacryl S-200 molecular sieve chromatography followed by DEAE-Sepharose column chromatography.

1327 THE dnaB GENE OF SALMONELLA TYPHIMURIUM, Russell Maurer, Annette Wong, and Leslie Kean, Case Western Reserve University, Cleveland, OH 44106

The dnaB gene of Salmonella typhiumurium has been cloned and sequenced and compared with the corresponding gene of E. coli, whose sequence has been reported by others (Nakayama et al., J. Biol. Chem. 259, 97-101 (1984)). The coding sequences are identical in length (1416 base pairs) and extensively homologous. The homology is 83% at the nucleotide level and 93% at the amino acid level. Most of the amino acid changes are conservative. This information readily explains how the E. coli dnaB gene can work in Salmonella and vice versa. In connection with the sequencing, we have developed a nested set of deletions from each end of dnaB. These deletions permit detailed mapping of dnaB mutations that restore growth to dnaC(Ts) mutants, using a heteroduplex mismatch repair approach. We will report on our mapping and sequence data for the suppressor mutations and discuss the implications of this data for the dnaB-dnaC protein interaction.

1328 POSITIVELY SUPERCOILED DNA EXISTS IN VIVO. Gilles Mirambeau, Marc Nadal, Patrick Forterre*, Christine Jaxel, and Michel Duguet, Université Pierre et Marie Curie, 96, Bd. Raspail, 75006 Paris, FRANCE. *Institut de Recherches sur le Cancer, BP n°8, 94802 Villejuif Cedex, FRANCE.

The importance of DNA topology in a variety of essential biological events has been stressed by a number of reports, in Eubacteria and in Eukaryotes. In Archaebacteria, the discovery of "Reverse Gyrase", an enzyme able to convert in vitro relaxed (or negatively supercoiled) DNA into positively supercoiled DNA, led us to ask whether this activity exists in vivo and if it is possible to isolate "natural" DNA in a positively superhelical state. The Archaebacterium Sulfolobus solfataricus can be infected by an UV inducible virus, which is called SSV1. The viral DNA is a covalently closed circular DNA of 16 KB. By using bidimensional agarose gels, we show that this DNA is positively supercoiled when extracted either from the infected bacterium, or from the virus particle. This finding strongly suggests the existence of positively supercoiled DNA in vivo.

1329 MAMMALIAN ENZYMES CATALYZING HOMOLOGOUS RECOMBINATION. Peter D. Moore and Linda Wallace, University of Illinois at Chicago, Box 6998, Chicago, IL 60613.

We are studying a homologous recombination reaction catalyzed by mammalian enzymes in vitro. Recombination between mutant plasmids is detected in a biological assay and requires co-incubation of homologous substrates with the enzyme preparation. The reaction carried out in gene conversion, sometimes accompanied by crossing over. Reconstruction experiments and co-factor requirements suggest the involvement of the equivalent of a recA-like recombinase and DNA polymerase activities. As has been found for purified recombination enzymes from other organisms, single-stranded, as well as double-stranded, DNA is efficiently utilized in the reaction and activity is largely dependent on the presence of strand breaks, single or double, in one of the substrates. Recombination activities have been recovered after partial fractionation of the enzyme preparation. Further purification of the enzymes involved is proceeding.

1330 PURIFICATION AND CHARACTERIZATION OF GENE PRODUCT 1.2 FROM PHAGE T7. J. A. Myers, B. B. Beauchamp and C. C. Richardson, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

Gene 1.2 of phage T7, located near the primary origin of DNA replication, encodes a protein essential for growth on E. coli optA1 strains (H. Saito and C. C. Richardson, J. Virol. 37, 343-351, 1981). Phage T7 1.2 mutants grow normally on E. coli optA$^+$ as do wild type T7 phage on E. coli optA1. E. coli optA1 cells infected with T7 gene 1.2 mutants are defective in T7 DNA replication. DNA synthesis is initiated at the primary origin but ceases prematurely, and unusually high amounts of DNA repair are observed. E. coli optA also does not support the growth of phage T4 CB120 having a mutation in gene 43 (DNA polymerase) that gives rise to an antimutator phenotype. Gene 1.2 has been cloned into pBR322 under the control of the tac promoter. Both T7 gene 1.2 mutant and T4 CB120 infections are supported by E. coli strains harboring the plasmid containing the cloned gene 1.2. The protein has been overproduced in the presence of ^{35}S-methionine and has been purified to homogeneity using radioactivity and gel electrophoresis as assays. Some evidence suggests that the gene 1.2 and optA proteins function in the degradation of host DNA.

I331 INTERACTIONS INVOLVED IN RNA-PRIMED DNA SYNTHESIS CATALYZED BY T7 GENE 4 PROTEIN AND DNA POLYMERASE, Hiroshi Nakai and Charles C. Richardson, Harvard Medical School, Boston, MA 02115

The gene 4 protein (primase and helicase) and DNA polymerase of bacteriophage T7 catalyze in vitro RNA-primed DNA synthesis on single-stranded DNA templates. To study interactions involved in this reaction, complexes composed of gene 4 protein, T7 DNA polymerase, and the M13 DNA template were formed in vitro, purified by gel filtration, and assayed for enzymatic activities. Gene 4 protein-M13 DNA complexes formed only in the presence of Mg^{2+} and dTTP. T7 DNA polymerase-M13 DNA, gene 4 protein-DNA polymerase, and gene 4 protein-DNA polymerase-M13 DNA complexes formed in the presence or absence of nucleotides. Purified gene 4 protein-M13 DNA complexes that were formed in the presence of CTP and ATP replicated without rNTPS upon addition of T7 DNA polymerase and dNTPs, indicating that RNA primers were associated with the complexes. Primer and DNA synthesis in the RNA-primed DNA synthesis reaction could be uncoupled by increasing the DNA template concentration without a proportionate increase in DNA polymerase. Gene 4 protein-DNA polymerase-M13 DNA complexes formed in the presence of dTTP were relatively more resistant to challenge by single-stranded DNA templates than complexes formed in the absence of nucleotides. The implications of these interactions to the mechanism of RNA-primed DNA synthesis at the T7 replication fork are discussed.

I332 PROTEIN-PROTEIN INTERACTIONS OF *ESCHERICHIA COLI* SINGLE-STRANDED DNA-BINDING PROTEIN IDENTIFIED BY SSB-AFFINITY CHROMATOGRAPHY, Fred W. Perrino, Diane C. Rein, Steven M. Ruben, Albert M. Bobst and Ralph R. Meyer, University of Cincinnati, Cincinnati, OH 45221

The single-stranded DNA-binding protein (SSB) of *Escherichia coli* plays a vital role in DNA replication, repair and recombination. SSB must, therefore, interact with many other proteins required for these processes. By using protein affinity chromatography, we have identified several proteins which interact directly with SSB. Purified SSB has been covalently coupled to Affigel-10. A DNA-free, wild-type [^{35}S]-labeled cell extract was passed over the column and washed with low salt buffer to elute nonspecifically bound proteins. High salt buffer was then used to elute three major proteins of M_r = 25,000, 32,000 and 36,000 plus a few minor proteins. All of these proteins were mapped by two-dimensional gel electrophoresis. SSB, itself, binds extremely tightly and can be eluted only with sodium dodecyl sulfate. The 25K protein was the most prominent, and retention of this protein was enhanced 4-fold by addition of DNA polymerase III holoenzyme to the extract. The 25K protein appears to be an accessory protein interacting with both SSB and holoenzyme. We are currently determining whether it may be the 25K ε-subunit of DNA polymerase III holoenzyme. All three proteins binding to the SSB column are being purified, and their roles in DNA metabolism are currently under investigation. Supported by grant GM31196 from the NIH.

I333 MOUSE PRIMASE AND ASSOCIATION WITH DNA POLYMERASE ALPHA, C. Prussak, M. Almazan, B. Tseng, University of California, San Diego, La Jolla, CA 92093

Mouse primase consists of 1 subunit each of 56 KDa and 46 KDa polypeptides that copurify in a 1 to 1 stoichiometry with activity. We have assessed the antigenic relatedness of the two subunits with antibodies raised against p56 or p46. By Western and dot blot analysis of separated subunits, we are unable to detect any cross-reactivity (<1%) between the subunits. We were also unable to detect any reactivity to larger polypeptides, although the analysis may be reduced in sensitivity due to the large amount of protein needed for detection.

Primase from eukaryotic cells has been isolated in either a complexed form with DNA polymerase alpha or as a homogeneous enzyme. To analyze the relation between these forms, DNA polymerase alpha was immunoprecipitated from a mouse cell extract with a monoclonal antibody (SJK 132-20) against human DNA pol alpha that cross-reacts with mouse DNA pol alpha. The immunoprecipitate when analyzed by Western analysis and immunodetection showed the same sizes and stoichiometry of polypeptides as purified primase. This indicates that only a single form of primase exists in mouse cells and can be isolated either in a free or complexed form with DNA polymerase alpha. (Research was supported by NIH grant GM 29091).

1334 ENHANCED FIDELITY OF DNA SYNTHESIS BY EUCARYOTIC HIGH MOLECULAR WEIGHT REPLICATING COMPLEXES. Mary E. Reyland and Lawrence A. Loeb, The Joseph Gottstein Memorial Cancer Research Laboratory, Dept. Pathology SM-30, University of Washington, Seattle, WA 98195.

Error rates for conventionally purified core enzyme of calf thymus DNA polymerase-α have been reported from our laboratory and others to be 1 in 30,000 nucleotides inserted. We have isolated a high molecular weight replicating complex of calf thymus polymerase-α by monoclonal antibody affinity chromatography utilizing anti-KB cell polymerase antibodies (S. Tanaka, et al., JBC 257: 8366-8390, 1982). DNA synthesis by this enzyme complex is five- to ten-fold more accurate when assayed by the ΦX am3 fidelity assay; the error rate is 1 in 270,000 nucleotides inserted. This is similar to the error rate reported for a high moelcular weight polymerase-primase replicating complex isolated from Drosophila by conventional methods (L.S. Kaguni, et al., JBC 259: 9314-9319, 1984), which we have confirmed to be one in 140,000. Like the Drosophila complex, our affinity purified complex is not highly processive as determined by primer extension analysis. Peptide analysis on SDS-PAGE shows a complex of several peptides, one band of which is approximately 200,000 daltons, corresponding to the undegraded catalytic core component of DNA polymerase-α. Analysis is underway to determine if, as with the Drosophila complex, this high degree of fidelity is achieved in the absence of an associated 3'→5' exonuclease activity, and if the enhanced fidelity is a result of an undegraded catalytic core, or association of other peptides in the complex.

1335 MAMMALIAN SINGLE STRANDED DNA BINDING PROTEINS DERIVE FROM hnRNP CORE PROTEINS S. Riva, G. Biamonti, C. Morandi, M. Pandolfo, P. Tsoulfas, K. Shäfer° and K. Williams* Istit. Genet. Bioch. Evoluz. CNR, Pavia, ITALY; °University of Bochum., FRG; *Yale University, (Conn.) USA.

As we previously demonstrated, mammalian single stranded DNA binding proteins (ssDBP) and heterogeneous nuclear RNA binding proteins (hnRNP core proteins) are antigenically and structurally related (NAR 13, 337, 1985). We also observed that ssDBP are proteolytic products of hnRNP core proteins (NAR 1985, in press). Proteolysis can be observed in crude extracts of HeLa cells, human fibroblasts and calf thymus extracts. A trypsin-like protease that cleaves purified hnRNP proteins to give ssDBP of Mr=24-28 Kd was purified from HeLa cells. A precursor-product relationship can be established between hnRNP core protein A1 (Mr=34 Kd) and a ssDBP of 24 Kd (UP1) (J.B.C. 251, 2124, 1976). In fact it has been found that the aminoacid sequence of a peptide from HeLa cell hnRNP core protein A1 perfectly overlaps the COOH-end of UP1 and extends further with a sequence rich in glycine (50%). Gene cloning and sequencing studies carried out in our laboratory demonstrate that UP1 gene is part of a longer open reading frame coding for a protein of about 34 Kd with an aminoacid composition identical to that of hnRNP core protein A1. Unlike their cleavage products (ssDBP), hnRNP core proteins do not stimulate DNA polymerase α in vitro. The significance of these findings in relation to DNA replication will be discussed.

1336 Adenovirus DNA Replication In Vitro: Characterization of the DNA and Protein Requirements, **Philip Rosenfeld, Ronald Wides, Edward O'Neill,* Mark Challberg, and Thomas J. Kelly, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205 (*Laboratory of Viral Diseases, NIAID, NIH, Bethesda, MD 20205)**

Nuclear Factor I (NFI), a cellular protein that enhances the initiation of adenovirus DNA replication in vitro, has been shown to bind a specific nucleotide sequence within the viral origin of replication. Using recognition site DNA affinity chromatography, we have purified NFI 2400-fold with a 57% recovery of specific binding activity from crude nuclear extract. A novel cloning strategy was used to prepare a plasmid that contains 88 copies of the NFI binding site for use on the specific DNA affinity matrix.

The terminal 67 bp of the adenovirus genome has been shown to contain the sequence required for optimal DNA replication. This sequence includes the minimal origin (1-18) and a region that greatly enhances the efficiency of adenovirus replication (19-67). To further characterize the correlation between enhanced replication efficiency and the ability of NFI to bind between nucleotides 18 and 45, we have constructed plasmid DNAs that contain a variety of mutations within the adenovirus origin of replication. Plasmid DNAs containing point mutations have been used to identify the optimal NFI recognition sequence as TTGGCNNNNNGCCAA. Moreover, we have altered the spacing between the minimal origin and NFI binding site. Analysis of these mutants has shown that optimal replication efficiency depends on a precise spatial relationship between these sequences.

1337 RAT LIVER DNA LIGASE J.M. Rossignol+, M. Mezzina+, R. Izzo*, M. Philippe+, U. Bertazzoni* and A. Sarasin+. +Department of Molecular Biology and Genetics - IRSC. BP N°8 - 94802 Villejuif - France and *Istituto di Genetica Biochimica ed Evoluzionistica del CNR - Pavia - Italia.

In prokaryotes and yeast, genetic data have indicated that a single species of DNA ligase is involved in replication, repair and recombination processes. In mammalian cells, conflictory results have been reported about the existence of one or two different ligases. In the later case, the two species called ligase I and ligase II would be involved in DNA replication and DNA repair respectively.

Partial purifications of DNA ligase from regenerating (in which DNA synthesis occurs) and normal adult rat liver have been done. The active structure of the DNA ligase was determined by the use of activity gels.

We showed that : 1) the active polypeptide of DNA ligase is a 130 Kda polypeptide both in normal or regenerating liver.

2) A trypsin treatment led to the disparition of the 130 Kda polypeptide which is converted into smaller active bands of Mr : 110, 100, 85 and 75 Kda.

3) On hydroxyapatite chromatography it is possible to obtain either one or two peaks of ligase activity depending of the procedure used. When the active structure of the two peaks is analysed, we found only one band with a Mr of 130 Kda. A 12 weeks storage of the 2 DNA ligases at 4°C led to the apparition in both cases of active bands with a pattern of 110, 85, 75 and 60 Kda. The 60 Kda band do not show any ligase activity.

From these results we suggest than in the cytosol extract of rat liver only one DNA ligase is present. Purification of DNA ligase from nuclei is now under progress.

1338 Supercoiling and E. coli single-stranded DNA binding protein are required for N4 virion RNA polymerase-promoter recognition. A. Glucksmann, C. Malone, P. Markiewicz, *J. Chase and L. B. Rothman-Denes. The University of Chicago, Chicago, Illinois and *Albert Einstein College of Medicine, Bronx, N.Y.

Coliphage N4 virion-encapsulated, DNA-dependent RNA polymerase is required for N4 early transcription and DNA replication. The enzyme is inactive on the linear double-stranded 72 kilobase pair N4 genome unless the DNA is denatured. We have shown that the enzyme recognizes in vivo sites of transcription initiation (promoters) on denatured N4 DNA. These sites share extensive sequence homology from -18 to +1 and two sets of inverted repeats (Cell 41, 597, 1985).

In vivo N4 early transcription requires the activities of E. coli DNA gyrase and single-stranded DNA binding (ssb) protein. Ssb activates N4 virion RNA polymerase transcription on single-stranded DNA and is absolutely required for transcription on supercoiled template. No other single-stranded DNA binding protein tested can substitute for ssb. In order to elucidate the role of supercoiling and ssb on promoter recognition, we have performed footprinting analysis of the initiation complex on single-stranded DNA. To our surprise, we detect specific interaction of ssb at the promoter region. DNAseI and neocarzinostatin hypersensitivity is observed at the first inverted repeat. The hypersensitivity is lost when the inverted repeat is destroyed by mutation. RNA polymerase binding protects this region from probe cleavage. We suggest that ssb binds near the promoter and stabilizes the stem-loop structure at the first inverted repeat, which is required for virion RNA polymerase activity. Footprinting experiments on supercoiled template are in progress.

1339 DNA POLYMERASE γ FROM DROSOPHILA: ISOLATION AND CHARACTERIZATION, Kengo Sakaguchi and James B. Boyd, Department of Genetics, University of California, Davis CA 95616

Previous studies in Drosophila have identified two DNA polymerases which are analogous to the α and β forms found in vertebrates. We have identified a third form which is designated polymerase γ by virtue of its mitochondrial localization. This enzyme has been purified to near homogeneity by fractionation on DEAE cellulose, phosphocellulose, isoelectric focusing and DNA cellulose. A 13,000 fold purification from 16g of embryos produced 126 μg of enzyme. The enzyme is stable for several months at -20 degrees C. A description of its subunit structure will be reported. Like related vertebrate enzymes, Drosophila polymerase γ is strongly inhibited by NEM but is relatively insensitive to aphidicolin and phosphate. In contrast to vertebrates, the Drosophila enzyme exhibits only slight reverse transcriptase activity, is strongly inhibited by ethidium bromide and is relatively insensitive to ddTTP. Rapid purification of a stable homogeneous enzyme should facilitate further analyses of mitochondrial replication.

1340 EXPRESSION AND FUNCTIONING IN YEAST OF A BACTERIAL SITE SPECIFIC RECOMBINATION SYSTEM, Brian Sauer, E. I. Du Pont de Nemours & Co., Inc., Central Research & Development, E328/136, Experimental Station, Wilmington, DE 19898

Phage P1 of E. coli encodes an efficient site-specific recombination system consisting of a 34 base pair site called loxP and a single protein called Cre which catalyses efficient DNA recombination between loxP sites. The cre gene was placed into the yeast Saccaromyces cerevisiae under the control of the inducible GAL1 promoter. An intact LEU2 gene flanked by loxP sites was also integrated into a yeast chromosome. In the absence of induction such yeast strains maintain a stable Leu^+ phenotype. When the cre gene is induced by galactose, all cells rapidly become Leu^-. This occurs by the precise deletion of the LEU2 gene at the flanking loxP sites. The ability of the cre-lox system to modulate the qaction of the endogenous yeast recombination system to generate gene conversion and reciprocal recombination events is being examined.

1341 CAPACITY OF RecA PROTEIN TO BIND PREFERENTIALLY TO UV LESIONS AND INHIBIT THE EDITING SUBUNIT OF DNA POLYMERASE III: A POSSIBLE MECHANISM FOR SOS-INDUCED TARGETED MUTAGENESIS. R. H. Scheuermann, C. Lu and H. Echols, Department of Molecular Biology, University of California, Berkeley, California.

In response to DNA damage, E. coli undergoes the induced SOS response. One aspect of this response is an increased frequency of mutations, most of which probably occur during replication across DNA lesions (targeted mutagenesis). In addition to its role in derepressing LexA-regulated operons, the RecA protein is required directly for mutagenesis. We have suggested that RecA might participate in targeted mutagenesis by binding preferentially to the inducing DNA lesions; DNA polymerase III might then replicate past the lesion more effectively but with reduced fidelity (Weigle mutagenesis and reactivation). We have investigated two major predictions of this model. With respect to lesion recognition, we have shown that RecA will bind preferentially to double-stranded DNA that has been UV irradiated. In addition, UV-irradiated, double-stranded DNA effectively activates RecA for cleavage of LexA. With respect to replication fidelity, Fersht and Knill-Jones have found that RecA inhibits the 3'-5' exonuclease activity of pol III holoenzyme. We have found that RecA inhibits the exonuclease activity of the purified editing subunit from pol III, the ε protein. Thus RecA might serve in targeted mutagenesis by binding to DNA lesions and inhibiting the editing capacity of the pol III.

1342 EARLY STAGES IN THE INITIATION OF oriC PLASMID REPLICATION, Kazuhisa Sekimizu, Tania A. Baker and Arthur Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

Prior to synthesis of primer RNA, three stages have been defined with the participation of purified proteins: (Stage I) An oriC complex of oriC DNA, dnaA protein and HU protein, is isolable by centrifugation. ATP at low concentration (<1 μM) is needed to stabilize dnaA protein and binds the protein with high affinity. (Stage II) A pre-prepriming complex is formed at an elevated temperature (>28°C), and requires ATP (>0.1 mM) and a complex of dnaB and dnaC proteins. The complex can be separated from free proteins by centrifugation or gel filtration. (Stage III) A prepriming complex is formed by extensive unwinding of the supercoiled DNA through helicase action of dnaB protein and swivelling action of gyrase (see Baker et al.).

1343 **REPLICATION OF SV40 DNA AND CHROMATIN ASSEMBLY DURING DNA SYNTHESIS IN VITRO.** Bruce Stillman, Gregory Prelich, Yakov Gluzman, Ronald Guggenheimer, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

Cell extracts prepared from the cytosol of human 293 cells support efficient SV40 DNA replication in vitro in the presence of purified SV40 T antigen. The DNA sequences within the SV40 origin region have been defined using mutant DNA templates. The T antigen functions required for efficient DNA synthesis in vitro have been examined. Five replication defective T antigen proteins have been compared to the wild type protein for origin binding, ATPase activity and DNA replication in vitro. Three proteins fail to bind to ori DNA and one is defective for ATPase activity. Interestingly, one altered T antigen, C8A, binds to ori DNA with wild type efficiency and retains ATPase activity, but still fails to support any DNA synthesis. All five defective T antigens inhibit the replication of SV40 DNA when added to extracts containing wild type T antigen. These results suggest that the SV40 T antigen has additional functions in DNA replication.
The products of the replication reaction consist of covalently closed, but relaxed circular DNA (form 1°), but addition of an extract from the nucleus of human cells promotes the negative supercoiling of the replicated DNA, but not the bulk of the non-replicated plasmid DNA. The negative supercoiling of the replication products is due to the assembly of the DNA into chromatin, a process that only occurs when T antigen is present and when the nuclear extract is present prior to the initiation of DNA synthesis.

1344 MOUSE L CELLS TRANSFORMED WITH EPSTEIN-BARR VIRUS DNA EXPRESS VIRUS-SPECIFIC THYMIDINE KINASE, Timothy Stinchcombe and Wendy Clough, Molecular Biology Division, Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089

Mouse L cells lacking thymidine kinase ($MLTk^-$) have been transformed to thymidine kinase positive (Tk^+) phenotypes by transfection with Epstein-Barr virus (EBV) DNA ($MLTk^+$ EBV). The viral DNA (obtained from producer B lymphocytes) was transfected intact or fragmented prior to transfection by treatment with MboI or BamHI endonuclease. The Tk^+ phenotype was selected for by growth in HAT (hypoxanthine/aminopterin/thymidine) medium. Membrane hybridization demonstrated that high molecular weight DNA isolated from $MLTk^+$ EBV cells hybridized to EBV DNA. Furthermore, cell extract from $MLTk^+$ EBV cells possessed two Tk activities which eluted separately from DEAE-cellulose columns. One of these Tk activities elutes in a manner identical to the cytosol Tk activity found in Tk^+ parental mouse L cells. The second Tk activity elutes in a manner similar to herpes simplex virus-encoded Tk and Epstein-Barr virus-specific Tk found in producer B lymphocytes. The Epstein-Barr virus-specific Tk found in these $MLTk^+$ EBV transformed cells shares with the herpes simplex virus-encoded Tk the ability to phosphorylate araT (9-β-D-arabinofuranosylthymine) and acyclovir (9-[2-hydroxyethoxy)methyl]guanine).

1345 DNA POLYMERASES AND DNA TOPOISOMERASES FROM GERMINATING MAIZE, Jorge Vázquez-Ramos, Luz Castellanos and Alejandra Vázquez. Dpto. Bioquímica Vegetal, Fac. Química, UNAM, México D.F. 04510.

DNA metabolism during germination of cereals has been poorly described and the enzymology of it is only beginning to be studied. We have been trying to isolate 2 types of enzymes essential for DNA replication from germinating maize embryos: DNA polymerases and DNA topoisomerases
Our results indicate that 2 types of DNA synthesis might take place during early germination of maize. Therefore we have partially isolated and characterized the major DNA polymerase activities from maize embryo axes imbibed for 3 and 24 hours. Both major activities have been purified at least 400 times. Our findings are that both are of the alfa-type of mammalian cells as shown by their response to substrate, ionic strenght, Mg requirement, temperature, pH and effect of inhibitors such as maleimides, AraCTP, aphidicolin and novobiocin. The electrophoretic mobility is also similar, with a calculated molecular weight of around 100Kd. So far we have no evidence of the presence of a beta-like enzyme, but we are in the search of it.
Additionally, we have partially characterized 2 types of DNA topoisomerases from 24 hours imbibed maize embryo axes, differing in their Mg and ATP requirements, ionic strenght and molecular weight, possibly equivalent to the type I and type II enzymes of mammals and we are currently trying to find out the relevance of these enzymes.

1346 CHARACTERIZATION OF DNA POLYMERASES ALPHA AND DELTA FROM CALF THYMUS, A.F. Wahl*, J.J. Crute+, L.W. Harwell, R.L. Marraccino, E.M. Lord and R.A. Bambara, University of Rochester, Biochemistry, Rochester, NY 14642. *Stanford University, Pathology, Stanford, CA 94305, +DNAX Research Institute, Palo Alto, CA 94304.

We have characterized three highly purified DNA polymerases from calf thymus: Alpha polymerase purified by immunoaffinity, and two forms of DNA polymerase delta. These differ in relative activity on activated calf thymus DNA. Both delta polymerases have an associated 3'-5' exonuclease which is inhibited by 5'AMP. Both delta polymerases are bound by columns of AMP-Agarose through their associated nucleases. DNA polymerase alpha, devoid of any nuclease activity, is not retained by the AMP resin. At sub-Km levels of dNTP substrate, inhibition of the delta polymerase I and II nucleases by 5'AMP increases the detectable nucleotide incorporation by the polymerase. This apparent stimulation by 5'AMP is not diminished by increased DNA concentration, suggesting a physical link between nuclease and polymerase. Each polymerase shows a similar dose response to aphidicolin, with 50% inhibition occuring a 2.0, 2.5, and 4.0 uM for alpha, delta I and delta II respectively, on poly(dA)·oligo(dT). Aphidicolin is a competitive inhibitor of dTMP incorporation such that decreasing the [dTTP] increases inhibition by the drug. Delta I and delta II polymerases show intermediate sensitivity to dideoxy-TTP compared to alpha polymerase (insensitive) and beta polymerase (sensitive). Both delta forms have little sensitivity either to monoclonal IgG directed against alpha polymerase or to butyl-phenyl dATP compared to alpha polymerase. Supported by NIH grants GM24441, CA11198, CA28322, CA09151 and ACS grant FRA220.

1347 STRUCTURAL CHARACTERIZATION OF IMMUNOAFFINITY PURIFIED DNA POLYMERASE α/DNA PRIMASE COMPLEX FROM KB CELLS, Scott W. Wong, Lisa R. Paborsky, Teresa S.-F. Wang and David Korn, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

We present a detailed description of the polypeptide structure of a highly purified DNA polymerase α/DNA primase complex that can be prepared from KB cells by an immunoaffinity protocol based on anti-polymerase α monoclonal antibodies of absolute specificity. On the basis of extensive immunochemical analyses with 5 independent monoclonal antibodies, 3 of which are potent neutralizers of polymerase α activity, peptide mapping studies, and the application of a sensitive immunoassay that permits detection of polymermase α antigens in crude lysates, we have established that the principal form of catalytically active DNA polymerase α in KB cells is a protein with a molecular mass of 180 kilodaltons. Utilizing synchronized cells, we demonstrate that the change in polymerase α activity that occurs during the cell cycle is due to the biosynthesis of that peptide. *In vivo*, this protein is extremely stable, with an estimated half life of ≥ 15 hours. In contrast, the polypeptide is extremely fragile to *in vitro* manipulation, and it readily generates a family of partial degradation products exemplified by polypeptides p165, p140, and p125 that sufficiently explain the "microheterogeneity" that is typically exhibited by polymerase α peptides in denaturing polyacrylamide gels. In addition to the catalytically active polymerase α polypeptide(s), the immunopurified enzyme fraction contains 3 other proteins, p77, p55, and p49, which by peptide mapping have been shown to be independent species that are unrelated either to the large polymerase α peptides or to one another. Labeling of crude lysates with [^{32}P]orthophosphate reveals that the polypeptides p180 and p77 are phosphorylated. Phosphoserine and phosphothreonine are identified after partial acid hydrolysis of the immunoprecipitated polypeptides. (Studies supported by NIH grant CA-14835, CA-09302 and George D. Smith Fund.)