MECHANISMS OF DNA REPLICATION AND RECOMBINATION

Nicholas R. Cozzarelli, Organizer

April 3 — April 10

Plenary Sessions

Poster Sessions

Structure of DNA and DNA-Protein Complexes

0662 LEFT-HANDED DNA HELICES, SUPERCOILING, AND THE B-Z JUNCTION, Robert D. Wells, Department of Biochemistry, School of Medicine and Dentistry, University of Alabama in Birmingham, Birmingham, Alabama 35294

Biological and physical studies on recombinant plasmids and restriction fragments containing tracts of (dC-dG) sequences which are 58, 32, 26, and 10 bp in length reveal the following: A) Left-handed Z-DNA can neighbor right-handed regions of DNA in close proximity on the same chain (1-7). B) Negative supercoiling (density greater than 0.072 is sufficient to convert the 58 bp (dC-dG) regions in pRW751 into a left-handed structure under physiological ionic conditions (200mM NaCl). Thus, left-handed DNA probably exists in vivo (6). C) Single-strand specific nucleases recognize and cleave aberrant structural features at the B-Z junctions (6,8). D) The B-Z junction is very short (several bp) as judged by ^{31}P-NMR, Raman spectroscopy, extent of relaxation of supercoils, and the sharpness of the products of single-strand specific nucleases (1-7). E) The left-handed conformation perturbs the right-handed backbone structure neighboring the B-Z junction for 3-4 helical turns as judged by Raman spectroscopy (5). F) (dC-dG) sequences exist in a family of left-handed conformations as stabilized by different conditions. Furthermore, spectroscopically identifiable conformational intermediates exist between the B and Z structures (7). G) A cloned segment of immunoglobulin gene containing $(dT\text{-}dG)_{31}\cdot(dC\text{-}dA)_{31}$ adopts a left-handed conformation under the influence of negative supercoiling (8). Also, the AAF-reacted polymer is left-handed in high salt solutions (4). H) The stabilizing effect of methylation on the Z-form in fragments and plasmids approximately offsets the free energy contributions of the B/Z junctions (9). I) The (dC-dG) tracts are specifically and highly susceptible to suffer deletions in vivo. Also, these tracts seem to enhance rec A mediated recombination (1,2).

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0663 STRUCTURE OF CRO REPRESSOR AND ITS IMPLICATIONS FOR PROTEIN-DNA INTERACTIONS, Douglas H. Ohlendorf[1], Wayne F. Anderson[2], Yoshinori Takeda[3], and Brian W. Matthews[1], [1]Institute of Molecular Biology, University of Oregon, Eugene, OR 97403, [2]MRC Group on Protein Structure and Function, University of Alberta, Edmonton, Alberta T6G247, Canada, [3]Chemistry Department, University of Maryland, Baltimore County, Catonsville, Maryland 21229

The structure of the cro repressor protein from bacteriophage lambda has been determined at 2.2 Angstrom resolution. From this structure, model building and energy refinement techniques have allowed the development of a detailed model for the presumed cro-DNA complex. This model is consistent with the known affinities of cro for its six binding sites in the lambda genome as well as for a number of mutant sites. In addition, the model suggests a mechanism for sliding along the surface of DNA duplex prior to the location of a specific binding site. Co-crystals of cro with DNA fragments have been obtained and are being examined by X-ray techniques.

Comparisons of both the primary and tertiary structures of cro and other DNA-binding proteins have indicated the presence of a homologous region of about 22 amino acids. This region corresponds to the two consecutive α-helices in cro, catabolite gene activator protein and lambda repressor that have been proposed to interact with DNA. The observed homology suggests that a bihelical DNA-binding unit is common to many gene-regulatory proteins.

Shaping of DNA

0664 IDENTIFICATION OF A TEMPERATURE-SENSITIVE YEAST DNA TOPOISOMERASE II MUTANT, Rolf Sternglanz and Stephen DiNardo, Department of Biochemistry, State University of New York, Stony Brook, N.Y. 11794

A collection of temperature-sensitive mutants of the yeast, Saccharomyces cerevisiae, has been screened for DNA topoisomerase defects. Recently a mutant has been identified which has much less DNA topoisomerase II activity than normal. The enzymatic activity from the mutant is itself temperature-sensitive. Mating of this mutant with a wild-type strain of opposite mating type, followed by standard tetrad analysis shows that a single nuclear mutation leads to temperature-sensitive growth and enzymatic activity. Thus, the mutation is probably in the structural gene for DNA topoisomerase II and the enzyme is essential for viability.

Phenotypes of the mutant are being studied in order to learn about the role of the enzyme in yeast DNA metabolism. Mapping of the mutation and cloning of the gene are underway.

0665 REGULATION OF BACTERIAL DNA SUPERCOILING, Martin Gellert, Rolf Menzel, Toshiro Adachi, and Kiyoshi Mizuuchi, Laboratory of Molecular Biology, NIADDK, National Institutes of Health, Bethesda, MD 20205.

We have studied the regulation of DNA gyrase synthesis in E. coli. Expression of the gyrase A and B proteins in intact cells has been quantitated by an antibody assay; synthesis directed by the cloned gyrA and gyrB genes in cell-free extracts has also been measured. We have also determined the DNA sequence of the region between dnaN and gyrB (including recF), and have used this information to help locate the promoter sites responsible for transcription of gyrB.

Expression of gyrA and gyrB is largely controlled by DNA supercoiling. When DNA gyrase activity in whole cells is blocked by novobiocin or coumermycin, or by incubating a temperature-sensitive gyrB mutant at 42°, the synthesis of GyrA and GyrB is considerably increased. Similarly, in the cell-free system, relaxed DNA is a much better template for GyrA and GyrB synthesis than supercoiled DNA. These genes are thus regulated so as to maintain a stable level of DNA supercoiling in the cell. Their regulation is different from that of most E. coli genes, whose expression is either stimulated by increased DNA supercoiling or is left unchanged.

We have mapped several promoter sites upstream of gyrB. Their function in the control mechanism will be discussed.

0666 INTERACTIONS OF EUKARYOTIC TYPE I AND TYPE II DNA TOPOISOMERASES WITH SV40 CHROMATIN, Leroy F. Liu, Eric M. Nelson, Brian D. Halligan and Thomas C. Rowe, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205

We have developed a sensitive and convenient assay, P4 unknotting assay, for type II DNA topoisomerases. Using the P4 unknotting assay, we have purified several mammalian type II DNA topoisomerases. Similar to T4 DNA topoisomerase, eukaryotic DNA topoisomerase II catalyze the ATP(or dATP)-dependent strand passing of double-stranded DNA, including relaxation, knotting/unknotting and catenation/decatenation. At high enzyme concentrations, DNA topoisomerase II induces both single-and double-stranded DNA breaks, and the enzyme is covalently linked to the 5'-end of the breaks. Several antineoplastic drugs, such as adriamycin and ellipticine strongly inhibit the catalytic activity of DNA topoisomerase II while DNA topoisomerase I activity is unaffected. In addition, ellipticine stimulates the double-stranded cleavage reaction of DNA topoisomerase II on DNA. We have observed that DNA topoisomerase II activity is associated with SV40 minichromosomes in a sucrose density gradient containing less than 0.1 M NaCl. Purified eukaryotic topoisomerase II can efficiently catalyze the ATP(or dATP)-dependent catenation and decatenation of SV40 minichromosomes. The enzymatic activity of topoisomerase II on SV40 chromatin is also inhibited by adriamycin and ellipticine. Furthermore, double-stranded cleavage of SV40 minichromosomes by high concentrations of topoisomerase II is also stimulated by ellipticine. Similar to topoisomerase II, topoisomerase I activity is also tightly associated with SV40 chromatin from virions and infected cells. Cleavage(single-stranded breaks) of SV40 chromatin by endogenous topoisomerase I can be efficiently induced. We have determined the nucleotide sequences of the cleavage sites induced by type I and type II DNA topoisomerases on SV40 DNA, SV40 chromatin(purified) and SV40 chromatin in vivo. We will discuss how topological changes of chromatin structures may influence gene expression. We will also discuss the possible roles of DNA topoisomerases in DNA replication and illegitimate recombination.

0667 DYNAMIC AND STRUCTURAL ASPECTS OF PROTEIN-DNA INTERACTIONS IN THE T4 DNA REPLICATION COMPLEX, Peter H. von Hippel, Leland S. Paul, Frederic R. Fairfield, Joel W. Hockensmith, Mary K. Dolejsi, Mary C. Dasso, and John W. Newport, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403.

A complex of seven bacteriophage T4 proteins, that catalyzes the elongation phase of DNA replication in vitro with close to physiological rates and fidelities, has been described and characterized by Alberts and Nossal and their coworkers (1,2). A five protein system that effectively carries out leading strand synthesis on either a single-stranded or a double-stranded (with displacement of the non-coding strand) DNA template has also been described (1, and references cited therein; 3).

We have examined some of the detailed molecular processes and interactions involved in the formation of this five protein system, which includes the T4-coded protein products of genes 43 (polymerase), 32 (single-stranded DNA binding protein), and 44, 62 and 45 (polymerase accessory proteins), as well as suitable primers and templates. We have investigated (and will report new results bearing on) the following questions: (i) How, and with what binding free energies and contact sites, is the five protein-primer-template complex assembled? (ii) What is the role of the ATPase activity of the polymerase accessory proteins in the assembly and function of the five protein complex in the single-stranded template and in the strand-displacement template synthesis modes? (iii) How does the processivity of synthesis depend on the sequence and structure of the template (and the primer), and on the participation of the single-stranded DNA-binding and polymerase accessory proteins?

A composite functional model of the five protein complex, reflecting partial answers to some of these questions, will be presented and discussed. [Supported by USPHS Research Grants GM-15792 and GM-29158 (to PHvH), USPHS Training Grants GM-00715 and GM-07759, and USPHS postdoctoral fellowship GM-08252 (to FRF).]

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0668 BIOLOGICAL ROLE OF DNA HELICASES I AND II OF *ESCHERICHIA COLI*, Hartmut Hoffmann-Berling, Gisela Taucher-Scholz and Mahmoud Abdel-Monem, Abteilung Molekulare Biologie, Max-Planck-Institut für medizinische Forschung, Jahnstrasse 29, 6900 Heidelberg, Federal Republic of Germany

Thus far three of the four DNA unwinding enzymes found in *E. coli* were without identified genes, namely the helicases I, II and III. Helicase I has now been shown to be the product of the *traI* gene of the F sex factor, one of some 20 transfer genes of F. We have further cloned the gene for helicase II in a λ vector starting from a representative bank of *E. coli* chromosomal DNA and using a radioimmune assay for detecting helicase II overproduction in phage plaques. The restriction pattern of the cloned DNA fragment resembles to that described for the *uvr*D gene of *E. coli* (1) which is known to specify a DNA dependent ATPase of M_r 75,000, similar to helicase II (2). Problems arise because *uvr*D mutants are not reported to be defective for DNA replication while antibody against helicase II interferes strongly with replication of the *E. coli* chromosome *in vitro* (3). The procedure used to select the gene for helicase II is, in principle, also applicable to other proteins including helicase III.

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Bacteriophage Replication Systems

0669 SINGLE-STRANDED (SS) PHAGES AS PROBES OF REPLICATION MECHANISMS, N. E. Dixon, L. L. Bertsch, S. B. Biswas, P. M. J. Burgers, J. E. Flynn, R. S. Fuller, J. M. Kaguni, M. Kodaira, M. M. Stayton and A. Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

Conversion of SS circular DNA of M13, G4 and ϕX174 to their duplex replicative forms (SS→RF) relies exclusively on host enzymes. These reactions are attractive probes for events in lagging strand synthesis in replication of the E. coli chromosome and plasmids that bear the chromosomal origin (oriC). Replication of SS DNA binding protein (SSB)-coated M13 SS DNA is primed by RNA polymerase, and G4 DNA by primase (dnaG protein). On ϕX174 DNA, the primosome, comprising 6 prepriming proteins (dnaB, dnaC, n, n', n" and i) and primase recognizes a unique site, and then moves along the template in the anti-elongation direction, laying down short RNA primers. In each system, the primers are extended by DNA polymerase III holoenzyme (holoenzyme).

Primase. Dependence on primase concentration of the initial rate of the G4 SS→RF reaction shows a pronounced sigmoidal response. Primase binds specifically to DNA containing the G4 complementary strand origin, with a stoichiometry of 2 monomers/circle. An isolated primase-SSB-DNA complex is fully competent for replication on addition only of holoenzyme, rNTPs and dNTPs.

DNA pol III holoenzyme. Involvement of ATP in holoenzyme action has been probed using isolated primed G4 DNA as template. In the absence of DNA, holoenzyme binds 2-3 molecules of ATP. Reaction of preformed ATP-holoenzyme and primed DNA results in hydrolysis of the bound ATP and formation of an isolable initiation complex. Holoenzyme action in the absence of ATP resembles that of pol III (the holoenzyme core) or pol III* (holoenzyme lacking the β subunit) with or without ATP, in sensitivity to salt and processivity of elongation. Elongation from the stable initiation complex formed with ATP resists a level of KCl (150 mM) that completely inhibits nonactivated holoenzyme, pol III or pol III*, and has a processivity at least 20 times greater. After synthesis of the RF, holoenzyme dissociates slowly, and in the presence of ATP, forms an initiation complex at other available primer termini. Thus, no essential subunits are lost during the cycle of initiation, elongation and dissociation. Transfer of holoenzyme to new primer termini is much more rapid if multiply-primed template is used, suggesting that facile intramolecular transfer of holoenzyme molecules (or dimers) from termini of Okazaki fragments may occur during lagging strand synthesis on duplex DNA. The sites of interaction of nucleotides and primer-template with holoenzyme have been examined by photoaffinity labelling.

0670 ENZYMATIC MECHANISMS OF T7 DNA REPLICATION, Steven W. Matson, Benjamin B. Beauchamp, Michael J. Engler, Carl W. Fuller, Robert L. Lechner, Stanley Tabor, John H. White and Charles C. Richardson, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

Studies with phage T7 have revealed the minimal requirements for the initiation and accurate replication of a duplex DNA molecule. The primary origin of T7 DNA replication, as determined by deletion mapping, is located at position 15 on the physical map. Using purified proteins we have obtained initiation at the primary origin of the T7 DNA molecule. In addition to T7 DNA polymerase, initiation requires T7 RNA polymerase and the 4 rNTPs; no initiation occurs on T7 LG37 DNA that lacks the primary origin sequence. Sequence analysis of the primary origin reveals two T7 RNA polymerase promoters (1.1A and 1.1B) and a 61 bp AT-rich region. Inactivation of the RNA polymerase promoters by in vitro mutagenesis forces DNA replication to initiate at secondary origins in vivo. Using plasmids containing the primary origin we have shown that DNA synthesis initiates at the origin but proceeds unidirectionally to the right in the direction of transcription for the 1.1A and 1.1B promoters. Analysis of newly synthesized DNA molecules reveals that their 5'-termini are covalently linked to RNA. These primer RNAs consist of two species, each having unique 5'-termini as a result of transcription from the two promoters. They are heterogeneous in length, however, their 3'-terminus being determined by the transition to DNA synthesis.

Two proteins, T7 DNA polymerase and T7 gene 4 protein, can account for most of the reactions occurring at the replication fork. The gene 4 protein has multiple activities: it is a single-stranded DNA dependent nucleoside 5'-triphosphatase, a helicase and a primase. The NTPase activity is coupled to unidirectional 5'→3' translocation of the gene 4 protein along a single strand of DNA, a reaction required for both helicase and primase activities. The helicase activity of gene 4 protein can be demonstrated directly as it will unwind a polynucleotide annealed to a circular, single-stranded DNA molecule, provided the fragment has a 3' tail. Using a preformed, topologically stable replication fork we have shown that the helicase activity of gene 4 protein specifically stimulates T7 DNA polymerase on duplex templates.

The rate of fork movement catalyzed by the two proteins is 300 bases/sec at 30°. As a primase gene 4 protein catalyzes the synthesis of RNA primers on single-stranded DNA. The predominant recognition sites for primer synthesis are 3'-CTGGG/T-5' giving rise to primers having the sequence pppACCC/A.

0671 STUDIES ON DNA REPLICATION IN THE T4 BACTERIOPHAGE *IN VITRO* SYSTEM. C. Victor Jongeneel, Jack Barry, Timothy Formosa, Kenneth N. Kreuzer, Maureen Munn, Carlos Zetina and Bruce M. Alberts, Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143

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

Both the 44/62 protein complex and the 41 protein catalyze a single-stranded DNA-dependent hydrolysis of nucleoside triphosphates to nucleoside diphosphates and inorganic phosphate; these hydrolyses can be differentially blocked by ATPγS and by GTPγS respectively, revealing that each hydrolysis has an essential role in a functioning replication fork. The DNA synthesis on the leading and lagging side of the fork are linked via the dual role of the gene 41 protein: this protein appears to use nucleotide hydrolysis energy to run along the lagging-strand template at the fork, acting as a DNA helicase to drive open the parental DNA helix (to permit rapid leading-strand synthesis), while serving as a mobile site at which new RNA primers are formed (to prime Okazaki fragment synthesis on the lagging strand). In a second reaction requiring nucleotide hydrolysis, the gene 44/62 and 45 proteins act in a synergistic manner to tie down the DNA polymerase, causing it to move through these double-helical templates in an enormously processive fashion. Kinetic studies reveal that the DNA polymerase molecule on the lagging strand likewise stays associated with the fork as it moves. Therefore, we conclude that the DNA template on the lagging strand must be folded so that the stop site for the synthesis of one Okazaki fragment is adjacent to the start site for the next such fragment, allowing the polymerase and other replication proteins on the lagging strand to recirculate continuously. Surprisingly, these replication forks are stopped by a single bound RNA polymerase molecule, unless an 8th T4 protein is present; this protein is the product of the dda gene and is a second DNA helicase in the complex.

Initiation of DNA Synthesis I

0672 THE E.coli ORIGIN OF REPLICATION: ESSENTIAL STRUCTURE FOR BIDIRECTIONAL REPLICATION AND A MODEL FOR THE REPLICATION OF oriC PLASMIDS, Mituru Takanami, Satoshi Tabata, Atsuhiro Oka, Kazunori Sugimoto, Seiichi Yasuda* and Yukinori Hirota*, Institute for Chemical Research, Kyoto University, Kyoto, and *National Institute of Genetics, Mishima

The replication origin of the E.coli K-12 chromosome has been isolated as autonomously replicating molecules (oriC plasmid), and the DNA region(ori) essential for replicating function(Ori function) has been localized to a sequence of 232-245 base-pairs(bp) by deletion analysis(1). Analysis of the sequence organization in ori further provided evidence that multiple recognition sequences have precisely been arranged by separated with spacer sequences. In order to elucidate the functional role of the ori sequence more precisely, the site and direction of oriC plasmids were investigated by using an in vitro replication system. The in vitro system used was essentially identical to those described by Fuller, Kaguni and Kornberg(2), except for the sources of enzyme fractions. Fraction II was prepared either from E.coli HMS83(F⁻ polA polB rha lys thyA lacZ str) or from JE107251(HfrP4X8 dnaA725 thy str), and a dnaA-complementing fraction(fraction III) was prepared from E.coli JE6087(F⊿proB lac) which harbors a dnaA gene-carrying plasmid pSY405. The extent of replication was limited either by the addition of a chain terminator ddTTP or by a brief incubation at reduced substrate concentrations, and labeled regions with (α^{32}P)dNTPs were analysed. The result of analysis clearly indicated that DNA replication started at a region near, but outside, the 245 bp ori box, and proceeded bidirectionally. The replicating function was reduced only by mutations introduced in the ori box, but not by the sequence replacements in the flanking regions. The result indicates that the 245 bp ori box contains information enough for inducing bidirectional replication at the vicinity of the ori box. Initiation of DNA replication at the restricted region became significant only when the dnaA-complementing fraction(fraction III) was

supplemented. Fraction II alone from the HMS83 strain had the activity to generate the ori-sequence dependent DNA synthesis, but at a reduced rate(about one third). The result of product analysis however indicated that all the regions of plasmid were rather uniformly labeled even at high ddTTP concentrations. The result implies that the component(s) contained in fraction III has at least two functions: one enhances the total DNA synthesis and the other restricts the initiation of DNA synthesis to a specific region. On the basis of these observations, a possible model for the replication of oriC plasmid will be discussed.

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0673 PRIMING OF DNA SYNTHESIS AT THE REPLICATION ORIGIN OF E. COLI, Tuneko Okazaki, Yuji Kohara, Tohru Ogawa*, Naoki Tohdoh, Kin-ya Yoda and Xiao-wan Jiang, Institute of Molecular Biology, Faculty of Science, Nagoya University, Nagoya, Japan 464

Replication of the E. coli chromosome is initiated at a fixed origin and proceeds bidirectionally. We are investigating the starting sites of DNA synthesis at the replication origin of E. coli. RNA-primed initiation sites of DNA synthesis (5' ends of RNA-DNA molecules and the RNA-DNA junction sites) in vivo[1][2] and in vitro[3] are precisely located on the nucleotide sequence of the E. coli origin. Within the minimal essential origin region (oriC)[4], many initiation sites are found in the counterclockwise strand (r-strand) but none in the clockwise strand (l-strand). Although the initiation sites in vivo and in vitro are mapped at the same loci, two of them are frequently used in vivo whereas all of them are used in similar frequency in vitro. Primer RNA currently detectable is quite short in length. The results suggest that initiation of DNA synthesis is asymmetrically started within the oriC and then bidirectional replication forks are established outside of oriC region.

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0674 ENZYMES AND PATHWAY OF INITIATION OF REPLICATION AT THE E. COLI CHROMOSOMAL ORIGIN, R. S. Fuller, L. L. Bertsch, N. E. Dixon, J. M. Kaguni, R. L. Low and A. Kornberg, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

Enzyme studies of replication of E. coli origin plasmids are pursued to define the pathway of initiation of replication at the chromosomal origin of replication (oriC) and its regulation. A certain ammonium sulfate fraction from the cell lysate supplemented with substrates, other small molecules and 7% polyvinyl alcohol (PVA) replicates plasmids containing oriC. The reaction requires RNA polymerase, DNA gyrase, SSB (single strand binding protein), and the products of the dnaA, dnaB and dnaC genes (1). Biochemical and EM evidence show that initiation occurs at or near oriC and proceeds bidirectionally from that point (1,2).

The in vitro replication system can be used to follow the purification of novel initiation factors. The dnaA protein, overproduced up to 200-fold in strains containing the dnaA gene cloned into a plasmid-expression vector, has been purified using an in vitro complementation assay. The purified protein (denatured molecular weight 52,000 ± 1000) is active in the replication assay when added in the monomer form, and binds specifically to plasmids containing oriC, as assayed by Millipore filter binding and by protection of the unique HindIII site within oriC.

A reconstituted (recon) system contains novel factors and the known purified replication enzymes (RNA pol, DNA gyrase, DNA pol III holoenzyme, SSB, the dnaB and dnaC proteins and proteins i, n, n' and n"). Unexpectedly, the known replication enzyme mix replicates, in a non-physiological way, a variety of supercoiled DNAs, including ϕXRFI, in the presence of 7% PVA. A "specificity protein" has been purified (denatured molecular weight ~100,000) which suppresses this dnaA-independent reaction but not the dnaA-dependent replication of oriC plasmids which also requires two fractions separable on DEAE cellulose and phosphocellulose. The specificity protein appears to be identical to topoisomerase I. The recon assay is currently being used in the purification of the additional novel factors.

Under the conditions of the dnaA-dependent oriC recon system at 30°C, a lag of about 5 minutes is observed before the onset of DNA synthesis. This lag is abolished if the mixture is preincubated in the absence of dNTPs and apparently corresponds to the time required for origin recognition and priming. DNA gyrase is absolutely required to reduce the lag, implying participation at an early stage, whereas with the omission of dnaA protein from the preincubation, there is still a lag of 1 to 2 minutes, limiting the time of action of the dnaA protein to an interval coincident with or just after the action of RNA polymerase.

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0675 REGULATORY CONTROL CIRCUITS IN COLE1 DNA REPLICATION

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Recent studies have shown that an RNA-RNA interaction between two plasmid-encoded molecules is a key element in ColE1 copy number control.[1,2] We have investigated the secondary structure of RNA1 from ColE1 and a compatible plasmid, JN87, using a variety of nucleases and chemical probes. These experiments have demonstrated that both RNA1 species have three stems supporting three nucleotide loops. The overall structures of the two RNA1 species are quite similar. Regions involved in possible tertiary interactions have been identified and are also similarly located in the two molecules. The results indicate that the loops of RNA1 are highly accessible and likely to be key regions of specificity in initiating contact with the complementary loops of the primer RNA. When labeled RNA1 is added to a transcription system specific for primer transcription, a complex between the two species can be detected on non-denaturing acrylamide gels. Formation of this complex is incompatibility specific; an RNA1 species from a ColE1-compatible plasmid does not interact with ColE1 primer. Primer RNA synthesized from a number of cis-dominant copy number mutants does not interact efficiently with wild-type RNA1 in this assay. The nature of the RNA1-primer complex will be discussed.

The rop gene product is known to specifically repress transcription from the primer promoter.[3] The rop gene is located 600 nucleotides downstream from the ColE1 replication origin. The rop gene has been cloned under the control of the ara promoter to permit controlled expression of this replication control element in vivo. Overproduction of the 63aa polypeptide rop gene product leads to loss of the ColE1 replicon containing it. This polypeptide has also been expressed at high levels under control of the lambda P_L promoter, and the polypeptide has been purified to homogeneity. Cells in which rop is overproduced cannot grow under selective conditions. A large number of plasmid mutants capable of growth under these conditions have been selected and will be described.

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Initiation of DNA Synthesis II

0676 THE DNA POLYMERASE-PRIMASE COMPLEX OF DROSOPHILA MELANOGASTER EMBRYOS, L. S. Kaguni, J.-M. Rossignol, R. C. Conaway and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

DNA polymerase α of Drosophila melanogaster was initially isolated as a 280,000 dalton protein composed of four non-identical subunits with molecular weights of 148,000 (α), 58,000 (β), 46,000 (γ) and 43,000 (δ) (1). Of the four subunits, only the isolated α subunit retained polymerase activity.

A DNA primase is closely associated with the α polymerase. The primase catalyzes the synthesis of ribo-oligonucleotide primers on single-stranded M13 DNA or poly(dT). The primers synthesized in a coupled primase-polymerase reaction with M13 DNA are of a unique size (15 residues); those synthesized with poly(dT) range from 8 to 15 nucleotides. As in the case of the DNA primase of E. coli, deoxynucleotides can substitute for ribonucleotides during primer synthesis (2,3).

Extracts of Drosophila embryos probed with antibody directed specifically against the α subunit, after transfer from an SDS polyacrylamide gel to diazotized paper, were shown to contain two antigenic polypeptides with molecular weights of 185,000 and 166,000 daltons. While these polypeptides were not detected in later enzyme fractions, the 148,000 dalton α subunit, not found in early fractions, was detected (4). Thus, the 148,000 dalton α subunit of the purified α polymerase is a proteolytic product of higher molecular weight polypeptides. A new simplified purification scheme has been developed which yields enzyme with an α subunit of 185,000 daltons in addition to other smaller subunits. It, like earlier α polymerase preparations, contains DNA primase activity.

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0677 INITIATION OF BACTERIOPHAGE λ DNA REPLICATION IN VITRO, Roger McMacken, Jonathan H. LeBowitz, Joanne B. Mallory, John D. Roberts, Marc S. Wold and Christine Loehrlein, Department of Biochemistry, Johns Hopkins University, Baltimore, MD 21205.

Replication of bacteriophage λ DNA is initiated at a unique site (oriλ) and requires the λ O and P replication proteins, as well as multiple E. coli replication proteins (1). We report the purification of the λ O and P initiators and the development of two in vitro replication systems that will facilitate identification of the molecular mechanisms involved in λ DNA replication.

Although the half-life of the λ O gene product in λ-infected cells is only 1.5 minutes, insertion of the O gene downstream from the powerful λ P_L promoter present on plasmid pKC30 enables amplification of the O protein to about 30% of the total cellular protein. Over 5 mg of homogeneous O protein can be isolated from a single gram of cell paste. Using an agarose gel-DNA binding assay, we have confirmed that the O protein binds specifically to DNA fragments containing oriλ (2). The λ P replication protein has been purified 3000-fold to homogeneity from cells containing amplified levels of P protein. We conclude that the λ P protein is a viral analogue of the E. coli dnaC protein. The two proteins have similar physical properties and each interacts with the bacterial dnaB protein to form an isolable complex. The purified λ P protein is a potent inhibitor of the DNA-dependent ATPase and general priming (3) activities of the dnaB protein, even in the presence of excess competing dnaC protein.

Addition of purified λ O and P proteins to a soluble enzyme system (4) containing partially purified E. coli replication proteins confers upon the system the capacity to replicate supercoiled plasmid DNAs (λdv) that contain oriλ (5). The properties of λdv replication in vitro, in regard to protein requirements, template specificity, and replication products, closely resemble the known features of λ DNA replication in vivo. Moreover, λ DNA replication both in vivo and in vitro, due to its dependence on RNA polymerase transcription of oriλ, is specifically blocked by the presence of the λ cI repressor. In other studies, we have developed a related in vitro replication system which also depends on the action of the λ O and P proteins, but which instead replicates the single-stranded chromosome of phage M13 to a duplex, supercoiled form. This rifampicin-resistant reaction,which is initiated at random sites, has been separated into two stages. In a pre-replicative stage, the λ O and P proteins, dnaB protein, and other E. coli proteins participate in an ATP-dependent reaction that converts the M13 viral DNA to an activated form. The activated M13 DNA (a pre-primosome?) is rapidly replicated upon the addition of primase, DNA polymerase III holoenzyme and dNTPs.

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0678 Mu DNA REPLICATION: IN VITRO CHARACTERIZATION OF A TRANSPOSING REPLICON. N Patrick Higgins, Department of Biochemistry, University of Wyoming, Laramie, Wyoming 82070 and Baldomero M. Olivera, Department of Biology, University of Utah, Salt Lake City, Utah 84112. When transposable elements move, one copy appears at the new position while one copy is retained at the original position. Thus, transposons are replicons and they can be described in the terms of the classic replicon model which postulates two controlling elements, an "initiator" protein and a "replicator" DNA sequence. Using the transposing bacteriophage Mu we have studied coupled transposition-replication in vitro with a film lysate technique.

The origin of Mu DNA replication is near the left genetic end of the virus. Replisomes traverse Mu sequences from left to right and replication is semiconservative; one parental strand remains at the original position while one parental strand is transferred to the new locus. Replication is also semi-discontinuous so that most of the Mu heavy strand is synthesized as Okazaki pieces. The Mu A gene product is one component of the "initiator" for transposition. Normally, initiation is tightly linked to protein synthesis and replication in vitro is inhibited by chloramphenicol added in vivo 5 min. before harvesting the cells. Replication events that were initiated in vivo are completed in vitro, but the efficiency of initiation of new rounds is very low. However, if the A protein is provided, reinitiation can occur in vitro.

Termination of replicative transposition occurs at the right end of Mu DNA which makes the replication event restricted to Mu sequences. Density shift experiments in vitro show that the hybrid density Mu sequences produced are bordered by completely light host sequences. In this respect Mu replication in a prokaryotic chromosome is akin to eukaryotic replicons in a eukaryotic chromosome. This is consistent with a Mu replication-transposition event in which bordering host sequences are not replicated. Late in the infectious cycle the residual Mu DNA synthesis that occurs in the absence of protein synthesis increases. Preliminary studies suggest that the latter is not coupled to transposition, but is "passive" replication by host replisomes and is not controlled by transposition proteins. In this mode neighboring host sequences are replicated.

Both the initiation and termination events are defined by element interactions with the DNA replication apparatus, i.e., a DNA sequence for initiating a replication fork and a sequence for termination. The principle distinguishing feature of the transposition-replication is the generation of new linkage for the transposing sequences. Our studies establish general criteria for distinguishing transposition replication from ordinary replication of transposable element sequences in the host chromosome.

0679 PRIMING OF THE INITIATION OF PHAGE Ø29 DNA REPLICATION BY THE TERMINAL PROTEIN.Margarita Salas,Miguel A. Peñalva,Juan A. García,Luis Blanco, Ignacio Prieto,José M. Lázaro,Rafael P. Mellado,Cristina Escarmís and José M. Hermoso. Centro de Biología Molecular (CSIC-UAM),Universidad Autónoma,Canto Blanco,Madrid-34, Spain.

Bacteriophage Ø29 has a linear, double-stranded DNA of 18 Kbp with a protein, p3, covalently linked to the two 5' ends by a phosphodiester bond between serine and 5'dAMP (1). The nucleotide sequence of the region containing gene 3 has been determined. Protein p3 is involved in the initiation of Ø29 DNA replication which starts at either DNA end and proceeds by strand displacement (2, 3). To solve the problem of the priming of replication at the 5'ends of Ø29 DNA a mechanism has been proposed by which protein p3 acts as a primer by reaction with dATP and formation of a protein p3-dAMP covalent complex that would provide the 3'OH group needed for elongation. In agreement with the model, protein p3 is present at the ends of the parental and daughter DNA strands in the replicative intermediates (4). Moreover, the incubation of extracts from Ø29-infected B. subtilis with {α-^{32}P}dATP gives place to the formation in vitro of a protein p3-dAMP covalent complex which can be elongated. These results provide a strong support to the model of priming by protein p3.

Protein p3 has been highly purified from E. coli cells transformed with a recombinant plasmid containing gene 3. The purified protein is active in the formation of the initiation complex when supplemented with extracts from B.subtilis infected with a sus3 mutant but not from uninfected cells, indicating that, in addition to protein p3, some other viral protein is required for the formation of the initiation complex in vitro. By using extracts from B. subtilis infected with mutants in genes 2,3,5,6 and 17, required in vivo for DNA replication, we found that in addition to p3, protein p2 is involved in the formation of the p3-dAMP complex. Cloning of gene 2 for the overproduction and purification of protein p2 to study its role in the formation of the initiation complex is in progress. Protein p3 mutants are being produced by in vitro mutagenesis to test the requirements for the formation of the initiation complex.

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Replicon Structure and Chromosome Mechanics

0680 SYMMETRY, INVERSION, AND SELF REPAIR IN ADENO-ASSOCIATED VIRUS DNA REPLICATION, Kenneth I. Berns, Nicholas Muzyczka, Richard J. Samulski, and Arun Srivastava, Department of Immunology and Medical Microbiology, University of Florida, Gainesville, FL 32610

The adeno-associated virus (AAV) genome contains an inverted terminal repetition of 145 bases (1). The first 125 bases are palindromic and hairpin to serve as a primer for DNA replication. We have recently cloned the intact AAV genome into pBR322 (2). Upon transfection of human cells in the presence of helper adenovirus infection, the AAV DNA is rescued from the recombinant molecule and replicated. In addition to the clone containing the genome intact, several of the clones contain deletions restricted to one or both terminal repetitions. Those clones with deletions in either the right or left terminal repeats can also be rescued upon transfection into human cells. The virus produced contain full length DNA in which the deletions in the terminal repetition have been repaired and the original sequence restored. Three of four clones with deletions in both terminal repeats could not be rescued. The fourth, which could be rescued, contained a 113 base deletion on one end and a deletion of 9 nucleotides on the other end. From these data a model for self repair via a circular intermediate in DNA replication has been developed. This system has also permitted us to begin the study of the requirements for specific terminal sequences in DNA replication.

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0681 STRUCTURE AND REPLICATION OF VACCINIA VIRUS TELOMERES, Bernard Moss, Elaine M. Winters, and Elaine V. Jones, Laboratory of Biology of Viruses, NIAID, NIH, Bethesda, Maryland 20205

Poxviruses are distinguished by their large size, ability to replicate within the cytoplasm of infected cells and encoding of enzymes for RNA and DNA synthesis. Vaccinia virus, the best studied member of this group, has a linear duplex DNA genome of about 180,000 base pairs (bp). Distinctive features of the vaccinia virus genome include a 10,000 bp inverted terminal repetition, arrays of tandem repeats and covalently linked ends. The precise nature of the joint between the two strands of DNA was determined by nucleotide sequencing (1). Loop structures of 104 nucleotides that are A + T-rich and incompletely base paired were found. Interestingly, two alternative loop structures that are inverted and complimentary (flip-flopped) in sequence are present at either end of the DNA. Accordingly, a vaccinia virus genome can be thought of as a single continuous polynucleotide chain resembling a rubber band. Proximal to the terminal loops are tandem repeats of a 70 bp sequence, a unique region of 325 bp, additional 70 bp tandem repeats, two 125 bp tandem repeats, and tandem 54 bp repeats. The numbers of 70 bp repeats are variable and occasionally unstable genome structures containing three or more sets of 70 bp repeats arise. Sequence homologies between the 70 bp, 125 bp and 54 bp repeats indicate that they arose by unequal cross-over events (2). Similar structures are present at the ends of other poxvirus genomes.

To examine the ends of replicating molecules, cytoplasmic DNA was isolated at varying times and digested with appropriate restriction endonucleases. After agarose gel electrophoresis, the DNA fragments were transferred to a nitrocellulose membrane and the ends were identified by hybridization to ^{32}P-labeled probes. Fragments, twice the length of mature ends, were detected. By synchronizing infected cells with 5-fluorodeoxyuridine, preparative amounts of the replicating ends were isolated. Restriction endonuclease mapping of these fragments indicated that they represent head to head concatamers. Replication models that account for concatamer formation and flip-flop loop structures have been proposed.

Genetic methods are being used to map enzymes involved in DNA replication. Vaccinia virus mutants that are resistant to phosphonoacetate direct the synthesis of drug-resistant DNA polymerase (3). Marker rescue experiments have localized the phosphonoacetate-resistance gene to a small DNA segment within the left third of the genome.

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0682 PROPERTIES OF CLONED TELOMERES AND ARTIFICIAL CHROMOSOMES. Jack W. Szostak, Andrew W. Murray and Toby E. Claus. Department of Biological Chemistry, and Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115

It has recently been found that the ends of the linear rDNA plasmid of Tetrahymena are able to function as ends on linear plasmids in yeast (J.W.Szostak and E.H.Blackburn (1982), Cell 29, 245-255). These DNA molecules contain a cluster of C_4A_2 repeats adjacent to a terminal hairpin loop; the hairpin loop is thought to be transformed into an inverted repeat after DNA replication. In order to investigate the reaction by which the inverted repeat is transformed into two new ends, we have constructed, in vitro, molecules that are similar to the inverted repeat made in vivo. Plasmid pTrel (from P.Challoner and E.H.Blackburn) contains a fragment of Tetrahymena rDNA that extends from an internal restriction site to a blunt end generated by S1 nuclease cleavage at or near the hairpin terminus of the rDNA molecule. This plasmid was deleted to form a derivative that contains only the most terminal 700 bp of the rDNA molecule. The fragment was excised by cutting at two restriction sites: one at the internal junction of the fragment, and one in pBR322, 9 bp past the S1 generated blunt end. This fragment was purified and ligated to itself to form an inverted repeat dimer in which the original end of each 700 bp fragment was near the center of symmetry. The inverted repeat was ligated to a circular yeast vector that had been linearized by restriction enzyme digestion, and used to transform yeast. The structure of the plasmids found in the transformants was determined by Southern blotting. Remarkably, the artificial inverted repeat had resolved within the cell, generating a novel linear plasmid in which the 700 bp Tetrahymena fragment is found at each end of the molecule. This experiment demonstrates that: 1)The DNA sequence of the hairpin loop is irrelevant to the resolution reaction, 2) there is no essential information upstream of the 700 bp site, and 3) the single strand interuptions found in the rDNA from Tetrahymena can be re-introduced after the DNA enters the cell. Furthermore, the inverted repeat seems to be a normal replication intermediate, since the cell is able to recognize and resolve an artificial inverted repeat into two new ends.

We have constructed a series of linear plasmids that incorporate a cloned yeast centromere (CEN3). This fragment stabilizes a variety of circular plasmids in yeast, but we have found that it does not stabilize any of a number of linear plasmids that are smaller than 15 kb. This surprising result may reflect some minimum length requirement for a yeast chromosome, perhaps due to the necesity of reaching from a telomeric attachment site (possibly on the nuclear membrane) to the spindle and back. We therefore constructed a longer (54 kb) linear plasmid with a centromere. This molecule appears to behave much like a real yeast chromosome. It is more stable than a CEN circle mitotically. Additional genetic analysis of such artificial chromosomes will be presented.

0683 FINAL STAGES OF DNA REPLICATION: MULTIPLY INTERTWINED CATENATED DIMERS AS SV40 SEGREGATION INTERMEDIATES, Alexander Varshavsky*, Engin Özkaynak*, Olof Sundin†, Mark Solomon*, Robert Snapka* and Richard Pan*, *Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139 and †Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

Terminal stages of SV40 DNA replication, from the latest cairns structure to the monomeric supercoiled SV40 DNA I have been shown to proceed via three discrete families of multiply intertwined catenated dimers (1,2). We will describe the results of further "DNA-level" studies of the structures and transitions involved, recent data on SV40 chromosome segregation in an in vitro system, and the results of biochemical and electron microscopic analyses of the nucleoprotein organization and protein composition of catenated SV40 chromosomes.

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Eukaryotic Replication Systems

0684 PARVOVIRAL DNA REPLICATION, M. Goulián, R. Kollek, W. Burhans, and C. Carton, Department of Medicine, University of California San Diego, La Jolla, CA 92093

The autonomous parvoviral (H-1) genome consists of ∿ 5 kb linear single stranded DNA with terminal foldbacks of ∿ 100 kb at each end.[1] It is converted to duplex replicative form (RF) in vivo, and by extracts of uninfected cells in vitro. The in vitro reaction with crude extracts is stimulated by ATP and involves participation of both DNA polymerases α and γ although purified preparations of either polymerase α and γ, alone, are capable of carrying out the reaction.[2,3] Duplex RF appears to replicate by a continuous, strand displacement mechanism that includes a dimer form of RF as an intermediate. In vitro experiments using inhibitors of DNA synthesis indicate that both DNA polymerases α and γ take part in the replication of RF.[4] Protein is covalently associated with the 5' termini of RF;[5] however, strand initiation may occur by extension from the 3' termini of the terminal foldbacks, rather than by a protein priming mechanism.

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0685 ADENOVIRUS DNA REPLICATION, Thomas J. Kelly, Jr., Jeffrey M. Ostrove, Philip Rosenfeld, Dan Rawlins, Mark D. Challberg*, Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD. 21205 (*Department of Molecular Biology and Microbiology, Tufts Medical School, Boston, MA. 02111)

The adenovirus genome is a linear, double-stranded DNA molecule containing about 35,000 base pairs. The nucleotide sequences at the two ends of the genome are identical over a distance of approximately 100 base pairs. The 5' terminus of each adenovirus DNA strand is covalently linked to a 55 kd protein via a phosphodiester bond to a serine residue. The terminal protein is synthesized in infected cells as an 80 kd precursor protein which is processed to the 55 kd form by proteolytic cleavage during virus assembly.

Initiation of adenovirus DNA replication takes place at the ends of the viral genome and involves the formation of a covalent linkage between the 80 kd terminal protein and the first nucleotide in the new DNA chain (dCMP). A daughter strand is then extended in the 5' to 3' direction displacing one of the parental strands. The displaced parental strand serves as template for the synthesis of the second daughter strand.

A cell-free system that carries out the replication of adenovirus DNA has been developed. This system requires components isolated from infected-cell nuclei and utilizes adenovirus DNA containing the covalently bound terminal protein as template. Several lines of evidence indicate that the mechanisms of initiation and chain elongation that operate in the in vitro system closely resemble those that operate in vivo. The system has provided a means to purify replication proteins and to explore replication mechanisms at the molecular level.

As an approach to the purification of adenovirus replication proteins we have developed in vitro complementation assays that make use of viral mutants defective in DNA replication in vivo. Extracts prepared from cells infected with H5ts36 or H5ts125, two such mutants belonging to different complementation groups, are defective for viral DNA replication in vitro, but replication activity can be restored by addition of appropriate purified fractions derived from wild-type-infected cells. Purified H5ts125 complementing activity consists of the 72 kd adenovirus single-stranded DNA binding protein. Purified H5ts36 complementing activity consists of the 80 kd terminal protein, two polypeptides with apparent molecular weights of 140 kd and 65 kd and some minor components. The complete in vitro adenovirus DNA replication reaction can be reconstituted from the purified H5ts36 complementing fraction, the adenovirus DNA binding protein, and a factor(s) present in uninfected cells.

Formation of a covalent complex between the 80 kd terminal protein and dCMP, the proposed first step in adenovirus DNA replication, can be detected in vitro in reaction mixtures containing dCTP as the only deoxynucleoside triphosphate. The reaction is dependent upon the presence of template which can be either adenovirus DNA containing attached terminal protein or certain single-stranded DNA molecules. With adenovirus DNA as template, optimal synthesis of the 80 kd terminal protein-dCMP complex requires purified H5ts36 complementing fraction and a factor from uninfected cells. The reaction is dependent upon specific nucleotide sequences in the template DNA. Experiments with mutant templates constructed by site-directed mutagenesis techniques indicate that sequences present within the first 18 nucleotides of the adenovirus genome are both necessary and sufficient.

0686 Dissection of the Reactions Involved in Adenovirus DNA Replication

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We have isolated 3 adenoviral encoded proteins which in the presence of two protein fractions isolated from nuclear extracts of HeLa cells support the replication of adenovirus DNA. We have devised a series of discrete reactions to define the role of each of the above proteins: a) Initiation reaction. preterminal protein (pTP) + dCTP ⟶ pTP-dCMP. This reaction requires Ad DNA-pro, ATP, Ad DBP, nuclear factor I (47K dal protein), the 80K dal pTP and the Ad induced DNA polymerase. The requirement for the Ad DBP and nuclear factor I are satisfied by the use of nuclear extracts from uninfected HeLa cells. In the presence of single-stranded DNA, only the 80K dal pTP and the Ad-polymerase are required for the formation of the pTP-dCMP complex. b) Formation of pTP-26 mer. The 26th nucleotide from the 5'-end of adenovirus type 2 DNA-pro is the first site requiring the incorporation of dGMP in the elongation reaction. In the presence of ddGTP and the other 3 dNTPs, this reaction shows an absolute requirement for ATP, Ad DBP, pTP and the Ad polymerase. Formation of the pTP-26 mer is stimulated by nuclear factor I. c) Replication of restriction fragments. Restriction enzyme digestion of Ad DNA-pro allows the analysis of the initiation and subsequent elongation process up to 25% of the full length adenovirus genome. Specific replication of the terminal fragments requires ATP, pTP, and Ad polymerase as well as the Ad DBP. Synthesis is stimulated by host nuclear factor I. d) Synthesis of full-length replication products. Synthesis of full length Ad DNA (35 kb) requires the combination of ATP, pTP, Ad DBP, Ad polymerase, nuclear factor I and nuclear factor II. Products formed in the absence of nuclear factor II have an average size of approximately 25% the length of Ad DNA.

0687 SIMIAN VIRUS 40 AS A MODEL FOR THE REPLICATION OF MAMMALIAN CHROMOSOMES, M.L. DePamphilis, L.E. Chalifour, M.F. Charette, M.E. Cusick, R.T. Hay, E.A. Hendrickson, C.G. Pritchard, L.C. Tack, P.M. Wassarman, D.T. Weaver and D.O. Wirak, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115, USA.

Simian virus 40 (SV40) provides an appropriate, but relatively simple, model to investigate how mammalian cells replicate their chromosomes, and how chromosome replication is related to the control of cell proliferation (1). We have utilized this system (i) to map the genomic locations and determine the structures of 5' and 3' ends of nascent DNA chains as a basis for understanding initiation and termination of replication (2,3), (ii) to analyze at single nucleotide resolution the action of DNA polymerases, with and without specific protein cofactors, on uniquely constructed primer-templates (4) and compare these results with similar studies on the movement of SV40 replication forks, (iii) to determine the structure of DNA (5) and the organization and mode of assembly of chromatin at replication forks (6,7), and most recently, (iv) to evaluate the role of cellular and viral factors in the activation of unique origins of replication during mammalian development by microinjection of SV40 and polyoma DNAs into mouse oocytes and eggs.

In general, bidirectional DNA replication originates at a specific site (2 bp) on one side of ori (65 bp) by initiating RNA-primed DNA synthesis at one of several possible initiation sites, but only on the ori strand that also encodes E-mRNA. With the exception of T-Ag, initiation of DNA synthesis in ori region is indistinguishable from initiation of Okazaki fragments found virtually exclusively on retrograde arms of forks. T-Ag is preferentially bound to replicating chromosomes less than 70% completed. DNA synthesis uses DNA polymerase alpha complexed with two protein cofactors that specifically stimulate this enzyme to initiate on RNA or DNA primers. As forks advance, old histone octamers are dispersed to both arms and immature nucleosomes are rapidly assembled with near-random phasing, then converted into mature chromatin. Replication forks are arrested at several specific DNA sites in the termination region that may be DNA polymerase arrest signals. At least two classes of DNA sequences arrest either alpha-polymerase or T4 DNA polymerase despite the presence of various accessory proteins.

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0688 IN VITRO RECONSTITUTION OF YEAST 2-μm PLASMID DNA REPLICATION, Akio Sugino, Francis Wilson-Coleman, Josef Arendes, Kwang C. Kim, Jennifer Motto, and Akira Sakai Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, and Department of Molecular and Population Genetics, the University of Georgia, Athens, GA 30602.

A soluble extract of yeast Saccharomyces cerevisiae carries out semiconservative replication of added 2-μm plasmid DNA, Escherichia coli chimeric plasmids containing the 2-μm DNA and the plasmid containing yeast ARS-1 sequence. Their replication is bidirectional and discontinous, and the origins are the same as in vivo origins. Furthermore, extracts from one of the DNA replication mutants, cdc8, show temperature-sensitive 2-μm DNA synthesis in vitro suggesting that this system resembles in vivo 2-μm plasmid DNA replication (1).

Using this system as a tool for complementation assay, CDC8 gene product has been purified from both wild-type and the cdc8 mutant cells and shown that it is a yeast single-stranded DNA binding protein (ySSB) which specifically stimulates the reactions catalyzed by yeast DNA polymerase I, which is a ture DNA replicase in yeast (2).

Because of lacking many DNA replication mutants in yeast, the extract has been fractionated by single-stranded DNA cellulose and phosphocellulose column chromatographies into 6 different fractions, some of which were further fractionated, to reconstitute the in vitro 2-μm DNA replication activity. These procedures have yielded at least 4 proteins (DNA polymerase I, DNA primase, ySSB, and a type II topoisomerase) and other 4 unresolved fractions.

The reconstituted in vitro DNA replication activity resembled or was the same as that observed in unfractionated crude extract.

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Transposition of DNA

0689 REQUIREMENT AND CONTROL FOR RECOMBINATION MEDIATED BY INSERTION SEQUENce IS1. Eiichi Ohtsubo, Chiyoko Machida*, Hisako Ohtsubo, and Yasunori Machida*, Institute of Applied Microbiology, University of Tokyo, Tokyo 113, Japan, and * Institute for Plant Virus Research, Tsukuba Science City, Yatabe, Ibaraki 305, Japan.

DNA dependent E. coli RNA polymerase was found to bind the restriction fragments containing either of the terminal regions of IS1 DNA. The binding of RNA polymerase to the left terminal region was stronger than that to the right terminal region. Foot print analysis using DNase I showed that RNA polymerase protected about 60 - 70 base pairs of each terminal region which included the left or right terminal inverted repeat sequence seen in IS1 (termed insL and insR, respectively) as well as the non-IS1 sequence of about 10 base pairs adjacent to each end of IS1. Analysis of transcripts from the left terminal region of IS1 showed that the insL sequence in this region completely overlapped with the promoter (named $insP_L$) and that RNA synthesis was initiated from a site, probably one in a stretch of five adenylate residues in the insL region and continued toward the interior region of IS1. Interestingly, most of the resulting transcripts contained polyuridylate residues (more than 5 U's) at their 5'-ends, presumably due to a slippage reaction of RNA polymerase at the five adenylate residues in the template. Analysis of transcripts from the right terminal region of IS1 showed that the insR sequence overlapped with the promoter (named $insP_R$) and that RNA synthesis was initiated from an adenylate residue within insR and elongated toward the interior region of IS1, thus against RNA synthesis from the promoter ($insP_R$) present at the other end. Transcripts from these two promoters contained nacent short transcripts of 8 - 14 bases long. Such transcripts were generated in a larger amount from $insP_L$ than $insP_R$. We propose that the $insP_L$ promoter is used to make the mRNA for the IS1-encoded genes, insA and insB, while the $insP_R$ promoter might be used to synthesize an anti-RNA and thereby negatively regulate the $insP_L$ promoter. We also discuss mechanisms of IS1-mediated recombination and of its control in general, based on the results obtained recently in our lab.

0690 MECHANISM AND REGULATION OF Tn10 TRANSPOSITION
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The tetR transposon Tn10 is 9300 bp in length and has closely related but non-identical inverted repeats of insertion sequence IS10 at its ends. All of the sites required for Tn10 transposition lie very near the ends of the element. Essential element-encoded functions, probably a single transposase protein, are encoded by IS10-Right. IS10-Left is non-functional.

Genetic analysis has established that the rate of Tn10 transposition in vivo is limited by the level of IS10-encoded functions; any participating host functions are not limiting. IS10 functions, probably the transposase, are preferentially cis-acting. They work more efficiently on transposon ends located near to the gene from which they are made than on ends located farther away. The property accounts for the fact that function-defective Tn10 mutants are not efficiently complemented in trans by a wild type element. Several lines of genetic evidence suggest that IS10 functions play an intimate role in breaking and joining both target DNA and transposon ends during the transposition process.

Tn10 transposition frequency also depends upon transposon length, as orinally observed for IS1-flanked transposons. Transposition frequencies decrease by about 40% for every 1000 base-pairs of transposon length. This may reflect the necessity for some aspect of the transposition process to proceed across the entire length of the element; that is for Tn10 to replicate.

Expression of IS10 transposition functions is controlled by determinants within IS10 itself; and this control is exerted at the level of translation. Multi-copy plasmids bearing IS10 depress transposition of an intact Tn10 element present in the bacterial chromosome. Only the outer 180 bp of IS10 must be present on the plasmid for inhibition to occur; and the presence of this information results in a decrease in translation but not transcription of the transposase gene on the chromosomal element. The outer 180 bp region encodes a strong promoter that directs transcription outwards towards the end of IS10. The transcript from p-OUT overlaps and is partially complementary to the transposase mRNA transcript; and the region of overlap includes the ATG start codon of the transposase gene. We have proposed that inhibition by multi-copy plasmids involves direct pairing between plasmid-specified p-OUT transcript and the transposase mRNA with resulting exclusion of ribosomes from the transposase message.

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0691 TRANSPOSABLE ELEMENTS IN DROSOPHILA, Gerald M. Rubin, Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210

The structure and properties of three distinct classes of Drosophila transposable elements will be discussed. The genetic behavior of mutations caused by insertion of transposable elements from each of these 3 classes into the white locus will be reviewed briefly (1-7). The results of recent studies on transposition (8,9), precise excision, and deletion formation will be presented (K. O'Hare, M. Collins, R. Levis, T. Hazelrigg, H. Roiha, R. Karess and G. Rubin, unpublished).

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0692 THE FUNCTIONS OF REPEATED DNA SEQUENCES, Gerald R. Fink, Biology Department, Massachusetts Institute of Technology and Whitehead Institute for Biomedical Research, Cambridge, MA 02139

Repeated DNA sequences account for a large proportion of the yeast genome, however, the function of only a few of these sequences is known. We have studied three different repeated DNA sequences in order to determine their role in the cell. One of these repeated sequences is crucial to the regulation of amino acid biosynthesis in yeast. Many genes which encode enzymes in different amino acid biosynthetic pathways are under a "general amino acid control" (GAC). Genes under this control increase their transcription when any one of a number of amino acids become limiting for growth. A nine base pair sequence is repeated in the 5' non-coding regions of several genes (HIS1, HIS3, HIS4, and TRP5) which are subject to the GAC type of regulation. This 9 base pair sequence does not appear in the 5' non-coding regions of yeast genes from other anabolic or catabolic pathways (i.e. CYC1, URA3, MAT, ADC1, etc.). Deletion analysis of this sequence is required for derepression by GAC.

The Ty sequences in yeast are repeated about 35 times in the genome. Unlike the 9 bp sequence required for GAC, the Ty sequences are found throughout the genome. Is Ty a "selfish" or "opportunistic" parasite or is it a functional part of the yeast genome? We have found a set of genes (SPM) which control the transcription and recombination of Ty elements. These genes appear to affect the Ty elements. In vitro mutagenesis of these genes suggests that they function to repress the activities of Ty elements. The existence of genes which act specifically on the transcription of Ty elements suggests that Ty elements have become domesticated by the cells that harbor them.

A third repeated DNA element, sigma, is found associated with tRNA genes. This element which is 341 bp in length is always 16 or 18 bp from the 5' end of the tRNA. The sigma appears to be a transposable element, but lacks the capacity to code for its own transposition. Sigmas are often found near Ty elements or parts of Ty elements. However, the Ty elements are at variable distances from the tRNA. These elements could provide homology for recombination between tRNA genes.

0693 RETROVIRAL PROVIRUSES AS INSERTIONAL MUTAGENS, Harold E. Varmus, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

The proviruses of retroviruses transpose through a viral RNA intermediate that serves as template for synthesis of unintegrated linear and closed circular DNA (1,2). Integration generally appears to occur at random within the host genome, always joining the same viral sequences (2 bp from the ends of linear DNA) to the host chromosome and duplicating 4, 5 or 6 bp of host sequences at the integration site; however, the immediate precursor to the provirus and the enzymes involved in integrative recombination have not been identified. Like other transposable elements, proviruses can act as insertional mutagens by disrupting or activating genes in the vicinity of integration sites; such mutations occasionally revert by deletion of most of the provirus by homologous recombination between its long terminal repeat (LTR) units (3). Proviruses also participate in a transduction phenomenon responsible for the acquisition of oncogenes from the host cell genome.

We have been exploring the behavior of proviruses with particular emphasis upon certain oncogenic retroviruses that lack their own oncogenes but can activate cellular genes by insertional mutation. In B cell lymphomas induced in chickens by avian leukosis virus (ALV), an elevated level of expression of a cellular oncogene (c-myc) is observed with three different arrangements of integrated ALV DNA within the c-myc locus (4). The results imply that proviral DNA can activate expression of host genes either directly, by providing a viral promoter, or indirectly, by affecting the efficiency of a host promoter. Analysis of DNA cloned from interrupted c-myc loci in B cell lymphomas and studies of the expression of the herpes simplex virus thymidine kinase gene joined in vitro to retroviral DNA (5) indicate that retroviral sequences sufficient for both the direct and indirect mechanisms lie within or immediately adjacent to an LTR.

Related mechanisms appear to operate during oncogenesis by the mouse mammary tumor virus (MMTV). Most of the MMTV-induced mammary tumors in C3H mice harbor proviral DNA within a 20 kb domain of the mouse genome that we have called int-1 (6). The insertions occur in several clusters throughout this region, on both sides of a transcriptional unit that is silent in normal mammary glands but expressed at a low level in tumors with an interrupted int-1 locus. We interpret these findings to mean that rare insertions of MMTV DNA into int-1 initiate tumorigenesis by activating a heretofore unrecognized cellular oncogene.

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0694 NATURE OF TRANSPOSITION IMMUNITY. F. Heffron, C. Lee, A. Bhagwat, M. McCormick* and E. Ohtsubo*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; *State University of New York, Stony Brook, N.Y.

Among transposable elements, the Tn3 family is unique in showing a phenomenon that limits multiple insertions of the same transposon into a plasmid (Robinson et al., 1977). This mechanism acts only in cis; transposition frequency to other plasmids in the same cell is unaffected. We have deletion mapped sequences required for transposition immunity. In most constructions only a single terminal repeat need be present to confer immunity to the plasmid. Complications and a model for transposition immunity will be discussed.

Site-Specific Recombination

0695 SITE-SPECIFIC DNA CONDENSATION AND PAIRING MEDIATED BY THE INT PROTEIN OF BACTERIOPHAGE λ, M. Better, C. Lu, R.C. Williams, S. Wickner*, J. Auerbach* and H. Echols, Department of Molecular Biology, University of California, Berkeley, CA and *Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20205.

Phage λ controls its integration and excision by differential catalysis of the forward and reverse reactions. The λ Int protein is required for both directions, but Xis for excision only. By electron microscopic observations, we have shown that Int protein forms a stable, condensed protein-DNA complex with the phage (attP) and prophage left (attL) substrate sites, butnot with the host (attB) or prophage right (attR) sites. We have found that Int and Xis together produce a stable, condensed complex with attR. The attP complex includes DNA from the P and P' regions on both sides of the crossover point (O); the attL structure involves the P' DNA to the right of O; and the attR complex includes the P region to the left of O. A single base-pair change in the P' regions destabilizes the condensed attP complex. In the presence of Int and Xis, the attL and attR sites form a paired structure. We suggest that a sequential cooperative interaction between bound Int or Int and Xis molecules provides for a distinct reactive DNA conformation and for pairing between substrate sites.

0696 THE MECHANISM OF PHAGE LAMBDA SITE-SPECIFIC RECOMBINATION. Nancy L. Craig and Howard A. Nash, LNC-NIMH, Bldg. 36, Rm. 3D-30, Bethesda, MD 20205.

During the establishment of lysogeny, phage lambda integrates into the E. coli chromosome by conservative, site-specific recombination between attP on the phage chromosome and attB on the bacterial chromosome (1). Strand exchange between these sites occurs within a region of homology called the core; the sequences that flank the core, called arms, are unique. The integrated phage chromosome is bounded by attL and attR, which are hyrid att sites containing information from both attP and attB. Integration requires a phage-encoded protein, Int, and a host-encoded protein, Integration Host Factor (IHF). Int is a single polypeptide of 40K and is the product of the lambda int gene. IHF contains two polypeptides which are the products of E. coli genes; the 11K polypeptide is the product of the himA gene and the 9.5K polypeptide is the product of the hip gene. Prophage induction results in the excision of the phage chromosome by conservative, site-specific recombination between attL and attR; this reaction regenerates attP and attB. Excision requires Int, IHF and another phage-encoded protein, Xis. Xis is the product of the lambda xis gene and is the single polypeptide of 9K.

How do the recombination proteins recognize att sites? Both Int and IHF recognize and bind to specific sequences in att sites. Int binds to both the core and arms of attP and to the core of attB (2). We have found that IHF binds to both arms of attP. Each IHF binding site contains the sequence AANNNPuTTGAT; some of the bases in this sequence are protected from chemical modification by IHF, indicating that at least part of this sequence is recognized by IHF. In addition to its direct role in recombination, IHF has a regulatory role in which it mediates gene expression (3). We have found that IHF also binds to the regulatory regions of genes whose expression is controlled by IHF. The binding of IHF to regulatory regions is directed by recognition of the same sequence that directs the binding of IHF to att sites.

Strand exchange requires the breakage and reunion of DNA within the core. Int has the capacity to break and rejoin DNA: it has topoisomerase activity (4). We have found that this activity acts at specific internucleotide junctions within the core. The positions of Int topoisomerase action coincide with the sites of strand exchange (5). This demonstrates that Int executes strand exchange during recombination and supports the hypothesis that recombination proceeds through a topoisomerase-like mechanism (4).

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0697 DNA STRUCTURES IN LAMBDA SITE-SPECIFIC RECOMBINATION, Arthur Landy, Monika Buraczynska, Wade Bushman, Noaman Hassan, Pei-Ling Hsu, John Leong, Wilma Ross, John Thompson and Sam Yin, Division of Biology and Medicine, Brown University, Providence, RI 02912

The site-specific recombination reaction of bacteriophage λ can proceed in either of two directions. In the integrative mode one partner, the attP site, is rather large (240bp) and has complex interactions with the two required proteins Int and IHF; the other partner, attB, is approximately one-tenth the size and has the capacity for a less complex interaction with Int protein. The products of this recombination, attL and attR, are also capable of recombining in the reverse direction to yield attP and attB. In this excisive mode, which additionally requires the protein Xis, the partners are more equal both in size and their complexity of protein interactions. For purposes of analysis, both reactions can be formally considered as consisting of four phases (some of which may be concurrent): 1) formation of the appropriate DNA-protein complexes, 2) synapsis of the recombining partners, 3) crossing over of the first pair of DNA strands (formation of a crossed-strand exchange or Holliday structure), 4) crossing over of the second pair of DNA strands (resolution of the Holliday structure). We shall discuss experiments pertaining primarily to the first and last phases of the reaction and to the problem of directionality.

0698 THE BIFUNCTIONAL ROLE OF γδ RESOLVASE IN DNA TRANSPOSITION, Randall R. Reed and Nigel D. F. Grindley*, Department of Genetics, Harvard Medical School, Boston, MA 02115, *Department of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, CT 06511.

The transposable DNA elements γδ and Tn3 each encode a site-specific recombination system required for the efficient conversion of intermediates in transposition to the normal end products. The recombination is mediated by an element-encoded protein, resolvase, acting at a particular site res located within the element. Additionally, resolvase plays a central role in regulation of transposition frequency, apparently by acting as a transcriptional repressor. The location of the res site within the intercistronic region between the two divergently transcribed genes involved in transposition (transposase, tnpA and resolvase, tnpR) suggested that resolvase may catalyze recombination and achieve repression by binding at a single region.

We have developed a purified, in vitro system[1] to study the recombination reaction and have determined the sites where transcription initiates for the tnpA and tnpR genes. These studies indicate that:

1) Resolvase binds at three distinct sites in the recombination region.[2] Interaction between resolvase and the DNA substrate at all three binding sites are important for efficient recombination.

2) Resolvase cleaves the DNA at a particular site within the res region and forms a transient, covalent linkage with the DNA substrate.

3) Divergent transcription initiates within the region where resolvase binds.[3]

These experiments, in conjunction with analysis of mutants and in vitro generated deletions suggest a model in which resolvase interacts with the res site in a complex manner to achieve both recombination and regulation of transposition functions.

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0699 DYNAMICS OF SITE-SPECIFIC RECOMBINATION BY THE Tn3 RESOLVASE,
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Transposition of Tn3 involves two sequential DNA breakage and reunion events. In the first there is a duplication of the transposon and a non-homologous recombination that links the donor and recipient DNA via Tn3 bridges. The second event, carried out by resolvase, is a reciprocal recombination at the res site of the directly repeated transposons which converts the cointegrate back to the separated donor and recipient which now both contain a copy of Tn3. We cloned the Tn3 resolvase downstream from the phage lambda P_L promoter and under cI^{857} control, and purified the enzyme to homogeneity from this overproducing strain. Sixty mg of the 21,000 dalton protomer enzyme were obtained from 150 gm of cells. Like the related enzyme from gamma delta (1), the enzyme efficiently recombines supercoiled plasmids containing two directly repeated res sites in the presence of Mg^{2+} and no other cofactor. Resolvase is also a strictly site-specific topoisomerase: plasmids containing res sites in a head-to-tail configuration are relaxed by resolvase, but not those containing 0, 1, or 2 sites in a head-to-head orientation. Even when proximity of res sites is guaranteed by catenation of two single res site plasmids, neither relaxation nor recombination occurs. With plasmids containing four directly repeated res sites, recombination between adjacent sites occurs 15 times as often as between opposing sites. Thus, resolvase can distinguish intra- from intermolecular sites and can distinguish the relative orientation and position of intramolecular sites separated by thousands of base pairs. These features of the enzyme are nicely tailored to its roles in vivo where it must resolve directly repeated transposons but would be undesirable to break and rejoin quiescent transposons in its role as a transcriptional repressor. Moreover, specificity insures the irreversibility of resolution. The two circular products of resolvase recombinaton are always catenated. The degree of interlinking is surprisingly simple: virtually all of the recombinants are linked only once. The simplicity of the interlinking implies that the DNA segments to be separated by recombination are not extensively intertwined at synapsis in the supercoiled plasmid. The homogeneity of the product catenanes shows that both the geometry of the DNA at synapsis and the dynamics of strand exchange are rigidly defined. A model in which one subunit of a functionally dimeric resolvase remains bound at one res site while the other tracks along adjacent DNA until it encounters a second site could account for its ability to distinguish intra- from intermolecular sites, sense the relative orientation and position of res sites, and produce singly interlinked catenanes. Although recombination necessarily involves duplex breaks, resolvase acts one strand at a time, since (A) it is a type 1 topoisomerase, and (B) transient intermediates in the breakage and reunion reaction are altered in only a single strand.

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0700 PROTEIN-NUCLEIC ACID INTERACTIONS IN P1 SITE-SPECIFIC RECOMBINATION: THE lox-cre SYSTEMS.
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The P1 site-specific recombination system consists of two elements: a recombinase gene, cre (1) and a recombination site, loxP (2). The gene is located 400 bp to one side of the site, is transcribed away from the site, and encodes a protein of 35 kd, Cre. Cre has been purified to homogeneity, and in the absence of any other cofactors it can promote efficient recombination between two loxP sites located on either linear or supercoiled circular DNA molecules. Intramolecular recombination is more efficient than intermolecular recombination, and inverted sites are recombined as efficiently as directly repeated sites are. No evidence of topoisomerase activity has been detected.

Localization of the minimum DNA sequence necessary for loxP recombination by deletion analysis indicates that a functional loxP site consists of a perfect 13-bp inverted repeat sequence separated by an 8-bp spacer (2). Support for this conclusion comes from two findings: The exchange of DNA between loxP and a less efficient site in the bacterial chromosome, loxB (3), occurs in the 8-bp spacer region; and the DNA protected by Cre in footprinting experiments consists of only the inverted repeats and the spacer. The small size of loxP (<34 bp) contrasts with the larger size of sites necessary for other site-specific recombination systems e.g., attP is >240 bp and res is >100 bp.

The most intriguing observation about the lox-cre system is that 50% of the products of recombination between two loxP sites on a supercoiled molecule are free supercoiled circles. For att-int and Tn3-res recombinations, more than 90% of the products are catenated circles. This property of the lox-cre system suggests that the DNA domains between loxP sites are separated before recombination occurs. We propose several models to explain how this might occur.

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General Recombination Mechanisms

0701 STRUCTURE AND MECHANISM OF THE recA PROTEIN OF E. COLI, D. A. Soltis, M. M. Cox, F. R. Bryant, J. N. Nakamura and I. R. Lehman, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

The recA protein of E. coli promotes the complete exchange of strands between full length linear duplex and homologous circular single-stranded (SS) DNA to yield an RFII-like molecule and a linear single strand. The reaction occurs in two phases: (i) formation of a short heteroduplex region termed a D-loop, and (ii) extension of the heteroduplex by branch migration. D-loop formation requires ATP but not its hydrolysis; branch migration has a constant requirement for ATP hydrolysis.

Strand exchange is stimulated five to ten fold by single-stranded DNA binding protein (SSB). Stimulation results from stabilization of recA protein-SS DNA complexes whose formation requires SSB and ATP. In the presence of SSB, recA protein does not dissociate from these complexes for up to 90 min. In its absence, recA protein equilibrates completely between two populations of SS DNA with a $t_{\frac{1}{2}}$ of 17 s. The complexes, which have sedimentation coefficients of >70 S, dissociate when the ADP/ATP ratio in the reaction approaches a value of 0.6-1.5. The recA protein can then participate in a second round of strand exchange upon regeneration of the ATP. While 100-200 moles of ATP are hydrolyzed per mole of heteroduplex base pair formed in the presence of SSB, this value is reduced to 16 at levels of ADP lower than that required to dissociate the complexes.

At higher salt concentrations (100 vs. 25 mM), strand exchange in the absence of SSB proceeds to completion at approximately one-third the rate observed in the presence of SSB. Preliminary results indicate that under these conditions, the strand exchange reaction occurs through a mechanism that does not include the formation of a recA protein-SS DNA complex. In addition, strand exchange in the presence of SSB is inhibited at 100 mM salt which again implies that the two reactions proceed by different mechanisms.

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0702 THE PATH OF HOMOLOGOUS PAIRING PROMOTED BY E. COLI REC A PROTEIN, C. M. Radding, R. Kahn, J. Flory, A. Wu, C. DasGupta, D. Gonda, M. Bianchi and S. S. Tsang, Depts. of Human Genetics and Molecular Biophysics & Biochemistry, Yale University, New Haven, CT

RecA protein in vitro promotes homologous pairing of single-stranded or partially single-stranded DNA with duplex DNA in three sequential phases: 1) During a slow presynaptic phase, which requires ATP, recA protein polymerizes on single-stranded DNA. ADP competitively inhibits presynapsis. E. coli single strand binding protein (SSB) accelerates polymerization and completion of presynapsis, stabilizes the product formed, and counteracts the inhibitory effect of ADP. 2) A set of rapid steps, called synapsis, aligns homologous sequences and creates a nascent heteroduplex joint. Although synapsis is non-polar and no free ends are required in any of the DNA strands to make the nascent heteroduplex joint, recA protein extensively unwinds the recipient duplex molecule and provisionally pairs an incoming strand with its complementary strand from the duplex DNA. These conclusions imply the existence of a novel paranemic synaptic structure in which base pairing occurs without topological interwinding of strands. Accordingly, when a free end permits interwinding of strands, the structure of the nascent joint would favor the formation of a true heteroduplex joint with interwound strands (a plectonemic joint), rather than reformation of the original duplex DNA. At 37°, recA protein causes a single strand to pair with its complement in duplex DNA at least as rapidly as such a strand can find a free complementary strand by annealing at T_m -25°. 3) Heteroduplex joints form initially whether the requisite free end is a 3' or a 5' end, but subsequently the joints either grow or dissociate, depending on the location of the free end relative to a slow unidirectional strand exchange that is driven by recA protein: the point of strand exchange, viz the point at which a new strand replaces an old one, moves 5' to 3' with respect to the displaced strand. Acting on linear duplex DNA and duplex DNA with a single-stranded end, recA protein causes not only an invasion of the fully duplex molecule by the single-stranded tail but also a reciprocal exchange of strands, thereby producing a Holliday structure, which has been implicated as an intermediate in both prokaryotic and eukaryotic recombination. RecA protein appears to promote reciprocal strand exchange efficiently and actively.

The mechanism of the pairing reaction is adumbrated by observations which show that recA protein polymerizes on single strands to produce a structure which binds and unwinds duplex DNA.

Refs.: DasGupta et al., Cell 25, 507-516, 1981; Flory & Radding, Cell 28, 747-756, 1982; Wu et al., Cell 30, 37-44, 1982.

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0703 THE UNWINDING OF DOUBLE-STRANDED DNA BY recA PROTEIN, Th. Koller, E. Di Capua, A. Stasiak, Institute for Cell Biology, Swiss Federal Institute of Technology, 8093-Zurich, Switzerland.

While the conceptual model of Holliday and Meselson & Radding for homologous recombination is generally accepted, no structural model exists so far for the molecular basis of homologous strand transfer, the reaction mediated by recA protein (for review see 1). This reaction has been shown in vitro to require ATP hydrolysis and stoichiometric amounts of recA protein (e.g. 2). In the presence of the non-hydrolyzable analog ATPγS, double stranded DNA is found covered with recA in stable complexes (3,4,5), very likely at a stoichiometry of one recA per 3 base pairs (6). These filamentous recA-DNA complexes as seen by electron microscopy are helical, suggesting that recA polymerizes in a helical structure in order to force the DNA into a conformation useful for some step of the recA-mediated reaction, maybe search for homology between DNA strands, or switch in base pairing between the DNA strands that are to be exchanged. We have shown that, in ATPγS-stabilized complexes with recA, double stranded DNA is unwound from 10.5 base pairs per turn (B-DNA) into 18.5 base pairs per turn, following the protein helix (7). The DNA is stretched by a factor 1.5. These data suggest an intercalative type of interaction. Ohtani et al. (8) have also found biochemically an extensive unwinding of double stranded DNA by recA protein in the presence of ATP; they have interpreted this result as reflecting regions of a fully unwound (melted) helix. Our aim is to describe the conformations of DNA involved in the homologous strand transfer reactions mediated by recA protein.

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0704 ENZYMATIC RESOLUTION OF HOLLIDAY RECOMBINATION JUNCTIONS, K. Mizuuchi, M. O'Dea and M. Gellert, Laboratory of Molecular Biology, NIADDK, National Institutes of Health, Bethesda, MD 20205 and B. Kemper, Institut für Genetik de Universität Köln, Weyertal 121, 5000 Köln 41, Federal Republic of Germany.

The multiple enzymatic steps involved in general genetic recombination between two homologous double-stranded DNA molecules can be separated into two major categories: formation and resolution of a recombination intermediate structure. A typical recombination intermediate has the structure called a Holliday junction. This structure is generated by cutting and exchange of a pair of strands between homologous DNA molecules. To regenerate a normal double-stranded DNA structure, another event that cuts a pair of strands must take place. These two sets of cuts can occur on the same pair of strands that was first exchanged, to restore the parental configuration of the outside DNA, or can occur on a different pair of strands to generate a recombinant configuration.

We have demonstrated that phage T4 endonuclease VII, the product of T4 gene 49 can carry out this second cutting reaction at the Holliday junction in a highly specific manner. Several kinds of DNA molecules all containing the local structure of a Holliday junction were cut at the junction by the endonuclease in such a manner that they can be resealed by DNA ligase to restore a normal double-stranded DNA structure (K. Mizuuchi, B. Kemper, J. Hays and R. Weisberg (1982) Cell 29, 357).

These experiments have suggested that the enzyme does not require a long stretch of unique sequence at the cutting site. We have now extended our experiments to address the question of detailed sequence specificity. The results of experiments on the action of the enzyme on different kinds of branched DNA structures will also be discussed.

The action of exonuclease V (recBC nuclease) of E. coli on Holliday junction structures was also studied. Exo V was unable to digest a DNA molecule containing a Holliday junction when the entire molecule was covalently closed. However, if the substrate DNA was cut first to generate ends, exo V could digest DNA with Holliday junctions to generate molecules with properties of the expected "resolution product."

0705 PARTICIPATION OF THE PACKAGING ORIGIN, cos, IN GENETIC RECOMBINATION IN PHAGE λ. Franklin W. Stahl, Ichizo Kobayashi, and Mary M. Stahl, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Generalized recombination in λ can be effected by either the Red system of λ, by one or another of the E. coli pathways, or by a combination of these. In all of these situations, cos, the locus at which λ's chromosome is cut during packaging, influences recombination. In all pathways except RecBC, recombination near cos is relatively independent of chromosome replication (1,2). In RecBC recombination, the effectiveness of Chi (5' GCTGGTGG) as a recombinator is dependent on its orientation relative to that of cos (3).

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Somatic and Specialized Recombination

0706 GENES OF THE MAJOR HISTOCOMPATIBILITY COMPLEX, L. Hood, K. Eakle, D. Fisher, L. Fors, R. Goodenow, J. Kobori, B. Malissen, M. Malissen, A. Orn, B. Sher, L. Smith, M. Steinmetz, I. Stroynowski, H. Sun, A. Winoto, M. Zuniga, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The major histocompatibility complex (MHC) of vertebrates encodes several gene families which mediate self and nonself recognition. The class I gene family encodes transplantation antigens and hematopoietic differentiation antigens such as Qa and TL. The class II gene family encodes the Ia antigens or the immune response genes. We have used class I and class II cDNA probes to isolate 36 class I genes encompassing 837 kilobases of DNA and six class II genes encompassing 250 kilobases of DNA. We have used genetic mapping by restriction enzyme site polymorphisms to map 13 different clusters of class I genes to their positions in the major histocompatibility complex. These studies suggest that class I genes can undergo gene expansion and contraction, presumably by homologous but unequal crossing-over in various inbred strains of mice. These studies also suggest that there are certain regions in the major histocompatibility complex that exhibit extensive restriction enzyme site polymorphisms and other regions that are much more limited in their polymorphisms. These restriction enzyme site polymorphisms correlate strikingly with the corresponding serologic polymorphisms that have been observed in gene products of the class I and class II families. We will discuss in detail the structure and organization of the class I and class II genes in the major histocompatibility complex.

We have used DNA-mediated gene transfer to analyze all 36 class I genes. Using this technique we have identified most of the serologically defined class I genes and we have identified at least ten novel class I gene products that heretofore had not been defined by serologic techniques. These transformed cells have also been used to study the mechanism of virus-restricted T-cell killing. We have demonstrated that in LCM virus-induced T-cell killing only the L^d class I molecule is used as a restricting element. This striking observation allows us to begin to analyze in detail the requirements for class I molecules to act as T-cell killing restricting elements through in vitro mutagenesis and exon shuffling with class I molecules that are incapable of carrying out this function. Thus, this system offers one of the first opportunities to study the molecules in genes involved in a cell-cell communication system.

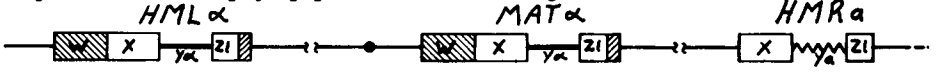
0707 MECHANISM OF MATING TYPE CASSETTE SWITCHING, J. Strathern, A. Klar, J. Hicks, J. Abraham, J. Ivy, M. Kelly, S. Weisbrod, C. Stephens, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Homothallic mating type switching in the yeast S. cerevisiae occurs by the transposition-substitution of genetic information from the silent donor loci, HML and HMR, into the expression locus, MAT. The end product description of this process has been well characterized both genetically and physically: The MATa and MATα alleles differ by an α specific sequence (Yα) and an a specific sequence (Ya); HML and HMR have regions of homology with MAT (W, X, Z1 Z2) in addition to whichever of the Y regions they carry; when a switch at MAT occurs the Y region that is replaced is discarded; the HML or HMR donor locus for the transposed Y sequence is not genetically altered by the event. In summary, there is a net replication of the donor and a loss of the Y region of the recipient.

We have demonstrated that switching is correlated with a double stranded cut near the Y-Z junction of MAT. Mutants at the site of cutting are incapable of switching. The same sequence is present at HML and HMR but is rendered inaccessible by the Mar/Sir functions which keep these loci unexpressed.

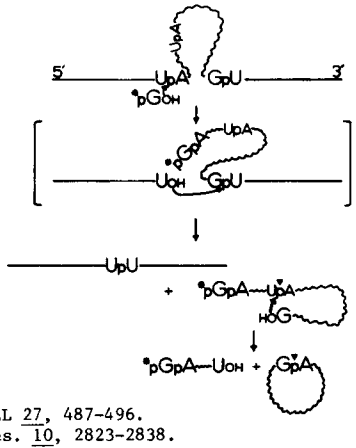
We have been able to mimic homothallic switching by transforming heterothallic, cells with replicating plasmids carrying MAT. Uncut plasmids transformed into these cells do not switch. In contrast, plasmids cleaved in the MAT sequence can switch alleles. The fraction of transformants that have had a switch on the plasmid is dependent on the position of the cut. The maximun switching rate is seen with plasmids cut between Y and Z. Up to 15% of mata plasmids cut at Y-Z switch to MATα by interaction with the HM loci.

While homothallic switching is formally a gene conversion event, it differs from most gene conversions in two ways: first it is directional in the sense that MAT is the recipient and the HM loci are the donors, and secondly, recombination of outside markers (which in this case would lead to the fusion of donor and recipient) is very rare. The first observation is now readily understood as a consequence of the fact that the initiating cut occurs only at MAT in Mar/Sir$^+$ strains. Recent evidence suggests that the second observation is also controlled by the Mar/Sir functions and is not merely a consequence of the topography of the donor recipient interaction.



0708 RNA SPLICING *VIA* RNA RECOMBINATION, Thomas R. Cech, University of Colorado, Boulder CO 80309

In eukaryotes, intervening sequences are removed from precursor RNAs by a process called RNA splicing. RNA splicing involves cleavage, strand switching, and rejoining of the RNA, resulting in a new combination of sequences. As such, it bears some similarity to the process of DNA recombination. The steps involved in splicing the ribosomal RNA precursor of Tetrahymena are shown below. Splicing requires a guanosine cofactor which must have free 2' and 3' hydroxyl groups.[1] The guanosine becomes covalently bonded to the 5' end of the intervening sequence during its excision.[1,2] No ATP or GTP hydrolysis is required for any step in the reaction. These features are consistent with a phosphoester transfer mechanism in which specific phosphodiester bonds are broken, then reformed with the 3'-hydroxyl of a different "acceptor" RNA. Bond cleavage and rejoining appear to occur in a concerted manner. The RNA splicing reactions require no enzyme or other protein, but are mediated by the structure of the RNA molecule itself.[3] It has frequently been suggested that RNA preceded DNA as the genetic material. This hypothesis is attractive because RNA has the same potential as DNA for storing information, but in addition is able to direct the synthesis of proteins. Therefore, the ability of an RNA molecule to rearrange its own internal structure may have provided a major mechanism of genetic recombination early in evolution.



[1]Cech, Zaug and Grabowski (1981) CELL 27, 487-496.
[2]Zaug and Cech (1982) Nucl. Acids Res. 10, 2823-2838.
[3]Kruger et al. (1982) CELL 31, 147-157.

DNA Structure; Topoisomerases; DNA Binding Proteins

0709 DNA SECONDARY STRUCTURE DIRECTS THE FORMATION OF DELETION AND ADDITION MUTATIONS, Barry W. Glickman and Lynn S. Ripley, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Determination of the DNA sequences at the endpoints of deletion mutations have frequently revealed the presence of repeated sequence. This correlation suggested a model for deletions in which the deletion resulted from the processing of inter-strand misalignments (e.g. mediated by slippage) permitted by the repeated sequences. However, the sequences of many deletions do not have repeated base pairs at their endponts and therefore cannot be explained by this model. We have proposed a model (1) that does account for the specificity of many deletion events and does not require repeated base pairs at the endpoints. In our model a palindromic or quasi-palincromic sequence permits intra-strand misalignments (DNA secondary structures) whose further processing is predicted to yield the observed deletion endpoints. The model also accounts for the specificity of certain addition and duplication events. In one sequenced addition, the concurrent occurence of multiple base substitutions suggests the formation of a DNA secondary structure intermediate.

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0710 ANALYSIS OF HAIRPIN STRUCTURES IN DOUBLE STRANDED DNA BY THE USE OF ENDONUCLEASE VII, Börries Kemper, Marianne v.Depka, Frank Jensch and Hans-J. Fritz, Institut für Genetik, Universität Köln, 5 Köln 41, F.R.G.

Endonuclease VII from phage T4 (Endo VII) has recently been shown to cleave branched, recombinant DNA by cutting across the Holliday junction. The enzyme is also capable of cleaving at the base of cruciform structures which closely resemble a Holliday junction. When circular plasmid pBR322 DNA was treated with the enzyme approximatly 20% of the molecules became linearised. provided the DNA was present in its supercoiled form. Sequence analyses of the termini revealed that the cut was located at a unique site . Two staggered nicks were simultaneously introduced into opposite strands. The nicks were located one nucleotide to the right and one nucleotide to the left of a hairpin occuring around position 3065. The hairpin consists of 11bp inverted repeats separated by a sequence of 3 non symmetrical nucleotides. When in addition to this hairpin a 31bp perfect inverted repeat was artificially introduced into the same plasmid, a new strong site was created for the enzyme. More than 80% of the molecules were now linearised at this site by the above mentioned mechanism. In summary our results suggest, that 1. Endo VII recognizes DNA-structures and has no sequence requirements, 2. the competition between different hairpins is influenced by the size and arrangement of the inverted repeats involved, and 3. the hairpin structure is formed over the entire length of the inverted repeat (in the two examples analysed).

0711 PROCEDURE USING PSORALEN CROSS-LINKING TO QUANTITATE DNA CRUCIFORM STRUCTURES SHOWS THAT CRUCIFORMS CAN EXIST IN VITRO BUT NOT IN VIVO, R.R. Sinden, S.S. Broyles, D.E. Pettijohn, University of Colorado Health Sciences Center, Denver, CO 80262

A new method for detecting cruciform structures in DNA has been developed, which is applicable to studies in living cells. This procedure could be used with any palindrome that can be identified in a relatively small restriction fragment. A 66-bp perfect palindromic lac operator DNA sequence, cloned into the EcoRI site of pMB9, can exist in plasmid DNA as a linear molecule or as two 33-bp cruciform arms. In the linear state EcoRI cutting produces a 66-bp linear fragment. When in the cruciform state cutting occurs at EcoRI sites now at the base of cruciform arms releasing two 33-bp "hairpin" fragments. Both configurations can be fixed by 4,5',8-trimethylpsoralen cross-links in DNA. Thus, cross-linking together with EcoRI cutting provides an assay that can quantitate the fraction of palindromes existing as cruciforms in DNA in vitro or in vivo. In vitro the fraction of palindromes existing as cruciforms is a function of the superhelical density of the DNA with transition mid-point occurring at $\sigma = -0.065$. Relaxed DNA contained no cruciforms. In molecules with $\sigma \geq -0.08$ at least 98% of the palindromic sequences existed as cruciforms. Once formed, cruciform structures were stable in covalently closed DNAs with σ as low as -0.02. Half lives of the cruciform and linear configurations were measured and both configurations were stabilized by Mg^{++}. Although the majority of the palindromic sequences existed as cruciforms in DNA as isolated from cells, few if any cruciforms could be detected in vivo. The results suggest that plasmid DNA as organized in vivo either lacks sufficient tension to drive the cruciform transition, or formation is prevented by other molecules interacting with the palindrome in vivo.

0712 SALT EFFECTS UPON THE Z-DNA FORMATION IN NEGATIVE SUPERCOILED PLASMIDS,F.Azorin, A.Nordheim and A.Rich.Massachussets Institute of Technology,Cambridge,MA.

It has been demostrated recently that the left-handed Z-DNA conformation could be stabilized by negative supercoiling(1-3).In this poster we use the antibody recognition technique to study the effect of different NaCl concentrations (whithin the physiological range)on the actual negative superhelical density required to stabilize Z-DNA.We used pBR 322 and two derivatives of it,pLP 32 and pLP 014,wich carry 32 bp and 14 bp long inserts of alternating CG,respectively.It is shown that as the NaCl concentration decreases,lower values of the negative superhelical density are required to stabilize Z-DNA.From the experimental data we can estimate the values for the free energy change per base pair(ΔG_{BZ}) and per junction (ΔG_j) fot the B to Z transition.It is shown that whereas ΔG_j is strongly dependent on the $NaCl^+$ concentration, ΔG_{BZ} does not depend on it.It is also shown that pBR 322 at bacterial superhelical density contains a DNA sequence stable in the Z-conformation at 100mM NaCl. Evidence is presented wich indicates that the actual sequence stable in the Z-conformation in pBR 322 is either a 9 base pairs long sequence of alternating purines and pyrimidines residues or a 14 base pairs sequence with one residue out of phase.

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2.-Singleton C.K.,Klysik J.,Stirdivant S.M. and Wells R.D. (1982)Nature 299,312-316.
3.-Nordheim A.,Lafer E.M.,Peck L.J.,Wang J.C.,Stollar B.D. and Rich A. (1982)Cell,in press.

0713 STABILIZATION OF Z-DNA NEAR PHYSIOLOGICAL IONIC STRENGTH, Leonard Klevan and Verne N. Schumaker, Department of Chemistry and Molecular Biology, University of California, Los Angeles, CA 90024 and Division of Molecular Biology, Bethesda Research Laboratories, Inc., P.O. Box 6009 , Gaithersburg, MD 20877

Binding of polyarginine to the Z form of poly d(GC) results in a protein-Z-DNA complex stable near physiological ionic strength. The percent DNA retained in the Z conformation at several protein concentrations was estimated from the C.D. profile of the polyarginine-poly d(GC) complex in low salt. C.D. spectra at amino acid to nucleotide ratios above 0.5 exhibit negative minimum ellipticities at 290nm and a positive C.D. band at 260-270nm, in qualitative agreement with the C.D. profile of Z-DNA.

The relative proportion of DNA retained in the Z conformation in the low salt complex was a linear function of protein concentration and after 24 hours in 0.17M NaCl was within 70-90% of the values obtained upon dilution to low salt. Treatment of this low salt complex with trypsin leads to inversion of the C.D. spectra to that of B-DNA. Association of poly d(GC)-polyarginine into stable Z-DNA-protein complexes may arise from a combination of polyarginine binding and Z-DNA aggregation or from association into Z*-DNA complexes of approximately 60-100 million daltons. The results of this study suggest that Z-DNA may be stabilized by specific protein interactions and offers an approach to the isolation of naturally occuring Z-DNA-protein complexes.

0714 TORSIONAL STRESS INDUCES LEFT-HANDED DNA STRETCHES IN DNA OF NATURAL BASE SEQUENCE: STUDY BY SPECIFIC ANTIBODY BINDING AND CIRCULAR DICHROISM Elisabeth DiCapua and Andrzej Stasiak, Institute for Cell Biology, ETH Hönggerberg, CH-8093 Zürich, Switzerland

Plasmid DNA is shown to contain stretches of left-handed DNA at specific linking differences σ = 0.1 and above. Antibodies raised against Z-DNA (a kind gift of Dr. F. Pohl) are bound in increasing amounts with increasing torsional stress. Mapping of the antibodies bound along the DNA by electron microsccpy reveals a correlation of the antibody binding sites with stretches of alternating purine-pyrimidine sequences of 9-10 base pairs.

While the antibody binding seems to be limited to 5-10 such long alternating stretches, the circular dichroism spectrum (obtained in collaboration with Dr. S. Brahms) indicates that a larger proportion of the base pairs are in a left-handed conformation: at σ = 0.24, the spectrum indicates 7% of the bp in a Z-like conformation (at σ = 1.0, even 30-40%), which cannot be accounted for by the antibody binding stretches alone. We conclude that some DNA stretches which cannot bind anti-Z antibody are nevertheless in a left-handed conformation; the question remains whether the stretches are Z-like (alternating), but too short to bind the antibody, or whether they are not alternating, thus displaying another kind of backbone. Since the energy of a junction is believed to be high, one is tempted to speculate that rather long left-handed stretches nucleate at the most favoured sites, the alternating Pu-Py stretches.

0715 MONOCLONAL ANTIBODIES TO UNUSUAL NUCLEIC ACIDS, Jeremy S. Lee, Dept. of Biochemistry, University of Saskatchewan, Saskatoon, Sask., Canada. S7N 0W0.

Monoclonal antibodies directed against nucleic acids are receiving considerable attention because of their potential use as probes of nucleic acid structure and because they may serve as models for protein/nucleic acid interactions in general (Lee et al. 1982, Biochemistry 21, 4940-4945). We have recently prepared monoclonal antibodies showing specificity for 'Z' DNA, $d(TG)_n.d(^{me}CA)_n$,$d(AGC)_n.d(GCT)_n$ and also the putrescinyl thymine containing DNA of phage ØW14. The specificity of these antibodies will be described in detail. For example the 'Z' DNA specific antibodies will only bind tightly to DNA which is in the 'Z' conformation and thus they can be used as probes for 'Z' DNA both *in vivo* and *in vitro*. Other antibodies will bind to $d(TG)_n.d(^{me}CA)_n$ in preference to $d(TG)_n.d(CA)_n$ or $d(TC)_n.d(GA)_n$ but the specificity is not absolute. The circular dichroism spectrum of $d(AGC)_n.d(GCT)_n$ suggests an unusual conformation which may be of the left-handed variety and the monoclonal antibodies are being used to assess the availability of this structure to other polymers. Finally the antibodies raised against phage ØW14 DNA are being used to map the distribution of the putrecinyl thymine bases by direct visualization in the electron microscope.

0716 THE PHYSICAL BASIS FOR DYNAMIC CONTROL OF THE O_R/O_L REGION OF BACTERIOPHAGE LAMBDA
Madeline A. Shea and Gary K. Ackers, The Johns Hopkins University, Baltimore, MD 21218

We have developed a quantitative model for the dynamic network of processes in bacteriophage λ which maintain stable lysogenic growth and drive the irreversible switchover to lytic growth and replication. These processes include the antagonistic binding of cI repressor and cro to O_R and their concerted binding to O_L, the binding of RNA polymerase to P_{RM}, P_R and P_L, and the degradative action of recA protein on cI during induction of lysis. The model has two major components: (a) a statistical thermodynamic theory for the probabilities of the various configurations of O_R and O_L, based upon the known protein-DNA interaction energies, and (b) a kinetic model for the coupling of these probabilities to the net production of cI, cro and N proteins. Promoter activities as well as protein levels are explicitly predicted. The model correctly predicts maintenance of the lysogenic state through synthesis of cI and repression of cro and N. During induction of lysis, it predicts a burst of cro and N protein synthesis followed by repression of P_R and P_L by cro. This turnoff of early genes is one of several predicted features not explicitly programmed into the model but which arise as a direct consequence of the variations in binding energies and fluctuations in concentrations of repressor and cro during induction of lysis. This model and the results obtained through its use demonstrate a powerful approach to understanding how complex dynamic properties in genomic regulatory systems may arise from fundamental interactions among their molecular constituents. The success of the model to predict physiological characteristics for mutant and wild-type phage provides support for the underlying physical assumptions used in its formulation.

0717 FORMATION AND PROGRESSION OF STABLE E. COLI DNA REPLICATION COMPLEXES, R. LaDuca*, J. Crute*, P. Fay*, C. Chuang*, K. Johanson†, C. McHenry†, and R. Bambara*, *University of Rochester, N.Y., 14642, and †University of Texas, Houston, TX., 77025.

Processive synthesis of DNA products greater in length than 100 nucleotides on a poly dA·oligo $(dT)_{10}$ primer-template results from the formation of a stable replication complex between Escherichia coli DNA polymerase III holoenzyme and the DNA. Most efficient formation of replication complexes capable of long product synthesis is achieved when the holoenzyme and DNA template are supplemented with ATP, excess β subunit and E. coli single-stranded DNA binding protein. Efficient reconstitution of holoenzyme complex forming activity from DNA polymerase III* requires excess β subunit at low ionic strengths (75 mM). At higher ionic strengths (155 mM), reconstitution requires ATP and excess β subunit.

The progression of four DNA polymerase III enzyme forms on specifically primed single-stranded fd DNA templates was also examined. DNA synthesis in vitro by each enzyme form generated products with the 3'-ends pausing at a limited number of sites located nonrandomly along each of the templates. The location and base composition of pause sites were determined at the resolution of a single nucleotide by comparison with products synthesized from the same primer-template using dideoxynucleotide sequencing methods. Many template sites where synthesis pauses correlate to regions of predicted secondary structure. Additionally, dA-rich regions on the template strand promote pausing. (This work was supported by NIH grants GM24441, CA-09363 and CA11198 and ACS grant FRA-220.)

0718 A BIOTIN-AVIDIN AFFINITY SYSTEM TO ISOLATE NEWLY REPLICATED NUCLEOSOMES, Timothy M. Herman, Mary L. Shimkus and Brian Castlemain, Medical College of Wisconsin, Milwaukee, WI 53226

A biotin-avidin affinity system is being developed to isolate nucleosomes containing newly replicated DNA. The feasibility of this approach has been demonstrated by nick-translating 145 bp DNA fragments (nucleosome length DNA) in the presence of ^{32}P-dCTP and biotin-labeled dUTP (Bio-dUTP). The Bio-dUTP-labeled DNA fragments, which quantitatively bind to avidin-agarose affinity columns, can be mixed with purified core histones in 2M NaCl and then dialyzed into 50mM NaCl to generate 11S nucleosomes. Thus, the presence of the biotin-labeled nucleotide in the DNA fragment does not prevent its in vitro assembly into a nucleosome. Furthermore, the Bio dUTP-nucleosomes are still quantitatively bound by an avidin-agarose affinity column. The avidin-agarose columns are highly selective for Bio-dUTP-nucleosomes in that 95% of the ^{32}P-Bio-dUTP-nucleosomes are bound to the column even in the presence of a 10,000-fold molar excess of unlabeled nucleosomes. Current efforts are directed toward the development of an in vitro DNA replication system that will incorporate the Bio-dUTP into DNA at active DNA replication forks. With such a system it should be possible to obtain a population of nucleosomes that have originated immediately downstream from active DNA replication forks. Analysis of this population of nucleosomes should give valuable information as to the molecular mechanisms of nucleosome assembly at DNA replication forks.

0719 Thermal Characteristics of Chromatin in Nuclei,Cells and Tumour Tissue, Horst H.Klump,Inst. Physical Chem. Univ. Freiburg,West Germany,D-78

Up to now a great body of calculated and of experimentally obtained data on the thermodynamic properties of dilute aqueous solutions of proteins and of nucleic acids have been accumulated. However ,knowledge of the physicochemical data of the individual biopolymers in solution is assumed to be of minor interest since most of them function in vivo as part of a complicated system.

Starting from isolated core particles and soluble chromatin as the reference system to determine the transition enthalpy of DNA bound to specific proteins this series of investigations covers also investigations on complete nuclei ,cell suspensions of rat liver cells ,and of transformed cells from mouse fibroblasts and from Hela cells.

Characteristic for the thermal denaturation of chromatin is a prtransition around 68°C and a main transition around 83°C.This can be monitored by either following the absorbance at 260nm as functionof the temperature or by measuring the additional heat capacity as a function of the temperature.The experimentally obtained value for the transition enthalpy of a base pair going from helical to the coiled state amounts to 11kcal/mbp as well for isolated core particles as for Hela cells.The results for rat liver nuclei and for mouse fobroblasts come close to the same result.This is the first example of continuous series from DNA to complete cells.

0720 COVALENT BINDING OF SUBSTRATE DEOXYNUCLEOSIDE TRIPHOSPHATE TO E. COLI DNA POLYMERASE I. Mukund J. Modak and Kakkudiyil Abraham. Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

We have developed a technique for the covalent attachment of unsubstituted deoxynucleoside triphosphates as well as 8-azido ATP to E. coli DNA polymerase I utilizing ultraviolet irradiation. The biochemical characterization of UV-mediated linkage of substrate dNTP and ATP to the enzyme revealed properties which were identical to those of substrate-enzyme complex formation, i.e., the presence of a divalent cation is required, competition for binding is exhibited with various dNTPs, and the sensitivity of binding to the presence of substrate binding site-directed reagents such as PPi and pyridoxal phosphate may be demonstrated. The UV-mediated cross-linking of substrate occurs independent of the presence of template-primer and the addition of a template-primer to the cross-linking reaction significantly reduces the quantity of non-substrate dNTPs and ATP bound to the enzyme. The UV-mediated cross-linking reaction is also significantly inhibited by the presence of o-phenanthroline. We have isolated, after cross-linking, a single peptide from proteolytic digests of E. coli DNA polymerase I that appeared to be the site of binding for both dTTP and azido-ATP.

0721 MINICHROMOSOME REPLICATION IN E. COLI MUTANTS WITH INCREASED OR DECREASED DNA SUPERCOILING, Alan C. Leonard and Charles E. Helmstetter, Roswell Park Memorial Institute, Buffalo, NY 14263

The role DNA supercoiling plays in minichromosome replication was investigated utilizing topoisomerase I-deficient mutants of E. coli. The minichromosome pAL1, comprised of a 9 kb. oriC-containing EcoRI fragment joined to a 7 kb. kanamycin resistance determinant, and a series of pAL1 deletion derivatives were studied. These minichromosomes were transformed into recA derivatives of topl0, topl0 gyrB225, and Δ(top-cysB) gyrB225 mutants. It has been determined that topl0 gyrB225 and Δ(top-cysB) gyrB225 mutants harbor DNA at decreased superhelical density with respect to top$^+$ strains. A topl0 mutant lacking the compensatory mutation in gyrB harbors DNA at increased superhelical density with respect to top$^+$ strains. The following results were obtained from minichromosome replication studies in these mutants: 1) the minichromosome pAL1 and its deletion derivatives replicated in the increased supercoiling mutant and were maintained at increased average superhelical densities. In addition, loss of topoisomerase I activity appeared to affect the rate at which minichromosomes passed through replicative intermediate forms. 2) pAL1 replicated in the decreased supercoiling mutants but all deletion derivatives examined thus far did not. Experiments were also performed to examine the replication of minichromosomes as a function of superhelical density in gyrB(ts) strains grown at various temperatures. All of these data support the notion that critical degrees of supercoiling are required for initiation of minichromosome replication and that the pAL1 deletion derivatives were not able to achieve this critical superhelical density in the decreased supercoiling mutant.

0722 MECHANISM OF TYPE 1 TOPOISOMERASES, Frank B. Dean* and Nicholas R. Cozzarelli,+ *Dept. of Biochemistry, University of Chicago, Chicago, IL 60637 and +Dept. of Molecular Biology, University of California, Berkeley, CA 94720.

Type 1 topoisomerases break and rejoin DNA segments a single strand at a time. Thus it was surprising that type 1 enzymes such as E. coli topoisomerase I catenate and knot circles of duplex DNA. The explanation emerged from the finding that nicked circles are required for these reactions. We propose that the enzyme makes a transient break in the strand opposite the nick and thereby creates the double strand break that allows formation of catenanes and knots. Consistent with this, we find that topoisomerase I acts with high preference on the DNA opposite a pre-existing nick. Topoisomerase I binds with greater affinity to even singly nicked plasmid DNA than to fully duplex DNA. Breaks in DNA resulting from trapping of the covalent DNA-enzyme intermediate are observed. Linearization of supercoiled circles occurs with covalent attachment of at least two topoisomerase molecules, but linearization of nicked plasmid circles requires the concomitant attachment of only a single enzyme molecule. Breaks observed on a fragment containing a uniquely located pre-existing nick occur near the site of the nick. Topoisomerase I may recognize a disordered structure in the DNA strand opposite the nick that is similar to single strand DNA. The breaks opposite a nick follow the same rule of site specificity found for single-stranded DNA. All are four nucleotides to the 3' side of a cytosine residue. The distance from the nick at which breaks occur can vary, but all occur at the same sites of breakage observed when the segment is single-stranded. Preference for binding a disordered region can also explain the heightened affinity of the enzyme for negatively supercoiled DNA. Thus all reactions of topoisomerase I are explained by a model in which DNA is passed through a transient enzyme-bridged break in single-stranded DNA.

0723 SUPERHELICAL DNA NICKING ACTIVITY ASSOCIATED WITH TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE, Martin R. Deibel, Jr., University of Kentucky, Lexington, KY 40536

An endonucleolytic activity has been detected and characterized in several homogeneous preparations of terminal transferase (TdT), a DNA polymerase which does not use a template. These TdT proteins were isolated from calf thymus gland and from the lymphoblasts of leukemic patients. The nuclease and polymerase activities co-purified during chromatography on oligo(dT) cellulose, co-migrated on isoelectric focusing gels, and co-eluted on gel filtration columns. Both activities showed comparable response to thermal denaturation, dilution and salt concentration, but were relatively insensitive to controlled proteolysis by trypsin. These data suggest that the nuclease activity is tightly associated with the TdT protein from at least two species, and suggest that it may be biologically relevant to the in vivo function of TdT, which is still an enigma. This nuclease activity, which converts superhelical DNA to nicked circular and linear forms, is shown by redigestion with single cut restriction enzymes of the linear DNA product to catalyze random nicks in supercoiled DNA. K_m and V_{max} values for this reaction (SV40 DNA, form I) were determined as 0.60 ± 0.12 ug (DNA) and 1 ug DNA nicked per hr per 80 ng of protein, respectively. Activity with circular or linear duplexes or single-strand ed DNA was not observed. Lack of sensitivity of the nuclease activity to N-ethyl maleimide, a potent inhibitor of the polymerase activity, and a lack of observable modulation of the nuclease activity by polymerase substrates, supports the premise that the two activities do not share a common active site. However, it remains to be determined if the nuclease catalytic site resides on the TdT protein. (Supported by a grant from the National Institute of General Medical Sciences (GM31076) and by a BRSG grant from NIH (RR 05374).

0724 DNA STRAND TRANSFER BY EUKARYOTIC DNA TOPOISOMERASES, Brian D. Halligan, Kenneth A. Edwards, and Leroy F. Liu, Johns Hopkins University, School of Medicine

We have studied the DNA strand transfer reaction of DNA topoisomerase I from HeLa cells and calf thymus as a possible mechanism of illegitimate recombination. In this reaction, single-stranded DNA can be fragmented spontaneously, and a covalent DNA-protein linkage formed via a 3'-phosphoryl bond. This DNA-enzyme complex can be covalently transferred to another double-stranded DNA fragment possessing a 5'-hydroxyl group or cyclize intramolecularly. To determine if this in vitro reaction may be analogous to the illegitimate recombination observed in vivo in eukaryotic cells, we have sequenced the cleavage sites of topo I on SV40 DNA. From an analysis of the sequence data, it appears that although there is no sequence specificity to the cleavages, cleavage occurs at specific sites on the molecule. There is a strong preference for the presence of a pyrimidine nucleotide at the topo I linkage on the 3' side of the cleavage site.

We have observed that DNA topoisomerase II can spontaneously cleave double-stranded DNA, although at a low frequency. Since DNA gyrase, a prokaryotic type II topoisomerase, has been shown to be required in cell free illegitimate recombination systems, it is possible that eukaryotic topoisomerase II may have a direct role in illegitimate recombination. Preliminary results indicate that after cleavage, topo II is covalently bound to the 5'-hydroxyl end of the fragmented DNA. We are currently determining if topo II can promote a strand transfer reaction, similar to that of topo I, and the sequence of the sites of topo II cleavage on SV40.

0725 A RAPID PURIFICATION OF A DNA TOPOISOMERASE I FROM MOUSE MAMMARY CARCINOMA CELLS AND ITS INHIBITION BY HEPARIN, Kazuyuki Ishii, Toshimitsu Hasegawa, Keiko Fujisawa, Sakurako Futaki, Gen-Ichiro Soma, Hideki Uchiyama,* Kinzo Nagasawa,* Nobuko Seno** and Toshiwo Andoh, Meiji College of Pharmacy, Tanashi, Tokyo 188, Kitasato University, Faculty of Pharmaceutical Sciences, Shirokane, Minato-ku, Tokyo 108* and Ochanomizu University, Faculty of Science, Ohtsuka, Bunkyo-ku, Tokyo 112**

We have previously shown that DNA topoisomerase I is inhibited by heparin. Taking advantage of this enzyme-heparin interaction we developed a rapid method of purification of this enzyme by two step column chromatography on heparin-Sepharose followed by pheny-Sepharose. The enzyme was purified to near homogeneity from chromatin extract of mouse mammary carcinoma cells with an overall yield of 60%. Molecular weight of the enzyme was estimated to be 96,000. We are currently under way in cloning of the gene for the enzyme from mouse genome. In commercially available heparin preparations more than 50% of the molecules lack the ability to bind to antithrombin III (AT-III) and is thus devoid of anticoagulant activity. The AT-III-binding sequence of the active heparin molecules was proposed to be a hexasaccharide of defined sequence. We found, however, that heparin fraction with low or no affinity for AT-III, and therefore with no anticoagulant activity showed a comparable inhibitory activity on DNA topoisomerase I as the fraction with high affinity for AT-III. This holds without regard to the molecular weight of the heparin fractions. This finding strongly suggests that heparin molecules contain another biologically active site within the molecule, i.e. topoisomerase I-binding sequence of oligosaccharide which is distinct from the well characterized AT-III-binding sequence and is distributed among heparin fractions with varing AT-III-binding activity.

0726 E. COLI TOPOISOMERASE MUTATIONS AND SUPERCOILING: A NOVEL STRAIN IN WHICH OXOLINIC ACID INCREASES THE LEVEL OF CHROMOSOMAL SUPERCOILING, Gail J. Pruss, Stephen H. Manes, and Karl Drlica, University of Rochester, Rochester, NY 14627

A mutation in the structural gene (*top*) for topoisomerase I which eliminates topoisomerase I activity causes an increase in DNA supercoiling *in vivo*; however, *top*⁻ strains frequently acquire mutations in *gyrB* or *gyrA* which reduce overall supercoiling to a level below that found in wild-type strains (Pruss *et al.*, Cell 31, 35-42, 1982; DiNardo *et al.*, Cell 31, 43-51, 1982). A *gyrA* (NAL^r) transductant of one such *top*⁻ strain, DM800 (=Δ*top*, *gyrB*225), is partially sensitive to the gyrase inhibitor oxolinic acid (oxo) and exhibits a novel response to that drug: treatment of cells with oxo causes chromosomal supercoiling to increase to higher than the wild-type level. We have used this strain to examine whether reduced supercoiling is responsible for inhibition of RNA synthesis by oxo *in vivo*. Oxo inhibits RNA synthesis in DM800*gyrA* (NAL^r) even though supercoiling increases. Therefore, loss of supercoiling is not necessary for inhibition of RNA synthesis by oxo. This experiment, like the discovery of gyrase mutations which eliminate the effect of *top* mutations on supercoiling, emphasizes that supercoiling must be measured before one can draw conclusions about the relationship between gyrase, supercoiling, and gene expression.

0727 SITE SPECIFICITY OF THE BACTERIOPHAGE T4-INDUCED TYPE II TOPOISOMERASE, Kenneth N. Kreuzer and Bruce M. Alberts, Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

Bacteriophage T4 induces a type II topoisomerase that is implicated in the initiation of T4 DNA replication. We examined the site-specificity of the enzyme to look for possible origin-specific binding. The topoisomerase inhibitor oxolinic acid results in near-quantitative recovery of a covalent complex of enzyme and broken DNA. A filter-binding assay was developed to purify these cleavage complexes, allowing an analysis of the enzyme binding sites on T4 DNA. The glucosyl modifications of native T4 DNA increased the specificity of topoisomerase, so that only about 6 to 8 major cleavage sites were recognized. One of these sites maps about 250 base pairs downstream from a strong early promoter in the region of the primary replication origin of T4. Recognition of an early promoter by host RNA polymerase is required for primary initiation,[1] and studies are in progress to look for interactions between these two enzymes at their adjacent binding sites. A second strong cleavage site maps near the best-studied recombination hotspot in the T4 genome. Recombination from this hotspot requires glucosylated DNA,[2] correlating to the in vitro effect of this modification on topoisomerase site specificity. Possible relationships between topoisomerase site specificity, recombination, and secondary initiation of replication forks (which depends on recombination[1]) will be discussed.

[1] Luder, A. and Mosig, G. (1982). Proc. Natl. Acad. Sci. USA 79, 1101.
[2] Levy, J. and Goldberg, E. (1980). Genetics 94, 519.

0728 UNIDIRECTIONAL ORIGIN INITIATION OF T4 DNA REPLICATION, DEFECTIVE RECOMBINATIONAL INITIATION AND ALTERED LATE TRANSCRIPTION IN TOPOISOMERASE MUTANTS, Gisela Mosig, Margaret Levin and Ruth Seaby, Vanderbilt University, Dept. of Molecular Biology, Nashville, TN 37235

Phage T4 genes 39, 52 and 60 code for a type II topoisomerase. DNA replication and progeny production in these mutants is delayed and reduced. It has been reported that residual DNA replication in the mutants depends on host gyrase and that T4 topoisomerase is an origin specific gyrase which can be replaced by host gyrase.

We have been unable to confirm these reports. We found DNA replication of T4 topoisomerase mutants to be indistinguishable in E. coli gyr$^+$ and ts gyrB hosts (as is DNA replication of wild type T4). DNA delay mutants produce, however, no progeny when host gyrB is defective. This defect is explained by differential alteration of late gene expression. Little or no major head protein (gp23) is synthesized by the T4 topoisomerase mutants in a gyr$^-$ host, whereas expression of other late T4 genes is unaffected or enhanced. Neither phage nor host mutants alone show similar effects.

The T4 topoisomerase mutants initiate the first round of DNA replication from the same origin, as wild type T4. In contrast to wild type, however, DNA replication remains unidirectional, in the same direction as early transcription, and initiation from recombinational intermediates is defective.

This finding has allowed mapping of a major origin near the junction of XbaI fragments 15 and 17, at 15.8 kb on the T4 map. The initiation in the absence of T4 topoisomerase indicates that topoisomerase-induced unwinding of an origin is not required prior to initiaton of replication.

0729 STABILIZATION OF DNA CONFORMATION BY SUPERCOILING AND DNA GYRASE, Richard Otter, David M. Lilley*, and Nicholas R. Cozzarelli, Dept. of Molecular Biology, Univ. of California, Berkeley, CA 94720 and *Dept. of Biochemistry, The University, Dundee, Scotland, U.K. DD1 4HN.

Supercoiling favors several alternate DNA conformations including single strand regions, Z DNA, and cruciforms. We used 2-D composite acrylamide-agarose gel electrophoresis to study structural transitions in small circular DNAs with varying superhelical density (σ). Topoisomers with high negative σ have aberrant mobility. In TBE buffer, the pAO3 topoisomer with σ=0.05 smears toward lower mobiity whereas topoisomer (σ=0.06) has the same moblity as topoisomers with σ=0.03. Further discontinuities in mobility vs. σ are seen at higher negative σ. In the presence of Mg^{2+}, several topoisomers run as 2 distinct bands. The major transition is interpreted as the formation of the cruciform with a 13 bp stem and 5 b loop as seen by Lilley (PNAS 77, 6468 1980) in the parental plasmid Col E1 because: 1) the predicted ΔWr of 3.1, compares favorably with the observed ΔWr of 3.5, 2) topoisomers with $\sigma > 0.04$ show nuclease S1 sensitive sites which map to the postulated hairpin loop, while topoisomers with $\sigma < 0.04$ are not cut, 3) such effects are not observed with positively supercoiled DNA where mobility increases monotonically with σ. These methods allow observation of cruciforms in the absence of a protein which may perturb the equilibrium between conformations.

DNA binds to DNA gyrase by wrapping around the outside of the enzyme which results in stabilization of positive supercoils. Use of small plasmids allows quantitation of the number of supercoils protected by gyrase binding. Initial experiments show about two positive supercoils protected per gyrase molecule.

0730 GYRASE-DNA COMPLEXES VISUALIZED AS LOOPED STRUCTURES BY ELECTRON MICROSCOPY, Claire L. Moore, Leonard Klevan, James Wang, and Jack Griffith, University of North Carolina, Chapel Hill, North Carolina 27514

Gyrase bound to duplex DNA in the absence of ATP is seen by electron microscopy as a nearly spherical particle frequently located at the intersection of two duplex DNA strands. Such looped structures with gyrase situated at the base of the loops are observed with both linear and circular DNA substrates, and two or three individual DNA molecules bound to the same protein are also seen at high DNA concentrations. Addition of the non-hydrolyzable β,γ-imido analog of ATP to the gyrase-DNA reaction mixture prior to sample fixation for microscopy reduces the frequency of gyrase molecules found at DNA intersections. Looped structures similar to those of the gyrase-DNA complex are also seen with the complex of DNA and the A subunit of gyrase. When negatively supercoiled DNA which has been partially relaxed by gyrase in the absence of ATP is fixed for electron microscopy, intermediate forms are observed that contain both supercoiled and relaxed loops in a single DNA molecule, with the enzyme located at the common base of the loops. These results suggest that gyrase possesses multiple DNA binding sites, a feature which allows the enzyme to hold DNA in constrained loops. The relation of these observations to the mechanism of gyrase action will be presented.

0731 REVERSIBLE DECATENATION OF KINETOPLAST DNA BY A UNIQUE DNA TOPOISOMERASE ACTIVITY FROM CRITHIDIA FASCICULATA, Joseph Shlomai and Anat Zadok, The Hebrew University, Jerusalem, Israel

An enzymatic activity detected in cell extracts of the hemoflagellate Crithidia fasciculata interlocks kinetoplast DNA minicircles into network forms resembling the natural kinetoplast DNA (kDNA) found in trypanosomatids. Partial purification and characterization of the enzyme responsible for this activity has revealed the existence of an ATP-dependent DNA topoisomerase in Crithidia fasciculata. Catenation of duplex kDNA minicircles is reversible, the equilibrium is affected by the ionic strength, and the reaction is sensitive to high levels of the drug novobiocin. The enzyme capability to discriminate between newly replicated and non-replicated kDNA minicircles as substrates for decatenation suggest that it could fit a role for the "release and reattachment" enzyme postulated by Englund and his colleagues (Englund, 1980) to function in the course of kDNA replication. Other catalytic properties of this enzyme are in support of such a role for the crithidial topoisomerase.

0732 DNA TOPOISOMERASE II FROM *DROSOPHILA MELANOGASTER*, Neil Osheroff, Earl R. Shelton, Denise Lew and Douglas L. Brutlag, Stanford University, Stanford, CA 94305

The type II DNA topoisomerase from *D. melanogaster* was purified from the nuclei of 6 to 18 hr old embryos. The enzyme is a homodimer with a subunit molecular weight of 166,000 and a frictional ratio of 1.66. In order to study the double strand-passage reaction of type II topoisomerases, a quantitative assay was developed to monitor the enzymic interconversion of supercoiled circular DNA to relaxed circular molecules. Under conditions of maximal activity, relaxation is processive and removal of supercoils is accompanied by the hydrolysis of either ATP or dATP to the corresponding nucleoside diphosphate and inorganic phosphate. Apparent K_m values are 200 μM for pBR322 plasmid DNA, 280 μM for ATP, and 630 μM for dATP. The turnover number for the topoisomerase is at least 190 supercoils relaxed per min per molecule of enzyme. The protein interacts preferentially with supercoiled over relaxed DNA and is strongly inhibited by single-stranded molecules. Kinetic and inhibition studies indicate that the β and γ phosphate groups, the 2'-OH of the ribose ring, and the C_6-NH_2 of the adenine ring are important for the interaction of ATP with the enzyme. The ATPase activity of the topoisomerase is stimulated 17-fold by supercoiled DNA and ∿4 molecules of ATP are hydrolyzed per supercoil removed. Based on these kinetic studies, a model has been developed to describe the processive *vs.* distributive nature of the relaxation reaction. In addition, antibodies against the *Drosophila* topoisomerase II have been raised in rabbits. These antibodies interact specifically with the *Drosophila* protein and inhibit relaxation by 50% at a 200-fold dilution of the antiserum. Supported by NIH grant GM-28079.

0733 DNA TOPOISOMERASES FROM RAT LIVER : PHYSIOLOGICAL VARIATIONS, Michel Duguet, Catherine Lavenot, Francis Harper, Gilles Mirambeau and Anne-Marie De Recondo, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France.

DNA topoisomerases are enzymes which catalyze the in vitro conversion of one DNA topological isomer to another. Their fundamental importance in nucleic acids metablolism has only begun to emerge as a consequence of the rapid accumulation of information on these proteins in recent years. DNA topoisomerases are presumably involved in a number of biological functions, including DNA replication, repair, recombination and chromatin condensation and decondensation.

Besides the nicking-closing (topoisomerase I) activity, we have isolated an ATP-dependent DNA topoisomerase from rat liver nuclei. The enzyme,partially purified, is able to catenate in vitro closed DNA circles in a magnesium-dependent, ATP-dependent, histone H1-dependent reaction, and to decatenate in vitro kinetoplast DNA networks to yield free minicircles in a magnesium-dependent and ATP-dependent reaction. It is largely similar to other eukaryotic type II topoisomerases in its requirements, and presumably belongs to this class of enzymes. Type I and type II activities were measured in rat liver nuclei as a function of regenerating time after partial hepatectomy : type I activity was not significantly changed during this process. In contrast, type II activity was considerably increased, suggesting a possible involvement of the enzyme in DNA replication.

0734 ISOLATION OF A DNA TOPOISOMERASE FROM HUMAN LEUKEMIC CELL MITOCHONDRIA, Frank and Gary M. Lazarus, University of Maryland Baltimore County, Catonsville, MD

The occurrence of complex mitochondrial DNA (mtDNA) has been well documented and o these species, the circular dimer, can be diagnostic for acute granulocytic leukemias normal monomeric mtDNA and these complex forms (circular dimers and catenanes) are re topological isomers, we have undertaken a study of the topoisomerase activity in mito from human leukemia cells.

To this end we have obtained white blood cells from patients who have undergone le resis. Leukocytes were prepared by Ficoll centrifugation, and disrupted mechanically multiple passes in a ground glass homogenizer. The nuclear and mitochondrial fractio separated by differential centrifugation. Mitochondria were lysed in phosphate buffe taining 1 M NaCl and 1% nonionic detergent NP40. The lysate was treated with 6% poly glycol to remove mtDNA. Using agarose gel electrophoresis, this mitochondrial PEG su was shown to relax supercoiled pBR322 in the presence or absence of added ATP. The a ture also contained faint bands of DNA species moving only slightly into the gel.

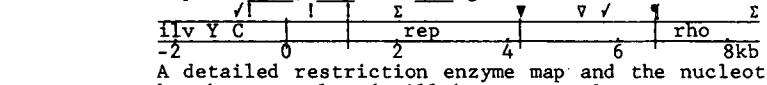
The extract was further fractionated on CM-cellulose, which results in an overall purification of an ATP-independent mitochondrial topoisomerase. Preliminary results permeation chromatography indicate that at least two activities of different molecula possess the ability to relax DNA in an ATP-independent reaction.

0735 TOPOISOMERASE III - A NEW ESCHERICHIA COLI TOPOISOMERASE, Martin M. Matzuk Martine Pastorcic+, and Nicholas R. Cozzarelli*, University of California, Berkeley, CA 9 and University of Chicago, Chicago, Illinois 60637+.

Until now, only three Escherichia coli topoisomerases, topoisomerase I (omega protein), topoisomerase II (DNA gyrase), and topoisomerase II ', have been identified. We have purified a n topoisomerase similar to topoisomerase I from both wild-type E. coli and a topoisomerase I deleti mutant, DM800. This new enzyme, topoisomerase III, consists of a single polypeptide chain and h molecular weight of about 75,000 and a sedimentation value of 4.9S. It is a type 1 topoisomerase requires only Mg^{++} to relax negatively supercoiled DNA and to catenate nicked DNA in the preser of spermidine. The enzyme also acts optimally at 52°C. Inhibitors of both DNA gyrase and topoisomerase II ' as well as anti-omega antibodies failed to inhibit topoisomerase III. In addition, cleavage specificities of topoisomerases I and III on single-stranded DNA are distinct. Topoisomerase III is a major E. coli topoisomerase present at about half the level of topoisomeras in wild-type cells.

0736 CLONING AND ANALYSIS OF THE E. COLI REP GENE, Hanna Bial Hobrzanska, Carol Gilchrist and David T. Denhardt, Cancer R Lab., University of Western Ontario, London, Ontario, N5X 2N6, Canada.

The E.coli rep protein is a ssDNA-dependent ATPase (DNA helicase III hashi et al. Can. J. Biochem. 57, 855, 1979) which is essential for un of duplex DNA during in vitro and in vivo replication of several ba phages (ØX174, fd, P2). E. coli rep deletion mutants isolated in thi as a result of MuctsX excision appear to have a reduced replication for city. We have isolated the rep gene of E. coli on a 2.9 kb EcoRI-Pst ment from the Clarke-Carbon plasmid pLC44-7, cloned it into pSC101, a cloned fragments into M13mp8. The coupled in vitro transcription-tran system of Zubay was used to show that the rep gene-containing plasmi encodes a protein that migrates in the position of 68000 daltons, whic agreement with the results of others obtained with purified rep protei map of ilvC, rep and rho genes is:



A detailed restriction enzyme map and the nucleotide sequence in so fa has been completed will be presented.

This research was supported by the National Cancer Institute of

0737 MECHANISM OF INHIBITION OF DNA SYNTHESIS IN VITRO BY A DOUBLE-STRANDED DNA BINDING PROTEIN INDUCED BY BACTERIOPHAGE T5, Robert K. Fujimura and Barbara C. Roop. Biol. Div., ORNL, Oak Ridge, TN 37830.

Product of gene D5 (gpD5) of bacteriophage T5 is a DNA binding protein that binds preferentially and cooperatively to double-stranded DNA and is essential for T5DNA replication. However, in vitro it inhibits both polymerization and 3'→5' exonucleolytic activities of the DNA polymerase that is bound to a primer-terminus of a primer-template. The primary cause of inhibition is not inhibition of binding of the DNA polymerase to gpD5-DNA complex. Studies with nicked, gapped, and primed single-stranded DNA as primer templates showed that inhibition occurs at 3'OH terminus of a primer. When duplex region of a primer-template is saturated with gpD5, translocation process of the polymerase appears inhibited. Competition experiments with primed-single stranded and double-stranded DNA showed that when double-stranded DNA is in excess of gpD5, there is no inhibition of synthesis from primed single-stranded DNA. These studies indicate that the role of gpD5 in vivo is to cover up duplex DNA, preventing initiation of DNA synthesis from any nicks and gaps, and to induce initiation and replication complexes to bind to specific sites that have lower affinity for gpD5. A subfraction of gpD5 is known to be phosphorylated, and it may also take part in initiation of DNA replication from a specific site. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with the Union Carbide Corporation.

0738 COAT PROTEIN AND DNA STRUCTURES IN FILAMENTOUS VIRUSES fd, If1, IKe, Pf1, Xf and Pf3, George J. Thomas, Jr. and Loren A. Day, Southeastern Massachusetts University, North Dartmouth, MA 027047, and The Public Health Research Institute, New York, N.Y. 10016.

Laser Raman and CD spectroscopy provide information about protein and DNA secondary structures and about molecular interactions involving protein and DNA subgroups of viruses. In this study we have obtained and compared laser Raman and CD spectra of six filamentous viruses of known composition. All viruses contain unorthodox DNA structures and coat proteins of substantial α-helix structure. The Raman spectra reveal remarkable variability of structure in each virus, particularly with respect to changes in temperature. The temperature profile of a given virus, as monitored by its Raman line frequencies and intensities, is usually different in solutions containing low salt (0.001M Na^+) than in solutions containing moderate (0.05M Na^+) or high salt (0.15M Na^+) concentration. The extent of α-helicity and its vulnerability to change with adjustments in temperature and salt concentration are significantly different in each virus. Refinement of the laser Raman spectra by computer averaging techniques and spectral subtraction routines allows identification of specific amino acid and nucleotide residues which interact in the native structures and which are involved in structure transitions.

Supported by N.I.H. Grants AI 09049 (L.A.D.) and AI 11855 (G.J.T.).

0739 "GENE 5 PROTEIN COMPLEXES" of Pf3, Pf1, IKe and fd BACTERIOPHAGES. Arturo Casadevall, Debra Gluck Putterman, Stephen A. Reisberg, and Loren A. Day. The Public Health Research Institute of the City of New York, New York, N.Y. 10016.

Filamentous complexes between viral DNA-binding proteins, called "gene 5 proteins", and circular single-stranded DNA molecules have been isolated from Pf3, IKe, Pf1 and fd infected cells. All the complexes are presumed to be products of DNA replication and precursors for virus assembly as has been shown in the fd system. They have been characterized according to sedimentation properties, DNA content, CD and absorbance spectra, contour lengths and mass-per-lengths by STEM (in collaboration with J. S. Wall, Brookhaven). The four complexes have similar overall morphologies in that they are unbranched yet very flexed filaments, with lengths between 0.5μ and 1μ. CD shows little α-helicity in the proteins and little base-base stacking in the DNAs. Most notable are differences in protein subunit molecular weights: 6200 for Pf3 and and 11000 for IKe as compared to literature values of 9600 for fd and 16500 for Pf1. The amino acid composition of the Pf3 "gene 5 protein" diverges widely from those in the literature for Pf1 and fd, which also differ widely. DNA structures in the complexes differ from each other and from the diverse DNA structures in the virions to which they give rise. As examples, the virions of Pf3 and Pf1 both appear to have highly unusual inside-out DNA helices with phosphates in (I-DNA), the axial nucleotide translations being 2.3 Å in Pf3 but 5.4 Å in Pf1, whereas their precursor structures have nucleotide translations of only 1.7 Å and 2.7 Å, respectively.

Supported through grants AI 09049 (L.A.D.) and T32-GM 07308 (A.C. and S.A.R.).

0740 SYNTHESIS OF COLE1 REPLICATIVE INTERMEDIATES CONTAINING INCOMPLETE LEADING STRANDS BY EXTRACTS OF E. COLI SSB MUTANT STRAINS, R.E. Bird and L. Fouser, Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235

We have studied the role of single-stranded DNA binding protein (ssb) in the replication of ColE1 DNA using extracts of E. coli strains carrying either the ssb1 or lexC113 mutations, two allelic ssb mutations. Analysis of ColE1 in vitro replication products by sedimentation on alkaline sucrose gradients demonstrated that mutant extracts synthesized large DNA fragments. Examination of these products, in the electron microscope revealed molecules containing large replication eyes with newly synthesized DNA on only one arm of the eye. These intermediates appeared to be initiated at the origin of replication since one end of the eye was 17% of unit length from an end generated by digestion of the intermediates with EcoR1. This was confirmed by gel electrophoresis of product which had been cleaved by HaeIII. The only bands containing newly replicated DNA were those immediately downstream from the origin. Product DNA hybridized only to isolated H strands of ColE1 DNA showing that only L-strands are made. Product was denatured in glyoxal and analyzed by gel electrophoresis to determine the size of the newly replicated DNA. The products contained newly replicated DNA strands which were an average of 4,000 bases for the ssb1 and 1,300 bases for the lexC113 strain. The missing activity in the mutant extracts was complemented by small amounts of extracts from a strain which overproduces ssb protein. We conclude that the E. coli ssb gene product is required for initiation of DNA synthesis on the lagging strand of ColE1 and for completion of the leading strand.

0741 EQUILIBRIUM AND KINETIC MEASUREMENTS OF THE INTERACTION OF THE E. coli SINGLE-STRANDED DNA BINDING PROTEIN (SSB) WITH SINGLE-STRANDED DNA (ssDNA) DETERMINED BY GEL ELECTROPHORESIS, James G. Wetmur, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Electrophoresis of ssDNA with a subsaturating amount of SSB leads to separation of various SSB-ssDNA complexes. When a 275-nucleotide ^{32}P-end labeled ssDNA is mixed with native SSB sufficient to protect 30% of a uniformly labeled ssDNA of identical concentration, six well resolved bands are detected following electrophoresis and autoradiography. The slowest moving band is the only band observed when the ssDNA is saturated with SSB. The fastest moving band is the only band observed in the absence of SSB. A double-label experiment using ^{14}C-labeled SSB was used to determine that each of more rapidly moving bands contained even numbers of SSB tetramers. An analysis of the levels of cooperativity in SSB-ssDNA interactions has been performed using various ssDNA and SSB concentrations as well as various ionic strengths. Additional studies have been performed to determine the rate of transfer of SSB from the SSB-ssDNA complexes to unlabeled recipient ssDNA molecules of various types. Parallel experiments using gel electrophoresis and filter binding assays have been performed for comparison.

0742 STRUCTURAL AND FUNCTIONAL HETEROGENEITY OF THE HELIX-DESTABILIZING PROTEINS FROM CALF THYMUS. Silvano Riva, Omar Valentini, Giuseppe Biamonti and Arturo Falaschi. Istituto di Genetica Biochimica ed Evoluzionistica del C.N.R. Via S. Epifanio, 14 27100 Pavia ITALY

We developed a new purification technique for the helix-destabilizing proteins from calf thymus which permits to evidence a high degree of heterogeneity within these proteins. The purified fractions are composed of many molecular species with molecular weight between 24 000 and 28 000 dalton and pI value between 6 and 8. Different fractions (containing different distributions of the various species) show significant differences with regard to the following properties 1) binding to single-stranded DNA 2) lowering of the melting temperature of poly [d(AT)] and 3) stimulation of DNA polymerase α on poly [d(AT)] template. Individual molecular species, separated as far as possible by isoelectric focusing in an ampholine-glycerol gradient, were submitted to limited tripsin digestion (in the presence or in the absence of SS-DNA) and analyzed by two-dimentional gel electrophoresis. Molecular species with different Mr and pI yielded apparently identical triptic fragments (same Mr and pI) which point to a remarkable homology in their primary structure. We hypothesize that the different species derive from one (or more) as yet unidentified large precursor by endogenous proteolysis.

0743 NUCLEIC ACID BINDING AFFINITY OF fd GENE 5 PROTEIN IN THE COOPERATIVE BINDING MODE, Albert M. Bobst, John C. Ireland and Elizabeth V. Bobst, University of Cincinnati, Cincinnati, Ohio 45221

The gene 5 protein product of bacteriophage fd is essential for DNA replication during the lifecycle of fd phage in E. coli. This laboratory has developed a sensitive electron spin resonance (ESR) methodology which allows a direct quantitative determination of nucleic acid binding affinities of proteins binding under physiologically relevant conditions to nucleic acids. This is achieved with spin labeled nucleic acids which are used as macromolecular spin probes in ESR competition experiments. The approach was first successfully applied to determine the relative affinity of gene 32 protein for various polynucleotides. Using the method on gene 5 protein resulted in the following affinity relationships:

$$K_{app}^{(dT)_n} = 10^3 K_{app}^{fdDNA} = 2 \times 10^4 K_{app}^{(A)_n} = 6.6 \times 10^4 K_{app}^{rRNA} = 1.5 \times 10^5 K_{app}^{R_{17}RNA}$$

The relative apparent affinity constants, K_{app}, were determined in 20 mM Tris HCl (pH 8.1), 1 mM sodium EDTA, 0.1 mM dithiothreitol, 10% w/v glycerol, 0.05% triton, and 125 mM NaCl. Increasing the [NaCl] from 125 mM to 200 mM caused considerably less tight binding of gene 5 protein to spin labeled $(A)_n$, $(\ell A,A)_n$, and a typical co-operative binding isotherm was observed, whereas at a lower [NaCl] the binding was essentially stoichiometric. A computer fit of the experimental titration data at 200 mM NaCl resulted in an intrinsic binding constant, K_{int}, of 1,300 M^{-1} and a cooperativity factor ω of 60 ($K_{int} \times \omega = K_{app}$) for $(\ell A,A)_n$ and gene 5 protein. (Supported in part by NIH grant GM-27002.)

0744 CROSSLINKING STUDIES OF THE T4 DNA REPLICATION COMPLEX IN VITRO, Joel W. Hockensmith, Frederic R. Fairfield, and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

The five gene products of T4 phage that participate in T4 DNA leading-strand synthesis have been crosslinked to the primer strand of a primer-template complex, using either broad-band ultraviolet radiation or coherent 266 nm radiation from a frequency-doubled YAG laser. For the protein complex associated with an oligo(dT)-poly(dA) primer-template junction, we find that the polymerase (gene product 43) and both types of subunits of the gene 44/62 polymerase accessory protein complex are crosslinked to the primer as determined by the co-migration of the protein and the 5' end-labelled primer DNA in SDS polyacrylamide gel electrophoresis. When complexed with the single-stranded oligonucleotide primer only, gene products 43, 44, 62, and 32 (single-stranded DNA binding protein) are all crosslinked to the DNA. If the laser is used as the source of photons, the efficiency of crosslinking increases three-fold relative to the broad-band procedure, and the irradiation time drops (by a factor of 10^9) to 6 nsec. This procedure is being applied in a study of the dynamics of synthesis of DNA using the five protein system with primed homopolymer and natural DNA templates (M13mp7 and related DNAs). (Supported by USPHS Research Grants GM-15792 and GM-29158 (to PvH) and USPHS Fellowship GM-08252 (to FRF).)

0745 PROTEIN-PROTEIN INTERACTIONS OF T4 GENE 32 PROTEIN, Junko Hosoda and Herb Moise Lawrence Berkeley Laboratory, University of California, Berkeley California 94720

T4 gene 32 protein (gp32) or its fragments lacking terminal regions have been covalently attached to agarose gel to study gp32 protein-protein interactions by affinity column chromatography. Over 20 (out of ~120) T4 early proteins bind to the intact gp32 column. When the column was made of 32*I, a proteolytic fragment lacking 51 residues from the carboxyl terminus, the majority of the gp32-binding proteins showed no or reduced affinities. When a column was made of Am-E1, an amber peptide lacking 15~20 residues, some gp32 binding proteins showed reduced affinities, but some had far stronger affinity than those for the intact gp32. The amE1 mutant was isolated by T. Minagawa as a pseudo-suppressor of tsC9 in gene 49 and it has phenotype of mini plaque and elevated UV-sensitivity. Its defective phenotype becomes more pronounced in a supressor-1 plus host, especially when the phage carries another mutation in gene 61. Further studies of amE1 to corelate its in vivo and in vitro activities are in progress.

GP32-gp32 binding constants (K) were calculated by the method of de Haseth et al (1977, Biochemistry, 16, 4777). Apparently 7~10% of the covalently attached gp32 molecules participate in a strong salt dependent gp32 binding with $k \simeq 1.2 \times 10^6 M^{-1}$ (0.05M NaCl) and $-\log K \simeq \log [Na^+]$. 32*II, a proteolysis product lacking 9 residues from the amino terminus showed no affinity for the gp32 column while 32*III, another proteolytic product lacking both the amino and carboxyl terminal regions showed reduced affinity of $K \simeq 1.5 \times 10^5 M^{-1}$ (0.05M NaCl).

0746 HELIX DESTABILIZATION BY BACTERIOPHAGE T4 DNA REPLICATION PROTEINS. Nancy G. Nossal, NIH, Bethesda, Maryland 20205

Unwinding the DNA ahead of the replication fork requires a minimum of five T4 proteins: the polymerase, gene 32 helix destabilizing protein, and the gene 44/62 and 45 polymerase accessory proteins, which keep the polymerase bound to the DNA. The rate is increased by the gene 41 priming protein, recently shown to be an ATP or GTP-dependent DNA helicase that unwinds short fragments annealed to single-stranded DNA beginning at their 3' termini [Venkatesan, Silver, and Nossal, JBC 257, 12426 (1982)]. Studies with the polymerase of the T4 antimutator mutant tsCB120 demonstrate that a point mutation in the polymerase gene decreases the ability of the T4 DNA replication complex to invade duplex templates. The mutant polymerase copies single-stranded homopolymers at rates comparable to the wild type enzyme, but is more inhibited by hairpins in poly d(A-T) [Gillin and Nossal, JBC 251, 5219 (1976)]. On restriction fragment primed single-stranded ϕX174 DNA the mutant enzyme is slowed down by pause sites not evident with the wild type enzyme, at temperatures from 30 to 42°. The CB120 polymerase is stimulated on this template by the 44/62, 45 and 32 proteins, but is still detained at pause sites to a far greater extent than the wild type polymerase. Moreover, at 30°, the CB120 polymerase is unable to carry out strand displacement synthesis on nicked duplex templates with the T4 gene 44/62, 45, 32 and 41 proteins. The replication of duplex DNA by T4tsCB120 at 30° *in vivo* may depend on additional helix destabilizing proteins. A likely candidate is the *E. coli* OptA gene product, since Gauss, Doherty and Gold (PNAS, in press) have shown that T4tsCB120 cannot replicate at any temperature in an OptA mutant host.

0747 NUCLEIC ACID SUBSTRATE RECOGNITION IN THE BACTERIOPHAGE T4 DNA REPLICATION COMPLEX, Leland S. Paul, John W. Newport, Mary C. Dasso and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

Several methods have been used to study nucleic acid interactions of the bacteriophage T4 protein products of genes 43, 44, 62, 45, and 32. We have made direct binding measurements of gene 43 protein (DNA polymerase) and gene 32 (single-stranded DNA binding) protein to several nucleic acid substrates. In addition, the intrinsic polymerase and nuclease activities of gene 43 protein and the nucleic acid-dependent ATPase activity of the accessory protein (gene 44, 62 and 45 proteins) complex have been examined using well-defined synthetic DNAs and RNAs.

For effective DNA synthesis to occur, the replication complex must recognize a primer-template junction. Our results indicate that the isolated polymerase has very limited capacity for primer-template recognition, and is effectively inhibited from enzymatically productive binding by single-stranded DNA or RNA. While gene 32 protein effectively coats single-stranded nucleic acids and thus reduces nonproductive polymerase binding, much of the specificity of the primer-template interaction is attributable to the accessory protein complex. A composite model describing some aspects of the functional binding of these accessory proteins to the primer-template junction, and their interaction with the polymerase and gene 32 protein will be described. (Supported by USPHS Research and Training Grants.)

0748 PROCESSIVITY STUDIES OF THE T4 DNA REPLICATION COMPLEX *IN VITRO*, Frederic R. Fairfield, John W. Newport, Mary K. Dolejsi, and Peter H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

We are examining the processivity of DNA synthesis catalyzed by the five protein (gene products 43, 44/62, 45, and 32) bacteriophage T4 DNA synthesis system. The effects of the nucleotide composition and sequence of the template on the ratio and length of products is being studied. Using an excess of primer-template complexes and a variety of assay conditions, we have determined the size distributions of the newly synthesized DNA by means of quantitative gel electrophoresis and autoradiography. When a homopolymer primer-template (e.g. oligo(dT)-poly(dA)) is used, a random distribution of size classes of extended primers is obtained. This is indicative of a constant "microscopic processivity parameter" (i.e. a constant probability of polymerase dissociation per polymerization event) under any particular set of assay conditions. When an M13 DNA primer-template complex is used as a natural template, the size distribution of extended primers at various times of synthesis becomes a complex function of the underlying DNA sequence, indicating that the "microscopic processivity parameter" varies with template sequence. Preliminary evidence suggests that the four nucleotides immediately prior to the site of polymerization primarily determine the local processivity. These results are being extended using multiple M13-derived sequences, including templates that contain various defined lengths and positions of duplex DNA, and templates that contain unpaired regions of DNA to simulate the active replication fork. (Supported by USPHS Research Grants GM-15792 and GM-29158 (to PvH) and USPHS Fellowship GM-08252 (to FRF).)

Phage Replication; Origins of DNA Synthesis

0749 CHARACTERIZATION OF A REGULATORY MUTANT OF THE dnaA REGION OF E. COLI, H. Eberle, N. Forrest and C. Stillman, U. of Rochester School of Medicine, Rochester, NY 14642
A phenotpyic revertant of a dnaA 508 temperature sensitive mutant, PR1, had been previously found to overproduce several proteins with molecular weights similar to those of products of genes in the dnaA region. The PR1 mutation was found to map in, or near, the dnaA gene. further characterization of this mutant by comparative two-dimensional gel analysis revealed that these proteins co-migrate with proteins coded for by λ tnaA transducing phages which carry the dnaA region. This finding confirms that these are indeed proteins coded for by the dnaA region which are overproduced in PR1. Attempts are being made to determine more precisely the location of the PR1 mutation and to characterize the transcription of genes in the dnaA region of this mutant.

This work is supported by Grant GM 28430 from the NIH and by Contract No. DE-AC02-76EV03490 with The U.S. Department of Energy at the University of Rochester Department of Radiation Biology and Biophysics.

0750 DasF MUTANTS OF Escherichia coli ARE STABLE DNA REPLICATION MUTANTS. Tokio Kogoma*, Ted A. Torrey* and Tove Atlung+. Department of Biology*, University of New Mexico, Albuquerque, NM 87131 and Institute of Microbiology+, Oster Farimagsgade 2A, DK-1353, Copenhagen, Denmark.

Stable DNA replication (sdrA) mutants of E. coli are capable of repeated, recA$^+$-dependent initiation of DNA replication in the absence of protein synthesis (Torrey and Kogoma (1982), Mol. Gen. Genet., in press). The sdrA mutations map between metD and proA. Recently, we have obtained evidence indicating that sdrA mutants can dispense with both the oriC sequence and the dnaA gene without losing viability (Kogoma and von Meyenburg, submitted). Thus, sdrA mutations are strong suppressors of dnaA mutations. Extragenic mutations (das) which suppress the defect in dnaA^{ts} mutants have been isolated and mapped at seven distinct loci (Atlung (1981), ICN-UCLA Symp. vol. 22, 361). One group of suppressor mutations (dasF) have been located near proA. In this study the possibility that the sdrA and dasF mutations are allelic has been examined. The results are as follows: (1) By P1 transduction two independent dasF mutations (dasF373 and dasF377) have been mapped between metD and proA. (2) dasF mutants can survive the inactivation of the dnaA gene by insertion of Tn10. (3) dasF mutants exhibit the stable DNA replication phenotype. (4) The stable DNA replication in the dasF mutants is recA$^+$ dependent. (5) In the absence of the dnaA function, the DNA replication and colony formation of dasF recA200(ts) double mutants becomes temperature sensitive.

0751 THE polC BY-PASS (pcb$^-$) MUTATION, Robb E. Moses and Sharon K. Bryan, Baylor College of Medicine, Houston, TX 77030
We have described a mutation, pcb$^-$, permitting replication of DNA at restrictive temperature in E. coli containing a polC$_{ts}$ gene. DNA replication in this case is dependent on a functional DNA polymerase I. The pcb$^-$ mutation will phenotypically suppress several different polC$_{ts}$ alleles, so the effect is not allele-specific. Other dna genes (dnaB, dnaC, dnaG, dnaZ) are required for by-pass replication dependent on pcb$^-$ and DNA polymerase I. The replication of pBR322, phages λ, G4 and φX174 is also supported by DNA polymerase I and pcb$^-$. The pcb$^-$ mutation has been mapped by inserting Tn10 in close linkage and locating the transposon near dnaA by P1 transduction. We have also inserted the pcb$^-$ gene into pBR322. Clones have been identified by converting E486 (polA$^+$, polC$_{ts}$) to temperature-resistance after transformation. Our results indicate that pcb is trans-acting, since the gene on a plasmid can allow cellular DNA replication. (Supported by grants from the USPHS and Robert A. Welch Foundation)

0752 SUPPRESSION OF E. COLI dnaA (Ts) MUTANTS BY P1 COPY MUTANTS, Barbara Froehlich, Kathleen Tatti, and June R. Scott, Emory University, Atlanta, GA 30322

Although prophage P1 is ordinarily a plasmid, maintained at about one copy/chromosome, it can suppress the heat sensitivity of an E. coli dnaA (Ts) strain by integrating into the host chromosome. In a recA strain, integration is site specific at loxP on P1 and loxB at about 66 min. in the host (Chesney, et al, 1979. J. Mol. Biol. 130, 161-173; Chesney and Adler, 1982. J. Bacteriol. 150, 1400-1404). Although P1 copy mutants are ordinarily maintained at 5-8 times the copy number of P1$^+$, the P1cop$^-$ suppressed dnaA lysogens grown at the nonpermissive temperature have no covalently closed circular plasmid DNA. When shifted down to the permissive temperature, 10^{-5} cells survive and many of these contain CCC DNA. The plasmid may be whole P1 or a small derivative of it.

DNA-DNA hybridization experiments show that in a dnaA recA strain, P1 copy mutants use the P1 loxP site for integration. However, none of the 7 copy mutant-suppressed lysogens tested uses the chromosomal loxB site. This suggests that P1cop$^-$ integrated at loxB may be lethal, possibly because of proximity to the E. coli origin.

0753 ANALYSIS OF CHROMOSOME REPLICATION IN INTEGRATIVELY SUPPRESSED HFr STRAINS: MORE PRECISE MAPPING OF TERC, AND EVIDENCE FOR FORK VELOCITY VARIATION. Bernard de Massey, Samir Bejar, Olivier Fayet, Jacqueline Louarn, Jean-Pierre Bouche, Jean-Michel Louarn
Center de Recherche de Biochemie et de Genegique Cellulaires du C.N.R.S., 118 route de Narbonne, 31062 Toulouse Cedex.

Evidence for a fixed terminus of chromosome replication in Escherichia coli stands on analysis of replication in dnaA (ts) strains phenotypically corrected by integrative suppression. It has been shown that Suppressive integration (the Sin phenotype) implies that the replication cycles are initiated at the origin of the suppressor plasmid (or prophage). In order to distinguish whether termination implies a fixed site or a constant region, we have undertaken a more precise description of the mode of chromosome replication in a number of Sin HFr's by measuring the relative frequence of various markers in exponentially grwoing cells, using DNA-DNA hybridization. Special emphasis was put on the terminus region taking advantage of the collection of cloned segments from the 30.5 to 34 min. region established in this laboratory by S. Bejar and J.P. Bouche. The main results so far obtained are:

1) replication forks are inhibited between 27.3 and 30.5 min.
2) when the plasmid integration site is close to terC, the terminus becomes transgresable by the replication forks.
3) in general, fork velocity is slow near the suppressor origin, then gradually increases up to nearly normal values as the forks move.

Recent developments of these experiments will be presented.

0754 ISOLATION OF MUTANTS OF ESCHERICHIA COLI WITH DELETIONS IN THE TERMINUS REGION OF THE CHROMOSOME, Joan M. Henson, Bede Kopp, and Peter Kuempel, University of Colorado, Boulder, Colorado 80309

Few genetic loci have been discovered in the interval between min 29 and min 35 on the genetic map of Escherichia coli, and no conditionally lethal mutations have been definitively mapped in this region. We have recently isolated a series of deletion mutants which suggest that the region from min 30 to min 34 is unessential for cell viability. One class of deletion strains was derived from a parental strain that contained λreverse (integrated at min 30.0) and trg-2::Tn10 (min 31.2). These strains were isolated by screening for the simultaneous loss of tetracycline resistance (present in Tn10) and phage production. Some of these strains have one endpoint of their deletion in λreverse and the other endpoint in Tn10. A junction fragment joins part of λreverse to part of Tn10, and the intervening DNA appears to be absent. Another type of mutant has a deletion that extends from λreverse to zdc-235::Tn10 (min 32.1). All of the above strains grow as well as their parent strains. However, a different deletion strain appears to be lacking,at a minimum, DNA from min 30.0 to min 34.1. This strain grows slowly and forms a wide variety of cell morphologies in YET medium. We are currently testing this strain to determine if it has deleted the terminus of DNA replication, which is thought to be located in this interval.

0755 ISOLATION AND CHARACTERIZATION OF E. COLI MUTANTS COMPLETELY DEFECTIVE IN RNASE H ACTIVITY, Takashi Horiuchi, Hisaji Maki and Mutsuo Sekiguchi, Department of Biology Faculty of Science, Kyushu University 33, Fukuoka 812, Japan.

A role of RNaseH in the priming step in ColE1 replication has been well established (see Ito & Tomizawa, PNAS, 77, 2450, 1980), and involvement in other cellular process is also implicated. To clarify the physiological role(s) of RNaseH, it is necessary to isolate mutants defective in the enzyme activity. Thus far, only a mutant with a moderately low level of RNaseH (30% of perental level) has been isolated (Carl et al., J. Bacteriol. 144, 28, 1980). In the present study, therefore, we have attemenpted to isolate mutants which are completely defective in RNaseH activity. The steps for the isolation of the mutants are as follows. (1) The structural gene for RNaseH (rnh) was cloned in a plasmid vector (Horiuchi et al, PNAS, 78, 3770, 1981). (2) Using the method developed by Kreshmer and Cohen (J. Bacteriol, 130, 888, 1977) a number of plasmids carrying transposon Tn3 were selected, from which we found six independent mutant plasmids whose rnh gene was inactivated by insertion of Tn3. (3) DNA of the resultant rnh::Tn3 plasmids was digested with EcoRI and subcloned into a vector λ phage ($\lambda G_{857}gt\lambda c$). (4) The λrnh::Tn3 phage was used to lysogenize wild type cells; the phage was integrated inside or near the rnh gene on the host chromosome by homologous recombination. (5) After heat induction temperature-resistant (Ts^+) and ampicilline-resistant (Ap^r) clone were selected, in which intact rnh^+ gene is thought to be replaced by rnh::Tn3. Biochemical analysis of the Ts^+ Ap^r cells indicated that Ts^+ Ap^r cells were indeed completely defective in RNaseH activity. Phenotypes of the mutants will be described.

0756 THE ROLE OF T4 DNA POLYMERASE IN SPONTANEOUS FRAMESHIFT MUTATION: A MUTATOR EFFECT PRODUCED BY AN ANTIMUTATOR POLYMERASE, L. S. Ripley, N. B. Shoemaker, A. B. Clark and J. G. de Boer, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Genetic studies of the specificity of spontaneous frameshift mutation in the N-terminal region of the rIIB gene of bacteriophage T4 have revealed a strong influence of the DNA polymerase on both the frequency and specicity of mutation. Genetic mapping of the frameshift mutations revealed a unique spectra for the mutator polymerases tsL56 and tsL88, the wild type polymerase, and the antimutator polymerase tsL141 (1). Polymerase tsL141 (and other antimutator polymerase alleles) reduce the frequencies of frameshift mutations that map into A:T-base pair runs, but substantially increase the frequency of frameshifts in a 30 bp region of the rIIB sequence assayed. A unique feature of this region is its ability to form a strong secondary structure that may have the potential to produce frameshift mutations (2). The DNA sequences of frameshift mutants in the rIIB gene produced by mutant and wild type DNA polymerases will be reported and discussed with respect to previously assayed properties of these enzymes.

1. Ripley, L . S. and N. B. Shoemaker 1983. A major role for bacteriophage T4 DNA polymerase in frameshift mutagenesis. Genetics (In Press).

2. Ripley, L. S. Model for the participation of quasipalindromic DNA sequences in frameshift mutation. Proc. Natl. Acad. Sci. USA 79: 4128-4132.

0757 ISOLATION OF AN ERROR-PRONE FORM OF DNA POLYMERASE I FROM E. COLI CELLS INDUCED FOR RECA/LEXA FUNCTIONS, David Lackey, Sharon Wald Krauss, and Stuart Linn, University of California, Berkeley, CA 94720

Expression of recA/lexA dependent functions in E. coli results in the appearance of a new DNA polymerase activity (pol I*), which we have identified as a form of DNA polymerase I on the basis of antibody inhibition, insensitivity to N-ethyl maleimide and polypeptide molecular weight. However, pol I* has a lower affinity for single-stranded DNA-agarose than does pol I, and pol I* has a sedimentation coefficient of 6.6s compared with 5.5s for pol I. The salient functional difference between pol I* and pol I appears to be the much higher error frequency of pol I* as determined by in vitro mis-incorporation assays utilizing a variety of synthetic polydeoxyribonucleic acid templates; this difference is also reflected in a reduced selectivity of pol I* for deoxyadenosine triphosphate over 2-aminopurine deoxyribotriphosphate with salmon sperm DNA as template. These observations may contribute to an understanding of the mutagenesis associated with recA/lexA dependent repair of DNA.

We are currently investigating the effect of certain mutations in the recA/lexA dependent genes on the expression of pol I*; our objectives also include examination of the possible role of pol I* synthesis past non-instructive DNA lesions by an application of DNA sequencing gel techniques.

This research has been supported by Grants GM19020 and AG00819 from the National Institutes of Health and Contract DE-AS03-76-SF0034 from the U.S. Department of Energy.

0758 PRIMER RNA IN DISCONTINUOUS REPLICATION OF ESCHERICHIA COLI IN VIVO AND IN VITRO, Tohru Ogawa*, Tuneko Okazaki* and Ken-ichi Arai**. *Nagoya University, Nagoya 464 Japan. **DNAX Research Institute, Palo Alto, California 94304

The chain length of primer RNA attached to the nascent DNA fragments isolated from Escherichia coli cells is mostly mono- to trinucleotide, even in polA strains which accumulate RNA-linked, short DNA fragments. Similarly, most of the primer RNA accumulated in rnh·polA4113 double mutant is mono- to trinucleotide, but some enrichment in longer primer (4 to 9 residues) is observed. These results suggest that the 5'→3' exonuclease of DNA polymerase I plays a major role in the removal of the primer and that RNase H functions auxiliary in the step.

The chain length of intact primer RNA synthesized in vitro by the primosome system (Arai, K., et al. (1981) Proc. Natl. Acad. Sci. USA 78: 707) was estimated using ϕX174 SS DNA as a template. The primers synthesized coupled to DNA replication are heterogeneous and shorter than decanucleotides. The primers start with ATP, followed mainly by purine nucleotides; internal sequences are heterogeneous. Size distribution of the primer is affected by the relative concentration of rNTPs and dNTPs. The proportion of mononucleotide (ATP) primer increases upon decreasing the concentration of rNTPs. Priming by a single ribonucleotide residue (ATP) has not been reported in other prokaryotic as well as eukaryotic systems.

0759 MECHANISM OF INITIATION COMPLEX FORMATION BETWEEN THE DNA POLYMERASE III HOLOENZYME AND PRIMED DNA. Charles S. McHenry and Kyung Oh Johanson, Department of Biochemistry and Molecular Biology, The University of Texas Medical School, Houston, Texas 77025.

The formation of an initiation complex between the DNA polymerase III holoenzyme and primed DNA requires ATP (or dATP) when equivalent ratios of enzyme and DNA are used. The hydrolysis resistant analog of ATP, ATP γS, will substitute for ATP in this reaction. We will describe its use to study the mechanism of ATP utilization. The initiation complex formation reaction can be blocked by an antibody directed against the β subunit of the holoenzyme; elongation is unaffected. Using an immunological method that we have developed for quantitating nanogram quantities of β in reaction complexes, we have demonstrated that β is present in all stages of the holoenzyme reaction. Upon initiation complex formation, the antigenic determinants of β become inaccessible to anti- β Immunoglobulin G. Even after conversion of a primed G4 single strand to the duplex replicative form, β does not readily dissociate. This creates a kinetic barrier to the overall holoenzyme replicative reaction. We have demonstrated that the subunit of holoenzyme when bound to the polymerase III core causes the polymerase to dimerize, forming a structure upon which a dimeric replicative complex could be constructed. We will discuss how such a structure may help overcome the aforementioned kinetic barrier.

0760 SPECIFIC INTERACTIONS BETWEEN E.coli REPLICATION FACTOR Y AND LAGGING STRAND ORIGINS OF DNA REPLICATION Kenneth J. Marians, Jeffrey Greenbaum, Walter C. Soeller, and Patricio Abarzua. Dept. of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, New York 10461

Recognition by E. coli factor Y of sequence elements within lagging strand origins of DNA replication governs the site-specific assembly of the primosome. Recombinant f1 bacteriophage DNAs containing the factor Y effector sites from pBR322 DNA which have had small (30 nucleotides) deletions constructed within them in vitro are totally inactive as effectors for the single-strand DNA dependent ATPase activity of factor Y and as templates for rifampicin resistant, dna B-, dna G-, and dna C-dependent complementary strand DNA synthesis. This co-inactivation of the two factor Y directed activities indicates that the sequences responsible for them are likely to be identical.

Construction of point mutations in vitro in the factor Y sites, DNA footprinting and methylation enhancement studies are being used to elucidate the interaction between factor Y and the DNA. These studies indicate that higher order structures of the DNA are involved in factor Y recognition and origin function.

0761 COLE1 MUTANTS DEFECTIVE IN RNA PRIMER FORMATION, Hisao Masukata and Jun-ichi Tomizawa, Laboratory of Molecular Biology, NIADDK, NIH, Bethesda, MD 20205

In vitro initiation of ColE1 DNA replication requires RNA polymerase, RNase H and DNA polymerase I (1). Primer precursors made by RNA polymerase are cleaved by RNase H to form the primer for DNA synthesis by DNA polymerase I (2).

Using in vivo or in vitro mutagenesis of composite plasmids of ColE1 with other replicon such as λ phage or pSC101, we isolated replication defective ColE1 mutants. All of 50 mutants thus obtained were defective in the initiation step of DNA replication. These mutants were characterized using an in vitro transcription system and classified into three groups. The first group is defective in the promoter activity for primer transcription. The second group forms transcripts from the primer promoter which cannot be cleaved by RNase H. The defect is caused by a single base change in various regions between the primer RNA starting site and the replication origin. In the third group, transcripts from the primer promoter are cleaved by RNase H at the same sites as those in wild-type, but inefficiently used as the primer for DNA synthesis by DNA polymerase I. This defect is caused by a mutation far upstream from the origin. These results demonstrate the importance of RNA secondary structures for the primer formation (3).

(1) Itoh,T. and Tomizawa,J.(1978). Cold Spring Harbor Sym.Quant.Biol.43,409-418.
(2) Itoh,T. and Tomizawa,J.(1980). Proc.Natl.Acad.Sci.USA.77,2450-2454.
(3) Tomizawa,J. and Itoh,T.(1982). Cell. In press.

0762 COPY NUMBER CONTROL OF PLASMID pT181: SEQUENCE ANALYSIS OF COPY NUMBER MUTANTS. Steven J. Projan, Stephen Carleton, Chandra C. Kumar and Richard P. Novick. The Public Health Research Institute of the City of New York, Inc., New York 10016

pT181 is a 4.4 kb multicopy plasmid which specifies tetracycline (Tc) resistance in Staphylococcus aureus. When pT181 is cloned onto a carrier plasmid, pE194, at a site which interrupts the coding sequence for a pT181 encoded protein (RepC) necessary for plasmid replication, it is passively replicated. The resultant cointegrate plasmid unilaterally excludes autonomous pT181. The locus of this unilateral incompatibility, inc3A, has been mapped by deletion analysis within the pT181 portion of the cointegrate. Several high copy number mutants of pT181, selected by spontaneous high level Tc resistance, have been sequenced. These mutations, which include deletions, insertions, and point mutations, all map within the inc3A locus. They fall into two classes: those sensitive to inc3A incompatibility and those which are resistant. The positions of these mutants identify putative "target" and "effector" loci. This effector is the postulated inc3A gene product, possibly one of two small RNAs transcribed from the inc3A region. The target is the putative site of action for the inc3A gene product. We propose that the inc3A gene product's function is to inhibit the production of RepC.

0763 CHARACTERIZATION OF TWO SMALL RNAs INVOLVED IN COPY CONTROL OF PLASMID pT181. C. C. Kumar, Steven J. Projan and Richard P. Novick, Public Health Research Institute, New York, NY.

pT181, a 4.4kb tetracycline resistance plasmid from Staphylococcus aureus, encodes a protein (RepC) which has been shown to be rate limiting for plasmid replication. Sequencing of the repC gene and its 5' leader region revealed a procaryotic transcription terminator directly before the start of the repC reading frame. This 5' leader region has also been shown to encode a determinant of unilateral incompatibility (Inc3A) that is expressed when pT181 is cloned to a carrier plasmid, pE194. Two small RNAs of about 90 and 140 bases in length have been shown to hybridize to DNA from this region of pT181. These two small RNAs have been characterized with respect to their precise start and stop points by hybrid selection followed by sequencing of the termini. A copy mutant, cop608, carrying a 180 base pair deletion in the leader region, overproduces the RepC protein and is insensitive to Inc3A incompatibility. Whereas the wild type plasmid, pT181, makes the two small RNAs, cop608 abolishes this synthesis. There is evidence which indicates that the two small RNAs are involved in Inc3A incompatibility and affect copy number control of pT181.

0764 INITIATION OF COLE2 AND COLE3 DNA REPLICATION, Tateo Itoh and Toshihiro Horii, Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

Colicin E2 and E3 plasmids (ColE2 and ColE3) are small, circular double-stranded DNA molecules with similar molecular weights and present in about 15 copies per colicinogenic E. coli cell. Extensive homology between these plasmids has been demonstrated by DNA-DNA hybridization and heteroduplex analysis, and recently, by analysis with various restriction endonucleases. Replication of ColE2 and ColE3 has been shown to require DNA polymeraseI(like ColE1), but to stop in the presence of chloramphenicol(unlike ColE1), indicating the involvement of some unstable protein(s). Some limited incompatibility between them has been demonstrated.

We have cloned fragments of these plasmids and shown that all the informations required for stable maintenance of them in growing bacterial cells are localized within corresponding 1.5 kb regions. Restriction and genetic maps and the nucleotide sequences of these regions will be presented.

We have developed an in vitro DNA replication system using a crude extract of E. coli cells which do not carry plasmids. The replication depends completely on exogenously added DNA, requires active DNA polymeraseI,RNA polymerase and DNA gyrase, starts from a fixed origin and proceeds unidirectionally through θ-shaped intermediates. A unique feature of this system is its requirement for concomitant protein synthesis, indicating the involvement of an essential replication protein(s), presumably an initiator protein(s), encoded by these plasmids. Various properties of this replication system will be presented.

0765 A COP⁻ MUTATION CAUSES A SPECIFIC 200 FOLD INCREASE IN INTERFERON LEVELS EXPRESSED FROM THE COLEI REPLICATION PROMOTERS, Nikos Panayotatos, Annick Fontaine and Kim Truong, Biogen S.A., 3 route de Troinex, 1227 Carouge, Geneva, Switzerland

Two promoters (P_I and Pm) which regulate RNA I and primer transcription have been used for the expression of human leukocyte interferons in E.coli. Plasmids containing two copies of the P_I (or the Pm) promoters, one copy as part of the regular pBR322 replicon and a second copy as a cloned fragment from ColEI controlling the expression of interferon genes, were found to replicate normally and synthesize 10,000 molecules of interferon per cell. A single point mutation in the small RNA I region of the replicon raised the copy number five fold and increased the levels of the plasmid-coded ß-lactamase by the same magnitude. In sharp contrast, the interferon levels synthesized under the control of P_I or Pm increased 200 fold relative to the parental molecule when the cop⁻ point mutation was introduced. This large increase in activity was not observed when interferon was expressed from plasmids containing a phage lambda replicon, with RNA I or its point mutant being provided in trans as part of a second plasmid. These results indicate that neither the point mutant RNA I nor a bacterial factor can, alone, be responsible for the observed phenomemon, despite the fact that the 200 fold increase in interferon levels (but not the five fold increase in ß-lactamase) was found to be host-cell specific.

0766 A COLEI SPECIFIED PROTEIN DETERMINES THE COPY NUMBER OF THE PLASMID, Tapan Som and Junichi Tomizawa, NIADDK, NIH, Bethesda, MD 20205

It was reported that the copy number of the plasmid ColE1 gets elevated by the deletion of its HaeII-C fragment. We have narrowed down the region to a 306 bp HpaII fragment which still causes the same effect. This region, when present in trans can also lower the copy number of a ColE1 plasmid devoid of the corresponding region. Plasmid pBR322 also demonstrate to have a similar system which also acts on ColE1. Comparison of the nucleotide sequences of the region of pBR322 corresponding to the 306 bp HpaII region of ColE1 indicates that a protein rather than a transcript is responsible for the lowering of the copy number. All the low copy number derivatives of ColE1 as well as pBR322 showed production of a protein in the maxi cell preparation which are absent in their derivative with the deletion in the 306 bp HpaII region. This region in trans suppresses the expression of Galk under control of ColE1 primer promoter, only if the fragment containing the promoter also contains the region that determines the sensitivity to the inhibitor.

0767 BASE PAIRING BETWEEN 7 BASES IN RNA1 AND THE COMPLEMENTARY SEQUENCE IN THE RNA PRIMER IS RESPONSIBLE FOR COLE1 INCOMPATIBILITY PROPERTIES, Rosa M.Lacatena and Gianni Cesareni, European Molecular Biology Laboratory, Meyerhofstraße 1, 6900 Heidelberg

Single base mutations in the region that codes for RNA1 create new incompatibility groups while preserving the mechanism of control of initiation of DNA replication in a ColE1 type plasmid.

By exploiting the properties of a phasmid phage hybrid we have isolated 73 independent ColE1 mutants which are insensitive to the wild type inhibitor but maintain the sensitivity to the mutant inhibitor of the same genotype: that is single base pair mutations in the target of the inhibitor of ColE1 replication causes a complementary alteration of the inhibitor itself so that the control mechanism remains fonctional despite the change in specificity. This prove that the inhibitory interaction is due to base pairing between RNA1 and the complementary sequence in the primer precursor.

By studying the interaction between the inhibitors and the targets of the 73 mutants we were able to subdivide them into 23 groups of specificity. Sequence analysis of at least one representative per each class suggests that 7 is very close to the total number of bases involved in the base pairing interaction.

0768 TRANSCRIPTIONAL AND TRANSLATIONAL CONTROL OF PLASMID NR1 DNA REPLICATION, David D. Womble, Ru Ping Wu, Verne A. Luckow, and Robert H. Rownd. Northwestern University Medical School, Chicago, IL 60611

The rates of in vivo transcription of the genes from the replication control region of the inc F II plasmid NR1 were measured. In vitro transcription experiments identified three major products, labeled RNA-A, RNA-C, and RNA-E by decreasing size. RNA-A and RNA-E are transcribed from opposite strands of the same DNA sequence, and are therefore complementary. Quantitative hybridization of in vivo labeled RNA to single stranded M13 phage derivatives with small plasmid DNA inserts in either orientation was used to compare transciption from wild type and copy number or incompatibility mutant templates. The rates of transciption were compared to the rates of β-galactosidase synthesis in phage λ lysogens in which the replication control genes were fused to lac DNA sequences. A comparison of wild type and mutant transcription and translation in various cis and trans configurations suggests a model for the control of inc F II plasmid DNA replication. We propose that plasmid copy number is proportional to the strength of the promoters for the rightward transcription of RNA-C and RNA-A, and inversely proportional to the leftward transciption of the 91-base RNA-E. Additionally we propose that the copy number is proportional to the rate of translation of RNA-A, and that this rate is inhibited by direct interaction of RNA-E with its complementary sequences on RNA-A

0769 Regulation of Plasmid R1 Replication, Søren Molin and Janice Light, Odense University, Odense, Denmark.

Plasmid R1 is a low copy number bacterial plasmid which encodes several antibiotic resistencies and is self-transferable. All genes required for controlled replication are clustered within a 2.5 Kb region. Besides a single replication origin three genes (repA, copA and copB) are located in this region. The repA gene is required for initiation of plasmid replication, and the two cop genes have been found to express replication control functions.

The copA gene product is a small (80-90 nucleotides) untranslatable RNA molecule which inhibits repA expression through interaction with the repA mRNA - an interaction which most likely interferes with translation of the repA mRNA.

There seems to be an inverse proportionality between the rate of repA translation - and hence of plasmid replication - and the concentration of CopA-RNA.

Transcription of repA is normally initiated constitutively from the copB promoter, which is repressed by the CopB protein, is located down-stream of the copB gene. The significance of the CopB regulatory loop is at present not known.

A quantitative analysis of transcription and translation of the repA gene has shown that most likely the RepA protein is rate limiting for plasmid replication, and that the control exerted on expression of the repA gene is the key element in the replication control system for plasmid R1.

0770 DNA SEQUENCE AND FUNCTIONAL ANALYSIS OF THE ORIGIN REGION OF THE ANTIBIOTIC RESISTANCE PLASMID R1, K.J. Burger, G.Röllich and L.J.Klimczak[+], Institute for Microbiology, Univ.of Würzburg, Röntgenring 11, D87 Würzburg, [+]Dpt. of Genetics, Univ.of Warsaw, Poland

The "basic replicon" of the incF$_{II}$ plasmid R1 consists of 3 PstI fragments comprising about 2.6 kb. This contiguous region carries the information for copy number control, incompatibility, a positive factor (repA), and the origin of replication(1,2). The nucleotide sequence of the largest PstI fragment, Pst-E (1.6 kb), is presented here. Combined with the DNA sequence from the R1 control region(3) the "basic replicon" from R1 wild type is now completely sequenced.

By electron microscopy the replication origin of incF$_{II}$ plasmids was localized between pos. 900 and 1500 on Pst-E(4,5). Our analysis of R1 recombinant plasmids deleted in vivo or in vitro shows that most of these sequences are dispensable. A region around pos. 1200 which has the potential to form extensive secondary structures and which was believed to be essential for R1 origin function (6) can also be deleted. Copy number determination and segregation analysis of such deleted R1 mini-replicons reveals that these plasmids though still being able to replicate do so with much lower efficiency as soon as certain sequences are removed. In addition to these studies experiments are described which are designed to determine the initiation site fo for leading strand synthesis in the unidirectional replicating R factor R1.

1) Burger et al., Molec.gen.Genet.(MGG) 182, 44-52 (1981); 2) Light and Molin, MGG 184, 56-61 (1981); 3) Stougaard et al., MGG 181, 116-122 (1981); 4) Ohtsubo et al., Plasmid 1,8-18 (1977); 5) Ohtsubo et al., MGG 159, 131-141 (1978); 6) Oertel et al., MGG 171, 277-285 (1979)

0771 THE ORIGIN OF DNA TRANSFER OF THE BROAD HOST RANGE PLASMID RK2. Donald G. Guiney, Emanuel Yakobson, and Gary Chikami, UCSD Medical Center, San Diego, Calif., 92103

Plasmid DNA transfer during bacterial conjugation begins at a fixed site, the origin of transfer (oriT). OriT is involved in several important biochemical events during the transfer process: 1) nicking of the DNA strand to be transferred and conduction to the recipient, 2) initiation of transfer DNA replication, and 3) recircularization of the plasmid DNA in the recipient. We have studied the transfer origin of the broad host range conjugative plasmid RK2, a member of the P incompatibility group. We have localized the RK2 oriT within a 760bp HaeII fragment which maps in the same region as the nick produced by the RK2 DNA-protein relaxation complex. The functional oriT can be subcloned as a 112bp HpaII fragment. The sequence of this 112bp oriT region has been determined, and contains a 19bp inverted repeat. The potential stem-loop structure formed by this inverted repeat comprises 40 bases with 16 of the 19 bases in each side of the stem able to form pairs. We propose that this inverted repeat is the binding site for the RK2 relaxation complex which initiates the transfer process. Southern hybridizations show homology between the RK2 oriT sequence and all other IncP group plasmids examined, although a group of 3 P plasmids show considerable sequence divergence from RK2. These latter plasmids also show sequence differences from RK2 in the trfA region required for vegetative DNA replication, indicating that divergence of both replication and transfer regions has occurred in the IncP group.

0772 STABILITY MUTANTS OF THE R PLASMID NR1, Yun-liu Fan and Robert H. Rownd, Northwestern University Medical and Dental Schools, Chicago, IL 60611

An unstable (stb) mutant plasmid pFY12 was isolated from a derivative of the R plasmid NR1 (pRR720) using in vitro hydroxyamine mutagenesis. The amount of covalently closed circular pFY12 and pRR720 DNA corresponded to plasmid copy numbers of 4.5 and 5.0 per chromosome respectively. Miki et al (J. Bacteriol 141, 87-99, 1980) reported that the stb gene of NR1 is located in restriction endonuclease EcoR1 fragment A, a part of which is carried by pRR720. An EcoR1-SmaI fragment located within EcoR1 fragment A of a wild type plasmid can stabilize the unstable miniplasmid pRR933 only in cis. pRR933 consists of the PstI 1.1 and 1.6kb replicator fragments of NR1 linked to a PstI fragment containing the chloramphenicol acetyl-transferase gene. The corresponding EcoR1-SmaI fragment from the mutant plasmid pFY12 cannot stabilize pRR933 in cis. Therefore, the observed instability of pFY12 appears to be due to mutation in the stb gene. pRR933, which does not contain EcoR1 fragment A, is not stabilized in trans by EcoR1 fragment A cloned on a ColE1 vector (pRR138).

The mutant plasmid pFY12 was stabilized in trans either by pRR138 or by plasmid pFYS-6 which consists of the vector plasmid pUC8 and the EcoR1-SmaI stb fragment of NR1. Thus, stb can function not only in cis, but also in trans. These findings suggest that pFY12 has a mutation in a diffusible product specified by the stb gene but not in its stability target site. pFY12 can therefore be stabilized in trans by a cloned stb gene. pRR933, on the other hand, has deleted the entire EcoR1 fragment A. This plasmid therefore does not contain the stb target site and cannot be stabilized in trans by the stb gene.

0773 CHARACTERISTICS OF THE IN VITRO REPLICATION OF PLASMID λdv, Marc S. Wold and Roger McMacken, Department of Biochemistry, Johns Hopkins University, Baltimore, Md 21205

We have developed a soluble in vitro system that specifically replicates plasmid DNA (λdv) bearing the replication origin (oriλ) of the phage λ chromosome (Wold et al. (1982) Proc. Nat. Acad. Sci. 79, 6176). Replication of supercoiled λdv DNA in this system depends on (i) the λ O and P replication proteins, (ii) Escherichia coli replication proteins (including primase, dnaB protein, single-stranded DNA-binding protein, and DNA gyrase), (iii) ATP and an ATP-regenerating system, and (iv) a hydrophilic polymer such as polyvinylalcohol. The products of λdv replication in vitro are full-length, catenated, circular daughter molecules that resemble known intermediates in the circular phase of phage λ DNA replication in vivo.

Initiation of λ DNA replication in vivo requires transcription at or near the viral replication origin, a process termed transcriptional activation. We have confirmed that λdv replication in the soluble enzyme system also depends on RNA polymerase transcription. Replication of λdv DNA is completely inhibited in the presence of rifampicin. Furthermore, addition of λ cI repressor protein to the complete system (20 dimers of repressor per molecule of template) specifically blocks initiation of λdv DNA replication. This indicates that the requisite transcription for activating oriλ emanates from the repressor-sensitive λ P_R promoter present on the plasmid. We have constructed λdv-like plasmids that carry the repressor-insensitive λ c17 promoter, from which transcription is directed across oriλ. The replication of these plasmids in the in vitro system is not inhibited by even a 500 fold molar excess of cI repressor.

0774 PRIMARY AND SECONDARY STRUCTURE OF THE BACTERIAL DNA REPLICATION ORIGIN, Judith W. Zyskind, San Diego State University, San Diego, CA 92182, and Douglas W. Smith, University of California, San Diego, La Jolla, CA 92093

A consensus sequence of the bacterial origin (oriC) which functions in Escherichia coli has been derived from comparison of the oriC sequences of E. coli, Salmonella typhimurium, Enterobacter aerogenes, Klebsiella pneumoniae, Erwinia carotovora, and the marine bacterium Vibrio harveyi. Nucleotide substitutions can occur at 122 positions within the 245 basepair oriC without loss of function, and these substitutions are mainly clustered. Four interrelated nine basepair repeats and eight of the dam methylation GATC sites are conserved in this consensus sequence. Very few relative insertions occur, and these are found at one end of oriC. Apparently, the size of the rest of oriC must remain invariant to be functional, even though base substitutions at half of the positions are oriC$^+$. Many translation stop codons and few start codons are present. Considerable potential intrastrand helical structure, arising from nucleotide dyad repeats, exists within oriC. Computer analysis using several programs is used to predict the energetically most favorable conformations. All of these include a helical structure formed between the two nine basepair inverted repeats at positions 80-88 and 260-268 and an attenuator-like structure between nucleotides 23 and 71. Intrastrand structure possibilities within the region between positions 89 and 259 show both similarities and differences between the six origins, and the similarities do not always involve identical nucleotide positions. This structural analysis of the bacterial origin leads to predictions for the location of protein binding sites and for RNA transcription events within this most complex regulatory region.

0775 SITE-SPECIFIC INTERACTIONS OF PROTEINS WITHIN AND NEXT TO THE REPLICATION ORIGIN OF E. COLI
H. Lother, T. Chakraborty, R. Lurz, W. Messer, and E. Orr
Max-Planck-Institut für molekulare Genetik, Abt. Trautner, Berlin, Germany

Looking for site specific interactions of various proteins using plasmids containing the replication origin (oriC) specific interactions of purified proteins such as a) a membrane protein (B'), b) purified RNA polymerase, c) dnaA gene product, and d) gyrase were localized either within or next to the minimal DNA segment required for autonomous replication. A model taking care of these data will be presented.

0776 CONTROL OF MINICHROMOSOME REPLICATION,
Walter Messer, Barbara Heimann, Trinad Chakraborty and Heinz Lother,
Max-Planck-Institut für molekulare Genetik, Berlin 33, West-Germany

Copy numbers of various minichromosomes were determined using dye-CsCl gradient centrifugation or hybridization techniques. Deletion of various segments around oriC has little effect on copy number, except for deletions in the atp operon. Deletions and rearrangements very close to oriC, however, do result in a reduction of the copy number. The implication of these results on the control of initiation of minichromosomes will be discussed.

0777 NUCLEOTIDE SEQUENCE OF THE REPLICATION REGION OF PLASMID pSC101, Karen Armstrong, Hisako Ohtsubo, Yasunori Machida, Elliot Ledner, Mark Pancotto and Eiichi Ohtsubo, SUNY, Stony Brook, NY 11794

pSC101 is a small (9.4 kb), tetracycline resistant plasmid of low copy number. All plasmid functions essential for autonomous pSC101 replication are encoded by a *Hae*II fragment 1.9 kb in size. Three types of replication mutants have been isolated from pSC101: Rep$^-$ mutants caused by IS*1*-mediated insertional inactivation, mutants with increased copy number and a temperature-sensitive replication mutant. Cloning experiments have demonstrated that the mutations resulting in increased copy number and ts replication map within the *Hae*II fragment, and we have determined the nucleotide sequence of this fragment. An open coding frame for a polypeptide approximately 32 kilodaltons in size is a prominent feature of this region. In confirmation of our cloning experiments, the lesions of the six Rep$^-$ insertion mutants, of the mutant with the highest copy number and of the ts replication mutant all occur within this coding region, the latter two lesions each causing an amino acid substitution. Upstream from this coding region are two 22 bp inverted repeat sequences preceded by three 20 bp direct repeat sequences. These repeated sequences all have sequence similarity to each other but not to repeated sequences found in other origin regions. Other intriguing properties of the pSC101 replication region will be described.

0778 IN VIVO STUDIES OF ØX174 DNA REPLICATION, Michael D. Strathearn, Robert Low* and Dan S. Ray, Molecular Biology Institute, University of California, Los Angeles, CA 90024 and *Department of Biochemistry, Stanford University, Stanford, CA 94305

An M13 phage deletion mutant, M13ΔE101, developed as a vector for selecting DNA sequences that direct DNA strand initiation on a single stranded template, has been used for cloning restriction enzyme digests of ØX174 RF DNA. Initiation determinants, detected on the basis of clear-plaque formation by the chimeric phage, were found only in restriction fragments containing the unique effector site in ØX174 DNA for the E. coli n' dATPase (ATPase). Further, these sequences were functional only when cloned in the orientation in which the ØX174 viral strand was joined to the M13 viral strand. A 181 nucleotide fragment containing this initiation determinant confers a ØX174-type complementary-strand replication mechanism on M13 chimeras. The chimeric phage is converted to the parental RF in vivo by a mechanism resistant to rifampicin, a specific inhibitor of the normal RNA polymerase-dependent mechanism of M13. In vitro, the chimeric single stranded DNA promotes the assembly of a functional multiprotein priming complex, "primosome", identical to that utilized by intact ØX174 viral strand DNA. Chimeric phage containing the sequence complementary to the 181 nucleotide viral strand sequence show no initiation capability either in vivo or in vitro.

0779 IN VITRO SYNTHESIS OF BACTERIOPHAGE ØX174 FROM PURIFIED PHAGE AND HOST COMPONENTS: ISOLATION OF A NEW E. COLI PROTEIN INVOLVED IN PHAGE FORMATION, Robert K. Hamatake, Akira Aoyama and Masaki Hayashi, University of California at San Diego, La Jolla, CA 92093
The morphogenesis of ØX174 has been analyzed with in vitro systems capable of synthesizing infectious phage. Our system, which previously used infected and uninfected cell extracts, has been refined to the point where viral DNA replication and phage production are achieved with purified phage and host components. The phage components are supertwisted replicative form DNA, the gene A protein, gene C protein, gene J protein, and the phage prohead composed of genes B, D, F, G and H proteins. When the E. coli rep protein and DNA polymerase III holoenzyme are added to the phage components and the mixture incubated in the appropriate buffer, viral DNA is synthesized and packaged into infectious phage. Using this system we have detected a protein factor from uninfected cell extracts that stimulates the formation of infectious phage. This protein, the α protein, has been purified to homogeneity. It's molecular weight of about 58,000 daltons under denaturing conditions distinguishes it from previously purified E. coli proteins involved in DNA synthesis. Data on the physical properties and effect of the α protein in our in vitro system will be presented.

0780 STRUCTURE AND FUNCTION OF INCOMPATIBILITY LOCI ASSOCIATED WITH DNA REPLICATION OF PLASMID PROPHAGE P1. Dhruba Chattoraj, Ann Abeles, Stuart Austin, Nat Sternberg and Michael Yarmolinsky, Basic Research Program-LBI, Frederick Cancer Research Facility, Frederick, Maryland 21701
Phage P1 is a stable plasmid in lysogenic cells and like other plasmids possesses determinants of incompatibility (inc). One or more extra copies of such a determinant destabilizes a P1 plasmid. Three inc loci have been mapped. incA and incC affect replication strikingly and are associated with the region essential for replication. incB, which maps outside the minimal replicon, seems to be involved in equipartitioning of daughter molecules during cell division. The structure of the incA and incC loci has been determined by DNA sequencing and cloning. The two loci are largely homologous and consist of several copies of a repeating sequence 19 base-pairs long. The sequence occurs 5 times in incC and 9 times in incA. Apparently, any three of the repeats from the incA locus can express incompatibility, but the degree of incompatibility increases with the number of repeats in the same cell. Thus the individual repeated sequences appear to act as competitive elements. Another striking feature of incA is that it only works in trans i.e., an extra copy of the incA locus has no phenotype when integrated into P1. The extinction of incA phenotype in cis makes it unlikely that incompatibility is a consequence of a trans-acting repressor of replication produced from incA. The repeating structure of the locus suggests to us that the locus could be a cooperative binding site for some component essential for replication, such as a protein encoded by a gene that lies between incA and incC. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO-CO-23909 with Litton Bionetics, Inc.

0781 BACTERIOPHAGE N4 DNA REPLICATION, J.K. Rist*, M.S. Pearle*, A. Sugino+, and L.B. Rothman-Denes*, +NIEHS, NIH, and *University of Chicago, Chicago, Il. 60615

Coliphage N4 contains 72kb of linear, double stranded DNA. N4 DNA replication is independent of the host replication genes dna A, B, C, E, and G, and requires the host genes dna F, gyr A and B, and pol A. N4 DNA replication requires expression of N4 early and middle genes. At least four N4 gene products are required directly for N4 DNA synthesis.

We have developed an in vitro system to study N4 DNA replication. DNA synthesis catalysed by extracts of N4 infected cells is dependent upon exogenous N4 DNA. Extracts of cells infected with mutants in the N4 genes dnp, dbp, and exo do not synthesize DNA unless they are complemented with the missing wild type protein. We have used this system to purify the N4 coded DNA polymerase (missing in dnp am25), DNA binding protein (missing in dbp 33A7), and an N4 coded exonuclease (missing in exo D11).

This in vitro replication system is very specific for N4 DNA. No other duplex DNA tested will support DNA synthesis. Hybridization of the material synthesized in vitro to Southern blots shows that synthesis begins at or near both ends of the genome, and proceeds toward the middle. We have recently tested recombinant clones containing N4 DNA for their ability to support DNA synthesis in vitro. Only clones containing N4 end fragments support DNA synthesis.

We are using this system to study the components and DNA sequences required for initiation of N4 DNA replication.

0782 BACTERIOPHAGE N4-CODED EXONUCLEASE, Diane Guinta and Lucia B. Rothman-Denes, University of Chicago, Chicago, Il. 60637.

An exonuclease has been purified to apparent homogeneity from extracts of bacteriophage N4 infected cells. The activity is an N4 middle gene product. The enzyme is a dimer of two 45,000 dalton subunits. It is active on double stranded and single stranded DNAs. It has no activity on RNAs but has RNase H activity. The predominant activity is in a 5'→3' direction although low 3'→5' activity has been detected.

We are investigating the role of the exonuclease in N4 development. Using an amber mutant which is N4 exo⁻, we have shown that this activity is not required for N4 recombination or processing of N4 Okazaki fragments. We are interested in the role of this enzyme in N4 DNA replication. N4 exo⁻ mutant infections have a DNA arrest phenotype; the time of arrest is temperature dependent.

An in vitro N4 DNA replication system has been developed in our laboratory which is specific for double stranded N4 DNA (J.K. Rist, A. Sugino, L.B. Rothman-Denes, unpublished results). In this system, extracts of N4 exo⁻ infected cells have no replication activity. Replication activity can be restored in these extracts by the addition of purified exonuclease. Replication complementing activity is coincident with exonuclease activity throughout the exonuclease purification. Phage λ exonuclease cannot substitute for N4 exonuclease in this in vitro replication system. The specific role of the exonuclease in replication is being studied.

0783 FUNCTIONAL DISSECTION OF BACTERIOPHAGE f1 ORIGIN OF DNA REPLICATION. Gian Paolo Dotto, Kensuke Horiuchi and Norton D. Zinder, The Rockefeller University, New York, NY 10021

The "functional origin" of DNA replication of bacteriophage f1 (defined as the minimal sequence that, when present in a plasmid, confers upon it the ability to enter the f1 mode of replication, provided that helper phage is present) contains several functional domains that are required for specific initiation and/or termination of f1 viral strand synthesis (Dotto, G. P., Horiuchi, K. and Zinder, N. D., 1982, Proc. Natl. Acad. Sci., in press). We report here: 1) a finer analysis of these functional domains; 2) the isolation of a phage mutation that reduces the length of the f1 functional origin from 200 to 40 nucleotide pairs.

0784 IN VITRO REPLICATION OF BACTERIOPHAGE Ø29 DNA: ISOLATION AND CHARACTERIZATION OF THE INITIATION COMPLEX, Kounosuke Watabe, Meng-Fu Shih and Junetsu Ito, Department of Molecular and Medical Microbiology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

We have developed an in vitro system for Ø29 DNA replication. Cell-free extract prepared from Ø29 infected Bacillus subtilis carries out the semi-conservative replication of exogenously added Ø29 DNA. DNA replication in this system closely mimics the Ø29 DNA replication in vivo. Thus, initiation of replication takes place at either terminus of the template Ø29 DNA. After initiation, a daughter strand is synthesized by strand displacement mechanism. Only Ø29 DNA-protein complex, but not protein free DNA, is active template in this system. The termini of the DNA products synthesized in this system are covalently attached to a 30,000 dalton terminal protein. Using this system, we isolated "initiation complex" from Ø29 infected-cell free extract through phospho- and DNA-cellulose chromatography. This initiation complex catalyzes specific complex formation between a 30,000 dalton terminal protein and dAMP in the presence of Ø29 DNA-protein. The initiation complex contains Ø29 terminal protein and other protein(s) which are different from the host DNA polymerase III. The properties of this complex and mechanism of initiation in Ø29 replication will be discussed.

0785 PRIMING OF DNA AT THE REPLICATION ORIGIN OF T7 PHAGE, Yuji Kohara, Kenji Sugimoto and Tuneko Okazaki, Institute of Molecular Biology, Faculty of Science, Nagoya University, Nagoya 464 Japan

Two models have been proposed for the initiation mechanism of T7 phage DNA replication[a]; (1) transcription from the primary origin by T7 RNA polymerase exposes the primase (gene 4 protein) recognition sites, allowing synthesis of an RNA primer that is then extended by T7 DNA polymerase, (2) the transcripts from the origin by T7 RNA polymerase itself serve as primer. To test these models, we have analyzed in vivo replicating DNA of T7 phage.

When $T7_{3}am,_{4}am,_{6}am$ or $T7_{3}am,_{6}am$ phages were infected to non-permissive cells, a small amounts of T7 DNA synthesis were occured and prematurely ceased. Hybridization experiments showed that DNA synthesized in $T7_{3,4,6}$ infected cells exclusively consisted of L-strand components, while those in $T7_{3,6}$ infected cells consisted of both strand components. Restriction enzyme analyses of $T7_{3,4,6}$ replicating DNA showed that the DNA synthesis initiated at or near the primary origin and proceeded rightward. Then we have tried to map the primer RNA-DNA junction sites in the region containing the primary origin. With $T7_{3,6}$, several sites, all of which corresponded to the gene 4 recognition sites, were detected on both strands. With $T7_{3,4,6}$, however, no junction site was detected on both strands. These results, favoring the model (2), suggest that T7 RNA polymerase synthesize primer RNA and the transition to DNA synthesis occur at some point located downstream. Preliminary data suggested that the transition sites were rather dispersed.

(a) Romano et al., Proc. Natl. Acad. Sci., 78, 4107 (1981)

0786 A SHORT NASCENT DNA MADE AT AN ORIGIN OF T4 DNA REPLICATION, Paul Macdonald and Gisela Mosig, Vanderbilt University, Nashville, TN 37235

Bacteriophage T4 initiates DNA replication sequentially by at least two distinct mechanisms: de novo initiation at genetically defined origins, and initiation via conversion of recombinational intermediates into replication forks. We would like to define both the sequence of events during de novo initiation and the components involved. Previous results from this lab have shown that RNA polymerase is required, presumably to make an obligatory RNA primer for leading strand synthesis. Therefore we have isolated nascent DNA linked to RNA from Cs_2SO_4 gradient fractions that have densities intermediate between those of DNA and RNA. A probable structure of this nucleic acid is shown below.

DNA ⟵〰〰 RNA

After alkaline hydrolysis of the gradient fractions (to remove RNA) we find several DNA species of precise lengths. Southern hybridization of the major DNA species to both cloned and uncloned T4 DNA restriction fragments reveals that this DNA is complementary to a region on the T4 chromosome (near the junction of XbaI fragments 15 and 17) that contains a strong origin of DNA replication. This DNA may therefore be an initiator DNA. We have sequenced the cloned DNA in this origin region. In vitro transcription of cloned DNA from this region reveals two promoters facing in the direction of early transcription, and separated by a potential rho-dependent termination site. Either of these promoters could initiate an RNA primer. We also find a promoter-like sequence facing in the opposite direction. Its location and orientation suggest that it might have a role in initiation of DNA replication.

0787 SUPPRESSION OF A TEMPERATURE SENSITIVE T4 DNA POLYMERASE, Linda J. Reha-Krantz and Jennifer K.J. Lambert, Dept. of Genetics, University of Alberta, Edmonton, Alberta T6G 2E9, Canada.

Suppressor mutations, new mutations which allow conditional-lethal mutants to survive under formerly non-permissive conditions, have been useful in enzyme characterization and in illucidating protein-protein interactions. We have isolated a suppressor mutation which allows T4 DNA polymerase mutants *am*B22·*ts*M19 and *ts*M19 to grow at 43°, normally a restrictive temperature. The new suppressor mutant, $J5_{43}°$, has been located near the amino terminus of the DNA polymerase gene by conventional mapping techniques.

Phage	Burst Size 30°	43°
wild type	300	65
*ts*M19	171	≤.01
*am*B22·*ts*M19	118	≤.01
*ts*M19·$J5_{43}°$	286	38
*am*B22·*ts*M19·$J5_{43}°$	367	44

The temperature sensitive DNA polymerase mutants, *ts*M19 and *am*B22·*ts*M19 are both strong mutators, increasing mutation frequencies more than 100-fold above wild type levels in both transition and transversion pathways. The $J5_{43}°$ mutation does not alter the high level of *ts*M19 induced transversion mutagenesis, but $J5_{43}°$ does lower the level of transitions. (Supported by the Alberta Heritage Foundation for Medical Research and the Natural Sciences and Engineering Research Council of Canada.)

Replication Proteins: Prokaryotic and Eukaryotic

0788 ALTERATION OF THE REVERSION FREQUENCY OF TRANSFORMED NIH/3T3 MOUSE CELLS, Robert F. Baker, Molecular Biology Division, University of Southern California, Los Angeles, CA 90089-1481

We have measured the frequency of reversion (to an anchorage-dependent phenotype) of spontaneously transformed (anchorage-independent, neoplastic) NIH/3T3 mouse cells. This reversion frequency can be increased from 5 - 10 revertants per 10^6 transformed NIH/3T3 cells (spontaneous reversion frequency) to a maximum of 70 - 80 revertants per 10^6 cells. This increase resulted after DNA-mediated transfection of a population of transformed NIH/3T3 cells, with DNA obtained in the following way: A first population of transformed NIH/3T3 cells was transfected with mouse or human whole cell DNA pieces and then immediately placed under selective pressure for reversion. (This selective pressure resulted from placing the transfected cells into media containing a low concentration of serum and then exposing the culture to cycles of 5-bromodeoxyuridine and UV light). Hirt supernatant (containing extrachromosomal DNA resulting from the transfection of the DNA pieces) from the revertants was spread into an increasingly larger number of transformed NIH/3T3 cells by cycles of transfection, of succeeding new populations of transformed cells, with Hirt supernatant DNA obtained from the non-passaged revertants selected at each previous cycle. Reversion activity in sixth cycle Hirt supernatant DNA was sensitive to EcoRI, but not to BamHI or Sal I. When the first population (cycle one) of transformed NIH/3T3 cells had been transfected with human DNA pieces (to initiate the extrachromosomal DNA in the Hirt supernatants obtained at subsequent cycles), the mouse cell revertants obtained at the sixth cycle contained chromosomally-integrated human Alu I-like sequences (Baker, R. F., Proc. Nat. Acad. Sci. USA, in press).

0789 CLONING OF *B.SUBTILIS* *polC*: THE STRUCTURAL GENE FOR DNA POLYMERASE III, Neal C. Brown and Marjorie H. Barnes, UMass. Med. Sch., Worcester, MA 01605

We have cloned in *E.coli* the *polC* region of *B.subtilis*. Cloning has exploited the plasmid p1949 and the following *polC* markers: *azp-12*, a mutation yielding an arylazopyrimidine - resistant polymerase and phenotype (azp^r), and *dnaF* and *ts-6*, distinct *polC* mutations specifying a temperature-sensitive (ts^-) phenotype. p1949, a hybrid constructed from plasmids pC194 and pMB9, carries a single EcoRI site and chloramphenicol resistance (cm^r), and replicates autonomously in *E.coli* (Haldenwang et al. J. Bact. *142*:90, 1980); in contrast, replication and expression of p1949 in *B.subtilis* requires its integration in the host genome. Integration requires *B.subtilis* *rec* functions and ligation of the plasmid to homologous *B.subtilis* "guide" sequences. The cloning strategy was: (1) to ligate EcoRI-derived fragments of DNA from *B.subtilis* *azp-12* to EcoRI-incised p1949; (2) to transform the (ts^-) *B.subtilis* *dnaF* with the hybrid product and select cm^r ts^+ azp^r clones; (3) from among these to isolate a *B.subtilis* clone with p1949 linked intimately to the *polC* region; (4) to isolate, to fragment, and to circularize the DNA of the latter clone; (5) to transform *E.coli* with the product, and (6) to isolate from cm^r *E.coli* transformants p1949 hybrids containing *B.subtilis* DNA encoding the *polC* region. One hybrid, p154T, was obtained. p154T contains a 6.5 kb fragment of *B.subtilis* DNA encompassing two of the three *polC* markers - *azp-12* and *ts-6*. With this fragment we have probed plasmid, cosmid, and phage-derived *E.coli* banks of *B.subtilis* DNA in an effort to isolate a complete copy of *polC*. The structure and further cloning of *polC* in *E.coli* will be discussed. Supported by USPHS grant CA15915 to NCB).

0790 ANALYSIS OF RNA PRIMER AT THE 5'-TERMINI OF NASCENT DNA CHAINS SYNTHESIZED IN PERMEABLE CELLS OF *B. SUBTILIS*, Nilima Sarkar and Gaspar Banfalvi, Boston Biomedical Research Institute and Harvard Medical School, Boston, MA. 02114.

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 [Bhattacharya and Sarkar, Biochem. (1981) *20*, 3029, J. Biol. Chem. (1982) *257*, 1610]. In the presence of 2'-3'-dideoxy ATP, nascent mercurated DNA synthesized in 1 min at 25°C was 33 nucleotide residues. Several lines of evidence indicated that at least 90% of the DNA thus isolated carried a terminally phosphorylated RNA moiety at its 5'-end: (1) The nascent DNA was resistant to degradation by spleen phosphodiesterase unless first hydrolyzed by alkali or RNAse; (2) the 5'-termini of nascent DNA could not be phosphorylated by polynucleotide kinase unless first treated with alkaline phosphatase or subjected to hydrolysis by alkali or RNAse; (3) alkaline hydrolysis of nascent DNA labeled with ^{32}P at the 5'-end released unlabeled DNA with a free 5'-terminus and ^{32}P-labeled prNp; (4) RNAse degradation of similarly labeled material produced an unlabeled DNA-containing polynucleotide fraction and ^{32}P-labeled ribooligonucleotides; (5) chromatography on dihydroxyboryl cellulose showed that the RNA moiety lacked a 3'-terminal cis-diol grouping unless first digested with T4 DNA polymerase. The ribonucleotide moiety was primarily 3 and 4 residues in length with the predominant sequence $(pp)pApG(pC)_{1-2}pDNA$. Our approach, pulse-labeling with mercurated nucleotides, thus represents an important advance in the study of early intermediates in DNA replication, permitting for the first time the partial sequence characterization of an RNA primer involved in bacterial chromosome replication [J. Mol. Biol., in press (1983)].

0791 CLONING AND IDENTIFICATION OF THE ESCHERICHIA COLI dnaY GENE PRODUCT, David A. Mullin and James R. Walker, University of Texas, Austin TX 78712

Temperature-sensitive (Ts) dnaY mutants of Escherichia coli K12 are defective in DNA polymerization at elevated temperatures. In order to identify the dnaY$^+$ gene product and examine its expression and function, we have constructed hybrid plasmids that complement a dnaY(Ts) mutant. pDM1 consists of pBR322 DNA joined to a 4.5 kilobase-pair dnaY$^+$ fragment derived from the E. coli chromosome. Tn5 insertional mutagenesis of pDM1 combined with SDS-PAGE analysis of radio-labeled plasmid-encoded proteins in minicells revealed that a 25,000 dalton protein product is not made when Tn5 insertion had inactivated dnaY$^+$ complementing activity. We suggest that this 25,000 dalton protein is the dnaY$^+$ gene product. Information on the function of the dnaY protein has been obtained by isolating and characterizing new dnaY mutants. One such mutant, DM2412, is temperature-sensitive for DNA synthesis and phage M13 growth at 43°C. Cell-free protein extracts prepared from DM2412 catalyze phage M13 DNA synthesis at 30°C, but lose about 80% of this activity following incubation at 46°C for 15 minutes. An extract prepared from the isogenic dnaY$^+$ parent strain shows no loss of activity after similar heating. This result suggests that the dnaY gene product is required for M13 DNA replication in vitro.

0792 NUCLEOTIDE SEQUENCE OF THE ESCHERICHIA COLI dnaB GENE AND THE PRIMARY STRUCTURE OF dnaB PROTEIN. Naoki Nakayama*, Naoko Arai**, Yoshito Kaziro*, and Ken-ichi Arai**.

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**DNAX Research Institute, Palo Alto, CA 94304

We have determined the nucleotide sequence of a 1.8 kilobase pair region of the Escherichia coli chromosome comprising the coding region for dnaB protein and about 400 base pairs of flanking sequence. The amino acid sequence of dnaB protein derived from DNA sequence is consistent with our protein sequence data. The coding region is 1410 base pairs long (470 amino acids) and is preceded by possible promoter sequences and a ribosome-binding site sequence. Initiator Met residue is removed, leaving Ala as the N-terminus of dnaB protein. Introduction of 5'-noncoding and coding sequences of the dnaB gene into a lac fusion plasmid (Casadaban, M.J., et al. (1980) J. Bacteriol., 143, 971) leads to the production of hybrid protein with β-galactosidase activity. These results suggest that the 120 bp preceding the dnaB coding region contain promoter and translational initiation signals. By limited proteolysis, 14 amino acids are removed from the N-terminus of dnaB protein to generate Fragment I (M.W. 48,000). Fragment I is further cleaved between residues 189 and 190 to yield the C-terminal Fragment II (M.W. 30,000) and the N-terminal Fragment III (M.W. 10,000). Fragment II elicits DNA dependent ATPase activity, and probably exists as a hexameric form (Arai, K. et al. (1982) AMBO Symposium. "The future of Nucleic Acid Research" Academic Press, in press).

0793 PURIFICATION AND PROPERTIES OF THE ESCHERICHIA COLI dnaK GENE PRODUCT, Maciej Zylicz and Costa Georgopoulos, University of Utah, Salt Lake City, Utah 84132.

E. coli mutants in the dnaK gene have been shown to block λ propagation at the level of λ DNA replication by specifically interfering with the function of the λ P gene product. In addition, some dnaK mutations exhibit a unique bacterial phenotype at high temperature, affecting both bacterial DNA replication and RNA transcription. Recently we have shown that the dnaK gene product (gpdnaK) is one of the major "heat-shock" proteins of E. coli. In order to better understand the many functions of gpdnaK we have proceeded to purify it. This was accomplished by cloning the dnaK gene on the mutant, runaway plasmid pMOB45, resulting in a 50-fold overproduction of gpdnaK. Starting with these extracts, gpdnaK was purified to homogeneity and shown to be an acidic, monomeric, asymmetric protein of 77,000-M_r and 87,000-M_r under denaturing and native conditions respectively. It exhibits an ATPase activity, largely independent of DNA. Surprisingly, about 50% of the ATPase activity is resistant to boiling for 10 min. Rabbit antibodies raised against purified gpdnaK quantitatively precipitate this heat-resistant ATPase activity from E. coli crude extracts. That dnaK is the structural gene that codes for this ATPase activity has been shown by the fact that the dnaK756 mutation results in the production of a heat-resistant ATPase with a more acidic isoelectric point.

0794 THE dnaB PROTEIN OF ESCHERICHIA COLI groP MUTANTS. Heinz Schuster, Eckhard Günther, Erich Lanka, and Michael Bagdasarian, Max-Planck-Institut für Molekulare Genetik, Berlin-Dahlem, Germany.

E.coli groP dnaB mutants are unable to replicate λ wild type phage. They are subdivided into groPA and groPB mutants by their ability to replicate certain λ P mutants, called λπA and λπB (1). Active dnaB protein could be isolated from groPA mutants by using a ØX174 DNA-dependent dnaB complementation assay. On the contrary, from groPB mutants active dnaB protein could only be isolated in the presence of ATP or ADP.

The dnaB protein of the groP mutant B612, thermosensitive in the groP character (1), was studied in more detail. In the absence of ATP the protein exists mainly in its monomeric form and does not bind to immobilized ATP. Upon addition of ATP, the dnaB monomers reassociate to active multimers. Active multimers composed of dnaB- and ban subunits can also be isolated from a P1bac crr lysogen of groPB612 in which the groPB character is suppressed by the prophage.

Multicopy plasmids carrying the groP gene B612 or B534 yield amplified levels of dnaB protein in the corresponding groPB mutant. Under these conditions growth of λ wild type and λπA mutants at 30°C is restored by the B534- but inhibited by the B612 dnaB protein, whereas growth of λπB mutants remains unaffected. The results indicate that the replication ability of λ is influenced not only by the dnaB- and P mutant types but also by the relative amounts of both replication proteins.

1. C.P.Georgopoulos & I.Herskowitz (1971) in THE BACTERIOPHAGE LAMBDA, Cold Spring Harbor Lab.

0795 PRIMER SYNTHESIS BY PLASMID-ENCODED DNA PRIMASE. Erich Lanka and Jens P. Fürste, Max-Planck-Institut für Molekulare Genetik, Abt. Schuster, Berlin-Dahlem, Germany.

The broad host range IncP plasmid RP4 specifies a DNA primase. The primase gene is located within the TraI region of RP4 between 41.5 and 44.8 kb relative to the unique EcoRI site. Two polypeptides of M_r = 118,000 and M_r = 80,000 resulting from overlapping genes were isolated and separated from each other. Both these polypeptides possess priming activity in vitro. The two forms of RP4 primases share homologous amino acid sequences as shown by immunological experiments. However, primases of different origins such as the E.coli dnaG protein and DNA primases of the IncI complex and of RP4 are not antigenically related with each other. Nevertheless, plasmid-encoded primases of the IncI- and IncP-type catalyze the same in vitro reaction which is distinct from that of the host primase. The enzymes synthesize short oligoribonucleotides on ssDNA in a process which is uncoupled from DNA synthesis. The products are complementary to the template and range in size from 2 to 10 nucleotides. Primer synthesis begins at the 5'-end with cytidine-5'-monophosphate or cytidine. Furthermore, the enzyme utilizes certain cytidine analogs for chain initiation but other than cytidine compounds could not substitute for them. The second nucleotide preferentially is AMP resulting in products with the unique dinucleotide sequence pCpA... or CpA... at the 5'-end of the primers. The enzyme recognizes 3'-dGdT... sequences on short oligodeoxynucleotides used as templates for the synthesis of complementary RNA primers. For efficient utilization by the primase this sequence could either be located within or at the 3'-terminus of a polydeoxynucleotide chain. Based on genetic and biochemical evidence, the idea is favored that in vivo the enzyme exerts its priming function during conjugation.

0796 OVERPRODUCTION AND PURIFICATION OF THE TERMINAL PROTEIN, p3, OF PHAGE Ø29 DNA. José M. Hermoso, Juan A. García, Ignacio Prieto, José M. Lázaro, Luis Blanco, Ricardo Pastrana and Margarita Salas, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, Madrid-34, Spain

The formation in vitro of a covalent complex between protein p3 and 5'dAMP by incubation of extracts from Ø29-infected cells with {α-^{32}P}dATP, supports the model of priming of Ø29 DNA initiation of replication by the terminal protein (see Abstracts by Salas et al. and García et al., this volume). To get further insight into the formation of the initiation complex we have cloned gene 3 to overproduce and purify the protein. A Ø29 DNA fragment containing gene 3 and other early genes was cloned in plasmid pKC30, under the control of the P_L promoter of bacteriophage λ. After induction, E. coli containing the recombinant plasmid produced the synthesis of four polypeptides. One of them, of Mr 27 000, which account for 3% of the de novo synthesized protein, was characterized as protein p3 by radioimmunoassay and by the in vitro formation of the initiation p3-dAMP complex when supplemented with extracts from B. subtilis infected with a sus3 mutant. Protein p3 has been highly purified from induced E.coli cells by DEAE-cellulose chromatography, eluting at 0.7 M KCl, and gel filtration through a Sephadex G200 column. No DNA polymerase activity copurifies with protein p3. The requirements for the in vitro formation of the initiation complex with the purified protein p3 will be described.

0797 IN VITRO INITIATION OF PHAGE Ø29 DNA REPLICATION. Juan A. García, Luis Blanco, Miguel A. Peñalva and Margarita Salas, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma,Canto Blanco, Madrid-34, Spain

Phage Ø29 has a protein, p3, covalently linked to the two 5' termini of the DNA. To test the model by which protein p3 acts as a primer in the initiation of Ø29 DNA replication (see Abstract by Salas et al.,this volume) we have developed an in vitro replication system. By incubation of extracts from Ø29-infected cells with {α-^{32}P}dATP, a covalent complex between protein p3 and dAMP is formed. This reaction requires ATP, although other nucleotides can be also used. The initiation reaction is not inhibited by novobiocine, nalidixic acid, aphidicolin, rifampicin or 6(p-hydroxyphenylazo)uracil, an inhibitor of the B. subtilis DNA polymerase III. The formation of the initiation complex requires Ø29-DNA protein p3 as a template; no reaction takes place with proteinase K-treated Ø29 DNA. Isolated protein p3-containing Eco RI fragments from the left or right DNA ends are active in the formation of the p3-dAMP complex while internal fragments are not. Protein p3-Ø29 DNA complex digested with the restriction enzymes Hpa II, Hinf I and the mixture of Mbo I plus RsaI, which produce shorter terminal fragments are also active.

The initiation complex formed in vitro can be elongated by addition of dNTPs. The product elongated in the presence of ddCTP releases, upon treatment with piperidine, the expected oligonucleotides, 9 and 12 bases long.

0798 IN VITRO STUDIES OF ROLES AND INTERACTIONS OF REPLICATION FACTORS IN λ DNA REPLICATION, Angelika Schäfer, Alfons Anderl, Walter Reiser and Albrecht Klein, Microbiology, University of Heidelberg, Im Neuenheimer Feld 230, 6900 Heidelberg, West Germany

In a soluble *in vitro* system λdv replication requires O and P protein besides other host cell factors. The role of helicase II in the *in vitro* system is under investigation. Inhibition of helicase II by antiserum results in the production of a characteristical DNA fragment. Attempts are made to localize this fragment on the λ DNA.

O protein binds specifically to DNA containing the origin of λ DNA. P protein forms a complex with the cellular replication protein dnaB. The possible interaction of O protein with the P protein is presently under investigation. Studies of mutants in the λ P gene which affect the binding with the dnaB protein show the possible site for interaction with the dnaB protein and strongly suggest the possible site involved in interaction with the O protein.

0799 DNA SYNTHESIS IN SLOWLY GROWING ESCHERICHIA COLI B/r A AND B/r K CELLS STUDIED BY FLOW CYTOMETRY, Kirsten Skarstad, Erik Boye, and Harald B. Steen, Norsk Hydro's Institute for Cancer Research and The Norwegian Radium Hospital, Montebello, Oslo 3,Norway.

DNA replication in Escherichia coli B/r A and B/r K was studied by flow cytometry. With this technique measurements of the cellular contents of DNA and protein in single cells can be made with a precision of a few percent and at a rate of 10^4 cells per sec. The resulting three dimensional histograms show the number of cells as a function of cellular DNA and protein contents and give detailed pictures of the cell cycle distribution with regard to these parameters. The DNA replication patterns of slowly growing E. coli (doubling time T >60 min) is described by the duration of the three periods C (DNA replication), B (pre-replication), and D (post-replication). Histograms of slowly growing chemostat cultures showed that B and C + D increase with decreasing growth rate, the B period occupying an increasing fraction of the cycle. Replication patterns of B/r A and K were quite similar. At extremely low growth rates (T=17h) B/r A cells had a B period of 0.8T, a C period of 0.1T, and a D period of 0.1T, and B/r K cells (T=16h) had a B period of 0.6T, a C period of 0.15T, and a D period of 0.25T. Information about mass increase was also obtained, revealing different patterns of protein synthesis for the two substrains. The DNA and cell size distribution of batch cultures in strict exponential growth was found to vary with time, indicating that such cultures are not suitable for studies of cell cycle kinetics.

0800 PEDIGREE ANALYSIS OF PLASMID SEGREGATION IN YEAST, Andrew W. Murray and Jack W. Szostak, Sidney Farber Cancer Institute,Harvard Medical School, Boston, MA, 02115

We have used pedigree analysis to invstigate the mitotic segregation of circular and linear DNA plasmids in S.cerevisae. This was done by following genetic markers on the plasmid in cell lineages where mother and daughter cells were seperated by micro-manipulation. We saw 3 types of cell divisions: S(symmetric),M(mother) and D(daughter)in which the plasmid is segregated to both progeny cells, only to the mother, or only to the daughter respectively. The segregation frequency (SF) represents the rate at which cells lacking the plasmid are generated and is the fraction of total divisions in which only one of the progeny receives the plasmid (SF=(M+D)/(S+M+D)). Segregation bias (SB) is the ratio of M to D divisions (SB=M/D) and is thus unity for a randomly segregating plasmid. For 5 different plasmids whose origin of replication is an ars element, segregation frequency varied from 0.39 to 0.53, and segregation bias from 12 to >20. Thus the segregation of these plasmids is markedly non-random. The plasmid YEp13 carries the replication origin of the 2μ circle and behaved like an ars plasmid when present in a strain which lacks endogenous 2μ circle (SF=0.44,SB>20). However in an isogenic strain containing intact 2μ DNA the segregation frequency and bias was drastically reduced (SF=0.07,SB=1.7).

The linear ars plasmid pSZ216 had a segregation frequency of 0.25 and a segregation bias of only 1.5 indicating nearly random segregation. The strong segregation bias for circular plasmids may reflect constraints which do not act on linear molecules. Further experiments will be discussed in the framework of a model which explains the above observations.

0801 SEQUENCE, HAIRPIN STRUCTURE AND RECOMBINATION AT TELOMERES OF THE PHYSARUM rDNA MINICHROMOSOME, Gerald R. Campbell, Peter Bergold and Edward M. Johnson, Rockefeller University, New York, NY 10021

The rDNA of Physarum polycephalum consists of multiple 61-kb palindromic molecules which replicate as discrete linear entities. We have cloned and sequenced rDNA termini, and provide evidence for participation of a complex hairpin structure in recombination and replication at the termini. The sequence of 840 nucleotides at the ends consist of a series of degenerate tandem repeats. These repeating units are themselves complex inverted repeats based on component repeats of 50 and 70 nucleotides. These inverted repeats can form hairpin secondary structures at ends of nucleolar rDNA molecules, as visualized by electron microscopy. Sequences associated with one-nucleotide single-strand gaps found in nucleolar rDNA are frequently present near the apices of the larger hairpin component of these repeats, suggesting the presence of gaps at these sites and the subsequent formation of a single-stranded tail from the hairpin loop. Recombination forms involving these repeats are seen by electron microscopy and may function during replication of the ends of the linear DNA molecule. Using rDNA terminal sequences we have constructed linear minichromosome vectors for use in Physarum and higher eukaryotic cells.

0802 INTRAMOLECULAR RECOMBINATIONS AND DELETIONS OCCURRING IN COMPOSITE CENTROMERE-2 MICRON PLASMIDS IN YEAST, Gary Tschumper and John Carbon, University of California, Santa Barbara, CA 93106

The yeast 2 micron plasmid exhibits high copy number and non-Mendelian segregation. By contrast, plasmids containing a cloned yeast centromere exhibit low copy number and Mendelian segregation in yeast. When both centromere and 2 micron DNAs are combined on the same plasmid, the copy number and segregation properties of the centromere are dominant. Combination of both DNAs on the same plasmid however generates an incompatibility which is sometimes resolved by intramolecular recombination and deletion in the composite plasmid in yeast. This is evident from recovery of some composite plasmids that contain a deletion of either the functional centromere DNA or a specific segment of 2 micron DNA. Composite plasmids in which one or the other type of deletion has occurred do not yield additional deletions upon transformation into yeast, indicating that the incompatibility is relieved by either type of deletion. Our results show that these deletion plasmids can only be recovered from yeast cells that contain a functional 2 micron FLP gene, however the gene need not be on the composite plasmid. This gene is known to catalyze site-specific recombination between two 599 base pair inverted repeat segments in the 2 micron plasmid DNA. Our results also suggest that these two inverted repeat segments must be present on the composite plasmids to recover deletion plasmids. In addition, we have found that plasmids containing the 2 micron deletion can only be recovered from yeast when the inverted repeat segments are in a specific orientation with respect to the deleted region.

0803 FIDELITY OF THE EPSTEIN-BARR VIRUS INDUCED DNA POLYMERASE. David Peim and Wendy Clough, Molecular Biology, University of Southern California, Los Angeles, CA 90089-1481.

The mechanism of fidelity of herpesvirus DNA polymerases and their interactions with nucleotide analogs are of particular interest because all currently used anti-herpes drugs are nucleotide analogs that function as inhibitors of viral DNA replication.

We have examined the interaction of the Epstein-Barr Virus (EBV)-induced intracellular DNA polymerase with the nucleotide analog 2-aminopurine deoxyribosetriphosphate (d2APTP). Unlike other polymerases used for comparison (phage T4, lymphocyte α and EB virion-associated), the EBV-induced polymerase was unable to distinguish between d2APTP and its natural counterpart dATP on artificial or natural templates.

Loeb and coworkers have developed an in vitro system for the measurement of replicative fidelity in which restriction fragment primed amber ϕX174 phage DNA is synthesized in vitro and subsequently transfected into bacteria to test for the reversion rate of the amber mutation. The fidelity of procaryotic DNA polymerases has been shown to differ significantly using natural templates as compared to synthetic templates. We are currently comparing the fidelity of EBV-induced polymerase with E. coli polymerase I and lymphocyte DNA polymerase α using the Loeb system. These experiments are being done both in the presence and absence of EBV polymerase-associated exonuclease activities to determine what role if any these nucleases play in the replicative process.

0804 PURIFICATION OF A DNA PRIMASE ACTIVITY FROM THE YEAST SACCHAROMYCES CEREVISIAE, Frances E. Wilson and Akio Sugino, Laboratory of Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

An activity which has primase-like properties has been purified from yeast. This activity is required for in vitro replication of yeast 2 μm plasmid DNA. It permits DNA synthesis by yeast DNA polymerase I on single-stranded circular ϕX174 and M13 DNAs and on synthetic poly(dT)$_n$. This activity, which is distinct from known RNA polymerases I, II, and III, synthesizes mainly oligoribonucleotides which are eight or nine nucleotides in length. The primase activity has a molecular weight of 65,000, and all but a small amount of primase activity can be separated from DNA polymerase I. The DNA products synthesized in the presence of primase plus DNA polymerase I on a single-stranded viral DNA are 300-500 nucleotides long and are covalently linked to oligoribonucleotides at their 5'-ends.

0805 A DNA PRIMASE ACTIVITY ASSOCIATED WITH YEAST DNA POLYMERASE I, David C. Hinkle and Russell J. DiGate, University of Rochester, Rochester, NY 14627

Yeast DNA polymerase I has been purified about 5,000 fold. In addition to its activity on primed DNA templates, our preparations of DNA polymerase I catalyze rNTP dependent DNA synthesis with single-stranded M13 DNA or poly (dT) templates. With the poly (dT) template, ATP is sufficient, but with M13 DNA all four rNTPs are required for maximal stimulation of DNA synthesis. Alkaline hydrolysis of the product formed in the presence of [α^{32}P] dATP and poly (dT) generates ^{32}P-labeled 3'(2') AMP, showing that a covalent ribo-deoxynucleotide linkage is formed. When M13 DNA is used as template ^{32}P is transferred to AMP (44%), GMP (27%), UMP (21%) and CMP (8%). When the product is labeled with [α^{32}P] or [γ^{32}P] ATP, DNase digestion releases ^{32}P-labeled oligonucleotides 6-13 residues in length. These results suggest that a ribo-oligonucleotide primer is synthesized by a primase activity present in our preparations of yeast DNA polymerase I. Since the ratio of activity on primed and unprimed templates remains constant throughout the later stages of purification, this primase activity may be closely associated with the DNA polymerase.

0806 PRIMASE AND DNA POLYMERASE α ACTIVITIES IN REGENERATING RAT LIVER. Michel Philippe, Geneviève Remones and Anne-Marie De Recondo, Institut de Recherches Scientifiques sur le Cancer. B.P. N°8, 94802 Villejuif Cédex. FRANCE.

By using aliquots corresponding to the different steps of DNA polymerase α purification (1) we have found a primase activity in all these fractions. As described by others (2,3) this activity catalyzed an ATP-dependent synthesis of DNA with single-stranded M13 DNA or poly deoxythymidilate templates. The reaction is completely dependent upon added template and ATP and is not inhibited by α amanitin. Strong arguments suggest that the primase activity is associated with a subspecies of DNA polymerase α present in a crude extract. Moreover, we have measured the variations of the primase activity in partially hepatectomized rat liver as a function of the regenerating time. We have found that the primase activity which is absent in normal rat liver did appear after hepatectomy : it increased as a function of time, reaching a maximum value 40 hours after. The curve of appearance of the primase activity as a function of regenerating time is roughly similar to that of DNA polymerase α. This observation strongly suggest that this activity play an important role during DNA replication.

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0807 PURIFICATION AND PROPERTIES OF DNA POLYMERASES α, γ AND MITOCHONDRIAL FROM WHEAT EMBRYOS.DNA SYNTHESIS IN ISOLATED WHEAT MITOCHONDRIA.

S.LITVAK; M.CASTROVIEJO; M.ECHEVERRIA; J.GRAVELINES; B.RICARD AND L.TARRAGO-LITVAK.
Institut de Biochimie Cellulaire et Neurochimie du CNRS. 1 rue Camille saint Saëns.33077 Bordeaux Cedex.France.

We have purified four DNA polymerases from quiescent wheat embryos or commercial wheat germ. Some properties of the enzymes are the following: All have molecular weight over 110,000 d although some of them may be found proteolyzed if no protease inhibitor is added during the purification procedure. Two DNA polymerases purified from the S_{100} are inhibited by aphidicolin and N-ethyl maleimide while ddTTP does not affect their activity. Thay have been named polymerases α_1 and α_2. A third polymerase purified from the soluble cytoplasm has many of the properties of the γ animal polymerase since it is strongly inhibited by ddTTP and ethidium bromide, it is resistant to aphidicolin and recognizes very efficiently a template poly rA.Howver this last enzyme is not confined to the mitochondria. The wheat mitochondrial DNA polymerase is an enzyme of 180,000 molecular weight resistant to aphidicolin and very sensitive to ddTTP and ethidium bromide.It uses very poorly a template poly rA. The conditions for optimal DNA synthesis with intact purified organelles will be described. The replication of the organelle genome using whole mitochondria follows a very similar pattern to that described for the solubilized mitochondrial polymerase. The association of DNA polymerase α_2 and the wheat tryptophanyl-tRNA synthetase will be shown.

0808 HIGH M_r POLYPEPTIDES FROM CALF AND MONKEY CELLS CROSS-REACT WITH MONOCLONAL ANTIBODY TO CALF DNA POLYMERASE α. Essam Karawya, Waltraud Albert, John Minna and Samuel H. Wilson. Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20205.

An IgM monoclonal antibody to calf thymus DNA α-polymerase was used to immunoprecipitate polypeptides in crude extracts from bovine (EBT) and monkey (BSC-1) cells. Immunobinding after transfer of immunoprecipitated polypeptides from SDS-polyacrylamide gels to nitrocellulose paper revealed that a polypeptide of M_r = ~ 200,000 reacted with the antibody. Using cells pulse labeled (30 min) with ^{35}S methionine, it was found that immunoprecipitates contained a ~ 200,000-M_r polypeptide that was predominantly labeled; after cells had been cultured for an additional 4 hr in medium containing unradioactive methionine, label in the immunoprecipitated ~ 200,000-M_r polypeptide was markedly reduced, whereas label in several lower M_r polypeptides increased. These results indicate that a ~ 200,000-M_r polypeptide in calf and monkey cells shares an antigenic determinant with α-polymerase and that this ~ 200,000-M_r polypeptide turns over with a half-life < 4 hr.

0809 REPLICATION OF M13mp7 DNA BY THE 9 S DNA POLYMERASE α FROM CALF THYMUS

Frank Grosse, Jörg Langowski and Gerhard Krauss, Med. Hochschule, Hannover, W.-Germany

We have investigated priming conditions and product distribution for the replication of single stranded M13mp7 DNA by the 9 S DNA polymerase α from calf thymus. Oligonucleotides of the deoxy- and ribo series comprising only 2-4 nucleotides can serve as primers. When replication is initiated with a specific primer comprising 15 nucleotides, barriers of replication show up, that can be correlated with stable secondary structures of the template DNA. Addition of the single stranded DNA binding protein (SSB) from E. coli helps the enzyme to proceed through the barriers. To achieve an optimal rate of synthesis the dNTPs have to be present at 300-400 uM. Under these conditions the turnover number is about 6 nucleotides / sec.
The 9 S enzyme contains a potent primase activity. Priming with rATP and/or rGTP occurs at random sites. The number of priming events is only 2-4 per template molecuele. Ap_4A is not able to initiate DNA synthesis.
The priming is sensitive to actinomycin D, it is not sensitive to α-amanitin.

Information on the assignment of the primase activity to the subunits of the 9 S enzyme will be given.

0810 STRUCTURAL AND FUNCTIONAL PROPERTIES OF CALF THYMUS DNA POLYMERASE α AND ORIGIN BINDING PROTEINS. Friedrich Grummt, Waltraud Albert, Gerd Zastrow, Andrea Schnabel, Jürgen Reinhold and Gabriele Erdelmeier, Institut für Biochemie, Universität Würzburg, Röntgenring 11, D-8700 Würzburg, GFR.

Calf thymus DNA polymerase α containes an abundant 118.000 M_r polypeptide as well as five lower molecular weight polypeptides in the range of 54.000 to 64.000 M_r. The structural interrelationships of these polypeptides were analyzed by tryptic peptide mapping. The high molecular weight polypeptide shared extensive primary structure homology with 57.000-, 58.000- and 64.000- M_r polypeptides and some limited homology with 54.000- and 56.000 M_r polypeptides. These results lead us to a model that predicts the existence of a common precursor with molecular weight > 140.000. Functional studies were carried out to analyze the catalytic and the Ap_4A-binding subunit as well as priming and proofreading acitivites of DNA polymerase α. In situ assay after SDS-polyacrylamide gel electrophoresis revealed that the 118.000- M_r polypeptide is the catalytically active subunit. The 57.000- M_r polypeptide has a high affinity binding site for Ap_4A. This was shown by affinity labeling as well as equilibrium dialysis experiments and Scatchard plot analysis with α polymerase holoenzyme. Calf thymus α-polymerase also exhibits priming acitivity using single-stranded circular M 13 DNA as a template. Ap_4A at a concentration of 10^{-4} M is a preferential substrate for this priming reaction being covalently incorporated into growing polynucleotides.

0811 STIMULATION OF CALF THYMUS DNA POLYMERASE ALPHA BY ATP, James V. Wierowski, Kathy G. Lawton, Joel W. Hockensmith and Robert A. Bambara, University of Rochester, Rochester, NY 14642.

Three distinct forms of DNA polymerase alpha (A,C and D) have been purified from calf thymus. ATP stimulates the synthetic rate of the A and C forms on several natural and synthetic primer-templates. In general, stimulation increased to a maximum at 4 mM ATP, followed by a decline at higher ATP concentrations. Stimulation of the A form ranged from 1.5- to 8-fold on poly(dT):oligo$(A)_{10}$, gapped bacteriophage fd RF DNA and poly(dA): oligo$(dT)_{10}$. The C form exhibited stimulation only on gapped fd RF DNA, with a maximum of 2-fold. dATP can substitute for ATP but is less effective. The non-hydrolysable ATP analogs AMPPNP and ATP-γ-S, and other deoxy- and ribonucleoside triphosphates are inactive for stimulation. Stimulation does not involve ATP mediated de novo synthesis of RNA primers.

In addition to the stimulation of synthetic rate, the size of the products synthesized processively by all three enzyme forms on the poly(dA): oligo$(dT)_{10}$ template is increased in the presence of ATP. Even at ATP concentrations where there is no stimulation of synthetic rate, 4 mM ATP with the C and D forms, there is an increase in processivity. Finally, in the presence of ATP the enzymes are more resistant to breakdown at 37°C. Supported by grants GM24441, CA11198, CA29324 and CA09363 from NIH, FRA220 from ACS, and a grant from the United Cancer Council of Rochester.

0812 DNA-PRIMASE FROM HUMAN (KB) CELLS, Teresa Shu-Fong Wang, Shi-Zhen Hu, and David Korn, Stanford University School of Medicine, Stanford, California 94305

Rapid protocols of immunoaffinity purification of KB cell DNA polymerase α, using monoclonal anti-pol α antibodies (Tanaka et al, J. Biol. Chem., 257, 8386 (1982)), yield enzyme preparations of very high purity that possess a DNA-primase activity. The polymerase is recovered as an immune complex and has a specific activity of 100K to 200K units/mg of non-IgG protein. The enzyme fractions contain 3 groups of peptides: (1) 185, 165, 145 and 120 Kd, that vary in their individual relative abundance in different preparations; (2) 80 Kd; (3) a variable cluster of 5-6 peptides between 45 and 60 Kd. The group of large peptides appears to comprise a closely-related family of proteins with almost identical tryptic maps, while the 80 Kd and 45-60 Kd peptides are largely unrelated to the large family or to one another. Immunoblots indicate that all 16 of our monoclonal antibodies recognize epitopes contained within the group (1) peptides, and renaturing gels strongly suggest that at least some of these peptides possess pol α catalytic activity. The primase activity is rNTP-dependent, α-amanitin-insensitive and synthesizes a de novo oligoribonucleotide primer that supports extensive DNA synthesis on single-stranded M13 DNA or on poly (dT). That the monoclonal IgG's recognize (i.e., bind or immuno-precipitate) a complex containing both pol α and primase activities argues for the close physical association of the respective catalytic peptides. However, the primase activity is strikingly resistant to aphidicolin, and the differential sensitivities of the primase and the pol α activities to several different neutralizing monoclonal antibodies suggest that the two catalytic centers are functionally distinct. (Studies supported by grant CA 14835 and the George D. Smith Fund).

0813 DNA PRIMASE FROM MOUSE CELLS, Ben Y. Tseng and Clarence N. Ahlem, Department of Medicine, University of California San Diego, La Jolla, CA 92093

We have previously reported the partial purification and characterization of a mammalian DNA primase from a human lymphoblastoid cell line (J.B.C. 257:7280-7283, 1982). The activity synthesizes oligoribonucleotides of predominantly 8 nucleotides in length that prime DNA synthesis. We have now purified a similar enzymatic activity over 5,000-fold, to near homogeneity, from another mammalian source, mouse hybridoma cells. Purified mouse DNA primase has a sedimentation value of 5-5.5S on glycerol gradients (with 0.2 M KCl) and no detectable DNA polymerase activity. Analysis by SDS polyacrylamide gel electrophoresis showed two components with approximate molecular weights of 55K and 45K and a 1 to 1 stoichiometry. These proteins co-fractionate along with primase activity in the final steps of the purification procedure and suggests that they are subunits of the enzyme. Characterization of primase and interactions with DNA polymerases will be described.

0814 BIOCHEMICAL GENETICS OF CHINESE HAMSTER OVARY CELL DNA POLYMERASE, Vishwanatha, J. K. and N. C. Mishra, Department of Biology, University of South Carolina, Columbia, South Carolina 29208.

Chinese hamster ovary cell line mutants resistant to the eukaryotic DNA polymerase inhibitors, aphidicolin, arabinosyl cytosine and arabinosyl adenine, show an altered growth with a longer doubling time, a slow rate of DNA synthesis and altered deoxyribonucleotide pool size. The specific activity of the enzyme DNA polymerase α is increased two fold although the activities of polymerase β and polymerase γ are unaltered. The polymerase α shows no change in sensitivity to the inhibitors in in vitro assays. Kinetic studies on the enzyme show an altered kinetics for inhibition by aphidicolin especially for the incorporation of dCMP. By genetic crosses, the dominance of genes conferring resistance to each of the three inhibitors has been established. By analysis of a large number of hybrid clones, the segregation of the genes conferring resistance to the inhibitors has been observed which has enabled us to construct a map for these three genes. Using the technique of DNA mediated gene transfer, we have introduced aphidicolin resistance from Chinese hamster cells to mouse L cells. Analysis of the transformants has revealed that aphidicolin resistance and ara-C resistance are controlled by different genes. Further studies on the transformants are being carried out aiming at cloning the genes for the resistance to the inhibitors in an attempt to understand the process of DNA replication in eukaryotic cells.
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0815 MAMMALIAN DNA POLYMERASE α HOLOENZYME FUNCTIONING ON DEFINED IN VIVO-LIKE TEMPLATES, Ulrich Hübscher, Dept. Biochemistry, University of Zürich, CH-8057 Zürich, Switzerland

A DNA polymerase α holoenzyme from calf thymus has been identified and purified by using defined in vivo-like templates (Hübscher et al., EMBO Journal (1982), in press). This enzyme complex has been compared to the corresponding homogeneous DNA polymerase α core from the same tissue. The holoenzyme is able to use single-stranded parvoviral DNA and M13 DNA with a single RNA primer as template. The core enzyme, on the other hand, although active on DNAs treated with deoxyribonuclease to create random gaps, is unable to act on these two long single-stranded DNAs. Escherichia coli DNA polymerase III holoenzyme and core enzyme were studied for comparison. The prokaryotic holoenzyme also copies the two in vivo-like templates, while the core enzyme is virtually inactive. The homologous single-stranded DNA-binding proteins from calf thymus and from Escherichia coli stimulate the respective holoenzymes and inhibit the core enzymes. These results suggest a cooperation between a DNA polymerase holoenzyme and its homologous single-stranded DNA-binding protein. The behaviour of Escherichia coli DNA polymerase III and DNA polymerase α holoenzymes on a variety of different chromatographic systems was similar: in nine of ten systems tested, they showed an analogous elution pattern, e.g. the holoenzymes forms of both sources either eluted intact or separated into at least two components. A protein factor has been separated from the mammalian DNA polymerase α holoenzyme by hydroxyapatite chromatography and this factor is able to restore holoenzyme activity. Data of the characteristics of this protein will be presented.

0816 DNA REPLICATION IN XENOPUS EGGS: IN VITRO AND IN VIVO STUDIES, Marcel Méchali, Richard Harland and Ron Laskey, MRC Laboratory of Molecular Biology, Hills Road, Cambridge UK.

A new cell-free system for DNA replication in eukaryotes, derived from the eggs of the frog Xenopus laevis, allows reproduction in vitro of the events acting at the replication fork during chromosomal replication (Mechali and Harland, 1982). Priming and elongation of DNA chains occurs with unusually high efficiency on single-stranded circular DNA templates. Up to 1.5 µg ss DNA are converted to the complete double-stranded molecule by 100 µl egg extract in 1 hr at 22°C. The replicated molecule is then ligated and made supercoiled by the extract. The overall rate of DNA synthesis is comparable to the fastest rate of chromosomal DNA synthesis during early embryogenesis.

The enzyme involved in priming of DNA chains resembles a eukaryotic primase. Priming occurs by the synthesis of a decaribonucleotide initiator. This process is sensitive to actinomycin D and resistant to α-amanitin. The elongation reaction is ATP dependent and requires DNA polymerase-α.

In vivo studies by microinjection experiments show that all DNA vectors tested replicate semiconservatively into Xenopus eggs (Harland and Laskey 1980). The efficiency of replication is proportional to the length of the template. The effect of some specific sequences as well as factors affecting the replication of microinjected DNA will be discussed.

0817 THE MACRONUCLEAR INVERTED TERMINAL REPEAT IN MICRONUCLEAR DNA, Glenn Herrick and Dean Dawson, University of Utah, Salt Lake City, Utah 84132

The macronuclear DNA molecules of the ciliate Oxytricha fallax are small and share the same inverted terminal repeat (ITR) sequence ($dC_4A_4C_4A_4C_4$). We are investigating the context of this sequence in the chromosomal micronuclear DNA (the DNA from which the small macronuclear molecules are derived). Hybridization to Southern blots of restriction digested micronuclear DNA (Nucleic Acids Res. 10, 2911-2924) have revealed two facts concerning the micronuclear context of this sequence: 1) much of it is found embedded in repeated long sequence blocks, 2) the portion of these repreated blocks which contains the ITR homology is 3-7 kbp long and is refractory to digestion with all restriction enzymes tested. Attempts to clone these refractory regions have been unsuccessful. Rather, a number of micronuclear clones homologous to the ITR have been analyzed and these contain dispersed copies of the sequence. Currently we are trying to determine the nature of the unclonable repeated sequence blocks which are homologous to the ITR. Preliminary results of restriction digests and Bal 31 experiments suggest they might be telomeric.

0818 COMPARISON OF THE DNA SEQUENCE AND SECONDARY STRUCTURE OF THE HSV L/S JUNCTION AND AAV TERMINAL REPEAT SEQUENCES. N. MUZYCZKA, M. RAYFIELD, G. MICHAELS, R. FELDMAN, and K.I. BERNS, University of Florida, Gainesville.

The defective parvovirus Adeno-associated virus (AAV) is absolutely dependent upon coinfection with either Adenovirus or Herpes Simplex Virus (HSV) for its multiplication. We have compared the terminal repeats of HSV-1F strain with the terminal 200 nucleotides of AAV. Our findings demonstrate an extensive homology between portions of the HSV inverted repeats found at the L/S junction and the termini of AAV. By computer analysis we have determined potential secondary folding patterns for both genomes. A cursory analysis of the sequences in HSV which are homologous to AAV reveals that they are not self complimentary and cannot easily be ascribed a secondary structure identical to that of AAV. The primary sequence within HSV will form a stable curciform secondary structure. The following points can be made about the a, b, and c repeats in HSV: 1) Regions b and c are complementary over a significant portion of their length with noncomplimentary stretches over this region being less than three base pairs in length or themselves forming stem and loop structures. 2) The ends of a can fold back on themselves to form large secondary structures. Moreover, when the b and c homology is used to align the ends of a, the b/a and c/a junctions are within 1 base of each other. 3) The short direct repeats within a are essentially a large loop with little secondary structure. The potential implications of these findings will be discussed.

0819 TIGHT BINDING PROTEINS ON HELA CELL DNA AT NUCLEAR MATRIX ATTACHMENT SITES, John W. Bodnar, Carol J. Jones, David H. Coombs, George D. Pearson and David C. Ward, Yale University, New Haven, CT 06510 and Oregon State University, Corvallis, OR 97331.

DNA-protein complexes have been isolated from HeLa cell nuclei and nuclear matrix. Proteins of 55kd and 66kd remain bound to HeLa DNA after treatment at 80°C in 2% SDS and purification by exclusion chromatography on Sepharose in SDS. These proteins, while tightly bound, are not covalently bound to HeLa DNA. They are distributed over the DNA every 40±11 kb and this spacing is constant throughout the cell cycle. The proteins are bound to the 2% of HeLa DNA which remains attached to the nuclear matrix after nuclease digestion when the bulk DNA has been reduced to nucleosome size pieces. This suggests that the tight binding proteins are involved in anchorage of DNA to the nuclear matrix.

0820 REPLICATION IN PARAMECIUM MITOCHONDRIAL DNA: FUNCTIONAL SEQUENCE REQUIREMENTS IN THE ORIGIN REGION. Arthur E. Pritchard and Donald J. Cummings, University of Colorado Health Sciences Center, Denver, CO 80262.

Replication of the linear mitochondrial DNA from Paramecium is initiated at a unique cross-linked terminus. The monomer cross-link is several hundred bases of single-stranded DNA arranged in an array of A-T rich direct tandem repeats (A.E. Pritchard and D.J. Cummings, 1981, Proc. Natl. Acad. Sci., U.S.A., 78, 7341-7345). Because of the cross-link, dimer length molecules are generated during replication. Restriction fragments containing the dimer form of the initiation region have been cloned and sequenced for five different species and several stocks within each species. The dimer clones contain head to head monomer fragments joined by the duplex form of the monomer cross-link sequence. The inter-species comparison reveals surprising sequence diversity except for a conserved "Goldberg-Hogness" box. The function of this sequence as a cross-link or as a replication origin apparently does not require a specific sequence, but all species examined contained direct tandem repeats. Adjacent to the cross-link sequence is a transcribed region which is highly conserved. The processing of dimer to monomer molecules was studied by determining the terminal sequence of a cross-linked monomer molecule. The monomer cross-link sequence is the same as the corresponding dimer sequence but two isomers, inverted complements of each other, were found. This supports the proposal that the dimer molecule is processed by staggered single strand nicks, on opposite strands, at either end of the cross-link sequence. The two strands fold back to form the two isomers of the terminal loop.

0821 INTERACTION OF AVIAN RETROVIRUS pp32 PROTEIN WITH VIRAL LONG TERMINAL REPEAT DNA. Duane P. Grandgenett, Ray J. Knaus and Paul J. Hippenmeyer. St. Louis University, St. Louis, Missouri 63110

The avian retrovirus pp32 protein possesses DNA endonuclease activity and unique DNA binding properties. We have investigated the DNA binding properties of pp32 using the nitrocellulose filter binding assay. Supercoiled DNA molecules rather than linear molecules are preferred by pp32 for binding or endonuclease activity. The pp32 protein was able to preferentially bind to viral long terminal repeat(LTR)DNA fragments over other viral and non-viral DNA fragments. Partial mapping of pp32 DNA binding sites on LTR DNA was accomplished by generation of deletions in LTR DNA sequences. The pp32 protein preferentially bound viral DNA fragments which contained only the viral promoter region and the adjacent "R" repeat sequences. Most of the LTR U_5, U_5-U_3 joint regions or the U_5-primer binding region did not enhance binding of pp32 to DNA. These above results confirmed previous published data from our laboratory describing pp32 binding to LTR DNA using the DNase I footprinting procedure. The biological role of pp32 is being investigated using site-directed in vitro mutagenesis of infectious cloned viral DNA. A variety of point, deletion and insertion mutations in the polymerase gene coding for the pp32 protein have been generated. Results demonstrating that pp32 is required in the viral replicative cycle will be presented.

0822 RAPID SPONTANEOUS CHANGES IN dhfr GENE COPY NUMBER, Randal N. Johnston, Robert T. Schimke, Stanford University, Stanford, CA 94305

Mammalian cells that survive selection with the chemotherapeutic agent methotrexate (MTX) display extra copies of the gene for dihydrofolate reductase (dhfr), the enzyme inhibited by MTX. We have determined whether changes in dhfr gene copy number can occur spontaneously, without selection or disruption of cell metabolism by MTX. Hamster CHO K_1 cells, not previously exposed to selection by MTX, were briefly stained with fluoresceinated MTX (F-MTX, which binds quantitatively to dhfr protein) in medium containing glycine, hypoxanthine and thymidine. With these staining conditions, cell proliferation is not inhibited by the F-MTX. Stained cells were sorted on a FACS instrument, and the cells with highest fluorescence retained for growth as a new population, in the absence of MTX or F-MTX. We find that after ten successive rounds of growth, staining, and sorting, the derived cells show 50-fold increases in staining intensity, are highly resistant to selection by MTX, and are amplified 60-fold for dhfr gene content. We also find that the rate of change in fluorescence varies among different starting populations. That is, cells already partially amplified can increase or decrease their fluorescence much more rapidly than do sensitive parental cells. For example, cells initially 50-fold amplified for dhfr can vary their fluorescence as much as 30-fold more rapidly than do MTX-sensitive cells. We therefore conclude that gene amplification can occur spontaneously, and that the rate of this occurrance may vary with initial gene copy number.

0823 ISOLATION OF THE INITIATION LOCUS OF THE AMPLIFIED DIHYDROFOLATE REDUCTASE DOMAIN IN CHO CELLS. J. L. Hamlin, N. H. Heintz, J. D. Milbrandt, and K. Greisen, Department of Biochemistry, University of Virginia, Charlottesville, VA 22908

We have been attempting to isolate a mammalian chromosomal origin of DNA synthesis that can be demonstrated to function in vivo. We have developed a Chinese hamster ovary cell line (CHOC 400) which has amplified the gene for dihydrofolate reductase (DHFR) approximately 500 times (J. D. Milbrandt, et al., PNAS 78, 6043, 1981). In addition to the DHFR gene itself, at least 100 kb of flanking sequences have been amplified. The homogenously-staining chromosomal regions in which these sequences are located initiate DNA synthesis at the very beginning of the S period. By pulse-labeling synchronized cultures at the onset of S, we have been able to show that, within the 30 restriction fragments comprising the amplified DHFR domain, initiation of replication is restricted to only three of these fragments (Heintz and Hamlin, PNAS 79, 4083, 1982). This result suggests that initiation occurs at fixed nucleotide sequences in mammalian replicons, and that at least one origin of DNA synthesis has been amplified as a part of the DHFR domain. In order to isolate the initiation locus (loci), we excised early-labeled fragments (ELFs) from preparative agarose gels, nick-translated them with ^{32}P-dCTP, and used them as hybridization probes on a genomic cosmid library prepared from CHOC 400 DNA. We found that virtually all the ELFs map together in a single recombinant cosmid (S21), indicating that these fragments represent a single early-replicating origin of DNA replication and its flanking sequences (Heintz et al., submitted). We have subcloned the HindIII fragments of cosmid S21 into poison-minus vectors containing the Herpes TK gene, and are testing these fragments for autonomous replication in CHO TK$^-$ cells.

Eukaryotic DNA Replication. Transposition of DNA

0824 STRUCTURE AND STABILITY OF LINEAR PLASMIDS IN SACCHAROMYCES CEREVISIAE, V.A. Zakian, G.M. Dani, and A.F. Pluta, Hutchinson Cancer Research Center, Seattle, WA 98014

Yeast cells transformed by circular plasmids containing an ARS can maintain these plasmids as self-replicating molecules. In contrast, transformation by linearized plasmids normally occurs by integration of the molecule into chromosomal DNA. Evidence will be presented that the termini of naturally occurring DNA molecules from two different ciliated protozoans (extrachromosomal rDNA from Tetrahymena thermophila and random restriction fragments from the macronuclear DNA of Oxytricha fallax) can enable recombinant DNA-ARS plasmids to persist as linear, extrachromosomal molecules in mitotic yeast cells, even though the sequence and structure of these DNA termini are different. We will also show that at least one of these DNAs (Tetrahymena rDNA) can provide telomere function during meiosis. In addition, the effect of linearity on the stability of recombinant DNA plasmids was determined. Most circular ARS plasmids are unstable (~20% loss/cell/generation): even plasmids containing yeast CENs are lost at a high rate (~3%) compared to a bonafide yeast chromosome (0.0005% loss; L. Hartwell, pers. comm.). We have found that linear plasmids without CENs are no more stable than the comparable circular plasmids (~20% loss). Moreover, linear plasmids containing CEN and Tetrahymena termini are less stable than circular CEN plasmids (~10% loss). Thus, linearization of a centromere plasmid has a detrimental effect on its mitotic stability, possibly due to spatial constraints on centromere and telomere function during mitosis, constraints which might not be satisfied by a small (12.7 kb) plasmid. In contrast, during yeast meiosis linear DNA molecules with yeast centromeres and Tetrahymena rDNA termini appear to function about as well as (but no better than) circular DNA molecules.

0825 NEW TEMPERATURE-SENSITIVE MUTANTS OF YEAST AFFECTING DNA REPLICATION Lawrence B. Dumas, Harinder Singh, Mark Pausch, Northwestern University, Evanston, Illinois 60201

We have isolated new mutants of yeast that are defective in mitotic DNA synthesis. This was accomplished by directly screening 1100 newly isolated temperature-sensitive yeast clones for DNA synthesis defects. Ninety-seven different mutant strains were identified. Approximately half had the fast-stop DNA synthesis phenotype; synthesis ceased quickly after shifting an asynchronous population of cells to the restrictive temperature. The other half had an intermediate-rate phenotype; synthesis continued at a reduced rate for at least 3 hr at the restrictive temperature. All exhibited protein synthesis resistant to the restrictive temperature. Genetic complementation analysis of temperature-sensitive segregants of these strains defined 60 new complementation groups. Thirty-five of these were associated with the fast-stop phenotype, 25 with the intermediate-rate phenotype. The fast-stop groups are likely to include many genes whose products play direct roles in mitotic S phase DNA synthesis. Some of the intermediate-rate groups may be associated with S phase as well. We are currently using these mutants as sources of soluble protein extracts, which both prime the synthesis of new DNA chains and elongate them, to screen for temperature-sensitive DNA synthesis in vitro.

0826 CONTROL OF REPLICATION OF THE YEAST PLASMID 2 MICRON CIRCLE, J.R. Broach, Y.-Y. Li, and M. Jayaram, State University of New York, Stony Brook, N.Y.
The yeast plasmid 2 micron circle and certain hybrid plasmids constructed from it are maintained stably and at high copy number in yeast cells. By examining the stabilities and copy numbers of various hybrid 2 micron circle plasmids, modified by specific insertions or deletion mutations, we show that these properties require the integrity of four plasmid loci. Two of these loci, designated REP1 and REP2, are active in trans and correspond to two open coding regions of 2 micron circle. The other two loci are active only in cis and correspond to a region spanning the origin of replication and a site, designated REP3, located several hundred base pairs away from the origin. REP3 is coincident with a region of the plasmid which consists of direct repeats of a 62 bp sequence and which is apparently depleted of nucleosomes in vivo. We also provide evidence which suggests that modulation of plasmid copy number does not involve transcriptional activation of replication even though we find that insertion of promoter sequences near the origin can enhance the stability of the plamsid and increase its copy number. On the basis of these results we suggest that the REP loci constitute a 2 micron circle copy control system, which promotes multiple rounds of plasmid replication when copy number is low. Since 2 micron circle replication is normally under strict cell cycle control, this system provides experimental access to the mechanism by which a eukaryotic cell normally prevents multiple initiation events at an origin during a single cell cycle.

0827 OLIGORIBONUCLEOTIDE PRIMED DNA SYNTHESIS BY A CRUDE CELL-FREE FRACTION FROM YEAST, Harinder Singh, Mark H. Pausch and Lawrence B. Dumas, Northwestern University, Evanston, IL 60201
A crude cell-free fraction has been prepared from S. cerevisiae that promotes oligoribonucleotide primed DNA synthesis on natural and synthetic single-stranded DNA templates. DNA synthesis on single-stranded circular M13mp9 and f1 templates has the following properties: (i) high efficiency, nearly 50% of input template copied in 60 min; (ii) formation of primarily open circular and linear duplex DNA products; (iii) stimulation (approx. 6-fold) by added rNTP's; (iv) insensitivity to α-amanitin at 1 mg/ml; and (v) partial inhibition by aphidicolin at 5 μg/ml. DNA synthesis on poly(dT) and poly(dC) exhibits an essential requirement for the complementary ribonucleoside triphosphate. Preliminary analysis of the presumptive RNA primer by direct (α-^{32}P rNTP) and indirect (α-^{32}P dNTP) labelling followed by DNase 1 digestion indicates an oligoribonucleotide, approx. 8 nucleotides in length. These observations suggest that yeast contains a DNA primase activity, which in concert with a DNA polymerase constitutes a system for de novo DNA synthesis. Similar systems have been recently described in other eukaryotic organisms. We are currently pursuing the biochemical fractionation of these activities as well as screening a collection of temperature-sensitive DNA synthesis mutants of yeast for defects in the same.

0828 APPARENT ARS DEPENDENT DNA SYNTHESIS IN YEAST EXTRACTS CAN BE MIMICKED BY T4 DNA POLYMERASE OR THE LARGE FRAGMENT OF E. coli DNA POLYMERASE I. John Scott and Ambrose Jong, Department of Microbiology, University of Illinois, Urbana, Illinois, 61801.
We have established DNA synthesis reactions in yeast cell-free protein extracts which exhibit strong (> 20 fold) apparent dependence on the presence of an ARS in the purified template DNA. The ARS elements were ARS1 from yeast chromosome IV and the ARS from the 2 μm yeast plasmid, each used as hybrid E. coli plasmids. However, the DNA preparation method was critical. ARS1+ plasmids prepared without chloramphenicol amplification from stationary-phase E. coli cells and those prepared using alkali treatment were inactive in the DNA synthesis reaction. TRP1 RI Circle prepared from log-phase yeast was also inactive. We compared activities of many of our ARS+ and ARS- template DNA preparations in yeast extracts with those found when the extract was replaced with either T4 DNA Polymerase or the large proteolytic fragment of E. coli DNA polymerase I. We found that the pattern of activity and inactivity of the particular template DNAs was comparable in all yeast extracts tried, and that the pattern was mimicked when the extract was replaced by either of the purified DNA polymerases. We also found that the size spectrum of product DNA fragments produced by the yeast extract was reproduced by the pure polymerase alone, and that the bulk of the fragments were released from the template in each case. These results strongly suggest that ARS dependent DNA synthesis reactions in yeast extracts observed by ourselves and by other laboratories may not represent DNA chain initiation reactions but rather may represent elongation of primer fragments present as base-paired strands on the template plasmids.

0829 REPLICATION AND EXPRESSION OF SV40 AND POLYOMA DNA DURING EARLY MAMMALIAN DEVELOPMENT, Dana O. Wirak, Lorraine E. Chalifour, Paul M. Wassarman and Melvin L. DePamphilis, Dept. Biological Chemistry, Harvard Medical School, Boston, MA 02115, U.S.A.

We have examined the ability of mouse cells at various stages of development to replicate and express simian virus 40 (SV40) and polyoma virus (PV) DNA when microinjected into mouse embryonic cells. Adult mouse somatic cells permit the complete transcription and replication of PV DNA, resulting in the production of new virus. In contrast, SV40 infected mouse cells express only the early viral genes (T,t-antigens); no SV40 DNA replication and little late gene transcription is observed. We have found that microinjection of 0.5-5 pg viral DNA into nuclei of growing oocytes containing about 12 pg cell DNA resulted in rapid conformational changes in the viral DNA consistent with DNA transcription and chromatin assembly. No significant DNA degradation occurred. The behavior of this viral DNA corresponded to that of the oocyte chromosomes. Viral DNA was not replicated, but early viral genes were expressed and viral coat proteins were absent. The synthesis of all forms of viral T-antigens implies accurate initiation and splicing of mRNA. Viral gene expression only occurred when the DNA was injected into actively transcribing oocyte nuclei. Gene expression did not occur when DNA was injected into the cytoplasm or when the oocyte was allowed to mature, arresting its own transcription. Experiments are now in progress to determine if viral DNA replication occurs in fertilized mouse eggs which are competant for cellular DNA replication. We hope to determine when permissive cell factors are expressed, whether viral gene products are always necessary for viral DNA replication, and whether specific DNA sites are utilized as origins of replication in the embryonic cell.

0830 CIS-ACTING SEQUENCES REQUIRED FOR THE REPLICATION OF POLYOMA VIRUS DNA: William J. Muller, Christopher S. Mueller, Anne-Marie Mes, and John A. Hassell. Department of Microbiology and Immunology, McGill University, 3775 University Street, Montreal, Quebec, H3A 2B4 Canada.

To define the minimal cis-acting sequences required for polyoma virus DNA replication we have constructed a number of recombinant plasmids carrying subgenomic fragments of viral DNA and measured their capacity for autonomous replication when introduced into mouse cells producing large T-antigen. The latter, named MOP cells, were isolated by transformation of 3T3 cells with a recombinant genome comprised of the polyoma virus early coding region fused to the SV40 early promoter. Plasmids containing a 250 base pair fragment of polyoma virus DNA that includes the region in which bidirectional replication begins replicate autonomously in MOP cells. Deletion analysis of this region reveals that it is comprised of two sets of sequences. Only plasmids that carry both regions replicate autonomously. One of these regions contains the AT rich sequence and a large T-antigen binding site. We refer to this element as a core region by analogy to SV40. The other is located towards the late region and bears no sequence homology to SV40 DNA. Surprisingly, polyoma virus DNA sequences farther upstream than these can substitute for the second element. The location of the cis-acting sequences required for DNA replication relative to the early promoter and T-antigen binding sites will be discussed.

0831 APHIDICOLIN ARREST IMPAIRS REPLICATING SV40 CHROMOSOMES IRREVERSIBLY Gail Dinter-Gottlieb and Gabriel Kaufmann, Weizmann Institute of Science, Rehovot, Israel 76100

Aphidicolin inhibits the growth of various eukaryotic cells and the replication of a number of animal DNA viruses through interaction with DNA polymerase α and the viral DNA polymerases. While in vitro inhibition of polymerase α is reversible, its in vivo effect on SV40 DNA replication, which depends on the cellular polymerase, was found to be irreversible. Infected cells, when exposed to aphidicolin, progressively lost their ability to incorporate ^{3}H-dT into viral DNA in a subsequent pulse without the drug. This loss increased with drug concentration, and was time dependent. Further, the ability of replicating SV40 molecules, pulse-labeled prior to drug exposure, to mature to Form I DNA after drug removal, was impaired. While no degradation of the replicating SV40 molecules was detected on neutral sucrose gradients following exposure to aphidicolin for up to one hour, breakdown of the arrested replicative intermediates, with concomitant resumption of viral DNA synthesis, was seen with longer exposure to the drug. SV40 replicons synchronized at the origin were affected less by the drug than ongoing replicons, SV40 tsA replicons, restricted at 40° for one hour and recovered at 32° for one hour prior to a pulse at 40° with ^{3}H-dT, incorporated the label to the same degree, whether or not the drug was present during the recovery period. Nevertheless, ongoing tsA replicons, similar to wt replicons, were impaired by drug exposure. It appears that the damaged SV40 replicons prevent initiation of new rounds of replication until their degradation, perhaps by occupying a fixed number of nuclear replication sites.

0832 REPLICATION OF BOVINE PARVOVIRUS DNA BY CELLULAR DNA POLYMERASES ALPHA AND GAMMA, Robert C. Bates, Alice T. Robertson, Laura L. Briggs, and Ernest R. Stout, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

The ability of DNA polymerases alpha and gamma to replicate bovine parvovirus (BPV) DNA in a defined in vitro reaction was investigated. DNA polymerase alpha from fetal calf thymus and from SV40 transformed bovine fetal lung (BFL) cells and DNA polymerase gamma from fetal calf liver were purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose, DEAE-trisacryl, phosphocellulose, and single-stranded DNA-sepharose. Each enzyme preparation was characterized on the basis of template specificity, inhibitor sensitivity and homogeneity as determined by PAGE. Partially purified polymerase alpha from calf thymus was able to replicate BPV single-stranded virion DNA to nearly unit length double-stranded form. A more highly purified alpha preparation from transformed BFL cells was capable of adding only a few nucleotides to the 3' end of the viral DNA primer-template. Both polymerase alpha preparations required ATP for activity. In contrast to DNA polymerase alpha, polymerase gamma from fetal calf liver was able to replicate BPV single-stranded virion DNA to a unit length double-stranded DNA product indistinguishable from BPV double-stranded RF DNA by restriction enzyme analysis. These studies are being extended to: a) determine the association between the various DNA polymerases, BPV DNA and the nuclear matrix and b) identify the viral DNA species produced in infected cells in the presence and absence of specific inhibitors.

0833 INITIATION OF ADENOVIRUS DNA REPLICATION IN VIVO, Ronald T. Hay and Nigel D. Stow, MRC Virology Unit, Glasgow, Scotland G11 5JR.

Initiation of adenovirus DNA replication takes place at the ends of the linear molecule and proceeds via a strand displacement mechanism. The objective of this work has been to define the *cis* acting DNA sequences which are required for the initiation of adenovirus DNA replication *in vivo*. To this end sequences from the termini of the Ad.2 genome have been cloned into the *E.coli* plasmid pUC9 such that the terminal C of the Ad.2 genome recreates an EcoRI site adjacent to the Ad.2 terminus. To assess the biological activity of these DNA sequences plasmids containing two Ad.2 termini have been constructed. Restriction enzyme cleavage of these plasmids generates a linear molecule bearing adenovirus termini at each end. These plasmids have been introduced into cells by calcium phosphate mediated transfection. Southern blotting and hybridization with plasmid DNA sequences has shown that, in the presence of helper virus, these linear plasmids are capable of autonomous replication. Circular plasmids or plasmids containing only one Ad.2 terminus are incapable of replication. Thus sequences required for Ad.2 DNA replication are located close to the termini of the linear molecules. The precise localization of these sequences will be presented.

0834 THE INTERACTION OF PROTEINS AND DNA SEQUENCES REQUIRED FOR ADENOVIRUS DNA REPLICATION *IN VITRO*, Bruce W. Stillman and Fuyuhiko Tamanoi, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 11724.

The initiation of adenovirus DNA replication is primed by a deoxynucleotide (dCMP) that is covalently linked to the virus coded terminal protein precursor (pTP). We have purified a virus coded DNA polymerase of 140,000 daltons which is required for the formation of the pTP-dCMP complex. The 140K DNA polymerase is inactive in extracts prepared from Ad5*ts*149 infected HeLa cells grown at the non-permissive temperature. In addition to the 140K polymerase and pTP, HeLa cell factors are also required for the initiation and elongation of DNA replication. We have begun to characterize these host cell proteins. Specific DNA sequences within the origin of DNA replication are also required for initiation. These DNA sequences have been identified by mutagenesis of a cloned copy of the origin DNA. They are located at the end of the adenovirus genome, within the highly A.T rich region that is conserved between all sequenced human adenoviruses.

0835 EVIDENCE FOR SINGLE BASE INSERTION-DELETION AND GENE CONVERSION EVENTS NEAR THE ORIGIN OF REPLICATION IN BOVINE MITOCHONDRIAL DNA. W.W. Hauswirth, P.J. Laipis, P.D. Olivo, and M.D. Van de Walle. Univ. of Florida, College of Medicine, Gainesville, FL 32610.

We have determined the nuclotide sequence of the 910 bp region containing the origin of replication (D-loop region) in mtDNA from fourteen maternally related Holstein cows. The types of sequence variation noted imply that both gene conversion and single base insertion and deletion events may occur during mtDNA replication. Within that portion of the D-loop region containing the triple-stranded D-loop structuré, the sequences fall into four families distinguished only by base transitions at four nucleotide positions. Consideration of analagous sequences from animals outside this lineage suggests that these and other related D-loop genotypes may exist within the germ lines of all domesticated cows. This conclusion, coupled with the above data, leads to a model for generating the present set of D-loop genotypes utilizing a gene conversion process. The model postulates exchange of variant, single-stranded 7S DNA creating a mismatched D-loop followed by mismatch repair. A second type of sequence variation involves length polymorphism at the extreme 5'-end of the D-loop region. Comparison of cloned molecules from individual animals shows that a stretch of L-strand cytosine residues can range from ten to fifteen bases in length. The mtDNA of the animal is made up of a population of molecules in which this cytosine homopolymer also varies in length from ten to fifteen nucleotides. These observations suggest that the observed length polymorphism may be generated by polymerase slippage within the homopolymer region during mtDNA replication. (Supported by NSF grant PCM 81-19266 and NIH grant AG-01636)

0836 THE LIGHT STRAND ORIGIN OF MAMMALIAN MITOCHONDRIAL DNA REPLICATION, Douglas P. Tapper and David A. Clayton, Department of Pathology, Stanford Medical School, Stanford, CA 94305

Mammalian mitochondrial DNA contains two distinct origins of DNA replication. The heavy (H) strand origin of DNA replication is maintained as a stable displacement loop (D-loop) structure in both human and mouse mitochondrial DNA. The light (L) strand origin of replication in both species is separated from the H strand origin by 60% of the genome in the direction of H strand synthesis. Here initiation occurs only after the nascent H strand has traversed this region. The 5' ends of nascent L strands have been precisely mapped on both genomes. The 5' ends of nascent L strands map within a region of approximately 32 nucleotides which has no coding function in contrast to the remainder of the genome outside the D-loop region. This region which can assume a striking stem and loop secondary structure is in the midst of a cluster of five tRNA coding sequences. Nascent L strands from human mtDNA replicative intermediates have two distinct 5' ends separated by 37 nucleotides. One falls at the base of the stem of the noncoding sequence, the other in the anticodon stem and loop of the downstream $tRNA^{cys}$ coding sequence. Both contain 5' dAMP residues. Nascent L strands from mouse mtDNA have at least 16 distinct 5' ends that map over a 37 nucleotide region, most separated by a single nucleotide. The majority of these 5' ends including the most abundant species map within the stem and loop structure of the noncoding sequence defining O_L. Strong evidence suggests that each of these nascent strands has two 5' rNMP residues. Preliminary data indicate that these nascent L strands are the result of processing of large RNA primers. At least three distinct triphosphate terminated 5' ends have been located upstream from mouse O_L.

0837 CHARACTERIZATION OF THE INITIATION LOCUS OF A MAMMALIAN CHROMOSOMAL REPLICON N.H. Heintz, J.D. Milbrandt, and J.L. Hamlin Department of Biochemistry University of Virginia School of Medicine, Charlottesville, VA 22908

Our laboratory has been studying the organization and replication of the 135 kb amplified dihydrofolate reductase (DHFR) domains in methotrexate-resistant CHO cells (CHOC 400). Pulse-labeling studies in synchronized CHOC 400 cells have shown that only 5 of the 30-35 Eco Rl restriction fragments that comprise the amplified DHFR domain become labeled during the onset of the S period (Heintz and Hamlin, PNAS 79, 4083, 1982). Two of these early-labeled fragments (ELFs) were isolated and used to recover recombinant cosmids containing the initiation locus for the DHFR replicon (Heintz et al., submitted). Comparison of the restriction maps of these clones with the in vivo labeling pattern of early-replicating DNA shows that all the ELFs are derived from a single locus, suggesting that each amplified DHFR contains only one origin of replication. By mapping overlapping cosmid clones, we have determined that the initiation locus lies 20 kb from the 3' end of the DHFR gene. This result correlates well with that of synchrony experiments which show that the amplified DHFR genes are replicated in the 3' to 5' direction. Southern blot analysis has shown that the initiation region contains a 1.6 kb Hind III fragments that is disbersed approximately 500 times throughout the parental CHO genome. This and other subclones of the DHFR initiation locus are being tested for: 1) ars activity in yeast; 2) autonomous replication in CHO cells; 3) the ability to increase the rate of transformation of TK^- CHO cells to the TK^+ phenotype; and 4) the ability to hybridize to the 5' ends of nascent DNA synthesized in both CHO and CHOC 400 cells.

0838 FLOW CYTOFLUORIMETRY STUDIES ON THE EXPRESSION OF NUCLEAR PROTEINS AS A FUNCTION OF THE CELL CYCLE : J.J. Lawrence*, A. Chabanas*, J. Imbert**, F. Birg**, DRF/CEN-GRENOBLE, FRANCE (*) and U 119 INSERM MARSEILLE, FRANCE (**).

We have studied the expression of two nuclear proteins as a function of the cell cycle by means of flow cytofluorimetry after double staining of the cells for DNA and protein (using intercalative dyes and indirect immunofluorescence respectively).

Histone H1° was found to be synthetized all along the cell cycle, showing that this protein is not involved in the arrest of cell growth. However, in cells arrested in G1 at saturation density the amount of H1° was found markedly increased as compared to the corresponding G1of cycling cells.

T antigen was studied in two types of ts A 30 SV 40 transformed 3T3 cells. In one type (type A) the protein was found to be present in an approximately constant amount in all the phases of the cell cycle, while, in the other type (type N) it was found only in the G2 phase. This experimental approach is a very convenient way to study the regulation of a protein as a function of the cell cycle, which avoid the use of synchronisation procedures which very often disturbs the cell cycle. Three types of regulation process were observed : one in which the amount of protein is proportionnal to the amount of DNA (H1°), the second in which the amount of protein is constant during the cell cycle and the third one in which the protein is expressed in only one phase (G2).

0839 ABNORMAL DNA REPLICATION IN HUMAN MUTANT CELLS, Martin F. Lavin, Jane Houldsworth and Miriam Ford, Department of Biochemistry, University of Queensland, Brisbane, Australia

Lack of suitable mutants and the increased complexity due to chromatin structure have made the study of DNA replication difficult in mammalian cells.

Exposure of mammalian cells to ionizing radiation leads to a preferential inhibition of initiation of DNA replication, at low to moderate doses of radiation. We have recently shown that γ-irradiation does not inhibit inhibition of DNA replication in ataxia telangiectasia (AT) cells at radiation doses up to 10 Grays. Since ionizing radiation reduces the normal level of negative supercoiling in DNA it may inhibit initiation of replication by preventing DNA-protein complexing. In AT cells a complex between DNA and protein may be achieved after irradiation due to a mutation in a protein normally involved in recognition or binding to supercoiled DNA. The description of this abnormality in DNA replication in AT cells presents a potentially useful model for the study of initiation of DNA replication. Results will be presented on inhibition of initiation of DNA replication, changes to DNA supercoiling and accessibility of both parental and newly synthesized DNA to nuclease digestion in control and AT cells. The use of this model for protein complementation studies will be discussed.

0840 DNA-DEPENDENT ATPase ACTIVITY IN EPSTEIN-BARR VIRUS-INFECTED LYMPHOCYTES. Wendy Clough and Mary F. Dougherty, Molecular Biology, University of Southern California, Los Angeles, CA, 90089-1481.

DNA-dependent ATPases are major components of procaryotic DNA replicative complexes in vivo and in vitro, and include as a group three or four separate types of enzymatic activity. Evidence has accumulated that mammalian cells contain similar enzymes, but precise breakdown of their specific enzymatic activities beyond the description of DNA-dependent ATPase is not available in most cases and their roles in the mammalian DNA replication process have yet to be defined.

We have identified and partially purified a DNA-dependent ATPase activity from lymphocytes infected with the human herpesvirus, Epstein-Barr (EBV). This activity copurifies with the EBV-induced DNA polymerase and nuclease previously found in virus producer cell lines. The ATPase is tightly bound to these viral enzymes and separates from them only after several purification steps. Upon preliminary characterization this ATPase activity has been shown to differ from the herpesvirus-induced protein kinase activity previously found in EBV and herpes simplex infected cells. Instead this EBV-associated ATPase resembles activities previously reported to be in calf thymus, KB cells and rat mitochondria. We are currently characterizing a highly purified preparation of this enzymatic activity for its DNA substrate preferences, interaction with circular DNAs and effects on EBV DNA polymerase activity when added to polymerase assay mixtures.

0841 A HERPESVIRUS REPLICON AS A SHUTTLE VECTOR, Jim W. Barnett, Deborah A. Eppstein, and Hardy W. Chan. Syntex Research, Palo Alto, CA 94304

Defective herpes simplex virus type I DNA is composed of head-to-tail tandem repeats of small regions of the genome. Monomeric repeat units of class I defective DNA were cloned into bacterial plasmids. The repeat units functioned as replicons since both viral and covalently linked bacterial plasmid DNA replicated (with the help of nondefective virion DNA) when transfected into rabbit skin cells. Recombinant plasmids were packaged into virions and were propagated from culture to culture by infecting with progeny virus. Replication was evidently by a rolling circle mechanism since plasmid DNA was present in a high molecular weight form in transfected cells. Circular recombinant plasmid replicated with a high degree of fidelity. In contrast, linear plasmid underwent extensive deletions of both viral and bacterial sequences when transfected into rabbit skin cells. Derivative plasmids, a fraction of the size of parental plasmid, were rescued by transforming E. coli with DNA from the transfected rabbit skin cells. These plasmids functioned as shuttle vectors since they replicated faithfully in both eukaryotic and prokaryotic cells. Since only a small fraction of viral DNA present in the parental plasmid was retained in the rescued plasmids, the viral origin of replication was localized to the TR_S sequences adjacent to the junction with unique DNA.

0842 HERPES SIMPLEX VIRUS DNA SYNTHESIS: REQUIREMENT FOR THE VIRAL SPECIFIC DNA BINDING PROTEIN ICP8 AS A COMPONENT OF DEOXYRIBONUCLEOPROTEIN COMPLEXES, Susan S. Leinbach, University of South Alabama College of Medicine, Mobile, AL 36688.

Extracts from nuclei of Vero cells infected with herpes simplex virus type 1 (HSV-1) were prepared by sonication and fractionated by sucrose gradient centrifugation. Large amounts of an HSV-1 specific protein with a molecular weight of about 133,000 were found associated with DNA. This protein is the HSV-1 DNA binding protein ICP8 based on its association with DNA in nuclear extracts, its molecular weight and its immunoprecipitation with monoclonal antibody to ICP8 (provided by Dr. M. Zweig). Cells infected with two temperature-sensitive mutants of HSV-1 which do not synthesize viral DNA at the nonpermissive temperature were examined for deoxyribonucleoprotein complexes containing ICP8 (ICP8-DNPCs). One mutant tsD9 (provided by Dr. P. Schaffer) has a lesion in the gene encoding the viral DNA polymerase. The other mutant tsl8 (provided by Dr. R. Sandri-Goldin) has a lesion in the region of the genome encoding ICP8. ICP8-DNPCs were found in cells infected with tsl8 at the permissive temperature but not in cells infected at the nonpermissive temperature, although ICP8 was synthesized and present in nuclear extracts at both temperatures. In contrast, ICP8-DNPCs were found in cells infected with tsD9 at both the permissive and nonpermissive temperatures. The results from the tsD9 infection suggest that ICP8-DNPCs can exist in the absence of HSV-1 DNA synthesis. Therefore, the results from the tsl8 infection suggest that tsl8 encodes a thermolabile ICP8 and ICP8 is required for HSV-1 DNA synthesis as a component of DNPCs. ICP8-DNPCs isolated from wild type and mutant infected cells are being examined for their ability to synthesize DNA.

0843 HERPES SIMPLEX VIRUS MUTANTS WITH ALTERED APHIDICOLIN-SENSITIVITY, Donald M. Coen, Phillip A. Furman*, Doris P. Aschman, and Priscilla A. Schaffer, Harvard Medical School, Boston, MA 02115 and *Burroughs Wellcome Company, Research Triangle Park, NC 27709

Sensitivity to aphidicolin is a hallmark of eukaryotic DNA polymerase α. Herpes simplex virus (HSV) DNA polymerase resembles cellular α-polymerase in several respects including aphidicolin sensitivity. We examined 15 mutants of HSV known or presumed to specify altered DNA polymerase (pol mutants). Twelve of these mutants exhibit hypersensitivity to aphidicolin. The DNA polymerase specified by one such mutant, PAA^r5, was examined for aphidicolin sensitivity; its activity was inhibited 50% (I_{50} value) by 0.7 μM aphidicolin whereas polymerase specified by its wild-type parent exhibited an I_{50} value nearly seven-fold higher (4.5 μM). The aphidicolin-hypersensitivity phenotype of mutant PAA^r5 has been mapped to an 800 bp region within the HSV pol locus, defining a region of DNA which when mutated affects the interaction of this interesting compound with polymerase. In addition, new mutants displaying aphidicolin resistance have been isolated. All mutants which exhibit altered sensitivity to aphidicolin also exhibit altered sensitivity to the pyrophosphate analog, phosphonoacetic acid (PAA). In contrast, the three pol mutants which do not exhibit altered sensitivity to aphidicolin exhibit little if any alteration in PAA-sensitivity compared with their wild-type parents. Since PAA appears to bind at the pyrophosphate exchange-release site of herpes virus DNA polymerases, this correlation suggests that aphidicolin binds in close proximity to this site or that aphidicolin and PAA somehow share common mechanisms of inhibition.

0844 ENZYMATIC MECHANISM FOR INITIATION OF RETROVIRAL RNA-DIRECTED PLUS STRAND DNA SYNTHESIS BY PURIFIED REVERSE TRANSCRIPTASE, Kenneth F. Watson and John C. Olsen, University of Montana, Missoula, Montana 59812.

Events of reverse transcription leading to initiation of plus DNA synthesis have been investigated in reconstructed reactions containing purified reverse transcriptase and a model 3'-poly(A)-containing fragment of avian retroviral RNA. Annealing $cDNA_{5}^{100}$ to a 15S poly(A)$^{+}$ viral RNA at the short terminal redundancy sequence adjacent to the poly(A) results in a template·primer directing double-stranded DNA synthesis. In the absence of the four deoxyribonucleoside triphosphates, only reverse transcriptase-associated RNase H activity is observed with release of the 3'-poly(A) segment. Upon addition of substrates for DNA synthesis, elongation of $cDNA_{5}^{100}$ and subsequent degradation of viral RNA in RNA·DNA hybrid proceed. Within a very short time, plus DNA synthesis is initiated at a specific site about 300 nucleotides from the site of poly(A) release. The (+)DNA product is a specie 400 nucleotides in length which contains a 5'-ribonucleotide sequence. Our results suggest initiation of (+)DNA synthesis with an RNA primer generated from degradation of viral RNA template by reverse transcriptase-associated RNase H. Similar results are obtained with genome-length RNA and $tRNA^{trp}$ primer.

0845 SYNTHESIS AND INTEGRATION OF AVIAN RETROVIRUS DNA, John M. Taylor, Teresa Hsu, Anita Cywinski and Julie Smith, Fox Chase Cancer Center, Philadelphia, PA 19111

We have studied the unintegrated linear viral DNA synthesized during the first day of infection of quail embryo fibroblasts by an avian sarcoma virus. By chromatography on benzoylated naphthoylated DEAE cellulose, it has been shown that more than 99.5% of the linears contain single-stranded regions distributed across the molecule. The exposed regions are of plus-strand DNA. No genome-sized plus strand DNA has been detected; it appears to be exclusively subgenomic. The subgenomic species are being examined for specificity at the levels of priming, initiation and termination.

To quantitate integration of viral DNA at early times after infection we have isolated high molecular weight cell DNA free of unintegrated viral DNA; we have applied cell fractionation, sedimentation and BND-cellulose chromatography. Also we have detected via the Southern procedure unintegrated circular DNA that is equivalent to viral DNA that has integrated on itself. This phenomenon was first described for MLV by Shoemaker et al. (Proc. Natl. Acad. Sci. USA 77: 3932 (1980)). We can now determine the extent of this phenomenon at various times after infection and the relative involvement of small versus large circular forms of viral DNA.

0846 MAPPING OF DNA REPLICATION ORIGINS OF DUCK HEPATITIS B VIRUS, Katherine L. Molnar-Kimber, Jesse Summers, and William S. Mason, Institute for Cancer Research, Fox Chase, Philadelphia, PA 19111

Duck hepatitis B virus (DHBV), which is similar to human hepatitis B virus, has a small circular DNA genome. In contrast to most circular DNA genomes which are supercoiled, DHBV has a relaxed circular structure with a full length minus strand (3 kb) and a plus strand that varies in length, up to 3 kb. The circular structure is maintained by a ∿200-300bp overlap between the 5' end of the plus strand and the 5' end of the minus strand. Two approaches are being used to determine (i) the exact location of the 5' ends on the nucleotide sequence of the virus (ii) whether these 5' ends found in the virion DNA correspond to the origin of DNA replication of the individual strands. First, it is known that DHBV replicating core complexes isolated from infected liver can synthesize both minus and plus strands. Using these complexes, elongation of minus or plus strand intermediates in the presence of ^{32}P-deoxynucleotides and dideoxynucleotides results in a sequencing ladder suggesting that the origin is at a unique site. Comparison of the minus strand ladder to a sequence generated from Maxam & Gilbert cleaved fragments of cloned DHBV shows that the origin of the minus strand is ∿112-117b from an Ava I restriction site present in the cohesive overlap. Second, S_1 mapping of the 5' end of the minus strand DNA isolated from virions shows that it is also ∿113-116b from the Ava I restriction site. These results locate the 5' end of the minus strand of virion DNA and show that it actually marks the origin of DNA replication. Similar studies are in progress with the plus strand.

0847 A TEMPERATURE-SENSITIVE (TS) MUTANT OF FROG VIRUS 3 (FV 3) IS DEFECTIVE IN SECOND-STAGE DNA REPLICATION. R. Goorha, Division of Virology & Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.

It has been shown recently that FV 3 DNA replication occurs in two stages (R. Goorha, J. Virol. 43: 519-528, 1982). First-stage DNA synthesis is restricted to the nucleus, where the replicating DNA ranges from genome to twice genome size; second stage DNA replication occurs in the cytoplasm exclusively; and the replicating DNA is concatameric.

Isolation and characterization of a temperature-sensitive mutant (ts 12488) have firmly established this mode of FV 3 replication. At nonpermissive temperature (30°), DNA synthesized in ts 12488-infected cells was present in the nuclear fraction only, but viral DNA represented the full complement of FV 3 genomic sequences. The size of the replicative complex (as determined by neutral sucrose gradient analysis) was between unit and twice unit length of mature FV 3 DNA molecules. These characteristics establish that at a nonpermissive temperature, ts 12488 is arrested in the first stage of DNA replication. Temperature shift-down (30°→25°) of ts 12488-infected cells at 4 hr postinfection showed that within 30 min of the shift the replicative complex became very large. Furthermore, newly synthesized DNA was now found in the cytoplasmic fraction also. These results suggest that ts 12488, upon shift-down, enters immediately into the second-stage of DNA replication, where progeny DNA is synthesized as a large concatamer. Pulse-chase experiments demonstrated that this concatameric DNA serves as the precursor for the production of mature FV 3 genomes. This genetic evidence corroborates previous biochemical findings and firmly establishes a unique two-stage replication scheme for FV 3 DNA.

0848 NEWLY ASSEMBLED AND CONSERVED NUCLEOSOMES AT THE DNA REPLICATION FORK OF SEA URCHIN EMBRYOS, Karl M. Jakob and Smadar Ben Yosef, Weizmann Institute, Rehovot, Israel.

^{3}H-thymidine pulses (20 sec) administered to blastulae, label nascent DNAs of two classes of deoxyribonucleoproteins (DNPs): 1. DNPs[1], containing nascent DNAs (∿300 bp, 450 bp etc) which have no bulk equivalents in polyacrylamide gels.- These DNAs are transiently hyperresistant to cleavage by micrococcal nuclease (MN), when compared to bulk nucleosome DNA[2]. Loss of this hyperresistance to MN is one of the first events during the maturation of these DNPs to protect their newly synthesized DNAs as bulk dimers (∿ 400 bp),trimers etc . These presumably closely-packed nucleosome cores with nascent DNA and their maturation products[2], can also be labeled by a 45 sec ^{3}H-leucine pulse. The DNPs contain the 4 newly synthesized core histones in the same relative amounts as found in long labeled bulk DNPs. 2. Regularly-spaced nucleosome cores with nascent DNA and linkers which are hypersensitive to MN. - The kinetics of cleavage and digestion of ^{3}H-thymidine pulse-labeled chromatin to "-H1" mononucleosomes indicates nascent linkers which are more sensitive to MN than the ^{14}C-thymidine, long-labeled (bulk) chromatin. In contrast, ^{3}H-leucine pulse labeled chromatin is cleaved to "-H1" mononucleosomes like bulk chromatin, suggesting that these may be maturation products of hyperresistant structures. There are therefore nucleosomes which cannot be pulse-labeled with ^{3}H-leucine(conserved) but contain nascent DNA and hypersensitive linkers. This will be demonstrated from: a) MN digests which contain nascent DNAs of bulk repeat size but exhibit hypersensitivity to MN, in the same gel with hyperresistant oligomers; b) DNA and DNP gels of MN digests from embryos treated with a protein synthesis inhibitor prior to and during ^{3}H-thymidine pulses./[1]Nucleic Acids Res 9, 3991, 1981. [2]Cell, 14, 259, 1978.

0849 MUTATIONS ASSOCIATED WITH REARRANGEMENTS OF THE GENES FOR THE VARIABLE SURFACE ANTIGENS OF TRYPANOSOMES. Allison C. Rice-Ficht, Kenneth K. Chen and John E. Donelson, The University of Iowa, Iowa City, IA 52242

Trypanosomes are protozoan parasites which escape the mammalian host's immune response by sequentially expressing a series of variable surface glycoproteins (VSGs). Each glycoprotein is antigenically unique and its expression appears to be controlled at the level of DNA transposition/recombination. Based on Southern hybridization data these transpositions involve either the production of an expression linked extra copy of the structural gene (ELC) or a rearrangement of sequences flanking the 3' end of the gene. We have compared the sequence of a cDNA representing the expression-linked gene copy with its corresponding basic copy gene. In this comparison we find that the noncoding sequences of 87 nucleotides between the termination codon and the poly A sequence are completely non-identical. The sequences extending 16 codons into the protein from the termination codon contain 10 nucleotide differences but no deletions or insertions. A comparison of 108 codons preceding this area in the coding sequence show no nucleotide changes while the remaining 150 codons contain single nucleotide changes in 3% of the positions. Since the serodeme contains a single basic copy gene of this VSG, point mutations appear to be actively introduced into specific areas of coding sequence during duplication and transposition of the expression linked copy.

0850 DNA JUNCTION SITE SEQUENCES OF A TRANSPOSON-LIKE MITOCHONDRIAL PLASMID. Donald J. Cummings and Richard M. Wright. University of Colorado Health Sciences Center, Denver, CO, 80262

During cellular senescence, specific regions of mitochondrial DNA from the filamentous fungus Podospora anserina are excised and amplified. The most frequently occurring of these autonomously replicating plasmids, termed α-sen DNA is 2600 bp in its monomer form and contains most of the oxi3 gene. During senescence this plasmid has been found integrated into the nuclear genome. We have cloned this plasmid and have sequenced the excision site junction sequences and compared them directly with the young mitochondrial genome(Hae 23 and Hae 14).

Hae23 3' CCGGTTCCACAAGTTATATAACGTC CACGCGGCAAATTGCACGCAAAATTCAGG 5' Msp

J1

α Hinf ←ATATATCTGATTCCTGACCGACGAATAGGATG CACGCGGCAAATTGCACGCAAAATTCAGG 5' Msp

J2

Hinf ←ATATATCTGATTCCTGACCGACGAATAGGATG TATTGGTTAATATATTATCGTAGTAAGTC—> 5'Hae14

The pertinent features of these sequences will be discussed.

0851 INSERTION-LIKE ELEMENTS IN THE GOAT β-LIKE GLOBIN GENE CLUSTER, Sally E. Spence, Regina M. Young, Karen J. Garner and Jerry B. Lingrel, University of Cincinnati, Cincinnati, OH 45267.

We have identified a family of repetitive sequences interspersed throughout the goat genome. Members of this family are found in the introns of the goat γ,β^C and β^A globin genes, as well as in other positions in the goat β-like globin gene cluster. As the insertion-like elements represent the only major difference between the developmentally regulated γ, β^C and β^A globin genes, their location and sequence organization is of primary interest. The relative position of the repetitive sequences in the introns of the globin genes indicates that they resulted from at least two independent insertional events. All three inserted sequences in the introns are flanked by perfect direct repeats. Members of the repetitive family have been mapped to the intergenic regions within the 160 Kb β-like globin gene cluster. Use of the repetitive sequences from the γ and β^A globin gene introns to probe a goat genomic "library" has resulted in an estimate of 200,000 copies of the repeat per cell.

Comparison of the nucleotide sequence of repeats within and flanking the β-like globin genes has pointed out some interesting features of their organization. The sequence of some of the repetitive elements shows striking homology to the origin of replication of human BK virus, simian virus 40 and polyoma virus. We are assaying the ability of the goat repetitive sequences to serve as origins of DNA replication. In addition, we are determining if the presence of these elements affects transcription of the goat β-like globin genes.

0852 **DEVELOPMENT OF AN ASSAY FOR TRANSPOSITION OF TY SEQUENCES IN YEAST.** V. M. Williamson and R. Wilson, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, California 94568.

The transposable element Ty of the yeast Saccharomyces cerevisiae has several structural similarities to certain transposable elements of Drosophila and retroviral proviruses. These similarities suggest that these sequences may share common mechanisms for transposition or integration. As a step toward analysis of eukayrotic transposition we are developing an assay system for Ty transposition. The majority of the characterized cis-linked mutations which result in constitutive expression of ADHII, the normally glucose-repressed enzyme of alcohol dehydrogenase, are due to insertion of the transposable element Ty into the region upstream from the structural gene. Since a positive selection is available to easily screen for mutants which express ADH constitutively, this system could be useful as an assay for Ty transposition. We have constructed a strain appropriate for isolation of such mutants and are determining the transposition frequency under various conditions.

0853 Transcription and Translation Signals in the Ty Elements of Yeast, Alan J. Kingsman, Benjamin A Bowen, Michael F. Tuite and Susan M. Kingsman. University of Oxford. England.

The transposable Ty elements of yeast are present at about 30 copies per haploid genome and together they encode about 1-5% of poly A-RNA. The function of Ty elements is unknown and there is no information available concerning the relative contributions of different Ty elements to total Ty gene products nor is there any evidence for Ty elements encoding proteins. In contrast to similar Drosophila elements, such as copia and 412, the study of Ty element expression is hampered by the marked heterogeneity of Ty elements within a single genome. This makes RNA and in vitro translation products difficult to analyse. In order to overcome some of these problems we have used heterologous genes lacking either transcription or transcription and translation signals as probes for expression signals in the two Ty elements, Ty1-15 and Ty1-17. These two elements differ by two 1kb substitutions. Using human interferon- 2 and HSV tk coding sequences in transcription fusions and E.coli -galactosidase in transcription and translation fusions we have identifed both transcription and translation signals within the two Ty elements. These have been localised to both the "5'" delta region and to a region 3.5kb from the "5" delta. The two elements differ markedly in their expression capabilities suggesting that different Ty elements contribute to the total Ty gene product pool to different extents. The more efficient Ty1-15 expression system is about 10% as efficient as the yeast PGK system. Mating-type control of these systems will be presented.

0854 THE P FAMILY OF TRANSPOSABLE ELEMENTS IN DROSOPHILA MELANOGASTER, Kevin O'Hare and Gerald M. Rubin, Carnegie Institution of Washington, Department of Embryology, Baltimore, Maryland, MD 21210.

The progeny of certain inter-strain crosses of Drosophila melanogaster show frequent germline abnormalities. This is known as hybrid dysgenesis and in the P-M system there is now evidence that P strains harbor a family of transposable elements (P elements) which are absent from M strains. Transposition of P elements is repressed in P strains but when a P male is mated with an M female, the elements become mobilised in the developing germ cells of the progeny. The consequences of transposition include mutations and chromosomal rearrangements. Several mutations generated by hybrid dysgenesis in the white locus have been shown to be due to DNA insertions (P elements). These insertions have been used to characterise P elements found in various strains. In the P strain, 2, there are a number of large well conserved elements and a collection of smaller heterogeneous elements. The large elements are probably functional and provide trans-acting functions for transposition of functional and defective elements and functions for the regulation of transposition. The structures of both functional and defective elements from various strains will be presented. The metabolism of P elements with respect to site specificity of integration, precision of excision, chromosome rearrangement, etc. will be discussed.

0855 STRUCTURAL AND FUNCTIONAL STUDIES OF TRANSPOSITION INTERMEDIATES OF THE DROSOPHILA ELEMENT COPIA. Andrew J. Flavell and David Ish-Horowicz, ICRF Laboratories,London,UK

Structural comparisons between the Drosophila mobile element copia and integrated retrovirus proviruses have indicated a familial relationship between these genetic elements. We have previously isolated from cultured Drosophila cells circular copia elements whose structures are strikingly similar to the unintegrated circular proviruses presumed to be intermediates in retroviral integration. These circular molecules contain either one or two long terminal repeats (LTRs). We have examined the LTR-LTR junctions of several 2LTR copia circles to ascertain their origin. In no case have we found circle junctions similar to those seen in circular retrovirus proviruses. We observe highly heterogeneous DNA sequence in this region with insertions of up to 15 nucleotide pairs between the LTRs. The sequence of these insertions bears no relation either to the TTAA insertion usually found in provirus LTR-LTR junctions or to any analogous sequence predicted from the reverse transcription of a putative copia retrovirus. We conclude that at least the majority of these circles derive by excision of genomic elements. We have also found a 2LTR circle in which a large fragment of the copia element, bounded at one end by the former LTR-LTR junction, has been precisely inverted with the duplication of 5bp of a sequence at the other inversion break point. Such a structure almost certainly derived from self-integration of a 2LTR copia circle, suggesting strongly that such molecules are capable of integration. We conclude that copia transposition need not require an intermediate RNA transcript but can proceed via DNA exclusively.

0856 INTRAMOLECULAR TRANSPOSITION EVENTS MEDIATED BY Tn903, T.A. Weinert, N.A. Schaus, and N.D.F. Grindley, Yale University, New Haven, Ct. 06510

The formation of adjacent deletions and inversion-insertions are two possible consequences of transpositional recombination if a transposon and its target site lie on the same DNA molecule. We have developed an assay that detects such intramolecular transposition events to allow us to systematically study recombination mediated by the transposon Tn903. Tn903 is a composite transposon that consists of identical copies of the insertion sequence IS903 flanking in inverted orientation a kanamycin resistance determinant. We have constructed plasmids that carry a constitutively expressed galK gene and a variety of derivatives of Tn903. E.coli galΔ cells that contain these galK plasmids are sensitive to galactose; by selection for resistance to galactose we can therefore detect all intramolecular transpositional events that inactivate galK. Each independent genetic rearrangement is readily determined by isolation and restriction analysis of plasmid DNA from individual galactose-resistant clones.

Deletion formation, at least formally, involves joining of only a single end of the transposon to a new target site since the DNA junction at the deletion-distal end of the element remains unchanged. Using IS903 derivatives in which the transposase gene is expressed from the lac promoter, we have tested whether both ends of IS903 are required for adjacent deletions. We find that removal of the distal end of IS903 reduces deletion formation by 100-fold. The requirement for both ends suggests either that deletions are formed by a "cointegrate" pathway or that both ends must interact before transposition can be initiated.

0857 IMMUNITY SEQUENCES OF Tn3. Chao-Hung Lee, Ashok S. Bhagwat and Fred L. Heffron. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

A plasmid carrying a Tn3 is immune to another Tn3 transposition. It has been shown to be a cis-acting mechanism, i.e., a sequence in Tn3 may play a role in regulating the immunity.

We have been able to show that tnpA and tnpR genes are not necessary for immunity. By cloning fragments of Tn3 sequences into pACYC184, we have demonstrated that sequences near the right-hand end inverted repeat are required for immunity.

0858 SYNTHESIS AND FUNCTIONAL PROPERTIES OF THE Tn3 INVERTED REPEAT. Ashok S. Bhagwat and Fred Heffron, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724

We are in the process of synthesizing the inverted repeat of Tn3 using chemically synthesized oligomers. The strategy for synthesis, cloning and functional testing of the synthetic inverted repeat will be presented.

0859 RNA POLYMERASE BINDING AND TRANSCRIPTION INITIATION SITES IN THE TRANSPOSON TN3, William Wishart, Chiyoko Machida, Hisako Ohtsubo, and Eiichi Ohtsubo, SUNY at Stony Brook, Stony Brook, NY 11794

We have analyzed the E. coli RNA polymerase binding and transcription initiation sites in the transposon Tn3 carried by the plasmid pMB8. Results from nitrocellulose filter binding assays indicate that there are two regions within Tn3(a 207 bp region containing the N-terminal coding sequence of the transposase(tnpA) and repressor(tnpR) genes and a 332 bp region containing the N-terminal coding sequence for the β-lactamase(bla) gene) capable of forming stable binary complexes with RNA polymerase. DNase I footprint analysis with the 207 bp and 332 bp fragments further defined an extended region of protection, approximately 110 bp long, located between the transposase and repressor coding regions, and an 80 bp region of protection near the N-terminal coding sequence of the β-lactamase gene, respectively. In vitro transcription studies with fragments containing these protected regions allowed us to determine the precise transcription initiation sites for the transposase, repressor, and β-lactamase mRNAs. The transposase and repressor mRNAs are transcribed divergently and their transcription initiation sites(Tn3 coordinates 3095 and 3176, respectively) are separated by precisely 80 bp. The -35 homology regions for the transposase and repressor promoters are separated by 10 bp and the -10 homology region of the transposase promoter is coincident with the recombination site(res) for the site-specific recombinase activity(resolvase) of the repressor protein, required for resolution of Tn3 cointegrates. We discuss the significance of this complex divergently transcribed promoter region with respect to regulation of Tn3 transposition and the resolution functions and propose a model for coordinated regulation of the tnpA and tnpR genes.

0860 IN VITRO STUDIES USING CLONED TN3 TRANSPOSASE, Sylvia J. Spengler, Stephen P. Gerrard, and Nicholas R. Cozzarelli, University of California, Berkeley, CA 94720

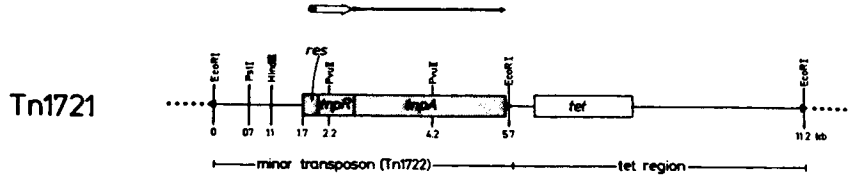
Transposition is a unique example of specific DNA replication and nonhomologous recombination. The bacterial transposon, Tn3, encodes three proteins: the transposase, product of the tnpA gene, a repressor-resolvase, product of the tnpR gene, and a β-lactamase which confers ampicillin resistance. The transposase is the only Tn3-specific protein required for replicon fusion in vivo. The level and activity of transposase are carefully regulated by cells. To avoid such control problems, we have cloned the tnpA gene under the control of the lambda promoter, P_L. Strains carrying the plasmid can be induced to overproduce transposase to 4% of the cellular protein. However, transposition of Tn3 is strikingly temperature sensitive, with the cloned transposase produced at 38° possessing very low activity in vivo at 30°. We have achieved significant overproduction for 2 hours at 32° following 15 min. at 42°. Using such a strain as the source of enzyme in replication competent extracts, we are currently investigating several probable Tn3 associated activities, including nicking and cutting at specific sites and specific replication of Tn3 DNA. In addition, we have defined the subunit structure of purified transposase using equilibrium sedimentation. The active dimer was the only form observed under all salt and concentration conditions.

0861 IN VITRO STUDIES USING CLONED TN3 TRANSPOSASE, Sylvia J. Spengler, Stephen P. Gerrard, and Nicholas R. Cozzarelli, University of California, Berkeley, CA 94720

Transposition is a unique example of specific DNA replication and nonhomologous recombination. The bacterial transposon, Tn3, encodes three proteins: the transposase, product of the tnpA gene, a repressor-resolvase, product of the tnpR gene, and a β-lactamase which confers ampicillin resistance. The transposase is the only Tn3-specific protein required for replicon fusion in vivo. The level and activity of transposase are carefully regulated by cells. To avoid such control problems, we have cloned the tnpA gene under the control of the lambda promoter, P_L. Strains carrying the plasmid can be induced to overproduce transposase to 4% of the cellular protein. However, transposition of Tn3 is strikingly temperature sensitive, with the cloned transposase produced at 38° possessing very low activity in vivo at 30°. We have achieved significant overproduction for 2 hours at 32° following 15 minutes at 42°. Using such a strain as the source of enzyme in replication competent extracts, we are currently investigating several probable Tn3 associated activities, including nicking and cutting at specific sites and specific replication of Tn3 DNA. In addition, we have defined the subunit structure of purified transposase using equilibrium sedimentation. The active dimer was the only form observed under all salt and concentration conditions.

0862 TN*1721*: ASYMMETRICAL TRANSPOSITION AND SITE-SPECIFIC RECOMBINATION, Rüdiger W. Schmitt, Josef Altenbuchner, Sabine E. Mötsch and Peter M. Rogowsky, Universität Regensburg, D-8400 Regensburg, Germany

The Tc-transposon Tn*1721* (Fig.) belongs to the Tn*3* family, but has features differing from Tn*3*. (i) Tn*1721* consists of a "minor transposon" encoding transposition functions and an amplifiable "tet region" encoding resistance. (ii) Transposition genes are arranged in the order *res* (resolution site) - *tnpR* (resolvase) - *tnpA* (transposase) and have identical polarity. (iii) Transposition events following the deletion of one terminal repeat of Tn*1721* suggest that transposition can proceed asymmetrically and that cointegrate formation is no necessary requirement. (iv) Resolvase catalyzes site-specific recombination at *res*; both, deletions and inversions are generated depending on direct or inverse orientation of two *res* loci in one replicon.



0863 BIOLOGICAL ROLES OF IS102 ENCODED PEPTIDES, Françoise Bernardi and Alberto Bernardi, Laboratoire d'Enzymologie du CNRS, 91190 Gif-sur-Yvette, France.

The complete sequences of the IS elements sofar obtained show a common organization : aside from the two inverted repeats two open reading frames are present, a larger one on one strand and a smaller one on the other strand included and in register with the larger one.

Our work on IS102 (a constituent of pSC101) is an attempt to assign biological roles to these peptides. We have taken advantage of the close similarities between IS102 and IS903 (a constituent of pML21). We have introduced internal deletions in vitro in IS102 (1 to 770 bp long) and complemented it with IS903. The ability of the mutated IS102 to promote deletion formation has been investigated both in the absence and presence of IS903. The small peptide is involved in transposition inhibition : no transposition of IS903 from pML21 to pSC101 occurs when the small IS102 peptide is intact. Loss of the COOH terminal of the large peptide allows deletion to occur, even in the absence of complementation by IS903. The frequency of the deletion formation is variable according to the number of aminoacids that have been removed or changed. Determination of the novel joint newly found in each case shows that the COOH terminal is necessary for the recognition of the inverted repeats and the new endpoints occur at other site on pSC101 or in the element itself. The level of deletion formation is drastically reduced when three aminoacids are deleted from the central region of the large protein, this fact could indicate the presence of a regulatory sequence on the element at this position. When large deletions have been created in IS102 abolishing completely deletion formation, IS903 carried by pML21 is able to induce deletion formation on pSC101 starting at the extremities of the mutated IS102, suggestion that some type of trans reaction is possible.

0864 THE TRANSPOSASE OF BACTERIOPHAGE MU CATALYZES ONLY A SINGLE REPLICATION EVENT, Martin L. Pato and Claudia Reich, National Jewish Hospital and Research Center, Denver, CO 80206

Transposition of bacteriophage Mu involves the coupled replication and integration of a copy of prophage DNA, catalyzed in part by the gene A product - the Mu transposase. When a population of cells is primed for replication of Mu DNA by induction of a lysogen in the absence of DNA replication, release of the block of replication results in a first round of replication of Mu DNA followed by subsequent rounds. If protein synthesis is inhibited before releasing the block on DNA replication, only the first round is observed. This result and our earlier results showing the functional instability of the Mu transposase (Pato and Reich, Cell 29:219-225, 1982) lead us to conclude that the transposase is used in only a single replication event and must be synthesized de novo for each round of Mu DNA replication.

0865 HOST MUTANTS OF E. COLI K12 WHICH AFFECT EXCISION OF TRANSPOSON Tn10, Victoria Lundblad and Nancy Kleckner, Harvard University, Cambridge, Massachusetts 02138.

Three illegitimate recombination events are associated with, but not promoted by, transposon Tn10: precise excision, nearly precise excision and precise excision of a 50 bp nearly precise excision remnant. All three are structurally analogous: excision occurs between two short direct repeat sequences, removing all intervening material plus one copy of the direct repeat. In each case, the direct repeats border a larger inverted repeat. We report here the isolation of host mutants of E. coli K12 which exhibit increased frequencies of precise excision of Tn10. In addition, all of the mutants display an increased frequency for nearly precise excision. However, none of the mutants are increased for the third excision event, precise excision of the 50 bp remnant, supporting the idea that precise and nearly precise excision occur by closely related pathways which are distinct from those pathways which promote the third type of excision event. Twenty five of the 39 mutants have been mapped to six distinct loci on the E. coli genetic map and have been designated texA through texF (for Tn10 excision). Mapping and genetic characterization indicate that each tex gene corresponds to a previously identified gene involved in cellular DNA metabolism: recB and/or recC, uvrD, mutH, mutL, mutS and dam. Previous proposals for excision pathways have suggested intrastrand snapbacks of the inverted repeat ends of Tn10 as possible intermediates. The role of these particular DNA repair and recombination genes in this process could be to mediate either the formation or resolution of such an intermediate.

0866 MECHANISM AND CONTROL OF TRANSPOSITION IN THE KANAMYCIN RESISTANCE TRANSPOSON TN903

Tn903 is a composite transposon consisting of two identical inverted repeats of the IS element IS903 [1050bp] bracketing a unique region [1000bp] which includes the gene for kanamycin resistance.One open reading frame,the putative tranposase gene,has been identified in IS903 by mutation.We are interested in three aspects of Tn903 structure and function. [1].Mechanism of transposition.The fact that Tn903 forms cointegrates as stable end products [10-30% of all transposition events] which are not resolved by Tn-encoded products suggests that Tn903 transposition is by a different pathway to that of elements of the Tn3 class. We hope to establish what controls cointegrate formation versus simple transposition. [2].Regulation of transposition.We are investigating the effect of transcriptional signals upon transposase production,which is limiting in transposition,and the possibility that use of promoters and/or tight binding of RNA polymerase to promoter sites may prevent productive interaction between transposase and the IS903 terminus.We have reason to believe that the stonger promoter activity across the inside end of IS903-right reduces the activity of this end in transposition events.[3].Evolution of transposons.Tn903 appears to be a primitive element in an early stage of development.The IS903 elements are capable of independent transposition,and the inefficient transfer of "young" transposons may be increased by mutations togive a more integrated unit by co-ordinating functions of the independent IS elements.

0867 THE PAR LOCUS OF pSC101, Christine Miller, William T. Tucker, Stanley N. Cohen, Stanford University, Stanford, Ca. 94305.

The par locus of pSC101 that codes for stable plasmid maintenance has been sequenced. Deletion mutants to delineate the regions which are responsible have been made. By Bal 31 digestions from unique restriction sites and by deletions generated from restriction sites within this region the sequences involved in partition have been limited to two areas. Both of these areas contain sequences that could form stable secondary structures. The par locus has been cloned and has been found to complement only in cis. These two facts might indicate that some sort of binding of the plasmid DNA is involved in the partition mechanism. Since plasmid DNA replication has been thought to involve membrane binding the attachment of the par^+ plasmid to the membrane and the lack of association in par^- plasmids has been studied. In connection with membrane binding the requirement for DNA replication also has been examined. The idea that replication of a plasmid during each cellcycle is required for correct partition suggested to us to look at partition during incompatibility studies. Par^- plasmids are always lost immediately in cells with an incompatible par^+ plasmid if no selection is maintained. This could be interpreted to mean that the replication step is a necessity before the par locus can act.

0868 TRANSPOSABLE ELEMENTS IN HALOBACTERIUM HALOBIUM, Felicitas Pfeifer, Mary Betlach, James Friedman and Herbert W. Boyer, University of California, San Francisco,CA 94143

H. halobium exhibits an unusual genetic instability. Phenotypic markers such as gas vacuoles, ruberins and purple membrane are lost with frequencies of 10^{-2} to 10^{-4}. Complex rearrangements, insertions and deletions in the cccDNA and the chromosome occur concurrently with these mutations. We characterized insertions causing mutations in the bacterio-opsin (*bop*) gene of *H. halobium* leading to purple membrane deficiency. Eleven out of twelve bop^- mutants examined have inserts ranging from 350 bp to 3000 bp either in the *bop* gene or up to 1400 bp upstream. Only some of these size-increments derive from the more G+C rich part of the chromosome while most of them originate from the more A-T rich part of the chromosome or from the cccDNA. Comparison of five of these insertions (350 bp, 500 bp, 900 bp, 1100 bp and 3000 bp) indicates that they lack homology and belong to different insertion families. Distribution and stability of these sequences in the genome of *H. halobium* wild type and mutants are discussed.

0869 THE RESPONSE OF TRANSPOSABLE GENETIC ELEMENTS TO GENOTOXIC AGENTS. Michael S. DuBow and Gayle Shinder, McGill University, Montréal, Québec H3A 2B4.

Movable genetic elements have been found in the genomes of all known organisms. These transposable elements can alter the genetic material both functionally by affecting the expression of adjacent host genes, and structurally by promoting insertions, deletions, inversions, or translocations of DNA adjacent to their termini. Many physical and chemical agents may act on the genetic material by causing non-specific DNA transpositions, or other subtle genomic rearrangements, through effects on the status or regulation of these mobile genetic elements. Furthermore, it is known that the major cause of spontaneous mutations (in E. coli) are due to the insertion and excision of transposable elements and not to simple base-pair changes.

In an effort to identify external agents which may affect the frequency or fidelity of DNA transposition, we devised several sensitive procedures to detect the induction and frequency of DNA transposition using bacteriophage Mu, a non-inducible, plaque-forming, transposable bacteriophage of Escherichia coli. We are currently screening a wide variety of potentially toxic agents to quantitatively determine their effects on Mu DNA transposition in vivo. Results to date indicate, for example, that many DNA damaging agents (e.g. dimethyl sulphate) actually reduce the frequency and extent of induction of Mu DNA transposition in vivo, while others (e.g. ethyl carbamate) have neither a stimulatory nor inhibitory effect. It appears from these, and other results that the response of cellular movable genetic elements to selected external agents is not a simple one and may reflect the presence of a compound regulatory pathway.

General and Site-Specific Recombination

0870 A DOUBLE STRAND BREAK MODEL FOR RECOMBINATION, Rodney J. Rothstein*, Terry L. Orr-Weaver+, Franklin W. Stahl# and Jack W. Szostak+. *New Jersey Medical School, Newark, NJ 07103; +Harvard Medical School, Boston, MA 02115; #University of Oregon, OR, 97403.

Current models for genetic recombination account for the observed association in fungi of gene conversion, post-meiotic segregation and crossing-over. The models involve hybrid DNA, Holliday structures and mismatch correction. We have been utilizing yeast transformation to study the interactions of hybrid plasmid molecules with the yeast genome. Plasmid molecules that contain double strand breaks or gaps within yeast sequences efficiently recombine with homologous sequences in the chromosome resulting in repair of the break or gap. Recombination, as detected by integration of the plasmid, occurs in approximately 50% of the interactions. In the other half, the plasmid break or gap is efficiently repaired without the subsequent integration of the plasmid. The repair of plasmid double strand breaks or gaps is blocked in strains carrying the pleiotropic recombination and repair deficient mutation, rad52. These observations suggest a model for gene conversion based on the repair of double strand gaps. We postulate initiation of recombination occurs by formation of a double strand break that is enlarged to a double strand gap. The two ends of the gap invade the homologous region of the recipient chromatid. Repair of the gap leads to gene conversion of the gapped regions. Failure to correct heteroduplex DNA formed at the boundaries of the repaired region results in post-meiotic segregation. The invading ends that prime the gap repair lead to the formation of two Holliday structures flanking the converted region. Resolution of the two Holliday structures in the same sense (cutting inside strands or outside strands) results in no recombination between flanking markers. Resolution of each Holliday structure in an opposite sense results in a crossover.

0871 YEAST MATING TYPE INTERCONVERSION IS INITIATED BY A SITE SPECIFIC ENDONUCLEASE, Richard Kostriken, Carolyn Moomaw, Fred Heffron and Jeff Strathern. Cold Spring Harbor Laboratory, New York 11724.

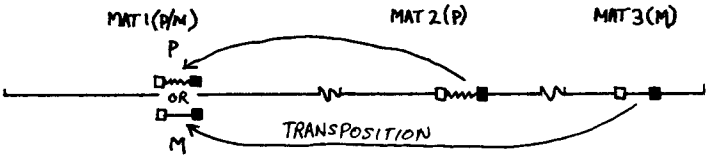
We have purified and characterized a site specific endonuclease from Saccharomyces cerevisiae. In vivo, we believe, this endonuclease catalizes an early event in the mating type switching process. Examination of the enzyme's substrate specificity has allowed us to explain the phenotypes of previously isolated MATinc (inconvertible) mutants as well as postulate the molecular basis of MATstk (stuck) mutations. We have determined the precise nature of the double strand break produced by this endonuclease and find it to be compelling evidence in favor of the model for mating type interconversion put forward by Strathern et al.

0872 SITE SPECIFIC RECOMBINATION ASSOCIATED WITH THE YEAST PLASMID, 2 MICRON CIRCLE, M. Jayaram, Y.Y. Li, and J.R. Broach. State University of NY at Stony Brook, NY 11794

The yeast plasmid, 2 μ circle, encodes a site-specific recombination system. A plasmid coded protein (FLP) catalyses recombination within the inverted repeats of the plasmid to cause an intramolecular DNA inversion. We have shown that this recombination is independent of the general mitotic and meiotic recombinational machinery of the yeast cell and that the recombination is confined to a region of less than 65 bp within the 600 bp long repeats. Apparently there are no recombinational sites on any of the yeast chromosomes. However, if a copy of 2 μ circle is integrated into a yeast chromosome, FLP mediated recombination occurs readily at this locus, leading to a high rate of chromosomal instability. Surprisingly, the bacterial transposon Tn5, which has invertedly repeated termini (each 1500 bp long), serves as a substrate for FLP mediated recombination. A region within the Tn5 repeat shows marked similarity to certain structural features that occur within the 2 μ circle recombination site. We have also noticed that the efficiency of Tn5 inversion is dependent on the location of Tn5 on the 2 μ circle genome. This may reflect inhomogeneities in chromatin structure or the presence of sequences that enhance recombination in their vicinity. We notice that a perfect "chi" sequence (which is known to enhance recombination in phage λ) is located in a region of the 2 μ circle where maximal inversion of Tn5 occurs. The formal analogy of the 2 μ circle recombination to prokaryotic DNA inversion systems suggests that it may act as a genetic switch between two alternate states of the molecule. To assess this we are evaluating flp^- derivatives of 2 μ circles to see how the absence of recombination affects transcription and propagation of the molecule.

0873 STRUCTURE OF THE MATING-TYPE LOCUS IN SCHIZOSACCHAROMYCES POMBE, David Beach and Amar Klar, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 10024.

Fission yeast alternates efficiently between the Plus and the Minus mating cell type in a fashion analogous to that observed in the budding yeast. The mating-type locus of fission yeast is a tightly linked cluster of unstable genes controlling conjugation and sporulation. The structure of this locus in homothallic (h^{90}) strains was obtained by restriction and heteroduplex analysis and is shown in the figure. The mating type of the cell is determined by which of the P(plus) or M(minus) cassettes is inserted at MAT1, the site at which the cassettes are expressed. Rearrangements at the mating-type locus have been discovered in the heterothallic strains, h^{+N}, h^{-u} and h^{-s}. Their structures, regulation and the mechanism of transposition will be presented.



0874 EVIDENCE FOR PRESENCE OF EXTRACHROMOSOMAL DNA IN CELLULOLYTIC FUNGI by Gene R. Petersen, Genadie G. Sverlow, and Greg Stephanopolous. California Institute of Technology & Jet Propulsion Laboratory, Pasadena, California 91109.

The enzymatic hydrolysis of cellulose by extracellular enzymes from bacteria and fungi is well documented. However, the understanding of even the rudiments of the genetic control and regulation of this secretion process are not known which hinders the study of this process by conventional or recombinant techniques. Research in our laboratories has been involved in isolating and characterizing possible extrachromosomal vectors in cellulolytic fungi. Conventional methods for identifying extrachromosomal DNA in yeasts and fungi are time consuming and laborious. An adaptation of a method applied to bacteria by Kado and Lin (J. Bacteriol 145:, 1365 (1981)) was used to screen several strains of yeasts and fungi for extrachromosomal DNA and isolate these elements for preliminary characterization. Data describing the methodology and the preliminary characterization of the isolated DNA by endonuclease digestion is presented. This data indicates that a relatively simple and rapid method for isolation of DNA from yeast and fungi for use in in vitro recombinant studies is possible.

0875 ANALYSIS OF A CLONED REGION WITH HIGH FREQUENCIES OF MEIOTIC RECOMBINATION IN YEAST. J. Haber, S. Stewart, R. Borts and M. Lichten. Brandeis University, Waltham, MA 02254

The integration of a 9.0 kb pBR322 plasmid containing both the yeast mating type (MAT) locus and the URA3 gene creates a tandem duplication of the MAT locus. Two haploid strains were constructed, one with the duplication MATα-URA3-pBR322-MATα, and the other with MATa-URA3-pBR322-MATa. Heterozygous diploids [α-URA3-α]/[a-URA3-a] were sporulated and meiotic tetrads were then dissected. More than 12% of the tetrads contained a reciprocal recombination in this 9 kb region, as evidenced by tetrads containing one [a-URA3-α] and one [α-URA3-a] segregant. Because MATa contains a BglII site not found in MATα or in the plasmid sequences, both parental duplications and their recombinants can readily be distinguished. Thus, a direct physical monitoring of recombination during meiosis can be achieved. We can also examine the effects of introducing various heterologies into the region. For example, another duplication with the LEU2 gene inserted at the SalI site has also been constructed. Diploids containing [a-URA3-a]/[α-LEU2-α] also yielded approximately 12% tetratype asci. In contrast, recombination within the duplication fell to less than 2% when one duplication contained URA3 at the HindIII site and the other contained the same URA3 fragment inserted at AvaI. These heterologous insertions appear to have disrupted pairing or branch migration rather than eliminated an essential sequence for recombination, because recombination again occurred in more than 10% of the tetrads when both duplications contained URA3 at the AvaI site. The ability to replace portions of the plasmid sequences in the duplication with various deletions and insertions makes it possible to determine if there is a specific initiation site for recombination within this region and to examine gene conversion events associated with meiotic recombination.

0876 RECOMBINATION ACTIVITIES OF DNA ENZYMES FROM USTILAGO. E.B. KMIEC*, M. BROUGHAM and W.K. HOLLOMAN, Department of Immunology and Medical Microbiology, University of Florida, Gainesville, FL 32610.

Our interest in the mechanism of genetic recombination has led us to examine the lower eukaryote Ustilago for enzymes important in the process. We have identified and purified two proteins that are candidates. One enzyme catalytically pairs homologous DNA molecules in a reaction dependent upon ATP. The purified protein also transfers a single strand from a linear duplex molecule to a complementary single stranded circular molecule. Initiation of the strand transfer reaction requires ATP as a cofactor, but does not depend on ATP hydrolysis. ADP suppresses the initiation. The protein also promotes branch migration in a reaction dependent on the continual presence of ATP, independent of accumulated ADP. The transfer of circular single strands to linear duplex DNA begins with pairing of the circular plus strand with the 5' end of the linear minus strand and is accompanied by displacement of the 3' end of the linear plus strand.

The other enzyme under study is a topoisomerase. Crude extracts from the rec-2 strain of Ustilago, a mutant defective in recombination, repair, and meiosis, have a reduced level of topoisomerase activity. Furthermore, the possibility that the topoisomerase from ths mutant is altered is suggested from its heat lability, as compared to the wild type enzyme. This possibility is being investigated by using an affinity assay fro the topoisomerase, based on its ability to from a covalent intermediate with nick translated single stranded DNA.

0877 CONSERVATION AND DIVERSIFICATION OF GENES BY MISMATCH REPAIR IN REPLICATIONAL AND RECOMBINATIONAL HETERODUPLEXES, Miroslav Radman, Département de Biologie Moléculaire, Université Libre de Bruxelles, B-1640 Rhode-St-Genese (Belgium) and Institut Jacques Monod, Université Paris 7, Tour 43, 2, Place Jussieu - 75251 PARIS CEDEX 05 (France).

Mismatched base pairs (mismatches) can occur in DNA as the result either of errors in DNA replication ("replication heteroduplex") or of DNA strand exchange between homologous but non-identical DNA sequences ("recombinational heteroduplex") as well as by specific chemical modifications of DNA bases. Evidence will be reviewed showing that in E. coli the pattern of mismatch repair in these two kinds of heteroduplexes is different : replicational heteroduplexes are subject to a highly efficient strand-directed mismatch correction leading to the conservation of DNA nucleotide sequences, whereas recombinational heteroduplexes are subject to a rather inefficient random mismatch repair leading to the diversification of nucleotide sequences. These two modes of mismatch repair appear to be due mainly to the differences in the chemistry of DNA substrate (state of methylation of GATC sequences and mismatch chemistry). The current status of the knowledge about mismatch repair mechanisms and the biological implications of mismatch repair will be presented.

0878 PURIFICATION AND CHARACTERIZATION OF EXONUCLEASE VIII FROM ESCHERICHIA COLI
Jackiel W. Joseph and Richard Kolodner, Department of Biological Chemistry, Harvard Medical School, and Laboratory of Molecular Genetics, Sidney Farber Cancer Institute, Boston, MA 02115

An indirect suppressor mutation, sbcA, restores recombination proficiency to recBC mutants and induces the synthesis of exonuclease VIII. To examine the role of this enzyme in recombination, exonuclease VIII has been purified to near homogeneity from a recBC sbcA ΔxthA endA strain (Exo III$^-$, Exo V$^-$, Exo VIII$^+$, Endo I$^-$). The protein has a subunit molecular weight of 140,000 and is present in the form of a multimer, most likely a tetramer. Linear duplex DNA is degraded by exonuclease VIII in a reaction requiring Mg^{++}, and which is inhibited by other divalent cations and by NEM. No nucleolytic activity is observed with any type of circular duplex DNA as substrate, even those containing single stranded nicks or gaps. A limited amount of activity is seen using single stranded linear DNA as substrate. Linear duplex DNA is degraded with a 5'→3' polarity, releasing 5'-dNMPs and leaving an equivalent region of single stranded DNA. Initiation of degradation can occur from either a 5'-phosphate or a 5'-hydroxyl residue. The reaction proceeds via a highly processive reaction mechanism. Our data suggests that linear duplex DNA molecules containing 3'-single stranded tails as a result of partial exonuclease VIII digestion may serve as recombination intermediates. Although sbcA strains promote a high level of recombination in plasmids, the role of exonuclease VIII in this process remains unclear, because of the lack of nucleolytic activity by the purified enzyme on circular molecules.

0879 THE ROLE OF recA PROTEIN AND ENDONUCLEASE VII IN MULTIPLICITY REACTIVATION OF PHAGE T4
Paul Hyman and R. Patrick McCreary, Department of Molecular and Medical Microbiology, University of Arizona, Tucson, Az. 85724

Substantial evidence on the genetic control of multiplicity reactivation (MR) in bacteriophage T4 indicates that MR is a recombinational process (C. Bernstein, Microbiol. Rev. 45:72-98, 1981). This leads to the expectation that intermediate structures similar to those found in normal recombination are probably involved in MR. The product of phage T4 gene 49, endonuclease VII, has been shown to cleave Holliday structures in vitro (Mizuuchi, et al, Cell 29: 357-365, 1982). However, using temperature sensitive and weakly suppressed amber alleles of gene 49, we have been unable to demonstrate a gene 49 dependence for MR of UV-damaged phage or for spontaneous recombination. In E. coli, the pairing of homologous DNA during recombination is promoted by the recA protein (Radding, Cell 25:3-4, 1981). This protein also regulates an inducible pathway of DNA repair by means of its proteolytic activity. Primer and Chan (Virol. 88:338-347, 1978) have shown a recA dependence for MR of UV-treated phage. Using recA mutants that are either recombination deficient and protease competent or recombination competent and protease deficient, we have shown a decrease in MR of Mitomycin C-damaged T4. This suggests that both activities of recA protein may have a role in MR. (This work is supported by NIH Grant #GM27219-03 and NCI NRSA Grant #CA09213.)

0880 INTERACTION OF REC A PROTEIN WITH NUCLEIC ACIDS AND ATP,
Stephen Kowalczykowski, Northwestern University Medical School, Chicago, IL 60611

In order to understand the molecular mechanism of the strand exchange reaction catalyzed by the E. Coli recA protein, the interaction of this protein with its various substrates (ATP, single-standed DNA, and double-stranded DNA) is being quantitatively investigated. The studies of ATP binding to recA protein have made use of a photoaffinity analogue of ATP, 8-azido-ATP, to investigate not only the binding of this analogue to the protein, but also of other nucleoside triphosphates and diphosphates. These quantitative analyses have shown that the order of binding affinity to recA protein is ATP-γ-S > dATP > ATP > AMP-P-N-P. In addition to the ATP binding studies, the interaction of recA protein with single-stranded DNA is being investigated by using DNA which has been modified to produce a fluorescent product. This modified single stranded DNA which contains 1, N^6 - ethenoadenylic acid residues shows a change in fluorescent properties when recA is bound, and is being used to quantify and study the nature of the recA protein - DNA complex. [Supported in part by USPHS grant AI 18987.]

0881 Abstract Withdrawn

0882 GENETIC EVIDENCE FOR RecBC ENZYME-CHI INTERACTION, Dennis W. Schultz and Gerald R. Smith, Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104

Chi recombinational hotspots stimulate the E. coli RecA-RecBC pathway of generalized recombination (Stahl, Annu. Rev. Genet. 13: 7 (1979)). Chi sites are specified by the sequence 5' G-C-T-G-G-T-G-G 3' (Smith, Kunes, Schultz, Taylor, and Triman, Cell 24: 429 (1981)). These results suggest that Chi is a recognition sequence for a protein specific to the RecA-RecBC pathway.

We have sought support for the hypothesis that RecBC enzyme recognizes Chi by isolating special RecBC mutants in which Chi is inactive. Four independent mutants were isolated following EMS mutagenesis of a recC73 (presumed missense) mutant. The pseudorevertants have regained to varying degrees recombinational proficiency and Exonuclease V (RecBC enzyme) activity; Chi has no detectable activity in λ vegetative crosses in the mutants. The mutations responsible for the pseudorevertant-phenotype map and complement like recC mutations. Recombination-proficiency of the pseudorevertants is independent of recE and recF but is dependent upon $recA^+$ and $recB^+$; this result indicates that the RecBC pathway has been restored in the pseudorevertants. The properties of these mutants are consistent with the hypothesis that wild-type RecBC enzyme recognizes Chi and that the mutant enzymes have regained the ability to promote recombination but have not regained Chi recognition. (Supported by NIH grant GM31693 and RCDA AI00547.)

0883 PHYSICAL AND BIOCHEMICAL ANALYSIS OF THE CLONED RECB AND RECC GENES OF ESCHERICHIA COLI K-12, Sidney R. Kushner and Christine C. Dykstra, University of Georgia, Athens, Georgia 30602

The recB and recC genes (exonuclease V) of E. coli have been cloned on a 17.5 kb BamHI fragment in both lambda and multicopy plasmid cloning vehicles. The multicopy plasmid pCCD3 complements recB, recC and thyA mutants of E. coli. In maxicell experiments plasmid encoded proteins of 140,000 (recB), 128,000 (recC), 97,000, 54,000, 51,000, 35,000 (thyA) and 20,000 daltons are observed. The 54,000 dalton polypeptide seems to arise from the 97,000 dalton species, since Tn1000 insertions that inactivate the larger species also eliminate the smaller protein. P97 appears to map between recB and recC and may in fact overlap the recB coding sequence. Strains harboring pCCDK3 produce between 20-30 times more exonuclease V activity than appropriate control strains. Preliminary purification results indicate that this observation represents a tru increase in enzyme synthesis. Overproduction of exonuclease V activity does not cause any phenotypic alterations in wild type bacteria. (This work was supported in part by grants GM27997 and GM28670 from the National Institutes of Health).

0884 RecBC ENZYME UNWINDING OF DNA: SUBSTRATE SPECIFICITY, Andrew F. Taylor and Gerald R. Smith, Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104.

Depending upon the reaction conditions the RecBC enzyme (exonuclease V) of E.coli can act either as a nuclease or as a DNA unwinding (and rewinding) enzyme. Under "nuclease" conditions it acts as an ATP-dependent double-strand exonuclease or as a single-strand exo- or endonuclease but cannot act at nicks or small gaps in duplex DNA. Under more physiological ("unwinding") conditions the enzyme can unwind and rewind linear DNA but not nicked circular DNA.

E.coli mutants defective in RecBC enzyme activity are relatively deficient in some types of genetic recombination (generalized transduction, conjugation, lytic phage recombination) but relatively proficient in other types (intra-chromosomal recombination, plasmid by chromosomal recombination).

In an attempt to rationalize these genetic and biochemical findings we are investigating the types of termini from which exonuclease V can unwind DNA. Flush-ended DNA and DNA with a 12 base 5' overhang are unwound at similar frequencies, but molecules with 3' or 5' overhangs of 25-50 nucleotides are unwound at least 20-fold less frequently. Circular DNA molecules with single-strand gaps of 500 nucleotides can be digested by the enzyme under "nuclease" conditions but cannot be unwound under "unwinding" conditions. We are investigating the action of the enzyme on shorter gaps (5-30 nucleotides) and will discuss the implications of the results for the enzyme's action in genetic recombination. (Supported by NIH grant GM31693 and RCDA AI00547.)

0885 CLONING AND EXPRESSION OF E. coli RecF RECOMBINATION PATHWAY GENES, A. J. Clark, K. D. Willis, L. W. Ream, S. Lovett and L. Herbert, Department of Molecular Biology, University of California, Berkeley, CA. 94720.

Three RecF pathway genes have been cloned with pBR322 as vector: recE, recF and recJ. recE is located on a 7.7 kb HindIII fragment largely derived from the defective prophage Rac. Expression of recE using a promoter from pBR322 does not occur unless part of the 7.7 kb fragment is deleted implying the presence of one or more transcription terminators upstream from recE. sbcA mutations which lead to expression of chromosomal recE occur either as large deletions of the Rac prophage and adjoining chromosome or as possible point mutations. Two of the latter group have been mapped to the segment containing the hypothetical transcription terminator(s). recF has been cloned on a 3.6 kb EcoRI fragment which includes three N terminal codons of gyrB on one side and the entire dnaN gene and three-quarters of dnaA on the other. Expression of recF on the plasmid seems to depend on dnaN or pBR322 promoters. recJ is located near serA and has been cloned on an 8 kb SalI BamHI fragment. Removal of 6 kb from the fragment does not affect recJ expression. recE determines the 130 kD DNA nuclease ExoVIII. Maxicell tests show candidates for recF and recJ proteins. The nucleotide sequence of recF (M. Blanar, E. Armengod and A. J. Clark, unpublished) helps to confirm the identity of the recF product. Genetic experiments show that one or more RecF pathway genes other than recA are part of the lexA regulon. Which gene(s) may be determined by examining Mud(lacZ bla) insertion mutants defective for RecF pathway recombination.

0886 PLASMID RECOMBINATION PATHWAYS IN ESCHERICHIA COLI K-12, Amikam Cohen and Avraham Laban, The Hebrew University, Hadassah Medical School, Jerusalem, Israel.

Interplasmidic and intraplasmidic recombination proficiencies have been determined in various mutants of E. coli K-12, using plasmids with mutation at different sites of the tet gene. Results indicate that the major recombination pathway in wild type E. coli cells depends on the recA and the recF gene functions. In recBrecC mutants, intraplasmidic recombination, but not interplasmidic recombination, is recF independent. Plasmidic recombination via the recE pathway proceeds at a higher proficiency than in wild type cells and is recA independent. The recBrecC gene product-exonuclease V does not play a catalytic role in any of the plasmid recombination systems which have been investigated.

Plasmidic recombination proficiencies have been determined in regions bounded at one side or bracketed by non homologous DNA sequences of various lengths. Results indicate that bracketing a short region with non homologous DNA leads to a substantial decrease in recombinants formation within the region. This observation is consistent with the notion that branch migration contributes to an increase in the number of recombination intermediates which mature within the region, and that in this system, sequences of non homology block branch migration.

0887 FORMATION AND RESOLUTION OF FIGURE-8 DNA STRUCTURES, Stephen C. West and Paul Howard-Flanders, Yale University, New Haven, CT 06511.

A key intermediate in the mechanism of general genetic recombination is a structure in which double stranded DNA molecules are covalently linked by two single strand crossovers (a Holliday junction). When the DNA molecules are circular, the recombinant structures take the form of a covalently closed figure-8.

We have prepared biparental figure-8 structures in vitro from homologous Cm^rTc^s and Cm^sTc^r plasmids, using E. coli RecA protein, DNA polymerase I and DNA ligase. The figure-8's made with purified enzymes are similar to those recovered from intact cells that carry plasmid DNA molecules in that 1) they are alkali stable, 2) the parental monomers are linked at a region of DNA homology, and 3) in many cases the single strand connections in the region of the crossover were visible by electron microscopy, and took the form of an open junction characteristic of a Holliday crossover. In addition, the structures are biologically active and transfect cells which then express the antibiotic resistance markers present on the figure-8. Following entry into the cell, the figure-8's are resolved into monomeric or dimeric circular plasmids that replicate to form progeny plasmids of both recombinant and parental types. A genetic and biochemical analysis of resolution will be presented.

0888 EFFECTS OF RECOMBINOGENIC SEQUENCES ON INTERMOLECULAR PLASMID RECOMBINATION IN ESCHERICHIA COLI, Anthony A. James and Richard Kolodner, Laboratory of Molecular Genetics, Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115.
Certain plasmids require a recombinogenic element distinct from the Chi sites of the phage lambda to recombine to form circular oligomers in E. coli (James, A.A., Morrison, P.T., and Kolodner, R., submitted). This type of recombination requires the functions of the recA, recF and recB recC genes to form circular oligomers but does not require the functions of the recB recC genes to break down circular oligomers (James, A.A., Morrison, P.T., and Kolodner, R., J. Mol. Biol. 160, 411-430). Recombination is independent of recombinogenic elements in strains containing an sbcA mutation. Deletion analysis of pACYC184, a plasmid containing a recombinogenic element, suggested that the recombinogenic element was located near the BamH1 site in the tetracycline-resistance gene. A series of genetic crosses between different mutations in the tetracycline-resistance genes of pACYC184 and pBR322 were carried out to locate the recombinogenic element. We found that recombination in a wild type strain is increased over 100-fold in a 351 base-pair region to the left of the BamHI site when compared to a 892 base-pair region to the right. Recombination in this region was increased only two-fold in a similar experiment carried out in a recA56, recB21, recC22, sbcA23 strain. In these experiments, the recombinogenic element has a strong effect on the recombination and may be located to the left of the BamHI site. In agreement with previous studies, plasmid recombination appeared to be independent of recombinogenic elements in strains with an sbcA mutation.

0889 TRANSPOSON-ENCODED SITE-SPECIFIC RECOMBINATION, Lorraine Symington,*Paul Dyson, *Paul Kitts and *David Sherratt. Sidney Farber Cancer Institute, 44 Binney St., Boston, MASS 02115 and*Institute of Genetics, Church St.,Glasgow, SCOTLAND.
The closely related transposable elements, Tn1/3/$\gamma\delta$, appear to transpose in two sequential steps; each step requiring a separate, element-encoded gene product. The first stage, mediated by the transposase protein, results in replicon fusion to form a transpositional cointegrate containing directly repeated copies of the element at the plasmid junctions. The cointegrate is resolved by recombination between the two copies of the element at a specific site, res. This reaction is catalysed by the resolvase protein. To study the recombination reaction in detail we have purified the resolvase protein to reconstruct the resolution reaction in vitro. The resolution reaction requires only resolvase, s/c substrate Mg^{++} and buffer. The reaction proceeds by a reciprocal break/rejoin event with no concomitant DNA synthesis, or requirement for high energy cofactors. Plasmid substrates containing two directly repeated copies of res are the optimal substrates for resolvase-mediated recombination. There is no recombination between inverted res sites or res sites in trans under the conditions employed. Reaction products are always catenated, suggesting that catenation is an intrinsic feature of the reaction. Resolvase has a high affinity for DNA, indicated by its specific and non-specific binding properties. Thes observations, in conjunction with detailed genetic analysis, have led to the formulation of a model for Tn3 resolvase-mediated site-specific recombination.

0890 ISOLATION OF INHIBITORS OF TN3 RESOLVASE-MEDIATED SITE-SPECIFIC RECOMBINATION, Michael A. Fennewald, University of Notre Dame and John Capobianco, Abbott Laboratories.
We have developed a genetic screening procedure for identifying inhibitors of Tn3 resolvase-mediated site-specific recombination. This procedure employs λXJS845 which is a lambda derivative that carries a gene for chloramphenicol resistance and a gene for streptomycin resistance gene which if flanked by two, directly-repeated copies of TnA ΔAp. Here, the Sm^R gene is in between two Tn3 resolvase recombination sites, and is lost if resolvase recombines the two sites. The assay involves a soft agar overlay with λ845 and then a second soft agar overlay of cells with resolvase on a chloramphenicol, streptomycin plate. Filter disc containing the possible inhibitors are then placed on the top layer. Infected cells which contain an inhibited resolvase will remain streptomycin resistant while cells with an uninhibited resolvase are streptomycin sensitive. Using this assay, we randomly screened over 6,000 different compounds and found 26 compounds that produced streptomycin reistant cells. When these 26 compounds were tested against purified Tn3 resolvase with an in vitro recombination reaction, 6 of them inhibited resolvase-mediated recombination. They also inhibited the topoisomerase activity of the Tn3 resolvase. We are currently undertaking mechanistic studies on these inhibitors to determine the point in the recombination reaction that is inhibited.

0891 A POSSIBLE SITE-SPECIFIC RECOMBINATION AND RESOLUTION SYSTEM IN PLASMID RP4. Peter T. Barth and Nigel J. Grinter, ICI Corporate Bioscience Group, Runcorn, England and Dept. of Biochemistry, University of Leicester, England.

Loss of The DNA primase specified by RP4 leads to a small loss of plasmid stability in E.coli (Lanka and Barth, 1981, J.Bacteriol 148, 769-781). Removal from such a pri mutant of the 6kb PstI fragment (at coordinates 28-34kb) leads to extreme plasmid instability(e.g. pNJ5000 is lost at a rate of about 6% per generation). Removal of various fragments from the 28-36kb region of pri$^+$ RP4 derivatives does not lead to instability however. We have cloned the 6kb PstI fragment onto suitable vectors but it does not stabilize pNJ5000 in trans. However, reinsertion of this fragment in either orientation and at different sites into pNJ5000 does re-establish stability. Thus a site on the PstI fragment is necessary for the stability of pri RP4 plasmids.

Agarose gel analysis of plasmid DNA shows that pNJ5000 gives an extra band (compared to RP4) at a position approximately where a plasmid dimer would be expected. In recA hosts, pNJ5000 is stable and this band is not visible. We therefore propose that RP4 may contain a recA$^+$-dependent site-specific recombination system which is blocked at an unresolved "dimer" stage in pNJ5000. (This disrupts normal partitioning leading to plasmid loss). In RP4, however, the "dimer" may be resolved by a system that involves a function of the RP4 primase or by a host system that requires a site in the 6kb PstI fragment. Loss of both options therefore leads to plasmid instability in recA$^+$ hosts.

0892 Host Genes Required for Site-Specific Recombination in E.coli, Harvey I. Miller, Genentech, South San Francisco, CA 94080.

The genome of E.coli contains two genes, himA and himD, which together, code for a sequence specific DNA binding protein called Integration Host Factor (IHF). The nucleic acid binding activity of IHF is manifested in several ways in E.coli:

1) It is required for site-specific recombination of phage lambda DNA in conjunction with the lambda Int protein
2) It is needed for translation of the lambda cII protein which, in turn, is required for Int protein synthesis
3) It participates directly or indirectly in the precise excision of several transposons
4) Phage Mu transcription requires IHF
5) IHF acts as a transcriptional repressor of the himA gene
6) The E.coli flagellar antigen gene, hag, is positively regulated by IHF.

Studies of the role of IHF in these systems suggest a mechanism of action of this important protein.

0893 cos, A SPECIAL SITE IN GENERAL RECOMBINATION OF BACTERIOPHAGE LAMBDA, Ichizo Kobayashi, Mary M. Stahl, and Franklin W. Stahl, Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403.

cos, cohesive end site, of bacteriophage lambda is recognized and cleaved during DNA packaging into a phage particle. cos stimulates general recombination of lambda DNA in two different pathways. In RecA RecBC mediated recombination of E. coli, cos helps another special site, Chi, to stimulate exchanges near Chi. Interaction of terminase with cos leads to activation of Chi that is correctly oriented with cos. In lambda's Red pathway cos enhances nearby exchanges even in the absence of DNA replication.

0894 GENETIC RECOMBINATION OF PHAGE T7 DNA STUDIED BY A SIMPLE IN VITRO ASSAY: Donald Lee and Paul Sadowski, Department of Medical Genetics, University of Toronto, Toronto, Canada.

We have developed a direct physical assay to study genetic recombination of bacteriophage T7 DNA in vitro. This assay is based on the use of restriction enzymes to detect recombination. Two T7 DNAs, each of which carries a unique restriction cleavage site, are incubated with a T7-infected cell extract in the presence of ATP and deoxynucleoside triphosphates. The DNA is purified, digested with restriction enzymes and analysed by agarose gel electrophoresis. Recombination generates a DNA species which contains both restriction sites: this DNA is readily detected by the presence of a new band after electrophoresis. We report here the use of such a restriction assay to study the genetic requirements for T7 recombinations in vitro.

By analysing extracts derived from cells infected with phage bearing single and multiple mutations, we found that in addition to the recombination promoted by the wild type extracts, there are two other pathways of recombination. Recombinants can be generated via the "exonuclease pathway" provided that the gene 6 exonuclease is functional. Furthermore recombination can also proceed by the "endonuclease pathway" which requires the absence of the gene 4 product but the functions of gene 3 (endonuclease) and gene 5 (DNA polymerase). The results here confirm those obtained previously using a 2-stage biological assay to detect recombination (Roeder and Sadowski, CSHSQB 43, 1023, 1979). The interactions between various T7 gene products in the in vitro assay will be presented. We are currently using this assay to investigate the molecular mechanisms of T7 DNA recombination.

0895 RECOMBINATION GENES OF PHAGE λ UNDER lac CONTROL ON A MULTI-COPY PLASMID, John B. Hays, Robert J. Zagursky and Stanley A. Friedman, University of Maryland Baltimore County, Catonsville, MD 21228

The phage λ recombination ("Red") genes, exo and bet, have been cloned under lacI^{q}lacPO control on PBR322. Prior induction with isopropylthiogalactoside (IPTG) yielded λ exonuclease (and, presumably, β protein) levels at least 100 times those produced by phage infection. Since recombination of λ Red$^-$ phages (in recA hosts) was elevated less than two-fold under these conditions, it appears that some other factor(s) are rate-limiting. Induction as late as 50 minutes after phage λ infection resulted in significant recombination, i.e. Red function can act very late in the lytic cycle. Induced exo$^+$bet$^+$ plasmids can replace the E. coli RecA system for at least one process, UV-stimualted recombination of nonreplicating λ DNA. Using methods developed for transferring Red mutations from phages to plasmids and vice-versa, complementation between Exo$^+$Bet$^-$ plasmids and Exo$^-$Bet$^+$ plasmids and phages (and vice-versa) was demonstrated. Plasmid induction affects short-term growth only slightly, but does reduce long-term cell viability; the plasmids may express λ Gam function.

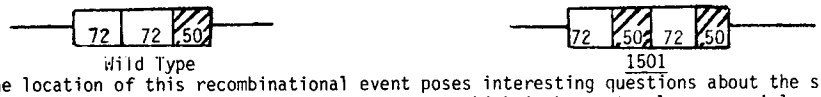
0896 INVOLVEMENT OF oriT IN THE ENCHANCED RECOMBINATION OF F42lac, H. Steven Seifert and Ronald D. Porter, The Pennsylvania State University, University Park, PA 16802

The F42lac plasmid of Escherichia coli recombines with a λplac5 transducing phage approximately thirtyfold more efficiently than a chromosomal lac gene recombines with λplac5. This enhanced recombination of F42lac is cis-dominant, recB dependent, and requires the constitutive expression of the tra regulon of F42lac. Mini F-lac plasmid derivatives do not show enhanced recombination, but the insertion of a BamHI fragment containing the tra region into mini F-lac restores the enhanced recombination.

Our working hypothesis is that the cis-acting site on F42lac is oriT, the origin of conjugational transfer. Everett and Willets have proposed that there is a nicking-closing equilibrium at oriT [J. Mol. Biol. 136:129 (1980)]. The recombination enhancement may result from this action at oriT as it may affect the supercoiling state of F42lac or provide access to F42lac for the RecBC enzyme. We have cloned a 1.1 kilobase BglII fragment containing oriT into miniF-lac and found that it does not restore the enhancement of recombination by itself. We are currently testing the recombination between miniF-lac-oriT and λplac5 when various combinations of tra proteins are provided in-trans. The results of this work will be presented.

0897 A TEMPERATURE-SENSITIVE MUTANT OF SV40, 1501, CONTAINS AN INSERTION LOCATED BETWEEN THE 72-BASE REPEATS. Jean K. Carr, Irwin Cohen, Henry Rothschild, Michael Murphey-Corb and J. Craig Cohen, Louisiana State University, New Orleans, LA 70112.

An SV40 mutant, 1501, which replicates at conditional temperatures in semi-permissive cells, was found by restriction mapping to have an insertion in the region of the 72-base repeats near the origin of viral replication. Dideoxy nucleotide sequencing of the region revealed a 50 base insertion precisely at the junction of the two 72-base repeats. The sequence was found to be identical to the 50 bases immediately adjacent to the 72-base repeats on the late side, thus creating 120-base repeat at the original site of the 72-base repeat, as diagrammed below:



Wild Type 1501

The location of this recombinational event poses interesting questions about the structure and function of the 72-base repeat, a sequence which is known to play a crucial role in activation of viral gene expression.

0898 HOMOLOGOUS RECOMBINATION IN CULTURED MAMMALIAN CELLS, R. Michael Liskay, Anthea Letsou, Gary Shapira, Lenore Soodak, Janet Stachelek, Yale University, New Haven, CT 06510

We are studying homologous recombination in mammalian cells using XhoI linker insertion mutants of the Herpes thymidine kinase (TK) gene. As expected these mutant genes transform mouse LTK$^-$ cells to the (TK$^+$) phenotype very inefficiently. If a co-transfer is performed with two different TK mutant genes HAT colonies appear at a frequency that is 2-5% of the wild-type (wt). Several of these transformants have been analyzed using Southern blots and appear to contain a normal wt TK sequence, i.e. one devoid of XhoI sites. Another goal is to study recombination between tandemly repeated and integrated TK genes. Therefore, we have inserted two different XhoI mutant genes into the vector PSV2-gpt which contains the dominantly selectable bacterial gene xanthine guanine phosphoribosyl transferase (gpt). LTK$^-$ were transformed with either of two plasmids of the above type, one with the TK mutant genes in direct orientation and one with them indirect, and selection for gpt was applied. Transformants were grown in gpt selective media and BUDR (to eliminate any recombination associated with transformation) and finally tested in HAT. Of the several lines tested so far all give HAT^r colonies (putative intrachromosomal recombinants) at frequencies ranging from 10^{-5} - 10^{-3} of cells plated. Southern blot analysis is underway to ask if these genetic events actually represent intrachromosomal recombination and if so whether it is reciprocal or non-reciprocal.

0899 SITES OF DIVERGENCE IN THE SEQUENCE OF A COMPLEX SATELLITE DNA AND SEVERAL CLONED VARIANTS. D.M. Skinner, R.F. Fowler and V. Bonnewell*. Biology Division and Univ. Tenn.-Oak Ridge Grad. School Biomed. Sci., Oak Ridge Natl. Lab., Oak Ridge, TN 37830

A complex G+C-rich (63% G,C) satellite DNA with a repeat unit (RU) of 2.07 kb accounts for 3% of the DNA of the Bermuda land crab. Satellite as isolated from various tissues or cloned into the Pst I or Eco RI sites of pBR322 is characterized by distinct, unrelated sequence domains. One of $\sim$ 300 bp is rich in adjacent T's or CT's, as indicated by the formation of pyrimidine photodimers. Other domains contain pure homocopolymer tracts, one begins with G_4, and is comprised of $(AG_3)_{10}$, two others are $(CA)_4$ or $(C_2T)_{15}$. There are five homopolymer tracts of $G_{8 \rightarrow 23}$. Three cloned variants, one of RU = 2.09 kb, another truncated (TRU = 1.69 kb), and a third extended (EXT = 2.7 kb), have some domains of perfect or close-to-perfect homology and other domains of major sequence divergence. An example of the type of sequence variation that occurs is in a prototype $G_4(AG_3)_n$ tract in the TRU that is modified in the RU to a seemingly scrambled AG-rich (98%) domain which, in turn, suffers a large ($\sim$ 60 bp) deletion in the EXT. There are also two distinct types of amplification: one is six tandem copies of a 142 bp sequence found as a single copy in the RU and TRU. The other is varying numbers (up to 14) of tandem repeats of CGCAC and its derivatives. The divergent regions are associated with and, in some cases, include domains containing the homoco- and homopolymers or two regions whose sequences "promise" the adoption of a Z-conformation. (*Postdoctoral fellow. Research supported by NSF grant PCM78-23373 and by the Office of Health and Environmental Research, U.S. D.O.E., under contract W-7405-eng-26 with the Union Carbide Corp.)

0900 PARVOVIRUS DELETION MUTANTS ARISE BY A SITE DIRECTED RECOMBINATION MECHANISM. Emanuel A. Faust and Aileen Hogan, University of Western Ontario, London, Ontario N6A 5B7.

Helper-dependent (AAV) and autonomous (MVM, H-1, BPV, LuIII, KRV) parvovirus genomes are linear, single-stranded DNA molecules consisting of 5100$\pm$ 50 nucleotides and possess short self-complementary sequences at their molecular termini. For both groups of viruses, replication of the DNA is accompanied by an unusually high frequency of spontaneous deletions. (E.A.Faust and D.C.Ward, J. Virol 32:276). In so far as deletions are heterogeneous, it is of interest to inquire as to the deletion mechanism by determining whether deletions are random or ordered. We have determined the nucleotide sequence of a 963 base segment of DNA extending in from the 3' terminus in DL-I mutants of minute virus of mice (MVM) which are deleted internally but retain information from both termini. This region was found to be free of deletions and was otherwise identical to wild type DNA, including a 3' terminal self-complementary sequence of 115 nucleotides. Since DL-I mutants are approximately 2000 nucleotides in length, deletions appear to be clustered within 1000 bases of the 5' terminus of the viral genome. Molecular cloning of DL-I mutants in the plasmid vector, pDE61, a derivative of pBR322, has yielded a collection of recombinant plasmids bearing MVM DL-I inserts of up to 2000 base pairs. The deletions in selected clones are currently being mapped and further analysis is being done to examine the DNA sequences at the recombination joints.

0901 GENETIC RECOMBINATION IN EUKARYOTIC CELLS CAN BE MEDIATED BY PARTIALLY HOMOLOGOUS SEQUENCES, Richard A. Anderson, Shingo Kato and R. Daniel Camerini-Otero, NIH, Bethesda, MD 20205

Herpes simplex virus thymidine kinase gene and pBR322 DNA were introduced into mouse L cells by DNA-mediated gene transfer. DNA fragments encompassing the junctions between the exogenous DNAs which have become linked in the eukaryotic cells and the junctions between exogenous DNA and cellular DNA have been cloned and their nucleotide sequences determined. Analysis of the sequences has shown that stretches of partial homology involving from 20 to 40 base pairs are characteristically present near the points at which joining occurs between the grossly non-homologous donor molecules. To explain our findings we envision that the recombination event involves the alignment of the donor molecules at the homologous regions with a subsequent asymmetric cutting and joining event. The cut in one sequence is always in the homology region while the cut in the second sequence occurs from 0 to 54 base pairs away from the first cut. In several of the junctions between exogenous DNAs that we have examined, the second cut occurs beyond the area of homology and a 17 to 19 base pair segment of a new DNA sequence separates the donor sequences in the recombinant. In one case three of the four ends resulting from a single recombination event have been located and sequenced. These results place limitations on the possible models for the mechanism of recombination in eukaryotic cells. Some of the requirements imposed on the models will be presented.

0902 THE USE OF polA MUTANTS TO DEFINE FUNCTIONAL DOMAINS OF DNA POLYMERASE I OF E. COLI, Catherine M. Joyce and Nigel D.F. Grindley, Department of Molecular Biophysics and Biochemistry, Yale University Medical School, New Haven, CT 06510.

An understanding of the role of DNA polymerase I in excision repair and in the processing of Okazaki fragments has largely come from the analysis of mutants in its structural gene, polA. Now that the sequence of DNA polymerase I is known and structural studies of the enzyme are in progress, the time is ripe for characterization of these mutants at the sequence level. We have therefore developed a method for the rapid mapping of point mutants relative to the polA physical map. By cloning and sequencing those mutants whose gene products have been studied biochemically, we hope to assign functions to various regions of the polymerase molecule.

Using a plasmid carrying the polA gene we have constructed, by standard in vitro techniques, a series of deletions whose ends are physically defined by restriction sites. We have cloned the resulting deleted polA genes onto phage λ. This series of phages with overlapping polA deletions provides us with an extremely simple and rapid procedure for genetically mapping any polA mutation to a precise DNA interval small enough to be easily sequenced. The first mutant we chose to study was polA6, whose gene product has a decreased affinity for its DNA substrate. We have shown that this mutation is a change from Arg(CGC) to His(CAC) within a cluster of basic amino acids which, we presume, constitutes part of the DNA binding region of the enzyme. Several other polA mutants have been mapped, and cloning and sequencing experiments are in progress. The results of these studies will be discussed.

0903 **REC A PROTEIN FORMS HIGHLY MISMATCHED HETERODUPLEX DNA,** Marco E. Bianchi and Charles M. Radding, Yale University, New Haven, CT 06510

E. coli recA protein promotes the pairing of circular single strands with linear duplex DNA and the subsequent formation of long heteroduplex joints. Single strand binding protein (SSB) and an ATP regenerating system enhance the yield of full length heteroduplex molecules, including those formed by M13 and fd DNA which contain about 200 mismatched base pairs. RecA protein therefore can make heteroduplex joints in which the local density of mismatches is 12 % and which include single-base mismatches of every possible kind, a single-base insertion/deletion and even a mismatch of three consecutive bases. Likewise, strand exchange was not blocked by heterologous insertions either in the duplex or in the single-stranded DNA up to 11 base pairs in length, a result that suggests that recA protein separates the strands of the duplex DNA in advance of the reannealing of complementary strands in the heteroduplex joint. SSB protein appears to play an important role in the relaxation of the requirements of homology during strand exchange, since it is essential for the formation of heteroduplex joints between the DNAs of phages G4 and ØX174, which are on the average 33 % heterologous. These observations confirm that strand exchange is not a stringent process since heteroduplex DNA containing significant structural deformations can be made. Correction of mispairings of this sort before replication is believed to underlie in part the classical genetic phenomena of gene conversion and aberrant meiotic segregation.

0904 THE TWO ADULT β GLOBIN GENES OF THE C57BL MOUSE HAVE UNDERGONE GENE CONVERSION. Steven Weaver, Mark A. Erhart & Kimberlee S. Simons, University of Illinois at Chicago, Chicago, IL 60680.

In Mus musculus, hemoglobin β chains are synthesized from a pair of tandemly duplicated genes. The species is dimorphic with respect to the number and kind of β globins produced by these two 'adult' genes. Animals homozygous for one alternative form of the gene cluster, the so-called 'single' haplotype, have but one molecular species of β globin, βsingle. Strains carrying the alternative 'diffuse' haplotype produce two distinct proteins, βdmajor and βdminor.

We have sequenced the two genes (designated βs and βt) of the 'single' mouse C57BL/10, and compared them to their alleles (βdmaj and βdmin) carried by the 'diffuse' strain BALB/c. As expected, the βs and βt genes encode identical polypeptides. Surprisingly, the two genes are virtually identical: no silent substitutions in any of the three exons, and almost no differences in either of the two introns. This is very different from the situation with the 'diffuse' haplotype. The βdmaj and βdmin genes are in the process of divergence. They have accumulated replacement changes at 9/146 codons, and their large introns have become radically different. (Konkel, et. al 1979 Cell 18,865-873)

The identity of βs and βt appears to be due to gene conversion. The gene conversion event(s) have occurred within defined interval, the limits of which can be discerned. The 5' boundary lies very near the ATG initiation codon and the 3' boundary lies very near the termination codon. Outside these limits, βs and βt are just as divergent as are βdmaj and βdmin, and in fact are almost identical with them.

0905 MICROINJECTION OF A HUMAN/RAT GROWTH HORMONE GENE INTO MOUSE EMBRYOS
Richard F Selden, Thomas E. Wagner*, Edmund Tischer, and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02138 *Departments of Chemistry, Zoology-Microbiology, and Biomedical Sciences, Ohio University, Athens, OH 45701

A human/rat hybrid growth hormone gene was cloned into pBR327. Approximately 2000 copies of the construct were microinjected into the male pronucleus of mouse zygotes, which were then transferred to pseudopregnant or pregnant mothers. Close to 15% of the mice born contained the injected DNA as evidenced by Southern hybridization analyses. In the animals studied thus far, the construct was present in 5-15 copies/cell as an extrachromosomal element. It appears that in at least one case, several copies of the construct were joined to produce a large element that yields restriction fragments not predicted based on the physical map of the injected construct.

0906 Structure of a mutant H-2 gene: generation of polymorphism in H-2 genes by gene conversion-like events, E.H. Weiss, A. Mellor, L. Golden and R.A. Flavell, Biogen Cambridge, Ma 02142

Mutant alleles of H-2 genes arise at a high frequency. We have cloned a mutant allele of the H-2K^{b} gene known as H-2K^{bm1}. DNA sequence analysis of this gene shows that it is the result of an intergenic exchange of DNA (geneconversion) which results in the conversion of a short internal segment of the H-2K^{b} to the corresponding sequence of another H-2 class I gene. We suggest that this type of gene conversion is widespread in the H-2 genes and that it is a major force in the generation of polymorphism in H-2 genes.

0907 REGULATION OF Ia ASSOCIATED INVARIANT CHAIN SYNTHESIS, Hans J.Rahmsdorf, Norbert Koch, Udo Mallick and Peter Herrlich, Institut für Genetik und Toxikologie, Kernforschungszentrum Karlsruhe, Postfach 3640, D-7500 Karlsruhe 1, Germany.

In B lymphocytes the invariant chain (I_i) is associated with the MHC coded class II antigens A_α, A_β, E_β and E_α. Class II antigens are involved in the interactions between B cells, T cells and macrophages. We describe here a first example of gene regulation in the MHC: The invariant chain is synthesized at a high rate only in resting lymphocytes. When they are activated by lipopolysaccharide, the synthesis of the invariant chain is shut off. This regulatory behavior seems to be reflected by the rates measured in corresponding cell lines. Some cell lines derived from early B cells synthesize I_i at high rate. Incontrast cell lines arising from differentiated B cells (myeloma cells, human plasmacytoma cells) do not synthesize I_i. The synthesis is reinduced, however, when these cells are treated with agents, which inhibit replication. This control works on the level of the gene or the RNA processing. mRNA levels were determined by in vitro translation. A I_i precursor is synthesized which is matured upon addition of cytoplasmic membranes. Rate measurements will be repeated using cDNA clones.

0908 A HIERARCHY OF IMMUNOGLOBULIN GENE RECOMBINATIONS IN HUMAN B-CELL PRECURSORS. S.J. Korsmeyer, A. Arnold, A. Bakhshi, J. Cossman, J.V. Ravetch, U. Siebenlist, P.A. Hieter, P. Leder, and T.A. Waldmann. Natl. Inst. of Health, Bethesda, Md. 20205 and Harvard Medical School, Boston, Mass. 02115

Prior to their effective expression immunoglobulin (Ig) genes must undergo site specific recombinations which precisely join the gene subsegments of variable (V_H), diversity (D_H), and joining (J_H) for heavy chains and variable and joining (V_κ/J_κ or V_λ/J_λ) for the light chain genes. We examined 25 human leukemias representing B-cell precursor stages of development with C_μ, J_H, D_H, C_κ, and C_λ probes. Fourteen cases had rearranged heavy chain genes yet retained germline light chain genes; whereas, 11 cases had both heavy and light chain gene reorganizations. All 7 cases with κ gene reorganizations retained germline λ genes while all 4 cases with λ gene reorganizations had no remaining germline κ genes. Despite the uniform presence of rearranged immunoglobulin genes only 5 cases produced cytoplasmic μ chain while one exceptional case produced γ chain and another λ chain. The error prone nature of these recombinations may frequently result in populations of precursor cells which possess ineffectively rearranged Ig genes. This may include some cells which are genetically trapped due to elimination of all available germline D_H or J_H segments necessary to assemble an effective heavy chain gene. Other cells within this series appear to have effectively rearranged genes that are only productive following the induction of cellular differentiation. The configurations of Ig gene rearrangements seen support a hierarchial model which proceeds from heavy chain gene recombinations to light chain genes and from κ to λ within the light chain genes.

0909 INTEGRATION AND EXCISION OF A VIRAL-CELLULAR HYBRID DNA MOLECULE, Pierre Chartrand, Alain Nepveu and John C. Wallenburg, Département de microbiologie, Université de Sherbrooke, Québec, Canada, J1H 5N4.

A natural recombinant molecule (RMI) consisting of a complete polyoma genome (Py) with an insertion in its late region of 1.6 kbp mouse cellular DNA (INS), is used to study integration and excision. This molecule is produced after induction of a specific clone of Py transformed mouse cells. In its integrated form, the INS sequences are found as flanking cellular sequences on one side of the integrated viral sequences. The free circular form is most likely generated by recombination between specific viral and cellular sequences. A comparison of the sequences at the two viral and cellular joints in the free form, reveal a direct viral repeat of 182 bp and an imperfect inverted cellular repeat of 7 bp. Thus the INS fragment is bordered by this imperfect inverted cellular repeat.

We have used this molecule to transform rat cells. The analysis of the integrated RMI structures in more than fifty clones have shown that recombination between RMI and the cellular genome can occur via different mechanisms. First RMI can integrate by seemingly illegitimate recombination as is usually found to be the case for Py. Secondly it can also integrate by homologous recombination involving the INS sequences. The INS sequences are found both in mouse and rat cells in very numerous copies that are scattered all over the genome. Thirdly, it also might be able to recombine by a site-specific mechanism that involves the viral-cellular joints of RMI. Experiments are now in progress to verify this last possibility.

0910 HOMOLOGOUS AND NON-HOMOLOGOUS RECOMBINATION IN MONKEY CELLS, Suresh Subramani and Paul Berg, Stanford University, CA 94305.

Though recombinational events are important for the proper functioning of most cells, little is known about the frequency and mechanisms of mitotic recombination in mammalian cells. We have used SV40-pBR322 hybrid plasmids constructed in vitro as substrates to detect and quantitate homologous and non-homologous recombination events in cultured monkey cells. Excision of wild-type or defective SV40 DNAs by recombination from these plasmids was scored by the viral plaque assay either in the absence or presence of DNA from a temperature sensitive helper virus. Several independent products of homologous and non-homologous recombination have been isolated and characterized at the DNA sequence level. Moreover, viral DNA replication and the SV40 large T antigen were not essential for either homologous or non-homologous recombination involving viral and/or pBR322 sequences.

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0911 Distribution of a Simple Sequence within Human DNA. Frank P. Johnston, Rona Hirschberg, Lyn Yarbourgh, and Oliver Smithies, University of Wisconsin, Madison, Wisconsin 53706

A simple sequence DNA consisting of a Run of $(TG)_n$ has previously been detected within the IVS2 of the human $^A\gamma$ and $^G\gamma$ globin genes. (Slighton et al., Cell 21:627-638, 1980). Southern transfer analysis reveals the presence of sequences homologous to it elsewhere in human DNA. Selected clones, designated as Zulus, from both a M13 and Charon 27 Library were analyzed by DNA sequencing and Dot Blotting. $(TG)_n$ runs were found to be universally distributed amongst different frequency classes and flanked by heterogenous DNA sequences.

0912 TEMPO AND MODE OF GENE CORRECTION IN THE ZETA GLOBIN REGION, Barbara S. Chapman and Allan C. Wilson, Biochemistry Department, University of California, Berkeley, CA 94720

By extensive restriction mapping of genomic DNA from many human individuals, we have characterized numerous length variants in the region between the two zeta genes (1 and 2) as well as within the first intron of the zeta 1 gene. Misalignments facilitated by the repetitious structure of this intron are probably responsible for the extremely high frequency of length variants observed there. It is also probable, we suggest, that a misalignment between a sequence in this intron and a closely related sequence in exon I allowed the zeta 1 gene to be converted to a pseudogene. This juxtaposition would occur during pairing of two zeta 1 length alleles. From our comparisons of individuals from human populations and primate species of approximately known divergence times, we estimate that length mutations are fixed at a rate of one per 10^5 - 10^6 years per polymorphic site. The maps also delineate two subregions, each corresponding approximately to a zeta transcription unit, which have often been corrected against each other during primate evolution. In addition, our analysis of linkage relationships among sites of length- and point-mutational polymorphism show that there has been recombination between the two sites of length polymorphism in the 5'flank and intron I. Finally, our mapping studies indicate that a dimeric Alu I sequence situated on the 5' side of the zeta 1 gene may be both a start point for the intergenic correction process and a mediator of equal recombination between the two sites of length polymorphism.

0913 SITE-SPECIFIC RECOMBINATION IN C. DIPHTHERIAE, Giulio Ratti and Rino Rappuoli, Sclavo Research Center, Siena, Italy.

The gram-positive bacterium Corynebacterium diphtheriae acquires the capability of producing diphtheria toxin upon lysogenization by temperate bacteriophages carrying the "tox" gene within their genome. Recent work has shown that the bacterial strain C7 possesses two distinct sites in its genome where the corynephage DNA can integrate with high, and approximately equal, efficiency.

A portion of phage DNA which was previously shown to contain the phage attachment site was cloned and sequenced. DNA fragments containing the junction between bacterial and integrated phage DNA and, subsequently, fragments containing the bacterial attachment sites have also been cloned and sequenced. A comparison of these data shows an overall sequence homology between bacterial and phage genomes extended for some 96 nucleotides; within this sequence, a stretch of 9 nucleotides, where bacterial and phage sequences diverge, is flanked by a 14 bp inverted repeat.