BACTERIAL CHROMOSOMES

Organizers: John Roth and Nancy E. Kleckner

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Replication Initiation-I and Cell Cycle-I

V 001 ASSEMBLY OF THE *E. COLI* ΦX-TYPE PRIMOSOME AND ITS ROLE IN CHROMOSOMAL REPLICATION, George Cyrus Allen, Jr. and Arthur Kornberg, Department of Biochemistry, Beckman Center, Stanford School of Medicine, Stanford, CA 94305-5307

The assembly of the *E. coli* ΦX-type primosome requires PriA, PriB, PriC, DnaB, DnaC, and DnaT acting at a *p*rimosome *a*ssembly *s*ite (*pas*) on an SSB-coated ssDNA. Assembly is initiated by the interactions of PriA and PriB with ssDNA and the *pas*. The DnaB•DnaC complex, PriC, and DnaT then act in concert with the PriA/PriB/DNA complex to yield the primosome which promotes synthesis of a primer by primase. Once assembled, the primosome blocks access to the *pas* preventing it from binding and activating additional PriA molecules. Assembly of a stable primosome requires a nucleotide for DnaB•DnaC complex (ATP or dATP) as well as one for the stable of maintenance of DnaB on the ssDNA (*e.g.* ATP or GTP). Such isolated primosomes contain a dATPase, the hallmark of PriA, and a GTPase indicative of DnaB. Further studies indicate that the isolated primosome contains the PriB replication activity in addition to PriA and DnaB.

The primosome assembles at a *pas* on a ssDNA, (*e.g.* the circular phage ΦX174) to provide a priming mechanism for replication of that template. In addition, the primosome can generate a rolling-circle replication product from this template. The structure of the rolling-circle product implies that the DnaB replicative helicase is transferred from its initial loading site on the leading strand to a displaced, lagging strand. This strand-switching process is actively promoted by components of the primosome. Isolated primosomes, containing only PriA, PriB, and DnaB, are sufficient for leading-strand priming and replication but fail to undergo strand switching and rolling-circle replication. Supplementation with PriC, DnaC, and DnaT is necessary to reconstitute rolling-circle synthesis. In addition, the rolling-circle stage of replication is sensitive to salt, whereas primosome assembly and unit-length synthesis are not. This difference implies that primosome assembly to allow leading-strand replication and strand-switching to promote rolling-circle synthesis represent two distinct reactions of the *E. coli* primosome. The implications of this strand switching of DnaB at a chromosomal replication fork will be discussed.

V 002 REGULATION OF oriC INITIATION IN Escherichia coli, Erik Boye[1], Anita Lyngstadaas[1], Anders Løbner-Olesen[2], Kirsten Skarstad[1], and Sture Wold[1], [1]Department of Biophysics, Institute for Cancer Research, Montebello, 0310 Oslo, Norway, [2] Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Campus Box 347, Boulder, CO 80309-0347.

Initiation of DNA replication from the chromosomal origin *oriC* in *Escherichia coli* is a tightly controlled and carefully regulated process. The factors necessary to carry out initiation *in vitro* have been characterized, but the molecular mechanisms involved in regulating initiation *in vivo* are largely unknown. Two aspects of replication control may be distinguished: (i) setting of the right time (in the cell cycle) for initiation, and (ii) making sure all origins in a cell are initiated at that time (synchrony). The only factor shown to influence the timing of initiation is the DnaA protein: a higher DnaA concentration results in earlier initiation and lower initiation mass. It is unclear whether DnaA is the main regulator of initiation, since the intracellular DnaA concentration has been reported to vary both with growth rate and with temperature, but also to be independent of growth rate.

Cell mass at initiation ("initiation mass") has been proposed to be a constant, independent of cell doubling time. We have employed flow cytometry to measure the initiation mass and found that it increases monotonously with doubing time and is 1.5 times higher at 100 min relative to 30 min doubling time.

Several factors have been suggested to be important for initiation control, but direct evidence is lacking. We have employed flow cytometry to investigate the possible role of the proteins RecA, IciA, Rob, Fis, and IHF as well as the presence of the gene neighboring *oriC*, termed *mioC*. Results with *recA* mutants were obscured by a specific and complete degradation of individual chromosomes in the absence of the recombination activity of RecA. IciA and Rob bind in the *oriC* region but their absence or overproduction only marginally affect initiation kinetics. Both Fis and IHF have binding sites in *oriC*, and absence of these proteins results in flow cytometry histograms compatible with a loss of initiation synchrony. However, specific chromosome degradation may be the reason for this phenotype in the *fis* mutant. Transcription from the *mioC* gene stimulates minichromosome replication, but initiation from chromosomal *oriC* was shown to be independent of the presence of the *mioC* gene.

V 003 CELL CYCLE EXPRESSION OF GENES INVOLVED IN *E. coli* CHROMOSOME REPLICATION, Patrick W. Theisen, Julia E. Grimwade, Joseph A. Bogan, Alan C. Leonard and Charles E. Helmstetter, Florida Institute of Technology, Melbourne.

We have undertaken a comprehensive analysis of the cell cycle-dependent transcription of genes involved in chromosome replication and cell division in *Escherichia coli*. Current analyses include *mioC* (both 5' and 3' ends of the transcripts), *gidA*, *dnaA*, and transcripts originating within the minimal *oriC*. Relationships between transcription and chromosome replication were compared by alignment of initiation of chromosome replication either through temperature shifts of *dnaC2* and *dnaA5* mutants of *E. coli* strains K12 and B/r from nonpermissive to permissive temperature, or by removal and restoration of thymine from *E. coli* B/r *thyA*. Studies of transcription throughout the cell cycle were performed by collecting samples of newborn cells from a baby machine and incubating each sample for different times in the cell cycle. The amount of each transcript present in the cells was determined by quantitative S1 nuclease protection. Oligonucleotides complementary to the transcripts of interest were end-labeled with ^{32}P, hybridized to total RNA isolated from each sample, digested with S1, and electrophoresed to separate the hybridized, protected fragment. Oligos were constructed so that each contained a noncomplementary tail of at least 10 bases which was digested by the S1, to separate the protected signal from the longer undigested, unhybridized radioactive probe. The protected lengths of each oligo was varied so that more than one transcript could be analyzed in each sample. Most assays also contained a probe for *rpoA* as a control for a gene whose transcription was not expected to show a cell cycle dependency.

At the nonpermissive temperature in the *dnaC* mutant, initiation of *mioC* transcription continued, but transcripts terminated before entering the *gidA* gene, which was not seen with the *dnaA* mutant. The shift of the *dnaA5* and *dnaC2* temperature-sensitive mutants from nonpermissive to permissive temperature resulted in a dramatic, transient reduction of all transcripts examined. The timing of resumption of transcription varied between the different transcripts and the two mutants. When the cells were aligned for initiation of chromosome replication by thymine starvation, a similar transient reduction in transcription was evident upon resumption of chromosome replication, but to a much lesser extent than with the temperature shifts. Similar periodicities were also detected in synchronous cultures, again associated with initiation of replication, but also to a much lesser extent. The timing of the reduced transcription in the cell cycle was compared to both the time of replication of minichromosomes and the appearance of hemimethylated *oriC* DNA. Similar experiments with genes involved in cell division are underway.

V 004 CONTROL OF THE *Escherichia coli* CELL CYCLE, Kurt Nordström, Rolf Bernander, Åsa Eliasson, Margareta Krabbe, and Thomas Åkerlund, Department of Microbiology, Uppsala University, Biomedical Center, Box 581, S-751 23 Uppsala, Sweden

Several different processes take place during the bacterial cell cycle, chromosome replication, nucleoid processing, and cell division. We are interested in how these processes are controlled to match growth in mass and how they are interrelated. The basic replicon of plasmid R1 was inserted into a small *oriC* deletion; this uncouples chromosome replication from its normal control[1]. Since plasmid R1 replicates unidirectionally, two sets of strains were constructed, *intR1*$_{CC}$ and *intR1*$_{CW}$, referring to the orientation of R1 replication (clockwise and counterclockwise on the chromosome map). The DNA content and cell size distribution of *intR1*$_{CW}$ strains are similar to that of normal strains. Replication is bidirectional and random in time, whereas it is coordinated in normal strains. In *intR1*$_{CC}$ strains, chromosome replication is unidirectional and they form filaments; this is counteracted by inactivation of the *tus* gene. A comparison between *intR1*$_{CC}$ and *intR1*$_{CW}$ strains permits an analysis of what determines uni- and bidirectionality. Conditional expression of the *copA* gene makes it possible to reversibly inhibit initiation of replication and to follow the kinetics and localization of cell divisions after resumption of replication. By inserting a runaway-replication derivative of R1, the copy number of the chromosome can be varied at will. Plasmid R1 replicates in the absence of the DnaA protein, but with reduced efficiency. By using the runaway replication system in a DnaA$^-$ host, the copy number of the chromosome can be varied from severalfold lower than normal to very high values[2]. Extensive over-replication is lethal to the host. Under- as well as over-replication cause inhibition of cell division. Our data suggest that cell division has its own control and that there are veto systems that prohibit cell division unless replication and nucleoid separation have taken place in proper time[3]. By growing the bacteria on microscopic slides, the nucleoid morphology can be studied *in vivo*. The nucleoids occupy a very large part of the cell volume. Over-replication disturbs nucleoid separation and leads to minicell formation and DNA-less areas appear at the cell poles. Presumably, this makes polar divisions possible. Therefore, we reexamined the function of the *minB* system. Division at the centre and at the poles were found to occur independently and to be random in time. Minicells were formed in cells in which the nucleoids had not separated, suggesting that *minB* is involved in nucleoid separation[4].

1. Bernander, R, Merryweather, A, Nordström, K: J. Bacteriol. 171:674-683, 1989.
2. Bernander, R, Nordström, K: Cell 60:365-374, 1990.
3. Bernander, R, Dasgupta, S, Nordström, K: Cell 64:1145-1153, 1991.
4. Åkerlund, T, Bernander, R, Nordström, K: Mol. Microbiol. 6:2073-2089, 1992.

V 005 THE FUNCTION OF *E. COLI* HEAT SHOCK PROTEINS IN PLASMID P1 DNA REPLICATION, Sue Wickner[1], Dorota Skowyra[1], Joel Hoskins[2] and Keith McKenney[2], [1]Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892 and [2]Center for Advanced Research in Biotechnology, National Institute of Standards and Technology, Gaithersburg, MD 20899.

We have been interested in the initiation and regulation of replication of plasmid P1. The P1 replicon exists with a copy number of about one per cell and consists of an origin of replication, a gene coding for the initiator protein, RepA, and a copy number control locus. P1 plasmids containing only the P1 origin and the *repA* gene are maintained at a copy number of about 8. The replication *in vitro* of *ori*P1 DNA requires host proteins, including DnaA initiator protein, DnaB helicase, DnaC, DnaG primase, DNA polymerase III holoenzyme, DNA gyrase and RNA polymerase. RepA, which binds specifically to five 19-bp direct repeats in the P1 origin region and to nine similar repeats in the plasmid copy number control locus, functions both in initiation of replication and regulation of replication.

Three *E. coli* heat shock proteins, DnaJ, DnaK and GrpE, are also involved in *ori*P1 DNA replication both *in vivo* and *in vitro*. Using purified proteins in an *in vitro* DNA binding assay, we found that DnaJ, DnaK, and GrpE stimulate the sequence specific DNA binding of RepA by about 100-fold in an ATP and Mg^{+2} dependent reaction. The requirement for GrpE can be bypassed *in vitro* by high Mg^{+2} concentration. We discovered that activation converts RepA dimers to monomers and that the monomer form binds with high affinity to *ori*P1 DNA; DnaJ and DnaK are not bound to the *ori*P1 DNA. The enzymatic activation can be mimicked by converting RepA dimers to monomers by urea denaturation followed by renaturation, suggesting that RepA is not necessarily modified.

We investigated whether the DnaJ, DnaK, and GrpE catalyzed activation of RepA DNA binding was required for *ori*P1 DNA replication *in vitro*. We found that the monomeric form of RepA bypasses the requirement for DnaJ, DnaK and GrpE for the *in vitro* replication of *ori*P1 DNA. Thus, the essential role of these three heat shock proteins in this replication system is to change the quaternary structure of a single protein, RepA. Surprisingly, they are not required at subsequent steps in *ori*P1 DNA replication.

We propose that in normal growth conditions, native proteins are identified as targets for DnaK via a protein tag. In our system, DnaJ is the protein specific tag. The RepA-DnaJ tetramer targets RepA for DnaK action. DnaK forms a complex with the RepA-DnaJ complex and RepA is monomerized in a reaction involving GrpE, ATP and Mg^{+2}.

Replication Initiation-II

V 006 REGULATION OF Tn10/IS10 TRANSPOSITION AND OF REPLICATION INITIATION AT oriC, Nancy Kleckner, Min Lu, Joseph L. Campbell, Ronald Chalmers, Janice Sakai and Laurence Signon, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA.

Transposition. During Tn10/IS10 transposition, interaction of the synapsed transposon ends with target DNA is intrinsically a random process involving collision of the synaptic complex with potential target DNA sites. These sites may be located either on a separate molecule or within the transposon itself. However, this basic target selection process is modulated by the bacterial host protein IHF. Under the influence of IHF, random collisions with target DNA are suppressed; instead, productive interactions of the synaptic complex with target DNA occur only with sites located inside the element itself and occur in a topologically constrained way. This type of regulation is especially appropriate for a composite transposon like Tn10 because it favors a certain type of intramolecular rearrangement that generates new composite transposons.

For bacteriophage Mu, biologically appropriate regulation of transposon/target interactions is also superimposed on an unregulated collision process. However, in the case of Mu, regulation of target selection has precisely the opposite consequence of regulation in the Tn10/IS10 case, and involves a very different mechanism.

Sequestration of *oriC*. Immediately after initiation of *E. coli* DNA replication from *oriC*, the origin region is sequestered from the replication initiation apparatus. This sequestration requires that *oriC* be hemimethylated, lasts for approximately one third of the cell cycle, and involves association of *oriC* with a cellular fraction that includes membrane components. The *dnaA* promoter, which is located near *oriC*, is also sequestered immediately following passage of the replication fork. Several lines of evidence suggest that an important role of sequestration is to block unwanted secondary replication initiations; it has also been proposed that sequestration might play a role in chromosome partitioning.

We have identified a gene which is required for sequestration of *oriC in vivo*. This gene, *seqA*, maps at about 15 minutes on the *E. coli* chromosome and has now been cloned and sequenced. It encodes a protein of 182 amino acids which exhibits no evidence of membrane association (signal sequence or membrane spanning domains). Initial characterization of the *seqA* null mutant phenotype and biochemical properties of the SeqA protein will be presented.

V 007 DAM METHYLATION AND THE INITIATION OF CHROMOSOME REPLICATION IN ESCHERICHIA COLI, Anders Løbner-Olesen[1], Erik Boye[2], Peter L. Kuempel[1], Martin G. Marinus[3] and Lene J. Rasmussen[3]. [1]University of Colorado, MCDB Box 347, Boulder CO 80309, [2]Institute for Cancer Research, Dept. of Biophysics, Montebello, 0310 Oslo 3, Norway, [3]University of Massachusetts Medical School, Dept. of Pharmacology, 55 Lake Avenue, Worcester MA 01655.

Initiation of DNA repliction in Escherichia coli occurs in a unique 245 bp sequence called oriC. This sequence contains four recognition sequences for the DnaA initiator protein, three 13-mer AT rich sequences where duplex opening is thought to occur, as well as 11 GATC sequences which are substrates for Dam methyltransferase.

Initiation of DNA replication is the key regulatory event in the cell cycle, and the timing of initiations from oriC is remarkably precise; all origins in each cell are initiated simultaneously. However, strains deficient in Dam methyltransferase show a loss of initiation synchrony, compatible with either random initiations throughout the cell cycle or synchronous initiations with an occasional skipping or reinitiation of one or more origins.

In cells where the amount of Dam methyltransferase could be varied, coordination of initiations was observed only when levels were close to that of the wild-type (estimated at 130 molecules per cell). These data are explained by a model where a newly replicated and therefore hemimethylated oriC is inert for reinitiation.

The observations above suggest a tight control of Dam methyltransferase synthesis. The dam gene containing the regulatory region was therefore cloned and the structure of the operon determined. Promoter and terminator sequences within this operon have been identified and sequenced. It has been found that the expression of the dam gene increases with decreasing doubling time. This is compatible with previous data on remethylation of oriC at different growth rates.

V 008 THE ORIGIN AND THE MEMBRANE, Moselio Schaechter, René Gallegos, Piotr Polaczek, James Fontaine and Ulrik von Freiesleben, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston MA 02111

The origin of replication is the only region of the *Escherichia coli* chromosome that binds to the membrane under certain *in vitro* conditions. For binding to take place, this stretch of DNA must be hemimethylated. Thus, the origin is only capable of binding to the membrane for a period of time after replication. The origin region of the chromosome is especially rich in the sequences (*GATC*) specific for the Dam methylase. These sites are methylated only after a considerable delay when compared to other sites on the chromosome. Methylation of the origin may, therefore, operate as an *interval timer* to define a period of time during which the chromosome is attached to the membrane. Because *dam* mutants survive, this mechanism is probably not essential for cell growth and division. However, *dam* mutants are defective in the timing of chromosome initiation and/or segregation. Consequently, binding of hemimethylated DNA to the membrane may be involved in the precise regulation of these processes.

A prediction of this model is that a membrane-associated protein that specifically binds hemimethylated origin DNA is altered in its binding ability in a cell-cycle dependent manner. Work on the isolation and biochemical and genetic characterization of such a protein is in progress.

Repair and Recombination-I

V 009 **ALTERNATIVE INITIATION MECHANISMS FOR *E. coli* CHROMOSOME REPLICATION: D-LOOPS AND R-LOOPS.**
Tokio Kogoma, Tsuneaki Asai, Xiankang Hong and Gregory Cadwell, Departments of Cell Biology and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM 87131.

There are at least two alternative initiation modes for chromosome replication other than the initiation at *oriC*; one is manifested after SOS induction and the other activated in RNase HI-deficient (*rnhA*) mutants. These are termed the inducible and constitutive stable DNA replication (iSDR and cSDR), respectively. iSDR is initiated primarily from the *oriC* and *terC* regions in a manner independent of protein synthesis, transcription and DnaA protein. The origin (*oriM1*) in the *oriC* region is localized within a 596 bp segment which includes the minimal *oriC*. Some of the mutations that completely inactivate *oriC* do not affect the *oriM1* activity. iSDR is blocked by a mutation in *recA* that inactivates the recombinase activity of RecA and by a mutation in *recB* or *recC*, but it is stimulated by *recD* mutations which inactivate the exonuclease V of RecBCD. Thus, initiation of iSDR is thought to occur at a D-loop that is generated by actions of RecA and RecBC proteins. The D-loop may be competed by two pathways, one leading to homologous recombination and the other to iSDR initiation because *ruvC*, *recG* or *ruvAB* mutations which block resolution of Holliday intermediates or branch migration greatly stimulate iSDR.

cSDR occurs from several sites (*oriK*s) on the chromosome other than *oriC* without DnaA protein but strictly requires transcription for initiation. It has been hypothesized that during certain transcription the transcript remains hybridized to the template DNA strand in a significant stretch leading to formation of an R-loop which consists of the DNA-RNA duplex and the displaced single-stranded (ss) DNA. The R-loop, stabilized in the absence of RNase HI activity, serves as a site for initiation. The following lines of evidence lend support to the contention that stable R-loops exist in vivo. 1) In *rnhA* mutants the SOS response is constitutively elicited, due perhaps to an increased amount of ss DNA associated with R-loops. 2) The response can be elevated by a nutritional shift-up from minimal media to broth which stimulates rates of certain transcription. 3) The response is also enhanced by a mutation in *recD*, *polA* or *recB*, and *rnhA recD* double mutants require *recA*$^+$ for viability, suggesting that removal of persisting R-loops requires the activities of Exo V and DNA poll and/or recombination repair functions. 4) Finally, mutants of RNA polymerase have been isolated that exhibit highly elevated levels of SOS response only when combined with an *rnhA* mutation. The mutations, which map at *rpoB*, render *rnhA* mutants extremely sensitive to broth.

V 010 CHROMOSOMAL REARRANGEMENTS DETECTED AS FRAMESHIFTS, Lynn S. Ripley, UMD-New Jersey Medical School, Newark, NJ.

Some mutational mechanisms include DNA intermediates in which DNA moves from its usual full association with its complement and moves to a misaligned position, which nonetheless serves as a substrate for processing by enzymes such as DNA polymerase. Using the bacteriophage T4 system we have detected frameshift mutations in the *rIIB* gene that arise from misalignments mediated by palindromic DNA sequences. These mutations are not limited to T4, but occur in other prokaryotes and eukaryotes, including humans. We have recently found that the mutations are recovered from *in vitro* DNA polymerization reactions at high frequencies, which have allowed us to begin to characterize the properties of mutagenesis depending on DNA misalignments with substantially improved description of the molecular specificity. The improved view of specificity has been used to develop biochemically testable models of how mutagenesis occurs *in vitro*. The results set the stage for a vastly improved attack on the nature of misalignment mutagenesis *in vivo*.

In vitro, we can now demonstrate that site-specific rates of mutagenesis depend on (at least) three features of misalignments and their processing. Analysis of a spectrum of ~1,000 frameshifts sequences in a 140 bp region arising after polymerization catalyzed by either *E. coli* PolI or its Klenow fragment revealed ~170 sequences whose sequence can be simply explained by palindromic sequence misalignments, but appear to be incompatible with other explanations. An unexpectedly large contribution is made by DNA polymerase to the specificity; the hotspots for PolI are distinct from the hotspots for Klenow polymerase.[1] Mutations produced by both enzymes share the characteristic of initiating at preferred polymerization pause sites; a characteristic compatible with a mass action effect leading to the accumulation of the first intermediate of the reaction.[2] The difference between the two enzymes is due to a later step in the reaction whose biochemical property has not yet been identified, although some possibilities have been eliminated.

The role of DNA structure in defining preferred mutational misalignments was unclear in the initial experiments. For example, although misaligned pairing was required, comparisons between sites revealed no quantitative relationship between extent of complementary base pairing during misalignment and mutagenesis was obvious. However, recent studies addressing the role of complementary pairing at a single site demonstrated that a single additional complementary base (4bp → 5bp) can increase mutation frequency by 8-fold (Wang and Ripley, submitted). Thus, both DNA structure and specific characteristics of polymerases influence the mutagenicity of palindromic DNA misalignments.

[1] Papanicolaou, C. and L.S. Ripley (1989) J. Mol. Biol. **207**:335-353.
[2] Papanicolaou, C. and L.S. Ripley (1991) J. Mol. Biol. **221**:805-821.

V 011 SITE-SPECIFIC RECOMBINATION AND CHROMOSOME SEGREGATION, David J. Sherratt, Lidia Arciszewska, Garry Blakely, Mary Burke, Nick Leslie, Richard McCulloch, Gerhard May and Jennifer Roberts, Institute of Genetics, Glasgow University, Glasgow G11 5JS, UK.

Linear chromosomes, for example those of eukaryotes, have had to evolve mechanisms to ensure that chromosome ends are protected from exonucleolytic attack and can be replicated completely. Circular chromosomes are not subject to these problems. However, their circularity makes homologous recombination a threat to their integrity and maintenance. Any number of homologous recombination events between linear homologous chromosomes generates linear products that have the same overall structure as their parents. In contrast, any odd number of homologous recombination events between homologous circles will generate fusions of the two circles. Such dimeric molecules may have difficulties in partition at cell division (or indeed in packaging when the circles are viral). It is therefore not surprising to find that circular chromosomes have evolved mechanisms to ensure that multimers can be effectively converted to monomers. In *E.coli* and its relatives, plasmids and the bacterial chromosomes use site-specific recombination to convert multimers to monomers. We have demonstrated that one such system, the XerC/XerD site-specific recombination system is used both by the E.coli chromosome and by multicopy plasmids to ensure normal replicon partition. The XerC/D recombinases share 37% identity and bind to opposite sides of the core recombination sites present at the replication terminus of the *E.coli* chromosome and in multicopy plasmids. The properties of these recombinase molecules and their recombination sites will be discussed in relation to the recombination mechanisms and the cellular function of these systems.

Termination of Replication

V 012 FUNCTIONS OF THE DIF LOCUS IN ESCHERICHIA COLI, Peter Kuempel, Marianne Tecklenburg, Lori Dircks, Olagappan Nagappan and Anders Lobner-Olesen, MCDB, Campus Box 347, University of Colorado, Boulder, Colorado 80309.

dif (deletion induced filamentation) is a newly identified locus that lies within the terminus region of the chromosome of Escherichia coli at 33.8 minutes. The locus was identified by deletion mutants which had a phenotype in which a subpopulation of the cells was filamentous and contained abnormal nucleoids, and the SOS system was induced. Studies of plasmids containing this locus demonstrated that dif functions as a recA independent recombination site. Interestingly, the Dif phenotype was suppressed by mutations that inhibited homologous recombination (recA or recBC) as well as lexA3. This suggested that dif was a resolvase site which functioned to reduce circular multimers to monomers at the end of the replication cycle, and that these monomers arose by sister-chromatid exchange. Consistent with its function as a resolvase site, dif shows homology over a 33 bp region with the cer site of plasmid ColE1. Further support has been obtained by Sherratt and co-workers, who have demonstrated that the XerC resolvase, which functions at the cer site, also functions at dif, and that xerC mutants have a Dif phenotype.

We are currently inserting dif at various locations in the chromosome to determine if the site is only effective in the terminus region, and how cells are affected if dif is located elsewhere. Using a plasmid system, we have integrated a 33 bp dif sequence into the site of a 12 kb dif deletion. By the criteria of cell morphology and SOS induction, this completely suppressed the Dif phenotype. This indicates that only the 33 bp sequence is needed for proper dif function, and that flanking sequences are not required. Procedures and results for integrating dif into other sites will be presented.

V 013 RECOMBINATION IN THE TERMINUS OF THE *Escherichia coli* CHROMOSOME Francois Cornet, Vincent Francois, Jacqueline Louarn, Josette Patte, and Jean-Michel Louarn. Centre de Microbiologie et de Genetique moleculaires du CNRS. 118 route de Narbonne, 31062 Toulouse cedex, France.

The frequency of deletion formation by homologous recombination between directly repeated sequences was found to be nearly constant and low along most of the *E. coli* chromosome, except in the region of replication termination where it is much higher. The reporter system makes use of a temperature-sensitive Lambda prophage harboring Tn5 and Tn10 sequences and able to integrate by homologous recombination into Tn5 or Tn10 transposons resident in the chromosome. The highest excision frequency (10^{-3} above the baseline) was found at a locus, *zdd263*::Tn5, located at 34 min between the normally used terminator *psrA* and the *dif* locus, which undergoes site-specific recombination controlled by the XerC/XerD recombinase. These observations led us to postulate a role of homologous recombination in the termination of replication and the separation of daughter chromosomes.

To test the model, we have analyzed the effects of various mutations affecting termination and *dif*-specific recombination on excision frequency at position *zdd263*. Termination was altered either by Tus$^-$ mutations or by displacement of the "replication fork trap". Exchanges at *dif* were inhibited by XerC$^-$ mutations or by small *dif* deletions. In all cases excisive hyperrecombination was still observed, suggesting that this phenomenon is not directly related to termination or dif-specific recombination.

However, we have observed that introduction into the chromosome of an additional terminator site significantly increases the frequency of excisive recombination in its vicinity. We now consider the possibility that the general 10-to-50 times increase in excisive recombination observed in the terminus region might be associated with termination, and that the 1000 times increase observed at locus *zdd263* might be due to some other other phenomenon.

In the course of these experiments, we observed a more complicated pattern for excisive recombination at position *zddz263*. Bacteria harboring a lambda prophage at this locus lose it according to 2 modes: mode "V", very high excision frequency (>10^{-2}/cell/generation), or mode "M", moderately high excision frequency (more than 10 times less frequent). These two modes are hereditary, and are found at about the same frequency among independently isolated lysogens, but the V mode is rather unstable, and shifts to the M mode at a frequency of about 10^{-2}/cell/generation. The reverse shift (M to V) has not been observed in a wild-type background. Experiments engaged to analyse these two modes (nature of recombination products, pathways involved, generalization to other systems and chromosomal locations) will be presented.

V 014 BIOLOGICAL ROLES OF TOPOISOMERASE IV IN *S. TYPHIMURIUM*, Molly B. Schmid, David J. Sekula, Amy L. Springer, Princeton University, Princeton, NJ 08544.

Bacteria possess four topoisomerases - two type 1 enzymes (topoisomerases I and III) and two type 2 enzymes (topoisomerase II (DNA gyrase) and topoisomerase IV). Both of the type 2 enzymes are essential for the growth of *E. coli* or *S. typhimurium*. Topoisomerase IV is composed of two subunits, encoded by the genes *parC* and *parE*, which show strong similarity in both DNA and amino acid sequence to the genes encoding gyrase, *gyrA* and *gyrB*. We have a large number of conditional lethal mutations in the *parC* and *parE* genes, and in the linked gene, *parF*. These conditional lethal mutations were noticed because they cannot segregate daughter nucleoids at the non-permissive temperature. These mutants allow us to assess the *in vivo* consequences of inactivating topoisomerase IV, and compare these phenotypes to those caused by inactivating DNA gyrase. In this way, we hope to describe the biological division of labor between the related enzymes, DNA gyrase and topoisomerase IV.

Conditional mutations in *parC* and *parE* do not change the superhelicity of the reporter plasmid pBR322, unlike alleles of *gyrA*. These mutations cause small differences in the transcription of certain mu*dlac* fusions, but these fusions are not those that are strongly altered by inhibiting DNA gyrase. Mutations in *parC* and *parE* do not alter the spontaneous duplication frequency, a measure of pseudo-legitimate recombination. In addition, *parC* and *parE* mutations do not block DNA replication after shift to non-permissive conditions. Thus, topoisomerase IV plays no obvious role in initiation or elongation of DNA replication, homologous or non-homologous recombination, transcription or maintenance of global superhelicity. These results suggest that topoisomerase IV is a highly specialized topoisomerase, specific for the task of aiding nucleoid partitioning. Presumably, topoisomerase IV allows partitioning by decatenating the daughter nucleoids, which then allows faithful segregation. This presumption has been substatiated by the finding that a portion of the cell's pBR322 is catenated in *parC* and *parE* mutants after a shift to non-permissive conditions[1].

Some topological tasks may be accomplished by more than one topoisomerase. We are assessing functional redundancy in the topoisomerases by construction of *gyrA parCE* and *topA parCE* double mutants.

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Repair and Recombination-II

V 015 RESOLUTION OF RECOMBINATION INTERMEDIATES, Robert G. Lloyd, Gary J. Sharples, Matthew C. Whitby, Lizanne Ryder, Tikshna N. Mandal, and Akeel A. Mahdi, Genetics Department, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

The formation and resolution of Holliday junctions are key stages in recombination. Studies of *E. coli* RecA protein have provided considerable insights into the homologous pairing and strand exchange reactions that lead to the formation of these structures, but the mechanisms by which the junctions are then resolved to produce mature recombinants are only beginning to emerge. The products of the *ruv* and *recG* loci have been linked recently with this stage in recombination. Three genes have been identified at the *ruv* locus. The products of all three have been overproduced and purified. RuvA and RuvB form specific complexes with Holliday junctions and have been shown to dissociate these junctions by catalysing branch migration[1,2]. RuvC is an endonuclease that is able to cleave junctions to produce recombinant products of the type expected from genetic crosses[3].

Strains defective for *recG* show reduced recombination and increased sensitivity to ultraviolet light, mitomycin C and ionising radiation. However, the mutant phenotype is very modest and is masked by the activities of the *ruv* genes. We have purified the *recG* product and shown that it is a DNA dependent ATPase. Like RuvAB, RecG binds to a Holliday junction and dissociates the structure by catalysing branch migration[4].

The functional overlap between RuvAB and RecG is consistent with the fact both that *ruv* and *recG* single mutants are reasonably proficient at producing recombinants in genetic crosses, whereas *ruv recG* double mutants produce less than 0.5% of the normal yield of progeny[5]. More recent studies with suppressor mutations have shown that the RuvAB and RuvC proteins are dispensable. Recombination in strains carrying suppressed *ruv* mutations depends very much on *recG*, and on the product of at least one other gene that probably provides an alternative to RuvC resolvase. A model for the repair of damaged DNA incorporating these alternative activities for the resolution of recombination intermediates will be presented.

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V 016 RECOMBINATION AND REPAIR OF THE *ESCHERICHIA COLI* CHROMOSOME BY THE RecBCD PATHWAY, Gerald R. Smith, Susan K. Amundsen, Sathyam Ganesan and Andrew F. Taylor, Fred Hutchinson Cancer Res Ctr, Seattle, WA 98104.

The RecBCD pathway is the major means by which linear chromosomal fragments recombine with the *E. coli* chromosome (*e.g.*, during conjugation, transduction and transformation) and by which chromosomes with double-stranded (ds) breaks are repaired (*e.g.*, following X-irradiation). In addition to RecBCD enzyme and ds DNA ends, this pathway requires RecA protein and single-stranded (ss) DNA binding protein (SSB) for homologous pairing and strand exchange, and DNA polymerase I and DNA ligase to fill gaps and seal nicks (reviewed in ref. 1). RecBCD enzyme binds tightly to ds DNA ends ($K_D < 10^{-10}$ *M*), with the RecB subunit contacting the 3'-ended strand and the RecC and RecD subunits contacting the 5'-ended strand (2). In the presence of ATP, RecBCD enzyme travels along the DNA at about 350 bp/sec and produces on the 3'-ended strand a ss DNA loop which grows about 100 nuc/sec. We hypothesize that DNA unwinding may result from the RecB subunit rolling along the 3'-ended strand at a slower rate than the RecCD subunits roll along the 5'-ended strand. Upon encountering a Chi site (5' G-C-T-G-G-T-G-G 3') from the 3' side, RecBCD enzyme cuts the Chi-containing strand to generate a ss DNA substrate for RecA and SSB proteins. As expected, during recombination Chi enhances the level of heteroduplex DNA, whose formation requires RecBCD enzyme and RecA protein (3). Upon cutting at Chi RecBCD enzyme is changed: it loses the ability to cut at another Chi on the same or a second DNA molecule and loses the ability to initiate unwinding on a second molecule (4). We have detected a small RNA molecule that is physically associated with RecBCD enzyme and that appears to be required for DNA unwinding and Chi-cutting. Loss or alteration of this RNA may be the change of RecBCD enzyme upon cutting at Chi. Cutting at a single Chi site would ensure single exchanges near each end of a linear chromosomal fragment and, hence, even numbers of exchanges (and viability of recombinants) in conjugation, transduction, and transformation. Analysis of previously published data indicates that double exchange recombinants incorporating the majority of the Hfr fragment ("long chunk" recombinants) predominate in conjugational recombination (5). A unified, non-reciprocal exchange mechanism can account for the RecBCD pathway-promoted recombination of chromosomal fragments ("ends out") and repair of broken chromosomes ("ends in") (5). We shall discuss the possibility that the *E. coli* chromosome is best represented as a collection of circularly permuted linear molecules continually recombining via the RecBCD pathway.

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V 017 THE ORF FUNCTION OF PHAGE λ, James Sawitzke, Mary M. Stahl and Franklin W. Stahl, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229

The Rec⁻ phenotype of *recBC* mutant bacteria is suppressed by mutations in *sbcB* and *sbcC*. The resulting recombination is dependent on a number of gene functions, including those of *recO, R* and *F*, and is said to proceed by the RecF pathway. When Red⁻ (Gam⁻) λ infects such bacteria, recombination of the phage is independent of *recO, R* and *F*. Deletion of Orf146 (hereinafter called *orf*), renders λ recombination dependent on each of those three functions. Thus λ's Orf function appears to replace the requirement for three separate *E. coli* proteins. Orf bears no sequence similarity to any of them, nor to any protein other than the obvious homolog found in the related phage P22 (1).

Crosses with partially blocked DNA replication (achieved by repression in the presence of heteroimmune "helper" phage) suggest that RecF pathway recombination (like Red recombination) acting on λ is stimulated by *cos*, the site of action of λ's double chain nuclease Ter, and by rolling circle DNA replication. In the presence of Orf function, recombination among unreplicated chromosomes is focused more sharply at *cos*. In the absence of RecO, RecR, or RecF, there is little or no focus of recombination at *cos* unless Orf is provided by the helper. When Orf is provided, recombination in these hosts (*recO* and *recR*)is also focused in the neighborhood of a double chain break introduced *in vivo* by the *Pae*R7 restriction enzyme acting at λ's unique *Xho*I site.

The phenomenology of Orf suggests that it binds to double chain ends, protecting them from excessive degradation and/or promoting their productive processing

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Population Questions

V 018 INTRON MOBILITY AND DISTRIBUTION, Marlene Belfort, Molecular Genetics Program, Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509.

Many group I introns are capable of mediating DNA-based mobility reactions, in addition to their ability to promote RNA splicing. The mobility process is initiated by an intron-encoded endonuclease that recognizes an intron-minus allele and creates a double-strand break in the DNA. Alignment of homologous exons, followed by double-strand-break repair using the intron-containing allele as template, results in acquisition of the intron by the cleaved recipient. The characteristics of this homing process have been defined with the mobile *td* intron of phage T4 as model system. Thus homing has been shown to be dependent on a double-strand break, and on exon homology, but independent of the nature of DNA ends or the intron sequences being transferred. In addition, both 5'-3' and 3'-5' exonucleases are required to process the cleaved DNA ends into viable recombination substrates. The recombination reaction also depends on recombinase activity. This activity can be provided by either *Escherichia coli* RecA function or by T4 UvsX recombinase.

The mobility process is reflected in the idiosyncratic distribution of introns. Thus closely-related species may have different intron profiles. Furthermore, whereas the core splicing structures of related introns are often extremely similar, the endonuclease ORFs can be highly variant. These observations are consistent with an independent origin of the intron core structure and the endonuclease ORF. We have proposed that the endonuclease genes are the primary agents of mobility, and that these have colonized pre-existing introns, thereby conferring mobile properties on the composite elements. Evidence supporting the intrusive nature of endonuclease-encoding elements will be presented.

V 019 CLONAL FRAMES: RECOMBINATION, SELECTION, AND THE MOLECULAR EVOLUTION OF THE *E. COLI* CHROMOSOME, Roger Milkman and Melissa McKane Bridges, Department of Biological Sciences, The University of Iowa, Iowa City.

Comparison of sequences in an 8.8 kb region of 35 ECOR (wild) strains of *E. coli* reveals 1) discontinuous similarities in randomly bounded segments up to 1 kb or more in length; 2) a majority sequence type, called a clonal frame, common to each of several strains; and 3) sequence divergence suggesting divergence times up to 2 billion generations.

The segmental affinities obviously result from recombination. An attempt to reconcile the observed segment lengths with the expected length of entrant DNA fragments has employed two approaches. First, P1 transduction from various ECOR strains into K12 containing *trpA33* (a non-reverting mutation consisting of two adjacent nucleotide substitutions). RFLP analysis of transductants determines the rough extent of donor DNA. Sequencing provides finer detail, determines whether the donor DNA is incorporated discontinuously, examines the distribution of breakpoints as well as their sharpness (complete at this writing). Second, computer simulation of successive overlapping, long, continuous recombinational replacements. It is has been easy to reproduce semi-quantitatively the observed pattern and distribution of segmental affinities.

We will report the quantitative results of selected simulated combinations of the following variables: numbers of up to 6 different initial sequence types; length of region observed; relation between length of entrant molecule and length of region observed (this is expressed as probability that the DNA in the observed region is totally replaced); the probability that an encounter between a donor of a particular sequence type and a recipient of a particular sequence type will result in a recombination event ("exchange"); and the total number of exchanges that take place (maximum is over 23,000). A given strain can, over time, serve as both donor and recipient. The relationship between number of exchanges (per lineage) and time will be addressed, as will that between exchanges and nucleotide substitutions.

As time permits, other details that have emerged from the sequence comparisons will be presented.

Milkman, R. and M. M. Bridges, 1993 The molecular evolution of the *E. coli* chromosome. IV. Sequence comparisons. Genetics 133 (in press).

V 020 EVOLUTION OF SPECIES-SPECIFC SEGMENTS OF BACTERIAL GENOMES, Howard Ochman[1] and Eduardo Groisman[2]. [1]Department of Biology, University of Rochester, Rochester, NY 14627 and [2]Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO 63110.

The most comprehensive information concerning the genetic structure and organization of bacterial genomes has come from the comparative analysis of *E. coli* and *S. typhimurium*. Although these species are well-conserved with respect to chromosome size and the order, orientation and spacing of genes, when their linkage maps are aligned, there are numerous regions ("loops") of the chromosome that are restricted to one of the species. Although loop regions are likely to harbor genes encoding the properties used to differentiate among species, the ancestry and function of most species-specific segments of the chromosome remains unknown.

To determine the molecular, genetic and phenotypic characteristics that differentiate bacterial species, we analyzed several cloned DNA fragments confined to *Salmonella*. These segments map to unique positions on the *S. typhimurium* chromosome, and all have very restricted phylogenetic distributions and G+C contents considerably lower than that of the entire *Salmonella* genome. To identify the potential coding sequences within these regions, we determined the nucleotide sequence of each of these clones and tested the phenotype of mutant strains harboring deletions in these regions.

Chromosome Structure-Global

V 021 FUNCTIONAL CONSEQUENCES OF NEW STRUCTURAL INFORMATION ON BACTERAL LP-CHROMATIN, Eduard Kellenberger, Bernd Bohrmann, Birgit Arnold-Schulz-Gahmen, Department of Microbiology, Biocentre of the University of Basel (Switzerland)

Thin sections of resin-embedded *E. coli* obtained after cryofixation and freeze-substitution (CFS), combined with immunocytochemistry, has provided new insights for the distribution of DNA and of the DNA-associated proteins HU and RNA-polymerase. The aggregation-sensitivity of bacterial chromatin is the consequence of its low relative protein content. LP-chromatins are also found in mitochondria, plastids, veg. phage DNA-pools and in the eukaryotic dinoflagellates (1).- Aggregation is prevented from occurring during subsequent dehydration, when the chromatin was gelled by producing chemical crosslinks. Aldehyde-crosslinking has to be mediated through protein partners, that are distributed all over the DNA. When below an estimated 50%, then aggregation occurs. - Radioautography (2) and immunocytochemistry (3,4) allow to distinguish a bulk-part of the nucleoid in which no RNA is synthesised. It occurs outside in the cytoplasm where polymerase and HU are located. CFS and immunostain (4) show excrescencies of the nucleoid which extend dendrite-like into the cytoplasm. - After CFS the texture of the DNA-plasm is now fine granular instead of fibrillar-reticular as with conventional fixations. It likely represents very labile selenoidal supercoil (1). It breaks down into "compactosomes" with little or no protein. By chemical fixation, likewise upon isolation, the supercoil is relaxed, leading to DNA-"crystallisation" into bundles. The latter occur after a first aldehyde-treatement that fixes only the cytoplasm, followed only later by an uranyl acetate fixation of the DNA. - The packing density has also been estimated by a new method (6) which complements and extends previous calculations: The bulk-nucleoid is 20 to 100 mg/ml, as is the veg.phage pool. It is around 200 to 300 in the permanently condensed chromosomes of dinoflagellates and in heterochromatin and 300 to 500 in metaphase chromosomes. It reaches over 800 in the mature T4-phage head.

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V 022 TRANSCRIPTION AS A DETERMINANT OF LOCAL DNA TOPOLOGY IN *E.COLI*, A. Simon Lynch and James C. Wang. Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

We are currently undertaking a series of studies to further elucidate specific physiological roles of the DNA topoisomerases and the division of labour between them in processes that are responsible for the maintenance and propagation of the *E.coli* chromosome.

The expression of plasmid-borne genes encoding either integral membrane proteins or exported proteins has been found to give rise to highly negatively supercoiled forms of the plasmids in cells lacking topoisomerase I. This result is consistent with predictions of the 'twin-supercoiled' domain theory of transcriptional supercoiling, wherein effective cellular anchoring of RNA polymerase can be mediated by co-transcriptional interactions of nascent polypeptides with factors involved in the integration into, or translocation across, the cytoplasmic membrane of the cell. Furthermore, the result also suggests that a major cellular function of topoisomerase I is the relaxation of negative supercoils which arise during transcription. Similarly, the accumulation of positive supercoils into plasmid DNAs following inactivation of DNA gyrase is indicative of a role of this enzyme in relaxing positive supercoils which arise during transcription.

The accumulation of high levels of negative supercoils into circular DNA templates undergoing transcription appears to require the presence of topological barriers which prevent the passive merging of domains of transcription-generated supercoils of opposite signs. We are currently characterizing sequences derived from the *E.coli* genome which may mediate the formation of such barriers. These studies employ site-specific recombination to generate non-replicating promoter containing DNA rings in vivo. Transcription-induced changes in template topology can thus be monitored as a function of the inclusion of candidate barrier sequences, in the absence of potential complications arising from the replication of the transcription templates. It is hoped that the characterization of such barrier sequences may indicate mechanisms whereby topologically-independent domains can be formed in the *E.coli* chromosome.

Global Aspects of Cell Regulation

V 023 ENERGY-DEPENDENT PROTEOLYSIS IN E. COLI, Susan Gottesman[1], William Clark[1], Valerie de Crecy[1], and Michael Maurizi[2], [1]Laboratory of Molecular Biology and [2]Laboratory of Cell Biology, National Cancer Institute, Bethesda, MD.

E. coli possesses at least two complementary systems for the rapid destruction of short-lived regulatory proteins and abnormal proteins. One of these, the Lon ATP-dependent protease, is responsible for the rapid turnover of SulA, a cell division inhibitor, RcsA, a positive regulator of capsule synthesis, and lambda N protein. In addition, Lon degrades abnormal proteins in a process which requires the presence of the heat shock chaperone proteins. The second ATP-dependent protease, Clp, does not have a substantial effect on turnover of the Lon substrates, but degrades a distinct set of specific substrates. Clp was originally identified *in vitro* as a two-component protein. The large final Clp protease complex is composed of 12 copies of a small subunit which contains the protease active site (ClpP) and 6 copies of a larger subunit which has ATPase activity (ClpA). A second ClpA-like protein, ClpB, is also made in *E. coli*, but evidence for a role of ClpB in proteolysis has not yet been published. ClpP subunit can apparently work with subunits other than ClpA. The gene just downstream of *clpP*, *clpX*, encodes a protein with a single ATP binding consensus sequence. Phenotypes found thus far in *clpP* mutant cells can be divided into those which depend on ClpX activity and those which depend on ClpA activity. ClpB may also interact with ClpP under some conditions. The accelerated death at high temperatures previously described by Squires *et al.* for *clpB* mutants is relieved by mutations in *clpP*. This suggests that ClpB may interact with ClpP at very high temperatures, and that in the absence of ClpB, ClpP is free to associate with other proteins and degrade inappropriate substrates. Therefore, it appears that these alternative ATPase subunits can reassort with ClpP *in vivo* to change substrate specificity. Such switches in substrate specificity may be particularly important as the cell switches from one mode of growth to another and needs to degrade different populations of proteins.

V 024 REGULATION OF GENE EXPRESSION BY METHYLATION PATTERNS, Xiangwu Nou, Bruce Braaten, Marjan van der Woude, Brad Hale, Linda Kaltenbach, and David Low, Department of Pathology, University of Utah Medical School, Salt Lake City, UT 84132.

Most of the estimated 18,000 "GATC" sequences in the *Escherichia coli* chromosome are methylated by deoxyadenosine methylase (Dam). However, under certain growth and environmental conditions, a subset of GATC sites are protected from methylation, forming specific methylation patterns. In the case of the pyelonephritis-associated pili (*pap*) operon, the methylation states of two GATC sites (GATC-I and GATC-II) upstream of the *pap* pilin promoter regulate a switch between transcriptionally active (ON) and inactive (OFF) states. Two regulatory proteins, PapI and leucine responsive regulatory protein (Lrp) are required for methylation protection of these GATC sites. Lrp binds near the GATC-II site in the absence of PapI, protecting this site from Dam methylation. This forms the phase OFF methylation pattern in which GATC-I is methylated and GATC-II is nonmethylated. In the presence of PapI, Lrp binds near GATC-I, protecting this site from Dam and forming the converse phase ON methylation pattern in which GATC-I is nonmethylated and GATC-II is methylated. These methylation patterns, in turn, modulate the binding of Lrp and PapI to *pap* DNA. Methylation of GATC-I prevents binding of Lrp-PapI to this site and thus maintains the phase OFF state. In contrast, methylation of the GATC-II site is required for *pap* transcription. Thus, *pap* transcription occurs only when GATC-I is nonmethylated and GATC-II is methylated. We hypothesize that switching from the OFF to ON methylation state occurs after DNA replication, when a hemimethylated GATC-I site is formed. Lrp-PapI binds to hemimethylated *pap* DNA, although with a reduced affinity compared to nonmethylated DNA. If Lrp-PapI binds to the GATC-I site before Dam, then after one more round of DNA replication the GATC-I site will be nonmethylated (the phase ON state). In summary, our results show that *pap* phase variation is controlled by an epigenetic mechanism in which Lrp and PapI regulate the methylation states of the GATC-I and GATC-II sites which, in turn, modulate the binding of these transcriptional factors to *pap* DNA. We find that Lrp controls the methylation states of other GATC sites in the *E. coli* chromosome as well and are currently analyzing the roles of DNA methylation in regulating the expression of genes near these GATC sites. Because binding of regulatory proteins to DNA can be affected by environmental factors, this could provide a type of "memory" in which gene expression of daughter cells is set by the milieu of the parent cell.

V 025 *E. COLI* FUNCTIONS REQUIRED FOR RESUMING GROWTH AFTER STARVATION, Deborah A. Siegele, Biology Department, Texas A&M University, College Station, TX 77843-3258.

Microorganisms have evolved a variety of mechanisms that allow them to survive in environments where the nutrient supply fluctuates. Under such conditions, the microbial life cycle can be seen as consisting of two phases: i) a growth phase where cells are growing and dividing and ii) a starvation or stationary phase where cells have left the cell cycle and growth is prevented by nutrient limitation. The transitions between these two phases of the life cycle involve dramatic changes in gene expression, cell physiology, and morphology.

In order to identify the mechanisms that allow *E. coli* to make the transitions between growth and stationary phase and to maintain viability during starvation, a genetic screen was used to isolate mutants defective in stationary phase survival (a Sur$^-$ phenotype) following glucose starvation. One of the mutants isolated, *surB1*, is a conditional mutant that remains viable during starvation, but is unable to exit from stationary phase and resume aerobic growth at high temperature (37°C). The *surB1* mutation does not appear to affect growth prior to entry into stationary phase; once growth has started at low temperature, mutant cells can be shifted to high temperature and growth continues at the same rate as the wild-type strain. SurB is not required for exiting stationary phase under all conditions; mutant cells can resume growth at low temperature and also at high temperature under anaerobic conditions. Thus, SurB function is specific for resuming aerobic growth at high temperature.

Sequencing of the cloned *surB* gene revealed that the predicted SurB protein is homologous to a family of ATP-dependent transport proteins, known as traffic ATPases or the ABC transport family. SurB is also predicted to have a similar topology to some other members of the family with six membrane spanning domains and a cytoplasmic carboxy–terminal ATP–binding domain. Work is in progress to determine the function of SurB and how loss of this putative transport protein results in a defect in exiting stationary phase and the inability to resume aerobic growth at high temperature.

Chromosome Structure-Local

V 026 SPECIFIC AND NON-SPECIFIC COMPONENTS OF A NUCLEOPROTEIN COMPLEX: HU VERSUS IHF, Howard A. Nash, Steven D. Goodman, Andrew E. Granston and Anca M. Segall, Laboratory of Molecular Biology, NIMH, Bethesda, Md. 20892.

IHF and HU are homologous proteins that bind to and deform DNA. IHF binds tightly to DNA at specific targets; in contrast, HU binds weakly to DNA and preferred binding sites have not been identified. IHF is an essential component for lambda integrative recombination, attP x attB; for this reaction IHF cannot be replaced by HU. However, we have recently shown that HU is a moderately effective replacement for IHF during lambda excisive recombination, attL x attR. The principal role of HU and IHF appears to be architectural because constructs in which sequence-directed bends have been engineered into attL and attR recombine in the absence of these proteins.

To investigate the basis for HU function during excisive recombination, we studied nucleoprotein complexes by PAGE. Landy and coworkers have shown that IHF cooperates with Int, the lambda-encoded recombinase, to produce a well defined complex at attL. At this site a specific IHF target lies between binding sites for Int. Despite the absence of a strong binding site for HU, we find that this protein cooperates with Int to produce an attL complex of similar stability and electrophoretic mobility. The features of this complex suggest that assembly is based not on contacts between HU and Int but on structural cooperation. We believe that DNA must be deformed to permit Int protein to maximally contact its preferred binding sites; HU-promoted deformation of DNA assists this process.

We have used the phenomenon of structural cooperation, in which Int protein traps HU at attL, to examine other non-specific DNA binding proteins. For example, several mutants of IHF that are defective in specific binding to isolated IHF sites can cooperate with Int to make complexes at attL. This may explain why such mutants retain partial function, such as the capacity to package lambdoid phages. Extracts of many eucaryotic cells contain an activity that also cooperates with Int to form attL complexes, as do purified HMG1 and HMG2 protein. Indeed, HMG1 and HMG2 behave like HU in recombination assays; they stimulate excisive but not integrative recombination.

While excision requires two IHF sites, H2 and H', integrative recombination requires both of these and an additional site, H1. We have tested whether the absolute requirement for IHF in integration is explained by the specific need for IHF at H1. We engineered substitution mutations to depress IHF binding to the H1, H2 or H' sites. As expected, the efficiency of integrative recombination fell in each case. We then supplemented recombination reactions with HU; recombination was improved substantially in all three cases. Thus, no single binding site absolutely requires IHF. However, certain combinations of two damaged IHF sites cannot be rescued by HU. This suggests that the requirement for IHF in integrative recombination reflects construction of a complex nucleoprotein array in which several deformations of DNA are coupled; in contrast, the architectural contribution of IHF to attL and attR behave as if they were independent.

V 027 INSERTION SEQUENCES MAY DICTATE CHROMOSOMAL DOMAINS, Bodo Rak[1], Karin Schnetz[1,2], Hans-Jörg Ronecker[1], and Caroline Welz[1], [1]Institut für Biologie 3, University, D-7800 Freiburg, FRG, [2]present address: Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The available data suggest that in the wild type, promoter *P0* of the *bgl* operon of *E.coli* is in an unfavourable topological state which results in low *P0* activity. The region upstream of *P0* is a hot spot for transposition of IS*1* and IS*5*. Transposition of these elements into the promoter region leads to a drastic enhancement of *P0* activity and thereby to activation of the operon. We have analyzed IS*5*-generated events and found that the presence of the termini of IS*5* are sufficient for *P0* activation, provided an intact IS*5* transposase gene is present within the same cell[1]. The data suggest that a product of this gene permanently interacts with the termini which leads to a change in the topological state of adjacent sequences.

IS*150* has a pronounced preference to transpose into an appr. 10 bp long target sequence. One such sequence motif is present within IS*1*. An IS*150*-flanked transposon forms transpositional cointegrates at an expected low frequency[2]. However, when a defective IS*1*, which carries a deletion encompassing the 5' end of gene *insA* together with its promoter, is present on a second replicon, IS*150*-driven transpositional cointegrates are found in almost 30% of all bacteria of an overnight culture. In about 90% of all cases cointegrates were formed within IS*1* and at the preferred target site. Thus transpositional activity of IS*150* can be stimulated to very high rates by a defective IS*1*-sequence. An intact IS*1*, however, does not have any stimulatory effect on IS*150* activity. Moreover, an intact IS*1* appears to be totally "immune" against integration of IS*150*. This immunity can be attributed, at least in part, to the activity of IS*1*'s InsA protein, which is known to bind to the termini of IS*1* thereby inhibiting transposition. The data suggest that the bound InsA protein imposes a topological constraint onto IS*1*, which would have regional consequences.

A structurally disturbed IS*1* sequence thus attracts integration and stimulates activity of a second insertion sequence, suggesting that the resident set of insertion sequences present in the *E.coli* genome are normally in an evolutionary balance which prevents high rates of transposition. In fact, there are numerous (mainly anecdotical) reports that insertion sequences are often found integrated into other, defective insertion sequences. The following observation lets us speculate that a transduction across a restriction barrier may transiently disturbe this balance leading to a transpositional avalanche: We have analyzed a region within the genome of an *E.coli* K-12 strain into which a marker has recently been transduced from *E.coli* B. To our surprise we found a cluster of eight different transpositional events.

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V 028 MODIFICATION OF RNA POLYMERASE BY THE PHAGE LAMBDA GENE Q TRANSCRIPTION ANTITERMINATOR AT THE PHAGE LATE GENE PROMOTER. Jeffrey W. Roberts, Mark Kainz, William Yarnell, Jennifer McDowell, Brian Ring, and Elizabeth Bartlett, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, N.Y. 14853, U.S.A.

Lambda late genes are regulated by the transcription antiterminator encoded by its gene Q. Q protein binds DNA between the -35 and -10 elements of the late gene promoter (1); however, RNA polymerase is modified by Q not in the open complex, but instead in an early elongation complex in which RNA polymerase pauses at nucleotides 16 & 17 (2,3) and exposes the Q binding site in the promoter region (1). Interaction of Q both modifies RNAP to an antiterminating form, and reduces the time of the pause. The pause is encoded mostly by nucleotides +2 and +6 (4,5), but is also affected by bases +7, +8, and +9, as well as bases at the downstream edge of the transcription bubble. Transcription of heteroduplex templates in vitro shows that bases in the non-template strand at positions +2 and +6, as well as +7 through +9, promote the pause. Q protein affects the DNA footprint in the region of these bases, inducing changes that possibly reflect supression of pausing by Q. The basic kinetic parameters of transcription elongation are changed by the interaction of Q at the pause to favor elongation, and we suggest that Q has a similar effect on an essential pause at terminators. Binding of Q at the qut site is detectable as changes in $KMnO_4$ reactivity in vivo not only at the promoter-distal end of the binding site found by footprinting in vitro (-30 to -10), but also at about +15 of the transcribed segment; furthermore, these reactions occur in the absence of RNA polymerase bound to the adjacent promoter. Presumably a more complex interaction exists in vivo than we detect in vitro, perhaps involving a bent DNA structure. In conditions of intense Q-mediated transcription, there exists a region of periodic $KMnO_4$ reactivity downstream of the late gene promoter that could represent transcription-induced topological changes in the transcribed DNA.

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Cell Division and Cell Cycle-II

V 029 MEMBRANE PROTEIN COMPONENTS OF THE E. COLI CELL DIVISION APPARATUS, Luz Maria Guzman, James Barondess, Michael Carson and Jon Beckwith, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115.

We have studied three membrane proteins that appear to be essential components of the cellular machinery for septum formation in E. coli. These proteins are coded for by the genes ftsI, ftsL, and ftsQ, which all map in the minute 2 cluster of morphogenes on the E. coli chromosome. The gene ftsL is adjacent to and precedes ftsI. Alkaline phosphatase fusion analysis of the three proteins suggests that they each contain a single membrane spanning stretch and hydrophilic cytoplasmic and periplasmic domains. Quantitation of the expression of the fusions indicates that the three proteins are made in only about 25-50 molecules per cell. In the case of ftsQ and ftsL, alkaline phosphatase fusions were recombined onto the chromosome to determine the effect of null mutations in these genes on cellular physiology. In order to study a conditionally expressed fts gene in the null background, we have constructed plasmids in which the two genes are under the control of the arabinose promoter. This promoter is highly expressed when cells are grown in arabinose, but is highly repressed when cells are grown in glucose. In cells carrying a null mutation in ftsL or ftsQ and the plasmid with the corresponding fts gene under arabinose control, removal of arabinose leads to cell death; these fts genes are essential. Depletion of FtsQ or FtsL from cells results in a strong filamentation phenotype. In addition, in FtsL-depleted cells, Y-shaped filaments are often seen. Overexpression of FtsQ leads to the formation of multiseptate filaments, while only extremely high levels of expression of FtsL interfere with cell division. The sequence of the newly characterized ftsL gene reveals a periplasmic domain that contains a sequence reminiscent of a leucine zipper. Five leucines in this domain are each separated by 6 amino acids. (A sixth is separated from the fifth by 13 amino acids.) The structure of FtsL and the potential leucine zipper raise the possibility that this protein is involved in some transmembrane signalling step of the cell division process. We are currently carrying out mutagenic studies on the potential leucine zipper to assess its function.

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V 030 THE ROLE OF FTSZ IN BACTERIAL CELL DIVISION, Joe Lutkenhaus, Amit Mukherjee, Kang Dai, Erfei Bi, Jian Hwang, and Xunde Wang, Department of Microbiology, Molecular Genetics and Immuology, University of Kansas Medical Center, Kansas City, KS 66103.

The essential cell division protein FtsZ forms a ring at the future division site, designated the FtsZ ring[1]. During cell division the FtsZ ring decreases in diameter at the leading edge of the septal invagination. Upon completion of division the FtsZ is dispersed into the cytoplasm and is not associated with the new cell pole. Expression of inhibitors of division, SulA or MinCD, prevent localization which would explain their ability to block cell division. Under conditions where the block to division by SulA is released cells are able to resume FtsZ localization and division, even in the absence of protein synthesis. This argues that the action of SulA is completely reversible and that FtsZ and the signal for localization are completely stable. The localization of FtsZ was also examined in various fts mutants. Mutations in ftsZ result in a smooth filamentation phenotype and prevent FtsZ localization. In contrast, filaments formed due to mutations in ftsA or ftsI have an idented filamentation phenotype. In these filaments some FtsZ rings are observed, however, there is not one for each division site. This result suggests that in these mutants the FtsZ ring forms and initiates division but it's progression is blocked by the lack of FtsA or FtsI which results in ring instability. Recently it has been determined that FtsZ has a unusual GTPase activity[2]. It will be of interest to determine what role this GTPase activity has in the dynamics of FtsZ localization during the cell cycle.

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V 031 THE GENETICS OF *mbr* MUTANTS OF *ESCHERICHIA COLI*, Nancy J. Trun and Jisoo Cha, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20892.

The *mbr* mutants of *E. coli* were isolated as resistant to camphor vapors, which selects for polyploid cells (Trun and Gottesman, Genes and Develop. 4:2036-2047, 1990). All of the mutants are unable to grow under a variety of conditions and have novel cellular morphologies when exposed to the nonpermissive conditions. Flow cytometry has shown that all of the mutants are diploid. We have been focusing on the characterization of *mbrA4*, which may be a checkpoint between chromosome replication and the cell cycle, and *mbrC17*, which is potentially involved in chromosomal partitioning. Starting with *mbrC17*, we have isolated and characterized a series of second site suppressors. One of these suppressors was found to be in the gene for adenyl cyclase (*cya*). Upon further study, deletions of *cya*, as well as deletions of *crp* (catabolite activating protein), partially suppress the growth defects of all of the *mbr* mutants. None of the suppressors identified was specific to *mbrC17*. However, these deletions completely and specifically suppress all of the phenotypes of *mbrA4*, including camphor resistance. This suggests that either *mbrA* is regulated by cAMP-CRP or that these genes function in the same place in the cell cycle with opposite effects. Because *mbrA4* is a dominant, gain of function mutation, we would expect any genes that function in the opposite direction from it would reverse the phenotypes of *mbrA4*. We are currently undertaking a molecular characterization of the *mbrA4* and *mbrC17* genes.

Distributing Chromosomes Prior to Division

V 032 REPLICATION AND PARTITIONING: AN ORIGIN SEGREGATION MODEL FOR REPLICATION CONTROL. Stuart J. Austin, Ann L. Abeles, Therese G. Brendler, Michael A. Davis, Finbarr Hayes, Lyndsay Radnedge, and Brenda Youngren-Grimes. ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702.

The plasmid prophage of bacteriophage P1 can be maintained at a copy number of 1 to 2 per *E. coli* cell with virtually no plasmid loss. Both highly accurate replication controls, and a precise mechanism for active partition of daughter molecules to daughter cells are operative. The adjacent but separable replication and partition cassettes have been studied in detail, and the nature of key cis-acting sites and their macromolecular interactions will be discussed.

Replication control appears to be exerted at two levels. Origin methylation is required for proper origin function and appears to modulate it by a mechanism that resembles the control of the host *oriC* origin (See Brendler et al. abstract). However, the primary control of the origin involves the action of a separate plasmid locus, *incA*. Considerable evidence supports the idea that a plasmid-plasmid pairing (DNA handcuffing) mechanism is operative. We have suggested the following model: Once replication occurs, the plasmid-encoded RepA protein promotes plasmid pairing, with the *incA* control locus of each daughter in contact with the origin of the other. This inactivates the origins of both daughter plasmids. Reinitiation does not occur until a partition event separates the copies in order to segregate them to the daughter cells. This automatically synchronizes replication control to the cell cycle. This "origin segregation" model implies that replication is positively controlled by partition, and that partition-defective plasmids should be far more unstable than predicted for a simple segregation defect. Measurements of loss rates of a variety of Par$^-$ mutant plasmids are consistent with this. Moreover, a recently discovered class of Par$^-$ point mutants appears to be unable to replicate at all.

P1 origin initiation is growth regulated like the host origin *oriC*. The number of plasmids (and therefore origins) per cell increases sharply with increasing growth rate. Consideration of how this might occur leads us to speculate that both origin types might be similarly controlled. Perhaps the well-documented sequestration of newly replicated *oriC* sequences to membrane sites is a manifestation of origin pairing and, subsequently, of origin segregation.

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V 033 GENES ESSENTIAL FOR CHROMOSOME PARTITIONING IN *E. COLI*, Sota Hiraga, Hironori Niki, Ryu Imamura, Jin Feng, Kunitoshi Yamanaka, Teru Ogura, and Mitsuhiko Kitaoka, Kumamoto University School of Medicine, Kumamoto 862, Japan.

Daughter cells almost always receive at least one copy of the chromosome; anucleate (chromosome-less) cells account for less than 0.03% of the population in growing cultures of wild-type strains of *Escherichia coli*. (1). To analyze molecular mechanism of the chromosome partition, we developed techniques to isolate mutants (*muk*) that were defective in chromosome partitioning and produced normal sized anucleate cells at high frequencies during cell proliferation. One of these mutants, *mukA1* had a defect in the *tolC* gene (61 min) encoding an outer membrane protein (2). Another mutant, *mukB106* was mapped at 21 min of the *E. coli* chromosome. Cloning and sequencing of the *mukB* gene revealed that the *mukB* gene codes for a 177 kDa protein consisting of 1534 amino acids (3). *MukB* null mutants were able to grow at 22°C and the size of nucleate cells was normal, but normal sized anucleate cells appeared at a high frequency in the population. The *mukB* null mutants were however unable to form colony at 42°C (3).

We recently purified the MukB protein of an over-producing strain by column chromatography of Sephacryl S-400, DEAE Sephacel, and DNA-cellulose (4). Throughout the purification steps, acyl carrier protein (ACP; 8.8 kDa) was co-purified with MukB. Results obtained by electron microscopic observation and the low angle laser light scatter analysis of the purified MukB protein showed that MukB forms a homodimer with a rod-and-hinge structure having a pair of small, N-terminal globular domains at one end and a pair of large, N-terminal globular domains at the opposite end; it tends to bend at a middle hinge site of the rod section (4). The center region (residues 339-665) between the globular domains was predicted to form an α-helical coiled-coil structure in a homodimer. The coiled-coil stretch may be interrupted at the hinge site of Gly_{487}-Pro_{488}. A possible ATP-binding sequence was found in the N-terminal globular domain. A leucine zipper sequence was found in the C-terminal domain. The C-terminal globular domain has a subregion (residues 1350-1534) rich in cysteine, arginine and lysine, suggesting that the subregion is involved in specific interaction with DNA or other cellular materials. Chromatography in a DNA-cellulose column and the gel retardation assay revealed that MukB possesses DNA binding activity. Photoaffinity cross-linking experiments showed that MukB binds to ATP and GTP in the presence of Zn^{2+} (4). The MukB protein exists about 1,300 molecules in a growing cell of the wild-type strain (5). We report here, in addition, that the *mukB* gene and other three genes constitute an operon (5).

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5. Feng *et al.* (unpublished data).

V 034 ON P1 PLASMID PARTITION: CONTEXT EFFECTS ON CENTROMERE FUNCTION Małgorzata Łobocka and Michael Yarmolinsky, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

The initial step in P1 plasmid partition appears to be the interaction of iterated sequences within the plasmid centromere analog, parS, with one of the two P1-encoded partition proteins, namely ParB. The context of parS is critical for the response of the plasmid. In certain cases, when Par A is supplied in addition to ParB, plasmid stabilization is achieved. In many others, we find that ParB causes active destabilization. This destabilization neither requires nor is overcome by ParA. It is influenced by transcriptional and structural features that are expected to affect the geometry of parS. The destabilization occurs at low physiological levels of parB and can be so dramatic that pSC101-derived and pBR322-derived plasmids bearing parS in particular sites can not be maintained at all in cells expressing ParB. We have taken advantage of this circumstance to isolate mutants altered in parB or parS that are affected in destabilization and partition. We find that of several ParB binding sequences in parS, only one need be altered to eliminate susceptibility to destabilization by ParB. We will report on experiments designed to distinguish between two potential sources of destabilization: dysfunction of partition and blockage of replication.

Presenters A-H

V 100 IS PARTITIONING OF CHROMOSOMES IN *E. COLI* RANDOM (OR WORSE)? S G Addinall, K J Begg, W D Donachie, Institute of Cell and Molecular Biology, University of Edinburgh, Scotland, UK

Spherical cells of *Escherichia coli* can be produced by mutations in *mreB*, *pbpA* or *rodA*[1,2]. Such mutant cells have been shown to have about six times the volume of wildtype rods at equivalent growth phases and also to have approximately six times the amount of DNA[3]. They therefore have about six times as many chromosomes per cell as do normal rods.

We used this observation to investigate how sister chromosomes are segregated into sister cells at division, by determining whether stable chromosomal heterozygotes could be maintained in spherical mutant cells.

We predicted that, if partitioning is mediated by a process in which each replicated chromosome is specifically separated from its sister into a different cell then a heterozygote should be entirely stable. Conversely, if partitioning simply results in random separation of half the total DNA (chromosomes) into each cell, then a heterozygote could be maintained under selection although it would be unstable in the absence of selection.

Our results suggest that a heterozygous spherical *Escherichia coli* cannot be maintained over even a few generations. We are therefore investigating possible explanations for this.

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V 101 *LISTERIA MONOCYTOGENES* DESCRIBED BY CONSERVED AND POLYMORPHIC *Eco*R1 FRAGMENTS CONTAINING RIBOSOMAL RNA SEQUENCES, Douglas A. Amorese, John A. Webster, Romeo J. Hubner, Eileen M. Cole, James L. Bruce, Joseph J. Neubauer, Channeary Iem, and Amy Jo McCardell, E.I. Du Pont de Nemours & Company, Experimental Station, Wilmington, DE 19880-0357

A method of detection and analysis of the set of genomic *Eco*R I fragments that contain portions of the rRNA genes has been developed for the description of bacteria. The method is based on obtaining a set of fragments from a single organism and assigning it a point in a mathematically defined space which can be compared to other points in space. 325 strains of *Listeria monocytogenes* have been analyzed using this approach to generate a data base used for identification. A pattern that quickly emerges is one in which several fragments are observed with a high frequency among strains of the same species. In addition to these conserved fragments there are also polymorphic fragments. When patterns obtained from *L. monocytogenes* were compared to patterns obtained from other *Listeria, Carnobacterium, Kurthia, Brochothrix, Erysipelothrix*, and *Bacillus* species, *L. monocytogenes* was distinguished from the other species. Use of this method allowed strains of *L. monocytogenes* to be identified according to species and classified into types with a single, standard procedure.

V 102 XerC AND XerD; TWO RELATED SITE-SPECIFIC RECOMBINASES INVOLVED IN *E.COLI* CHROMOSOME PARTITION AND PLASMID STABILITY, Lidia Arciszewska, Mary Burke and David Sherratt, Institute of Genetics, Glasgow University, Glasgow G11 5JS. U.K.

Normal partition of the *Escherichia coli* chromosome at cell division requires a site-specific recombination event at the replication terminus region. The same recombination system functions in multicopy plasmid stability by converting multimers to monomers. Two related recombinases XerC and XerD, sharing 37% identity, participate in this recombination reaction. These two integrase family proteins are encoded by unlinked genes located at 4024 kbp and 3050 kbp on the *E.coli* genome. Each protein binds to different sides of the recombination site. Binding of both proteins is cooperative.

In order to determine the precise role of XerC and XerD in the site-specific recombination we have constructed derivatives of both proteins carring mutations at two of four residues that are conserved throughout the integrase family enzymes: XerCR243Q, XerCY276F, XerDR247Q and XerDY280F. All four mutants bind the recombination site but do not mediate recombination *in vivo* indicating that both enzymes are catalitically involved in the reaction. We are currently studying interactions of wild type and mutant proteins with linear DNA substrates and with Holliday intermediates. While both YF mutants bind the recombination site with similar affinity to wild type proteins, the RQ mutants bind less tightly. The XerC RQ mutant protein generates protein-DNA complexes with XerD that have different properties to wild type XerC/D-DNA complexes.

V 103 HOMOLOGOUS RECOMBINATION-DEPENDENT INITIATION OF DNA REPLICATION FROM DNA DAMAGE-INDUCIBLE ORIGINS IN *E. coli*, Tsuneaki Asai and Tokio Kogoma, Department of Cell Biology, University of New Mexico School of Medicine, Albuquerque, NM 87131

Escherichia coli cells induced for the SOS response express an alternative mode of DNA replication (inducible stable DNA replication, iSDR). Initiation of iSDR is independent of protein synthesis, transcription and DnaA protein, which are essential for initiation of replication from *oriC*, but is dependent on some functions of the *recA*, *recB* and *recC* genes. We found that a *recA* mutant, which is defective in recombination but is proficient in SOS induction, could not elicit iSDR. In contrast, iSDR was enhanced by *recD* mutations that inactivate the Exonuclease V activity of the RecBCD enzyme, suggesting that recombination intermediates (i.e. D-loop structures) created by the action of RecA recombinase and RecBC helicase are involved in the formation of the initiation complex for iSDR. In support of this, mutations in the *ruvA*, *ruvB* and *ruvC* genes, which are thought to preserve D-loop structures by blocking the migration and resolution of Holliday junctions, were also found to enhance iSDR. A similar result was obtained with a *recG* mutant which is suggested to have a defect in the resolution step of homologous recombination. iSDR starts primarily from the *oriC* and *terC* regions on the chromosome. Using minichromosomes, iSDR was shown to occur in a 596-bp region which includes the minimal *oriC*. Precise mapping of the origin for iSDR in this region was carried out by inserting various *oriC* fragments into a plasmid vector derived from pSC101, which does not undergo iSDR by itself, and by measuring the copy number of these chimera plasmids in the presence of chloramphenicol and rifampin in SOS-induced cells. The result so far obtained suggests that there are at least two origins for iSDR within the minimal *oriC*; one is between the *Bam*HI(1) and the *Ava*II(155) sites, and the other between the *Ava*II(155) and the *Hin*dIII(244) sites. We are currently analyzing the origin activity of these fragments on the chromosome by using a λ phage vector.

V 104 BACTERIAL INTERSPERSED MOSAIC ELEMENTS (BIME) ARE PRESENT IN THE GENOME OF *KLEBSIELLA*, Sophie Bachellier, David Perrin, Maurice Hofnung and Eric Gilson, Programmation Moléculaire et Toxicologie Génétique, CNRS URA 1444, Institut Pasteur, 25 rue du docteur Roux, 75015 Paris, FRANCE

Bacterial Interspersed Mosaic Elements constitute a family of highly repeated sequences containing the previously described Palindromic Units (PU), also called Repetitive Extragenic Palindromes (REP). BIME were originally described in *Escherichia coli* and *Salmonella typhimurium*, but were never detected in *Klebsiella*, a closely related enterobacteria. The function of these sequences is still unclear, but evidences for specific interactions of BIME with DNA polymerase I and DNA gyrase suggest that they could play a role in the organization of the bacterial chromosome.

The search of BIME in *Klebsiella* genome is presented; it was made by the determination of the nucleotidic sequence of some intergenic regions, known in *E. coli* and *S. typhimurium* to contain BIME, and then by a computer search using the determined sequences as probes. These sequences were also used in Southern hybridization experiments on several *Klebsiella* genomic DNAs. It has revealed that the genome of *Klebsiella* possess BIME-like sequences. These sequences are different but clearly related to *E. coli* and *S. typhimurium* BIME.

These findings confirm the high species-specificity displayed by BIME. In addition, their conservation across species reinforces the idea that these sequences play a major role in the organization of the bacterial chromosome.

Reference : Bachellier *et al.* Mol. Microbiol. In Press.

V 105 CLONING, SEQUENCING AND EXPRESSION OF DNA GYRASE GENES FROM *STAPHYLOCOCCUS AUREUS*. Peter T Barth and Sarah M V Brockbank, Biotechnology Department, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, U. K.

DNA gyrase is an important target for antibacterial agents such as the 4-quinolones. Resistant strains of clinically important pathogens, such as *Staphylococcus aureus*, are rapidly emerging. We have cloned the *gyrA* and *gyrB* genes of a drug-sensitive clinical isolate of *S. aureus*. Oligonucleotide probes were designed from a published partial sequence (Hopewell *et al*, 1991, J Bact 172: 3481). Restriction analysis of positive clones produced a contiguous map of 8.9 kb containing adjacent *gyrB* and *gyrA* genes. Appropriate restriction fragments were cloned into a T7 promoter vector to express these genes separately. N-terminal sequencing confirmed the identity of the GyrB and GyrA subunits by homology with other known gyrase proteins. Other peptide fragments were also sequenced, matching internal GyrB and RecF sequences.

The nucleotide sequence of a 5.9 kb region has been determined. This consists of part of an upstream *recF* gene, through *gyrB* and *gyrA*, plus about 1 kb unknown downstream sequence. Expression of the gyrase genes appears to be from a promoter in *recF* to a terminator just downstream of *gyrA*. The open reading frames for *gyrB* and *gyrA* predict proteins of 886 and 644 residues respectively. The mature proteins have their initial methionines clipped but GyrB is found mainly with five N-terminal residues removed. The *S. aureus* GyrB has homologies of 57 and 69% with GyrB from *E. coli* and *B. subtilis*. The equivalents are 52 and 65% for GyrA. Both *S aureus* gyrase proteins are structurally closer to the *B. subtilis* than the *E. coli* ones. Residues that have been shown to be important in gyrase structure and function are conserved in all three species.

After completion of our sequence determination, a similar sequence was published (Margerrison *et al*, 1992, J Bact 174: 1596). Our predictions for RecF, GyrB and GyrA differ by a total of 40 residues from theirs. Comparison suggests that they may have made a few short frame-shift errors although a few discrepancies may be due to strain differences. Our predictions are supported by peptide fragment sequence data and match other alignments better.

V 106 PATHWAYS OF TRANSDUCTIONAL RECOMBINATION IN *SALMONELLA TYPHIMURIUM* AND SUPPRESSORS OF *recBC* INSERTIONS, Nicholas R. Benson and John Roth. Department of Biology, University of Utah, Salt Lake City, UT 84112

Recombinational mechanisms are believed to be very similar across diverse species. In order to explore this belief we have begun a study of transductional recombination in *Salmonella typhimurium* with the idea of comparing the results to what is known about recombination in *E. coli*, a species closely related to *S. typhimurium*. The similarity of these species might predict that recombinational mechanisms would be nearly identical. If this were the case the strength of the salmonella genetic system and the versatility of the transducing phage P22 might allow us to answer previously unanswered questions concerning recombination in *E. coli*. Alternatively, differences in recombination between closely related species would be an interesting demonstration of how recombinational mechanisms might diverge. We report that Salmonella contains genes analogous to the *E. coli* genes *sbcB* and *sbcC* (suppressors of recBC insertions). The alleles of *sbcB* that we have isolated differ in their phenotype compared to similar mutations isolated in *E. coli*. The *sbcB* alleles we have isolated in Salmonella are rec^+ and UV^r in contrast to the rec^-, UV^r alleles isolated in *E. coli* (recombination is measured by the ability act as a transductional recipient). We have also isolated "*xon*" like mutations (deletions of *sbcB*). These mutations are rec^- and UV^r. The addition of *sbcC* mutations to these *sbcB* mutations improves the viability of these strains and in the case of the *sbcB* alleles improves thier transductional competence to 16-fold better than wild-type salmonella. Thus, it seems that while *S. typhimurium* has a recombinational architecture very similar to E. coli, the specific details of gene function and mechanism could be different. We present the results of experiments that reveal the structure of the transduced intermediate (homoduplex or heteroduplex) and compare this intermediate to that formed by the recBCD pathway. We also discuss: 1] the nature of recF pathway genes; 2] the existence of an insertion tightly linked to *sbcB* with a phenotype different than *xon* or *sbcB* and 2] present a model for recombination in *S. typhimurium*.

V 107 A NOVEL *ESCHERICHIA COLI* GENE INVOLVED IN BICYCLOMYCIN RESISTANCE. J. Bentley, L.S. Hyatt, K. Ainley, J.H. Parish, R.B. Herbert[1] and G.R. White[2], Department of Biochemistry & Molecular Biology and [1]School of Chemistry, The University, Leeds, UK. [2]SmithKline Beecham Pharmaceuticals, Cold Harbour Rd, Harlow, Essex, UK.

Bicyclomycin (Bc) was discovered in the culture broth of *Streptomyces sapporonensis*. It has weak antibiotic activity against Gram-negative organisms including *Escherichia coli* and species of *Klebsiella, Salmonella* and *Shigella*. The structure of Bc is based on a diketo-piperazine ring, no other antibiotic has this unusual structure, also organisms that show resistance to all major classes of antibiotics do not show resistance to Bc. These facts imply a novel mode of action. Structure-activity studies with Bc have not led to a clear understanding of its mechanism of action. In the course of our investigations into the mechanism of bacterial resistance to Bc we have discovered a previously unreported *E. coli* gene that confers resistance to Bc. Our research started with a spontaneous Bc resistant mutant of *E. coli* C600. A recombinant plasmid derived from this mutant confers Bc resistance. The 2 kb insert DNA was sequenced and analysis revealed an open reading frame (ORF) of 1131 nucleotides, disruption of which results in the loss of Bc resistance. Studies using the minicell expression system show a polypeptide of 31 kDa is produced from this cloned region and by using the Kohara gene mapping membrane we have shown that the ORF maps at 47.1 min. on the *E. coli* chromosome. Sequence comparisons show the ORF has 26.5% sequence homology with the chloramphenicol and tetracycline resistance proteins in a number of species.

We have also cloned the equivalent wild-type gene from a Bc sensitive *E. coli* DH5, this gene is currently being sequenced. It is hoped that comparison of the wild-type and mutant sequences will reveal the mutation that causes the Bc resistance phenotype.

V 108 **RecA-INDEPENDENT PLASMIDIC RECOMBINATION IN *E. coli*.**

Xin Bi, Mark Drolet and Leroy F. Liu, Department of Biological Chemistry, Johns Hopkins Medical School, Baltimore, MD 21205

Recombination between directly repeated sequences in plasmids was studied using pBR322 derivatives with certain parts of the *tetA* gene duplicated in tandem. Recombination between the repeats regenerates the functional *tetA* gene. Recombination between these tandem duplications shows virtually no RecA dependence. However, increasing the size of the repeated sequence gradually restores RecA dependence. In addition, inserting a sizable DNA sequence in the middle of the tandem duplications also confers RecA dependence. Detailed analysis of the RecA-independent recombination has indicated that (1). Different from RecA-dependent recombination, RecA-independent recombination is adversely affected by the size of the DNA sequence separating the homologous sequences. (2). RecA -independent recombination occurs readily between tandem duplications as short as 14 bp long. (3). RecA-independent recombination between direct repeats does not occur by a mechanism involving slippage.

V 109 *E. COLI* HU PROTEIN IS THE FUNCTIONAL ANALOG OF MAMMALIAN HMG1 PROTEIN, Marco E. Bianchi, Monica Beltrame and Andrea Pontiggia, Dip. di Genetica e Biologia dei Microrganismi, Universitá di Milano, via Celoria 26, 20133 Milano, and DIBIT, H San Raffaele, via Olgettina 60, 20132 Milano, Italy

HMG boxes are DNA binding domains present in eukaryotic chromatin proteins, general transcription factors for nucleolar and mitochondrial RNA polymerases, and gene- and tissue-specific transcriptional regulators. We and others have shown that they interact specifically with sharply bent DNA, or alternatively they recognize specific sequences in linear DNA and produce a sharp bend (see Lilley, 1992, Nature 357:282-283, for a short review).

Up to now, no HMG boxes have been found in bacterial proteins. We looked for similar proteins in *Escherichia coli* by screening for the ability to interact with four-way junctions — DNA molecules with the shape of an X, which are selectively bound by HMG boxes. We purified and microsequenced the main four-way junction binding protein of *E. coli*, and found it to be the well known "histone-like" HU protein. HU protein recognizes with high affinity one of the angles present in the junction. Other DNA structures characterized by sharp bends or kinks, like bulged duplex DNAs containing unpaired bases, are also bound. HU protein appears to inhibit cruciform extrusion from supercoiled inverted repeat (palindromic) DNA, either by constraining supercoiling or by trapping a metastable inter-conversion intermediate. All these properties are analogous to the properties of HMG1, a mammalian protein which is by weight a major component of chromatin. We suggest that HU, despite the lack of sequence similarity, is a prokaryotic HMG1-like protein.

V 110 PARTITIONING FUNCTIONS OF PLASMID F AND DNA SUPERCOILING. Donald Biek and Joquetta Strings. Department of Microbiology & Immunology, University of Kentucky Medical Center, Lexington, KY 40536.

The partitioning region of the low copy number F plasmid is required for effective segregation of plasmid molecules in *E. coli*. Mutations in the *sop* (stability of plasmid) genes result in plasmid loss during growth owing to a defect in segregation. The mini-F partitioning functions consist of two protein coding regions, *sopA* and *sopB*, and a cis-acting region *sopC* which may function as a centromere. The host-encoded genes which participate in plasmid partitioning have not been identified. The SopB protein has been shown to bind a series of twelve tandem repeats that comprise *sopC*.

We have found that the binding of SopB to *sopC* is reflected in a change in DNA super-helicity in vivo, perhaps reflecting the wrapping of the *sopC* DNA about the SopB protein thus restraining positive superhelical turns. Mutations that inactivate SopB or its sites of binding (*sopC*) lead to increased negative superhelicity of the plasmid because of the supercoiling activity of DNA gyrase in vivo. We have performed experiments to analyze the effects of the *sop* system on supercoiling of mini-F to try to elucidate the role of supercoiling in plasmid partitioning.

V 111 **ROLE OF *dif* CORE SEQUENCE ARRANGEMENT IN XERC/D-MEDIATED SITE-SPECIFIC RECOMBINATION.**

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The terminus of DNA replication in the chromosome of *E.coli* K12 is a 400kb region that is not essential for cell growth or viability. A site-specific recombination locus, *dif*, within this region has been implicated in normal chromosome segregation possibly through resolution of dimeric chromosomes, (formed by homologous recombination), to segregatable monomeric chromosomes. Although this locus is dispensable, its absence produces cells with a filamentous phenotype and aberrant nucleoids that appear unable to undergo partitioning during cell division. The proteins required for functional recombination at this locus are XerC and XerD; both are related members of the integrase family of recombinases. The 32bp minimal *dif* DNA sequence consists of single left (XerC) and right (XerD) binding sites which each contain 5bp of dyad symmetry, but differ over the rest of their 12bp sequences. The recombinase binding sites flank an asymmetric 6bp overlap region. Construction of a *dif* site with a symmetric overlap has shown that asymmetry of this region is not essential for ensuring correct site alignment. The function of each binding site is not interchangeable, again demonstrating that both left and right half sites are required for correct site alignment. The interactions between the recombinase proteins and the *dif* locus were investigated at the molecular level using both high and low resolution footprinting techniques. Interference footprinting has shown that both proteins interact with specific residues within the major groove of the regions of dyad symmetry.

V 112 THE *E. coli dnaX* GENE PRODUCES BOTH THE τ AND γ SUBUNITS OF DNA POLYMERASE III HOLOENZYME, BUT ONLY τ IS ESSENTIAL FOR GROWTH,
Alexandra Blinkova, Christine Hervas and James R. Walker, Department of Microbiology, The University of Texas, Austin, TX 78712-1095

DNA polymerase III holoenzyme consists of a core (α,ϵ,θ) plus seven accessory factors ($\beta,\tau,\gamma,\delta,\delta',\chi,\psi$), which are necessary for efficient recycling during lagging strand synthesis and for the high processivity characteristic of holoenzyme. In the simplest system for highly processive replication of a primed single-strand template, $\tau\delta$ (or $\gamma\delta$ or $\gamma\delta\delta'\chi\psi$) transfers β to the primed template. β acts as a sliding clamp on the duplex to tether $\alpha\epsilon$ which catalyze polymerization [O'Donnell, M. E., J. Biol. Chem. 262 (1987) 16565-16588; Stukenberg et al., J. Biol. Chem. 266 (1991) 11328-11334]. Two of the accessory factors, τ and γ, are products of one gene, *dnaX*. τ is the full length translational product of the 643 codon *dnaX* messenger. The shorter γ is initiated at the same start codon as τ but its translation is terminated within the reading frame by a programmed ribosomal frameshift. About half the ribosomes shift back one nucleotide over codons 428-430 and, after incorporating one unique amino acid, encounter a stop codon in the new frame. The τ:γ ratio in whole cell extract and in holoenzyme is about 1:1.

The roles of τ and γ in vivo have been tested by replacing the chromosomal *dnaX* gene with mutant alleles which encode only τ or only γ. Strains which produce only τ can be constructed but strains producing only γ could not, indicating that τ is essential but γ is not. Perhaps the τ subunit can substitute for the activity or activities normally performed by γ.

V 113 **BINDING OF IHF TO SMALL REPETITIVE ELEMENTS OF THE E.COLI GENOME,**
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The Integration Host Factor (IHF) of *E.coli* is a small histone-like protein which bends the DNA sharply upon binding to its specific sites. Most *ihf* sites found so far in the *E.coli* genome were located in promoter regions. We have recently observed that *ihf* sites can also be found at the 3' end of a large number of *E.coli* genes. A particularly interesting class of such sites lies within "REP" elements, which are small, abundant, highly conserved repetitive sequences usually found at the 3' end of genes. We are now characterizing the functional significance of this interaction.

V 114 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE pT181 REPLICATION ENHANCER,
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cmp is a DNA replication enhancer found in the plasmid pT181 of *Staphylococcus aureus*. The presence of *cmp* on the plasmid increases the ability of a linked replication origin to utilize the plasmid-encoded initiator protein, Rep C.
The *cmp* sequence is approximately 100 bp in length and contains a loosely repeated sequence motif as well as intrinsically bent DNA. The initial goal of this research project was to examine the DNA requirements for full *cmp* function. To this end, a series of base pair changes and deletions were carried out within the minimal *cmp* sequence, and the activity of the mutated *cmp* sequence was observed. The first set of experiments involved substitution of each repeated sequence motif, one at a time. Oligonucleotide-directed mutagenesis without phenotypic selection was used. These sites were mutagenized in such a way as to be recognized by a unique restriction site. Results showed that these substitutions had no visible effect on *cmp* activity. In contrast, preliminary experiments showed a correlation between insertions or deletions within the *cmp* minimal sequence and loss of *cmp* activity. This observation suggests that the spacing between structure motifs may be critical for wild-type behavior.
It was previously shown that deletion of either end of the *cmp* minimal sequence has little effect on *cmp* activity, while deletion of both ends eliminates *cmp* function. We are in the process of testing the effect on *cmp* activity of combining the deletion of one end with any of the internal substitutions. These experiments might provide further insight about the effect of *cmp* ends on function. We are also testing the effect of multiple internal substitutions on *cmp* activity.
A question was raised as to whether supercoiling was affected in the obtained mutants. DNA electrophoresis of *cmp* mutants on agarose gels containing chloroquine in order to separate topoisomers revealed that while mutants with substitutions displayed no change in supercoiling, mutants with insertions or deletions in *cmp* had significantly reduced levels of supercoiling as well as decreased *cmp* activity. These results support a correlation between *cmp* and supercoiling.

V 115 FtsZ FUNCTIONS APPROACHED BY AN ANALYSIS OF DELETIONS AND POINT MUTATIONS.
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The essential division gene ftsZ encodes a GTP-binding protein capable of forming a ring-like structure bound to the inner membrane at the site of cell division. Interactions of FtsZ with proteins FtsA and MinC have been inferred from the analysis of mutations and/or suppression effects. Our results have suggested that FtsZ inhibits nucleoid partitioning prior to the stage of septum formation (1). To isolate ftsZ mutations affecting this inhibition, and to provide a wider basis for the study of the interactions of FtsZ with other proteins, we have selected deletions and point mutations in ftsZ.
Truncated FtsZ proteins produced from a plasmid were tested for their ability to compete with the chromosomal protein. On this basis, four regions: 1-70, 70-210, 210-310 and 310-381 aa could be distinguished. Region 70-210 appears to contain most of the determinants involved in FtsZ-mediated division inhibition.
To isolate point mutations, we devised a system in which the chromosomal copy may be turned off by the antisense inhibitory action of DicF-RNA, while a plasmid copy may be induced and tested as replacement gene. Among the mutants isolated so far, one, ftsZ29V, leads to viable cells with "curved" division sites. This phenotype, reminiscent to that caused by a 6-aa insertion recently described (2), provides independent genetic evidence in favor of a cytoskeletal role involving the association of many FtsZ monomers at the site of division.

(1). Tétart, F., Albigot, R., Conter, A., Mulder, E. and Bouché, J.P. (1992). Mol. Microbiol. 6:621-627.
(2). Bi, E. and Lutkenhaus, J. (1992). J. Bacteriol. 174:5414-5423.

V 116 **Formation and Stability of Complexes Involving ssDNA, DnaB and DnaC Proteins of *E. coli*.**

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The hexameric DnaB helicase is the central actor at an *E. coli* replication fork. It unwinds the duplex DNA and guides priming of the discontinuous strand. Delivery of DnaB to single stranded DNA (ssDNA) exhibits a lag of 50-100 seconds. This lag is similar for an 8 base oligonucleotide and for viral circles. Efficient delivery of a DnaB hexamer requires the formation of an ATP-stabilized complex with six subunits of DnaC protein. This complex can interact rapidly with ssDNA, whereupon the ATP is hydrolyzed and the DnaC protein released into solution. When ATP hydrolysis is blocked, intermediates in this process can be isolated by gel filtration. These intermediates contain both DnaB and DnaC (in approximately equimolar ratios) bound tightly to ssDNA. The intermediates are fully capable of completing the delivery of DnaB so it can subsequently direct priming of the template for replication. Once delivered to DNA, DnaB is extraordinarily stable, remaining bound for 30 min or more without dissociating. In vivo results suggest that a single hexamer of DnaB usually remains at a replication fork without dissociating, all the way from the origin to the terminus. DnaB can be abstracted from a complex with ssDNA by certain mutant DnaC proteins which retain the ability to interact with DnaB. Abstraction of DnaB presumably involves a ssDNA-DnaB-DnaC intermediate.

V 117 CONTROL OF P1 *oriR* AND HOST *oriC* REPLICATION ORIGINS BY DNA ADENINE METHYLATION. Therese G. Brendler, Ann L. Abeles, and Stuart J. Austin. ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702

The P1 prophage is a stringently controlled autonomous plasmid. Adenine methylation of the multiple GATC sites clustered within the origin core is required for normal function. This feature is shared by the *oriC* system that replicates the *E. coli* host chromosome. Wild-type P1 miniplasmids are unable to replicate in Dam$^-$ strains, and Dam methylation is required for origin function in vitro. In contrast, *oriC* functions in Dam$^-$ strains, and origin control involves sequestration of newly replicated (hemimethylated) origin DNA to membrane sites. We show that this difference is superficial. High-copy-number variants of mini-P1 do replicate in Dam$^-$ strains, and appear to respond to hemimethylation control in the same way as *oriC*. Moreover, in the complete absence of origin methylation, both origins function very poorly. Thus both *oriC* and *oriP1* appear to respond to two levels of control by methylation: one involving a methylation requirement for efficient origin firing, and the second involving negative control by sequestration of hemimethylated products.

Mutational analysis of the P1 origin core shows that function depends not only on methylation of the GATC sites but also on the integrity of the heptamer repeats in which they are nested. We hypothesized that a host protein might recognize the heptamer repeats only in their *dam* methylated state. Gel-retardation studies show that crude extracts of *E. coli* contain a protein which binds to the origin core region only when it is *dam* methylated. This factor has been purified about 2000-fold and appears to have a native molecular weight of 100,000 Da. It is not DnaA, P1 RepA, Dam methylase or MutH protein. Preliminary results suggest that the protein may also recognize *oriC* in a methylation-dependent manner. Thus the protein may be a common component involved in methylation regulation of P1 and host chromosome replication.

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V 118 PLASMID DNA SUPERCOILING AFTER AN UPSHIFT IN TEMPERATURE AND CELL EXPOSURE TO ETHANOL AND CADMIUM CHLORIDE IN E. COLI. Rafael Camacho-Carranza, Jorge Membrillo-Hernández, Alejandra Núñez-de la Mora and M. Carmen Gómez-Eichelmann, Department of Molecular Biology, Instituto de Investigaciones Biomédicas, UNAM, México City 04510. MEXICO

The effect of heat shock upon the synthesis and/or function of DNA topoisomerases is unclear. The level of plasmid supercoiling increases when the temperature is raised abruptly from 17°C to 37°C. The upshift of temperature is presumed to decrease supercoiling and as a consequence, to favor gyrase activity (Goldstein and Drlica, PNAS 81:4046, 1984). In this context, we decided to analyze topoisomerase activities during the heat-shock (HS) cellular response. The selected temperature upshift was 30°C to 47°C, since cells growing in Luria broth are still viable at this temperature. During the HS response an increase in the level of plasmid supercoiling (IPS) was observed. Novobiocin, an inhibitor of gyrase, blocked the heat-induced IPS. In contrast, rifampicin did not. Chloramphenicol added simultaneously to the temperature upshift, inhibited the IPS. This was not so when the antibiotic was added five min. after the upshift. Other inducers of HS proteins, such as ethanol and cadmium chloride, were unable to induce the IPS. In strain K165 rpoH (am) SupC (ts), although the normal heat-shock response is inhibited, the IPS was present. However, under our experimental conditions proteins GroEL and GroES were induced in this strain. Our results show that the heat-induced increase in IPS is catalyzed by gyrase, requires the synthesis of proteins during the first minutes of the HS and is probably independent of sigma-32 directed transcription. These results suggest that gyrase or gyrase together with some heat-shock proteins are required for the heat-induced IPS in a mechanisms that links the HS response with topoisomerase activities during temperature stress.

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V 119 REQUIREMENT OF ACTIVE DNA POLYMERASE I FOR SUPPRESSION OF RecA DEPENDENCE OF cSDR IN *E. coli rnhA* mutants, Yang Cao, Robert R. Rowland and Tokio Kogoma, Departments of Cell Biology and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM 87131

E. coli rnhA mutants (RNase HI-deficient) initiate DNA replication at several origins other than *oriC*. This type of replication, termed constitutive stable DNA replication (cSDR), can sustain chromosome replication and renders the *oriC* system dispensable for cell viability; *rnhA dnaA*::Tn*10* or *rnhA oriC-del* mutants are viable. cSDR requires active RecA protein and thus *rnhA dnaA*::Tn*10 recA*(Ts) mutants are temperature sensitive (Ts). We previously reported that *lexA*(Def) mutations suppress this RecA requirement and render *rnhA dnaA*::Tn*10 recA*(Ts) mutants temperature resistant. We speculated that derepression of a gene under the control of the LexA repressor leads to activation of a process (Rip for *recA*$^+$-independent process) which allows cSDR to operate in the absence of active RecA protein. In order to analyze the Rip pathway, we have isolated a Ts mutant after random mutagenesis of *rnhA dnaA*::Tn*10 recA*(Ts) *lexA*(Def) cells with a miniTn*10*. The insertion mutation has been cloned and sequenced. The miniTn*10* insertion occurs at the end of the *polA* gene. The effects on DNA polymerase I are twofold; the change of an amino acid residue at the 871 position and the truncation of the polypeptide by 57 amino acid residues at the C terminus. The mutation is expected to abolish the polymerizing activity but not the 3′ -> 5′ exonuclease activity. The *polA*::miniTn*10* mutants are as sensitive to UV and MMS as *polA1* mutants which lack both activities. Since the *lexA*(Def) mutation can also suppress the lethality of *recA* and *polA* double mutants, it appears that the *polA*::miniTn*10* specifically affects the Rip pathway. In addition, we have found that whereas the *lexA*(Def)::Tn*5* mutation can be introduced into the *polA*::miniTn*10* mutant, it has not been possible to construct a *polA1 lexA*(Def)::Tn*5* double mutant.

V 120 **VARIATION IN PLASMID DNA TOPOLOGY AMONG MESOPHILIC AND THERMOPHILIC EUBACTERIA AND ARCHAEBACTERIA.**

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Prokaryotes are divided into two separate domains: eubacteria and archaebacteria. Archaebacteria have several distinct features, and some of these are a consequence of their growth in extreme habitats, such as high temperature for thermophiles and high salt concentration for halophiles. There is a special interest in the study of archaebacterial chromatin in order to know how chromosome structure is maintained under such extreme conditions, such as 100°C for hyperthermophiles or 3 to 4M KCl for halophiles. Histone-like proteins or other DNA-binding proteins might play important roles. Specific DNA-topoisomerases may exist to change local constraints on DNA in order to permit biological phenomenoms such as replication or transcription to occur in extreme conditions.

In eukaryotes and mesophilic eubacteria, all native DNA molecules previously purified exhibit a negative supercoiling. In these microorganisms, the DNA-topoisomerases either relax or introduce negative superturns into DNA. On the contrary, in hyperthermophilic eu- and archaebacteria, the major DNA-topoisomerase activity is due to reverse gyrase (1). This is an ATP-dependent type I DNA-topoisomerase wich introduce positive superturns into DNA. This finding raised the possibility that DNA is positively supercoiled in order to be protected against thermal denaturation.

We have purified plasmidic DNAs from mesophilic and thermophilic eu- and archaebacteria, and determined what might be their superhelical densities at the physiological growth temperature of their host strain. The equation which relates the change of the rotation angle of the DNA double helix with temperature was validated up to 95°C (2).

Our results suggest that the presence of a strong reverse gyrase activity in hyperthermophilic microorganisms is correlated with a plasmid DNA in a relaxed state at physiological temperatures. This is the case of the pGT5 plasmid from the new hyperthermophilic archaebacterium GE5 (2). On the contrary, halophilic plasmids seem to be more negatively supercoiled than mesophilic eubacterial plasmids.

We will discuss these results and propose some explanations for so different DNA topology in these microorganisms.

1- Bouthier de la Tour *et al.* 1991. *J. Bact.* 173: 3921-3923. / 2- Charbonnier *et al.* 1992. *J. Bact.* 174: 6103-6108.

V 121 THE CHROMOSOMAL DNA OF *STREPTOMYCES LIVIDANS* 66 APPEARS TO BE LINEAR. Carton W. Chen[1], Yi-Shing Lin[2], Helen M. Kieser[3], and David A. Hopwood[3]; [1]Institutes of Genetics and [2]Biochemistry, National Yang-Ming Medical College, Taipei, Taiwan; [3]John Innes Institute, Norwich, UK.

It has been commonly accepted that most bacterial chromosomes consist of covalently closed circular DNA molecules. The only bacterium so far shown to possess linear chromosomal DNA is the spirochete *Borrelia* (946 kb). Many species of the filamentous bacteria streptomycetes, including the well-studied *Streptomyces coelicolor* A3(2) and the closely related *Streptomyces lividans* 66, also exhibit circular genetic maps. A circular restriction map of *S. coelicolor* A3(2) for two rare cutting enzymes has recently been constructed (Kieser *et al.*, J. Bacteriol. 174:5496, 1992). However, here we show that the 8-mb *S. lividans* chromosomal DNA is apparently a linear molecule. The termini are located in the 'silent region' that contained few genetic markers and exhibited high degrees of structural instability. The termini appear to contain covalently bound proteins, which might mediate circularization of the DNA as in the cases of phage ϕ29 and adenovirus. The *right* terminus of the *S. lividans* chromosome and the *right* terminus of a linear plasmid SLP2 of *S. lividans* share a 16-kb homologous sequence. A transposon Tn*4811* is located at the junction between the homologous sequence and the remaining of the chromosome. We postulate that the linearity of the *S. lividans* chromosome arose by interaction with the linear plasmid SLP2. Alternatively, SLP2 acquired the terminus from the chromosome.

V 122 SITE-SPECIFIC RECOMBINATION IN A GENETIC AMPLIFICATION PROCESS IN *M. HYORHINIS*.

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Genomic variation may have particular importance in mechanisms of mycoplasma pathogenesis and evolution. Examples of high frequency genomic variation have been reported for *M. hyorhinis*, including the identification of multiple copies of an insertion sequence (IS3)-like element and variable surface lipoprotein genes that undergo recombinations leading to phase and size variations. A novel amplifiable DNA region (RS-2) has recently been isolated from *M. hyorhinis* strain GDL-1. The copy number of RS-2 has been shown to vary within an *M. hyorhinis* cell population. Sequence data identified a specific 1348 bp DNA region which varies from 2-4 copies in different clonal populations and is flanked by 20 bp directly repeating "cassettes", suggesting the involvement of site-specific recombination in the initial RS-2 duplication. All "cassette" sequences are characterized by two copies of the hexanucleotide sequence 5'-TTCTTG-3' separated by three nucleotides, except in the last copy, where an extra six nucleotides are present. The 20 bp cassettes from all clonal isolates have identical features, with all the downstream copies having 20 nucleotides and the upstream cassette having 26 nucleotides. If these sequences represent the site of recombination in the amplification/reduction mechanism controlling RS-2 copy number, these sequence features suggest that the hexanucleotide repeat unit may serve to bind recombination factors or serve as the crossover point for such an event. Three ORFs were found in this sequence, including a large ORF (ORF3) containing multiple repeated domains of 456 amino acids in length. These domains directly result from RS-2 duplication. Further examination of the encoded polypeptide sequences showed that these domains have considerable structural similarity to cysteine-rich motifs characteristic of zinc-finger proteins and metal-binding proteins. The multiple types of characteristic cysteine-rich motifs are repeatedly present within the amplified domain, and thus, amplification of the RS-2 DNA sequences result in increased copy number of these motifs. The data suggest that ORF3 is transcribed and translated in *M. hyorhinis*. Initial data indicated that the copy number of RS-2 in a cloned cell population is correlated to its ability to infect a murine T-lymphoblastoid cell line. Trypsin sensitivity and flow microfluorimetry experiments showed that this protein is not on the cell surface of *M. hyorhinis* and its intracellular functions are discussed.

V 123 DETECTION OF A NOVEL DNA SWITCH IN *BORDETELLA BRONCHISEPTICA* USING Tn*phoA*.

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In *B. bronchiseptica*, the Tn*phoA* mutagenesis system allowed random mutagenesis of the genome and detected secreted gene products were at a frequency of 3% of all genomic insertions. One Tn*phoA* mutant has an insertion in a metastable piece of DNA, the orientation of which determines whether a surface protein is expressed or not. The expression of this protein is also growth phase dependent - expression is five fold greater in stationary phase than in the early exponential phase of growth. In other bacteria the role of histonelike proteins in DNA inversion systems has been well documented. A search was therefore carried out using oligonucleotide probes specific for the most conserved parts of the coding regions of the HU family members from *Escherichia coli* and *Salmonella typhimurium*, and the genes coding for the *Bordetella* counterparts were detected. Investigations are currently underway to establish the role (if any) of HU and other histonelike proteins in this DNA switch.

V 124 DNA SUPERCOILING DURING TRANSCRIPTION, Marc Drolet, Xin Bi and Leroy F. Liu, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

In vivo studies have shown that transcription can cause extreme heterogeneity of the plasmid DNA linking number when one of two major DNA topoisomerases is inactivated. In the present study, we have investigated the effects of transcription on the plasmid DNA linking number *in vitro* in the presence of different ratios of bacterial DNA topoisomerases I (ω) and II (DNA gyrase). We show that, as is the case *in vivo*, the linking number of pBR322 becomes much more heterogeneous during transcription. When topoisomerase I is in sufficient excess over topoisomerase II, pBR322 DNA becomes positively supercoiled, while in the reverse situation, in excess of topoisomerase II, pBR322 DNA becomes hyper-negatively supercoiled. The extreme heterogeneity in the linking number of the plasmid DNA is shown to be linked to transcription elongation. The similarity between *in vitro* and *in vivo* results suggests that transcription is a significant factor in supercoiling dynamics in bacterial cells. The hyper-negative supercoiling of pBR322 DNA was further studied *in vitro*. In contrast to the formation of hyper-negatively supercoiled pBR322 DNA in *topA* mutants, the generation of hyper-negatively supercoiled pBR322 DNA topoisomers *in vitro* is not dependent on transcription of the *tetA* gene. In addition, the formation of hyper-negatively supercoiled pBR322 DNA topoisomers involves R-loops. Our results suggest a possible mechanism for hyper-negative supercoiling of plasmid DNAs *in vivo*.

V 125 TIMING OF REPLICATION OF LOW-COPY-NUMBER PLASMIDS AND MINICHROMOSOMES DURING THE *ESCHERICHIA COLI* CELL CYCLE

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Initiation of replication of the *Escherichia coli* chromosome is strictly *timed* during the cell cycle. Initiation only occurs when the ratio between the cell mass and the origins present in the cell reaches a particular value known as the *initiation mass*. Correct timing of replication is necessary to avoid the production of DNA-less cells, and to prevent unnecessary DNA synthesis.

We are interested in the timing of replication of various low-copy-number plasmids, such as R1, P1 and F. These plasmids are maintained at a copy number of about 1-2 per chromosome equivalent for F and P1, while R1 has a slightly higher copy number (3-4). The timing of R1 replication is random, *i. e.* replication can be initiated at any time during the cell cycle. In *intR1* strains, where part of the origin of replication of the *E. coli* chromosome has been deleted and the R1 basic replicon is inserted in its place, the timing of chromosome replication also becomes random.

We will analyze the timing of F and P1 replication since conflicting results have been obtained as to their timing. Therefore, we are constructing *intP1* and *intF* strains similar to the *intR1* strains. That is, an essential part of *oriC* is deleted and the basic replicon of the F or P1 plasmid is inserted in its place, such that chromosome replication is controlled by either F or P1.

The timing of replication can be analyzed by density shift experiments. The strains are first grown in medium supplemented with heavy isotopes for many generations to obtain chromosome DNA of high density. The cells are pulse-labelled with ^{3}H-methyl-thymidine and then shifted to medium containing lighter isotopes, and samples are collected. The samples are centrifuged on CsCl-gradients to separate replicated (heavy-light, HL) from non-replicated (heavy-heavy, HH) DNA. By analyzing the kinetics with which the ^{3}H DNA starts to appear in the HL band, it is possible to draw conclusions about the timing of replication.

A minichromosome, *i. e.* a plasmid carrying the minimal origin of replication of the chromosome, is replicated at the same time as the chromosome in a wild-type strain. Thus, an interesting question is what happens to the timing of replication of a minichromosome if it is introduced into an *intR1* strain. In addition we would like to study timing of replication in the absence of the DnaA protein, which is an important regulatory factor for initiation of replication in many replicons. Taken together, the results may give information about the cell cycle-clock for timing of initiation of replication.

V 126 THE RecB PROTEIN OF *E. coli* COUPLES ATP HYDROLYSIS TO 3'TO 5' MOVEMENT ALONG SINGLE-STRANDED DNA, Peter T. Emmerson, Paul E. Boehmer, David Hickleton and Robert Phillips, Department of Biochemistry, Medical School, The University, Newcastle upon Tyne, UK.

The *E. coli* RecBCD, RecB, RecC and RecD proteins have been purified to homogeneity from strains harbouring overexpression plasmids. Mixing of the RecB, RecC and RecD subunits results in a reconstituted RecBCD protein with all of the known activities of the native RecBCD holoenzyme. We have tried to dissect the many different activities of the RecBCD enzyme by studying the properties of the individual subunits both alone and in the various combinations. Although both RecB and RecD proteins have consensus "G-K-T" ATP binding sites, and both have been shown to bind ATP, only RecB protein on its own has DNA dependent ATPase activity. This ATPase activity is coupled to the ability of the RecB protein to track along single stranded DNA and unwind any annealed oligonucleotides as it does so. Studies with synthetic short oligonucleotides with and without non-complementary "tails" at either end, annealed to ssDNA M13 circles, suggest that the recB protein tracks along ssDNA in the 3' to 5' direction. This conclusion is supported by studies with synthetic DNA substrates in which 20-mers are annealed at either the 5' end or the 3' end of a 60-mer. In this case, only the 20-mer at the 5' end is removed. RecD protein does not unwind any of these substrates under the same conditions, but it is possible that this protein behaves differently when part of the RecBCD complex. As expected, RecC protein, which does not hydrolyse ATP, shows no helicase activity.

V 127 CHROMOSOMAL REARRANGEMENTS STIMULATED BY SPECIFIC TN*10*-CATALYZED DOUBLE STRAND BREAKS, Timothy P. Galitski and John R. Roth, Department of Biology, University of Utah, Salt Lake City, UT 84112

We have set up a system for the observation of chromosomal rearrangements stimulated by the excision of a defective Tn*10*-derived element, Tn*10*d-tet. Plasmids provide inducible expression of mutant Tn*10* transposase that catalyzes excision, but not transposition, of Tn*10*d-tet. This allows the regulated introduction of a double strand break to a single defined site in the bacterial chromosome. Previously, we have described the use of large (>1 minute) chromosomal duplications whose presence is indicated by a Mud(Lac^{+}) element at the duplication join point. By scoring loss of the Lac^{+} phenotype, one can assay recombination between large direct repeats. We can stimulate these events by inducing a double strand break in one copy of the tandem repeats. This assay permits us to observe the pathways and classes of events that are stimulated by a structurally and positionally predetermined type of initiating lesion in the bacterial chromosome.

V 128 DNAA PROTEIN: EFFECTS OF ppGpp ON *dnaA* GENE EXPRESSION, EFFECTS OF METHYLATION ON DNAA BINDING TO DNA, Inés Gil, Amy L. Svitil, R. Allyn Forsyth, Victoria G. Newman, Aaron V. Ponstler, and Judith W. Zyskind, Department of Biology, San Diego State University, San Diego, CA 92182

DnaA protein is required for initiation of DNA replication from *oriC*, the chromosomal origin of replication. The concentration of DnaA in the cell appears to be a critical determinant of when in the cell cycle initiation occurs. A detailed understanding of the transcriptional regulation of the *dnaA* gene will be crucial for delineating how a critical concentration of DnaA protein is achieved in order for the initiation event to occur. We examined the effects on *dnaA* transcriptional activity after increasing the intracellular concentration of ppGpp by overproducing RelA protein. To increase the synthesis of ppGpp, a shortened *relA* gene was placed under control of the *tac* promoter so that expression could be induced with IPTG. The shortened *relA* gene encodes a truncated RelA protein that is not bound to the ribosome and synthesizes ppGpp independent of amino acid starvation. The two promoters of the *dnaA* gene were affected differently by ppGpp; *dnaAp1* expression was unchanged while *dnaAp2* expression was almost eliminated after 20 minutes of exposure to IPTG. A purified transcription system for the *dnaA* promoters has been developed to study the mechanism of this transcriptional control. Preliminary results with this purified system indicate that the transcription from *dnaA*P2 is repressed by ppGpp and *dnaA*P1 transcription is unaffected, in agreement with the in vivo results. We intend to use this system to determine the stage in transcription from the *dnaA*P2 promoter affected by ppGpp and to determine the combined effects of DnaA binding and methylation on the activities of the two *dnaA* promoters (see below).

We have examined the binding of DnaA to its promoter and find that, of the three sites that bind to DnaA, two sites lose their affinity for DnaA after methylation of GATC sites contained within them. Furthermore, DnaA protein binds with very different kinetics to an unmethylated *dnaA* promoter sequence containing all three sites as compared to a methylated *dnaA* promoter sequence. The methylated *dnaA* promoter sequence binds with first order kinetics and the unmethylated with second order kinetics. The two binding sites that are sensitive to methylation appear to inhibit the binding activity of the third site when all three sites are active, which is the case for the unmethylated *dnaA* promoter sequence. Extrapolation of these binding studies suggests a cascade mechanism for binding to *oriC*, a sequence with 4 DnaA binding sites.

V 129 EXTRAGENIC SUPPRESSORS OF A *dnaX*(Ts) POLYMERIZATION MUTANT LOCATED IN THE INITIATION GENE *dnaA*. Edwin Gines-Candelaria, Alexandra Blinkova and James R. Walker, Department of Microbiology, The University of Texas, Austin, TX 78712-1095

To discover relationships between *E. coli* DNA replication factors, extragenic suppressor mutations were sought among temperature-resistant revertants of a temperature-sensitive (Ts) *dnaX* mutant. The rationale was that a change in a second replication factor which normally interacts with the Ts mutant protein could result in suppression of the Ts defect but sometimes would create a Cs defect in the second factor. To be able to study the suppressor mutations directly, only those suppressor mutants which concomitantly became cold-sensitive (Cs) were chosen for further study.

dnaX encodes both the τ and γ subunits of DNA polymerase III holoenzyme. The 643-residue τ is the full length product of the reading frame; the shorter γ is formed by a programmed ribosomal -1 frameshift over codons 428-430 followed by a stop codon in the new frame. Mutation 2016(Ts) converted codon 118 from glycine to aspartate and altered both τ and γ.

Of about 5,000 temperature-resistant revertants of a *dnaX*2016(Ts) mutant, about 30 were Cs. A series of conjugation, transduction, and marker rescue mapping experiments and complementation tests with a *dnaA*$^+$ plasmid indicated that about 10% of the Cs suppressor mutations were located in the initiation gene *dnaA*. Three mutant alleles, designated *dnaA*(Cs,Sx), were either cloned directly from the mutant chromosome or PCR amplified and cloned and sequenced. All three mutations were single base changes within the *dnaA* reading frame. One mutation changed codon 213 from alanine to aspartate. The other two were in the C terminal region of the 467 residue DnaA protein; one altered codon 432 from arginine to leucine and the third changed codon 435 from threonine to lysine. These results demonstrate that alteration of the DnaA protein can suppressor a mutation in the *dnaX* gene.

V 130 SEQUENCE-DEPENDENCE OF DNA BENDING INDUCED BY FIS INTERACTIONS WITH RECOMBINATIONAL ENHANCER DNA-BINDING SITES, Anna C. Glasgow*, Reid C. Johnson, and Melvin I. Simon, *Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30322

The active recombinational enhancer in Hin-mediated site-specific DNA inversion has Fis protein bound to two dissimilar sequences, the proximal and distal domains, within the enhancer. Fis, which binds DNA as a dimer, has a nearly 5-fold higher affinity for the distal domain than for the proximal domain. Characterization of Fis-enhancer DNA complexes revealed that Fis can induce directed DNA bending. We have used circular permutation assays to determine the location of sequence-directed and Fis-induced DNA bending within the enhancer complex. In addition, we have estimated the degree of bending induced by Fis using gel electrophoresis techniques. These analyses show that the sequence of the Fis binding sites determines the degree of the directed DNA bend, i.e. Fis binding to the high affinity distal domain induces a greater degree of bending than binding to the proximal domain and sequences adjacent to the binding site influence the degree of bending. This data suggests that the DNA structure, as well as sequence, contributes to DNA recognition and binding by Fis. This is consistent with models of the Fis-DNA complex based on the X-ray crystal structure of Fis, chemical and nuclease protection assays and protein/site mutagenesis analyses.

V 131 REPLICATION KINETICS OF P1 AND F IN THE PRESENCE AND ABSENCE OF MINICHROMOSOMES, Julia E. Grimwade, Alan C. Leonard, and Charles E. Helmstetter, Department of Biological Sciences, Florida Institute of Technology, Melbourne FL 32901

P1 and F are stably inherited, extremely low copy number plasmids whose replication timing during the E. coli cell division cycle is not fully understood. There are reports showing these plasmids to replicate both at specific cell ages and throughout the cell cycle. These conflicting results may be due to differences in the growth rates and plasmid content of the host cells used. We have examined the replication timing of P1 and F derivatives in the presence and absence of minichromosomes in B/r F_{26} hosts grown at a variety of growth rates. The cultures were pulse-labeled for 1/10 generation with ^{3}H-thymidine and age fractionated on the baby machine. Radioactivity incorporated into plasmid DNA in cells of different ages was analyzed following agarose gel electrophoresis of whole cell lysates. We have found that at rapid growth rates (glucose/casamino acids minimal medium, τ=27 min) both mini P1 (pZC176) and mini F (pML31) replicate at all cell ages, regardless of whether or not a minichromosome is harbored in the cell. At slower growth rates (τ=40 min in glucose minimal medium or 50 min in glycerol minimal medium) cells without a minichromosome replicated the stable low copy plasmid throughout the division cycle. However, in cells harboring both the stable plasmid and the minichromosome growing at slow growth rates, replication of pZC176 and pML31 was reduced during the period of the cell cycle when minichromosomes and the chromosome initiated replication. These cells also had longer generation times (τ=45 min for glucose and 60 min for glycerol minimal media), suggesting that the cells required more time to accumulate sufficient mass for inititation of chromosome replication. A model explaining these results in terms of a competition for replication factors will be presented.

V 132 *IN VIVO* ASSAY FOR SYNAPTIC COMPLEX FORMATION

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Heritable stability of *E.coli* plasmid ColE1 requires efficient dimer resolution mediated by four chromosomal proteins (ArgR[1], PepA[2], XerC[3] and XerD[4]) which act at the *cer*[5] site to convert plasmid dimers to monomers by site-specific recombination. While XerC and XerD together comprise the heterodimeric recombinase, the roles of ArgR and PepA are not clear. *cer* recombination is topologically constrained and recombination occurs only between direct repeats on the same molecule.[6] However, a hybrid site generated by recombination between *cer* and the *parB* site of CloDF13 (typeII hybrid) is unconstrained; it supports both intra- and intermolecular recombination and requires neither ArgR nor PepA.[7]

We have devised an assay to detect the formation of *cer* synaptic complex *in vivo* by exploiting the observation that interaction between a wild type *cer* site and unconstrained typeII site *in cis* traps the latter into a synaptic complex that inhibits its ability to recombine *in trans*. When *cer* and typeII are in inverted repeat, inter-molecular recombination by the typeII site is still blocked but the sites form a non-productive complex that cannot be further processed. We use this assay to demonstrate that deletions in the left hand end of *cer* destabilise the complex between tandem *wt. cer* and typeII site. Our assay demonstrates that ArgR, and PepA are absolutely required for the formation of *cer* synaptic complex.

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V 133 DNA LOOPING MEDIATED BY HU AND HMG's,

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Site-specific DNA inversion by the Hin recombinase requires the formation of a complex nucleoprotein structure called an invertasome. In this structure the two recombination sites bound by Hin are assembled together at the Fis-bound recombinational enhancer with the requisite looping of the intervening DNA segments. We have analyzed the efficiency of invertasome assembly in the presence or absence of the HU protein *in vivo* and *in vitro* when the enhancer is located at variable positions close to one of the recombination sites. In the absence of HU *in vitro* or in *hupAhupB* mutant cells, invertasome assembly was very inefficient with less than 104 bp of DNA between the enhancer and the recombination site. In the presence of HU invertasome assembly displayed a periodicity *in vitro* and *in vivo* beginning with 60 bp of intervening DNA that reflected its helical repeat. Using this data, the helical repeat of DNA was calculated by Fourier transformation and autocorrelation analysis to be about 11.2 bp per turn both for supercoiled DNA in the presence of HU *in vitro* and in *hup*$^+$ cells *in vivo*. HU is the only protein in *E. coli* that can potentiate invertasome formation with short DNA length between the enhancer and recombination sites. We have, however, found activity similar to HU in HeLa nuclear extracts, which we identified as high mobility group (HMG) proteins 1 and 2, and found that they are even more efficient than HU in stimulating invertasome assembly. To determine the function of HU and HMG's in the inversion reaction we employed ligase-mediated circularization of short DNA fragments to measure bending activity. HMG 1 and 2 were even more active in this assay than HU. The smallest circles formed in the presence of HU were 78 bp, while circles as small as 59 bp could be facilitated by HMG 1. We also purified protease-generated DNA-binding domains of HMG 1 and found that they have different activities: domain B is fully functional in the inversion and ligation assays while domain A is inactive. We conclude that HMG proteins 1 and 2 appear to be eukaryotic functional equivalents of HU in terms of non-specific DNA bending activity and facilitation of nucleoprotein complex assembly.

V 134 *ESCHERICHIA COLI* TOPOISOMERASE III, BUT NOT TOPOISOMERASE I, CAN SEGREGATE DAUGHTER DNA MOLECULES DURING *oriC* AND pBR322 DNA REPLICATION *IN VITRO*.

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oriC and pBR322 DNA replication, reconstituted with purified replication proteins *in vitro*, has been used to study the functional activities of *Escherichia coli* topoisomerase I, DNA gyrase, and topoisomerase III during the segregation stage of DNA replication. In the *oriC* system, DNA gyrase could segregate some, but not all, of the replicating daughter DNA molecules, whereas topoisomerase III could catalyze complete segregation. In the pBR322 DNA replication system, almost all the daughter DNA molecules could be segregated by DNA gyrase alone in the absence of salt. However, the segregation activity of DNA gyrase in the pBR322 system was completely inhibited, without a decrease in DNA synthesis, by the addition of moderate concentrations of salt. Topoisomerase III, however, could segregate all of the daughter DNA molecules in the pBR322 system even in the presence of high concentrations of salt. A similar effect could not be observed in the *oriC* system, since the addition of salt inhibited DNA synthesis. No segregation could be catalyzed by topoisomerase I under any conditions examined in either the *oriC* or pBR322 replication systems. The addition of topoisomerase I to the replication systems resulted only in an inhibition of DNA synthesis. Our previous report that topoisomerase I could catalyze segregation in the pBR322 replication system can be attributed to a less than 1% (w/w) contamination of topoisomerase III in the topoisomerase I preparations used previously.

V 135 DETRMINATION OF THE EFFECTIVE CONCENTRATION OF DNA IN *ESCHERICHIA COLI*.

Emily R. Hildebrandt and Nicholas R. Cozzarelli, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The contents of an *E.coli* cell are of necessity compact and crowded. The concentration of DNA in *E.coli* is 10-30mg/ml whereas typical *in vitro* experiments use 10ug/ml DNA. Therefore, one would expect that collisions between distant DNA sites or domains would be very frequent events *in vivo*. To the contrary, activities such as catenation of DNA rings by gyrase, which are easy to detect *in vitro*, are rarely if ever observed *in vivo*. Indeed, decatenation is the predominant activity *in vivo*. This disparity led us to ask whether the effective concentration of DNA, a value reflecting the sum of influences from the cellular environment (e.g. chromatin structure, molecular crowding, sequestration, and entrapment), is different from the chemical concentration of DNA *in vivo*. Our approach is to measure the reaction [2 monomer plasmid$\leftrightarrow$dimer plasmid] mediated by a site-specific recombinase, over a range of DNA concentrations *in vitro* and to compare the extent of the reaction with that obtained for the same reaction *in vivo*. If the effective concentration of DNA is a general property of the cell it should hold when a variety of site-specific recombination systems are utilized.

The first system examined was Cre recombination. We have established a calibration curve *in vitro* for the intermolecular reaction between two loxP containing plasmids over a variety of conditions. Our initial results show that at the DNA concentrations expected inside the cell for the particular plasmid used (copy number~45), 400-1000ug/ml, the extent of fusion is 40%. However, this fusion reaction by Cre *in vivo* only reaches about 5%, corresponding to an "effective" concentration 20-40X lower than the chemical concentration. Further work will be done to test this result including varying the concentration of plasmid DNA sites *in vivo*, using another recombination system, and testing catenation by topoisomerases.

V 136 USE OF THE DNA REPLICATION ARREST SITES TO EXAMINE THE COORDINATION OF DNA REPLICATION AND CELL DIVISION IN E. COLI, Thomas Hill and Bela Sharma, *Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104*

The *Ter* sites of the *E. coli* chromosome function in a polar fashion and are normally oriented so as to allow replication in the origin-to-terminus direction but prevent replication in the terminus-to-origin direction. We supposed that if an inverted *Ter* site was introduced into the chromosome, it would prematurely arrest DNA replication and prevent completion of the chromosome, thereby delaying the onset of cell division. To test this hypothesis, we constructed a cassette that contains a spectinomycin-resistance gene flanked on either side by an inverted *Ter* site and inserted this cassette into the terminus region of a *tus* strain. We then introduced a functional *tus* gene by transforming the cells with a plasmid carrying a copy of the *tus* gene under control of an arabinose-inducible promoter. These cells grow normally in the absence of arabinose, when *tus* gene expression is repressed. However, in the presence of 0.2% arabinose, these cells form long filaments. This suggests that activation of the inverted *Ter* sites by induction of *tus* gene expression is arresting DNA replication and delaying the onset of cell division. We are currently characterizing this strain to identify the factors that prevent cell division when DNA replication is prematurely halted.

V 137 H-NS: A NOVEL DNA-BINDING PROTEIN INVOLVED IN MAINTAINING CHROMATIN STRUCTURE AND REGULATING GENE EXPRESSION. **Jay C. D. Hinton**, Graham D. Pavitt, Tom A. Owen-Hughes, Diogenes S. Santos, Alex Seirafi, Julie M. Sidebotham and Christopher F. Higgins. Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.

We have been analysing the structure and function of the nucleoid-associated protein H-NS. H-NS binds to DNA, exhibiting an increased affinity for curved DNA (Owen-Hughes *et al.*, 1992). Mutants lacking full-length H-NS protein exhibit pleiotropic phenotypes, including loss of motility and altered regulation of the *proU* operon (Hinton *et al.*, 1992).

We have used two approaches to study the H-NS-DNA interaction; first, we are conducting electron microscopic analysis of H-NS bound to DNA. Second, DNase footprinting of the DNA binding sites of H-NS has revealed no sequence specificity, and has confirmed that H-NS binds cooperatively to DNA, with a five-fold greater affinity for curved DNA. Possible mechanisms for the binding of H-NS to DNA will be outlined. Furthermore, we have been determining the affinity of H-NS protein for the bacterial nucleoid *in vivo*, when cells are grown under various environmental conditions.

Studies have been initiated to determine the tertiary structure of the H-NS protein; circular dichroism analysis of H-NS protein suggests that H-NS contains a significant amount of alpha helix, which is consistent with sequence-based structural predictions. The implications of these various approaches for the function of H-NS will be discussed.

Hinton *et al.*, (1992) Mol. Micro. **6**: 2327-2337; Owen-Hughes *et al.*, (1992) Cell **71**: 255-265; Hulton *et al.*, (1990) Cell **63**: 631-642; Higgins *et al.*, (1988) Cell **63**: 631-642

V 138 CLASSIFICATION OF *LISTERIA MONOCYTOGENES* STRAINS BY DETERMINING THE POSITIONS OF *Eco*R1 CLEAVAGE SITES RELATIVE TO RIBOSOMAL RNA SEQUENCES, Romeo J. Hubner, John A. Webster, James L. Bruce, Eileen M. Cole, Joseph J. Neubauer, and Channeary Iem, E.I. Du Pont de Nemours & Company, Experimental Station, Wilmington, DE 19880-0357

Analysis and comparison of *Eco*R I fragments containing portions of the rRNA operons has been used to type a variety of bacteria. Through the use of finely controlled electrophoresis conditions, molecular weight markers, and algorithms for mobility adjustments, a system has been developed that has sufficient precision and reproducibility that it is capable of distinguishing slight variations in fragment mobilities and is therefore capable of distinguishing otherwise identical bacteria within the same species. As an example, this system has the ability to distinguish between 36 different pattern types in the genus *Listeria*, 18 of which are *L. monocytogenes*. The *L. monocytogenes* description is comprised of a set of fragments with high frequencies of occurrence among strains of the species and additional sets that contain polymorphic variants. The size polymorphisms of a given fragment, termed the polymorphic set, suggested the positions of the *Eco*R1 recognition sites relative to ribosomal RNA sequences. All possible combinations of fragments, one from each polymorphic set, were compiled into patterns and the probability of occurrence of each pattern was calculated. This permitted formation of taxonomic groups or types on the basis of both observed and predicted sets of characters. One hundred additional strains of *L. monocytogenes* were studied with this classification scheme to test for the occurrence of patterns that were unseen but predicted, with high probability, to exist. Analysis revealed that one of them belonged to a predicted pattern type with a calculated abundance of 1 in 566. By extending the classification beyond the observed patterns in the initial data base, a theoretical, but more complete, description of the species is possible. This extended description was tested for specificity by comparison to equivalent patterns from other *Listeria* species.

Presenters I-Q

V 200 METHOD FOR RAPID IDENTIFICATION OF BACTERIA BASED ON POLYMERASE CHAIN-REACTION AMPLIFIED RIBOSOMAL DNA SPACER POLYMORPHISMS, Mark A. Jensen*[1], John A. Webster[1] and Neil Straus[2], [1]Central Research and Development Department, E. I. du Pont de Nemours & Company (Inc.), Wilmington, DE 19880 USA and [2]Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

Identification of a broad range of bacteria is demonstrated using a unified set of primers and polymerase chain-reaction conditions to amplify spacer regions between the 16S and 23S genes in the prokaryotic ribosomal RNA genetic loci. A generic pair of priming sequences was selected for the amplification of polymorphic spacer regions from highly conserved sequences in the 16S and 23S genes occurring adjacent to these regions. This single set of primers and reaction conditions was used for the amplification of 16S/23S spacer regions for over 300 strains of bacteria, consisting of 8 genera and 28 species or serotypes, including *Listeria, Staphylococcus, Salmonella,* and additional species related to these pathogenic organisms. Resolution of the spacer amplification products by electrophoresis resulted in patterns which could be used to distinguish all of the species of bacteria within the test group. Unique elements in the amplification product patterns generally clustered at the species level although some genus-specific elements were also observed. Based on results obtained with our test group of 300 bacterial strains, amplification of the 16S/23S ribosomal spacer region is a suitable process for generating a database for use in a PCR-based identification method, which can be comprehensively applied to the bacterial kingdom.

V 201 MECHANISM FOR OVERINITIATION BY DnaAcos PROTEIN,
Tsutomu Katayama and Arthur Kornberg, Department of Biochemistry, Stanford University, Stanford, CA 94305

DnaA protein plays a key role in replicational initiation at the chromosomal origin (*oriC*).

A cold-sensitive *dnaAcos* mutant causes lethal chromosomal overreplication at 30°C, but grows normally at 42°C[1]. (The *dnaAcos* gene is an intragenic suppressor of the *dnaA46* mutation[2]). Overreplication in this mutant was not caused by oversupply of the initiator protein, suggesting that a change in form of DnaA protein is responsible for excess initiation. Purified DnaAcos protein expressed an activity similar to that of wild-type DnaA protein at 30°C, yet was almost inert at 42°C, a permissive temperature for growth of the mutant. Whereas wild-type DnaA protein binds ADP and is rendered inert, DnaAcos protein was defective in binding ATP or ADP. Further, while wild-type DnaA protein lost activity upon exposure to a crude protein extract, DnaAcos protein did not. These findings suggest that DnaAcos protein lacks a negative controlling element in chromosome initiation, and that repression of DnaA function may be needed to regulate initiation frequency *in vivo*.

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V 202 CcdB PROTEIN OF F PLASMID TRAPS DNA GYRASE IN CLEAVABLE COMPLEXES, Katherine E. Kézdy, Philippe Bernard, Martine Couturier, and N. Patrick Higgins, Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294 and Laboratoire de Genetique, Department de Biologie Moleculaire, Universite Libre de Bruxelles, rue des Chevaux, 67, B-1640 Rhode Saint Genese, Belgium.

The genes *ccdA* and *ccdB* of the F plasmid of *Escherichia coli* are responsible for linking cell division of the host to segregation of the plasmid. If replication of the F plasmid is blocked, the formation of viable plasmid free segregants is inhibited by CcdB protein. CcdA protein acts to suppress this inhibition as long as the F plasmid copy number remains greater than one copy per cell. Cells expressing *ccdB* but not *ccdA* are filamentous with masses of nucleoids distributed throughout the cytoplasm. This perturbation of DNA metabolism combined with the formation of small anucleate cells suggests that CcdB interferes with chromosome replication and/or segregation. Recent genetic studies identified gyrase as the target of CcdB protein killing *in vivo* and biochemical studies showed that CcdB protein traps gyrase in a cleavable complex. *In vitro*, CcdB is unable to trap gyrase in a cleavable complex in the absence of ATP or in the presence of novobicin or AMP-PNP, a non-hydrolyzable ATP analogue. CcdB only traps gyrase when the enzyme is bound to rare specific sites. A strong gyrase site in phage Mu (Mu-SGS) is an example of such a site. A complex containing the Mu-SGS, gyrase, and CcdB protein has been isolated which can be induced to cleave DNA by the addition of SDS. This complex is not dissociated by heating to 65° or by high concentrations of salt. Incubation of these complexes with novobiocin or AMP-PNP prior to the addition of SDS does not inhibit SDS induced cleavage. In contrast, enoxacin, a quinolone antibiotic, traps gyrase in cleavable complexes at the Mu-SGS in the absence of ATP or in the presence of AMP-PNP. We propose that the CcdB protein is a natural toxin that attacks gyrase only when the enzyme is poised in a specific conformation.

V 203 CONSERVATIVE DOUBLE-STRAND BREAK REPAIR BY *E. coli*, Ichizo Kobayashi+, Kohji Kusano+, Tomoki Yokochi+, Noriko Takahashi*, Hiroshi Yoshikura*. +: Dept Molecular Biology, Institute of Medical Science, Univ. Tokyo, Shiroganedai, Tokyo 108 Japan. *: Dept Bacteriology, Medical School, Univ Tokyo, Tokyo 113 Japan.

Double-strand (ds) break repair model of homologous recombination predicts ds gap repair by gene conversion. We demonstrated such repair in a *recBC sbcA* strain (Genetics 119: 751) and have found the following: (i) This conservative (2 products from 2 parents) repair can not be explained by successive rounds of non-conservative (1 product from 2 parents) recombination proposed for RecF pathway (PNAS 89: 5912. MGG 234: 1). (ii) This conservative recombination takes place in a *recA* deletion derivative. (iii) This conservative recombination is not a minor route from a ds break. Non-homologous DNA attached to the break specifically inhibits this conservative recombination and reveals non-conservative recombination. (iv) *recE* exonuclease (5' to 3' from a ds end) and *recT* gene (Dr. A.J. Clark) product are necessary. (redα exonuclease and redβ annealase are necessary for ds break repair by λ (PNAS 89: 2790)). (v) Mutations in *recJ* exonuclease (5' to 3' on single-strand) or in *recQ* helicase decreased association of flanking crossing-over. (vi) Several mutations block double-strand break repair and cause non-homologous rearrangements (Kusano et al., this symposium).

V 204 DNA TOPOISOMERASES FROM HYPERTHERMOPHILIC PROKARYOTES, Sergei A. Kozyavkin*, Regis Krah*, Martin Gellert*, James A. Lake°, Karl O. Stetter• and Alexei I. Slesarev°, *Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892; •Lehrstuhl für Mikrobiologie, Universität Regensburg, D-8400 Regensburg, FRG; °Molecular Biology Institute, UCLA, Los Angeles, CA 90024

Little is known about the structure and function of chromosomes in hyperthermophilic prokaryotes, the organisms which live up to 110°C. Recent discoveries of eukaryotic-like enzymes in hyperthermophiles raise intriguing questions about possible relations among the molecular mechanisms of genome functioning in these group of prokaryotes and eukaryotic nuclei. We focus on the structure and biochemistry of thermophilic topoisomerases, the enzymes controlling DNA supercoiling. The primary aspects of their biochemistry are the influence of DNA melting on topological relaxation, the specificity of DNA cleavage and the mechanism of positive supercoiling by reverse gyrase. Trapping of the covalent complex between DNA and protein was used to identify which part of the enzyme cleaves DNA. The blot-overlay assay used previously for the identification of GTP-binding proteins was applied to reverse gyrase. After SDS-PAGE and blotting to a membrane reverse gyrase can bind labeled ATP during incubation at high temperature. These methods can be used to localize catalytic sites of the enzyme.

V 205 INITIATION OF DNA REPLICATION AT THE PLASMID R1 ORIGIN *IN VIVO* , **Margareta Krabbe, Rolf Bernander, and Kurt Nordström, Department of Microbiology, Biomedical Center, Uppsala University, Box 581, S-751 23, Uppsala, Sweden.**

We have previously mapped the *in vivo* start site for leading strand DNA synthesis in the unidirectionally replicating R1 plasmid. The mapping was performed by primer extension analysis of total DNA isolated from exponentially growing and from stationary phase cells. The sensitivity of the mapping was considerably increased when cycles of repeated primer extensions (RPE) were performed, using a polymerase chain reaction (PCR) protocol. The start point of replication was found to be located 400 bp downstream of the minimal origin, within an *ssi* site (a sequence at which initiation of DNA replication on single-stranded templates can occur). We identified the same 5' start sites in strains lacking the DnaA protein, which indicates that DnaA does not participate in the determination of the start point for leading strand DNA synthesis in R1.

We have recently also mapped the free 3' end which is formed *in vivo* as a replication intermediate in the origin region during lagging strand replication. The mapping was perfomed with strand-specific probes hybridized to restriction-cleaved DNA, which was prepared from exponentially-growing and from stationary-phase cells. The 3' end was located near the *oriR* region, approximately 460 bp upstream of the 5' end. This position corresponds to the rightmost border of the RepA protein binding-site within *oriR*, suggesting that the bound RepA protein may block further lagging-strand replication. The positions of both the 5' and 3' ends were in good agreement with *in vitro* results from other laboratories.

We are at present also investigating the role of a second *ssi* site, *ssiB* located 1700 bp upstream of the minimal origin of R1. In preliminary experiments a free 5' end has been detected also within this *ssiB* region.

Furthermore, we have constructed a set of *intR1* strains in which a mini-R1 plasmid is inserted into the chromosome within the minimal origin of replication, *oriC*, which has been inactivated. We are currently performing two-dimensional gel electrophoresis to analyze the replication patterns of these strains in the attempt to identify differences between uni- and bidirectional replicons.

V 206 DNA SUPERCOILING AND REGULATION OF TRANSCRIPTION: ANALYSIS OF THE *gyrA* PROMOTER. Regis Krah[1], Rolf Menzel[2] and Martin Gellert[1]. [1]Laboratory of Molecular Biology, NIDDK, NIH, 9000 Rockville Pike, Bethesda MD, 20892. [2]Bristol-Myers Squibb, P.O. Box 4000, Princeton, NJ 08543-4000.

DNA gyrase is responsible for negative supercoiling in *E. coli*. The promoters for the *gyrA* and *gyrB* structural genes are sensitive to DNA supercoiling, with transcription increased upon DNA relaxation. PgyrA and PgyrB are also responsible for unexpectedly high readthrough at a rho independent terminator (λ T_{oop}); this readthrough increases upon DNA relaxation (eg. for *gyrA*, 24% anti-termination increases to 60%). The antitermination ability of 16 *gyrA* promoter mutants was investigated to determine whether relaxation stimulation of transcription (RST) is related to antitermination. 14 promoter mutants maintained a high basal antitermination ability (>15%). For 13 of the mutants a correlation was found between the relaxation induced promoter activity and the corresponding increase in antitermination of transcripts initiated at these promoters. With purified RNA polymerase transcription complexes initiated at P_{gyrA} and mutant promoters are all terminated with 90% efficiency, as is normally found for λ T_{oop} *in vitro*. This observation suggests that the antitermination ability of *gyrA* transcription complexes is caused by a *trans*-acting factor . We interpret the strong correlation between induced promoter activity and antitermination ability to indicate this factor is necessary for normal RST regulation of the *gyrA* promoter.

V 207 RECOMBINATION-DEPENDENT DNA REPLICATION TRIGGERED BY A DOUBLE-STRAND BREAK, Kenneth Kreuzer, Marion Saunders and Helen Engman, Department of Microbiology, Duke University Medical Center, Durham, NC 27710

Most replication of the bacteriophage T4 genome is initiated by a process that requires phage-encoded recombination proteins. In addition, plasmids with fragments of the T4 genome, but no phage replication origin, replicate in a recombination-dependent manner following phage infection. This plasmid replication provides a convenient model system to study the mechanism of T4 recombination-dependent replication. We have used the plasmid model system to answer two questions:

1) Does T4 recombination-dependent replication require any specific sequences from the T4 genome? When T4 infects a cell that carries pBR322 with no insert, the plasmid is unable to replicate. However, we found that a T4-pBR322 cointegrate phage induces the replication of a pBR322 plasmid resident in the infected host cell, and that this replication requires T4 recombination proteins. Thus, specific T4 DNA sequences are not required for recombination-dependent plasmid replication, but plasmid-phage homology is required.

2) Is recombination-dependent DNA replication triggered by a double-strand break? The Mosig model for recombination-dependent phage replication proposes that an end of the T4 genome invades an homologous segment of DNA, and that the invading 3' end serves as the primer for leading-strand synthesis. We generated a T4 phage that carries a synthetic cleavage site for the *td* intron endonuclease in the *frd* gene. Because this phage produces intron endonuclease, it cleaves its own *frd* gene in a site-specific manner. We tested whether the double-strand break in the *frd* gene of the phage would increase replication of a pBR322 plasmid that contains wild-type *frd* DNA (i.e., no cleavage site in the plasmid). The double-strand break in the phage DNA increased plasmid replication by several fold, demonstrating that a double-strand break can indeed trigger recombination-dependent DNA replication during T4 infections.

V 208 **GENETIC BLOCK OF DOUBLE-STRAND BREAK REPAIR LEADS TO NON-HOMOLOGOUS REARRANGEMENTS,**
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We looked for genes responsible for the late steps of double-strand break repair in *E. coli* RecE pathway (Genetics 119: 751. Kobayashi et al., this symposium). Some double-strand (ds) break repair models propose that "double Holliday structure" is resolved in two ways to produce "without flanking crossing-over" pair or "with crossing-over" pair. However, mutations of two groups of Holliday-interacting proteins, *ruvABC* and *recG*, did not affect efficiency or outcome of the repair by themselves or in combination. We isolated several mutations that block this double-strand break repair. They resulted in enhancement of non-homologous rearrangements. These two effects were eliminated by *recE159* mutation.

V 209 ASSOCIATIONS AND ACTIVATION OF THE *BACILLUS SUBTILIS* INITIATION PROTEIN -*dna*B.
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The *dna*B gene of *Bacillus subtilis* is essential for initiation of DNA replication and membrane binding of the chromosome and some plasmids. Northern blot analysis shows that the *dna*B gene is transcribed as an operon with two other genes, ORF-Y and *dna*Z. We have p.eviously demonstrated that both the *dna*Z and the *dna*B proteins are involved in initiation by an *in vitro* replication assay.

The *dna*B protein *in vivo* appears to be translated as an inactive 55 kD protein then processed into an active form of approximately 32 kD. Temperature-sensitive mutants show gradual loss of the small form at the restrictive temperature, which reappears rapidly upon returning to the permissive temperature.

The *dna*B gene has been cloned separately into the inducible expression vector pGEX-2T (Pharmacia). Purified polyclonal antibodies raised against the expressed protein were use in immunoprecipitations to identify associated proteins. The protein was present in both the cytosol and the membrane. The cytosolic fraction showed only the 55 kD form that was associated with many other proteins, while the membrane fraction showed both the small and large forms which were associated with *dna*Z.

*Dna*B seems to be a very strong non-specific single-stranded DNA binding protein. The protein shows a low affinity ATP binding, possibly two cooperative sites. The Bacillus *dna*B protein appears to be unrelated to the *E. coli dna*B helicase.

The phylogenetic distribution of the *dna*B operon is being examined via Southern hybridization. While *dna*B does not appear to have a homolog in *E. coli*, many other organisms show homologous DNA.

V 210 THE SOS RESPONSE AS A PROBE FOR IS*1* ACTIVITY.
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IS*1* is a 768bp transposable element present at 6-7 copies in the *E.coli* chromosome. When IS*1* transposition is measured by the mating out assay it is seen to proceed mainly by formation of donor-recipient cointegrates coupled with replication of the IS*1* element. It is not expected to involve simultaneous double-strand breaks, as in the case of direct, "cut-and-paste" transposition. Nevertheless we have found that the transposase of IS*1*, like that of Tn*10* (Roberts & Kleckner; PNAS 85, 6037-6041; 1988) can induce the SOS response in a RecBCD-dependent manner. We have exploited this observation to isolate transposase mutants and to investigate transposase cleavage of the left and right inverted repeats (IRL, IRR) of IS*1*.

High concentrations of wild-type transposase were obtained by replacing the IRL with pL(λ) and expressing the two ORFs of IS*1* as a fused-frame construction on a plasmid vector: SOS induction was monitored using a chromosomally-located pR(434):*lacZ* fusion. We found that the type of cleavage at IRR is highly dependent on its orientation relative to the ORFs: the natural orientation fails to induce SOS, the inverted orientation induces strongly. No effect of swapping IRL for IRR was seen, despite their sequence differences, but the relative abilities of mutant repeat sequences to provoke an SOS response allowed us to distinguish between the binding and cleavage steps of transposition. In the complete absence of plasmid-borne IRs, substantial *in trans* activity directed at chromosomal IS1 elements was observed.

The SOS reaction has also been used to demonstrate that when the distance between IRL and IRR is reduced to <150bp the activity of transposase depends on the number of helix turns between the repeats: SOS response is highest for an integral number of helices between the apparent centres of the InsA binding sites. We are exploiting the SOS system as an *in vivo* monitor for following the interactions of transposase with its binding sites in each repeat sequence.

V 211 IN VIVO FOOTPRINTING OF IHF AND FIS BINDING SITES IN ORIC,
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Accessibility of sites within the oriC region of E. coli minichromosomes to in vivo methylation was examined using the dimethyl sulfate (DMS) footprinting technique. Although dimethyl sulfate preferentially modifies guanines, CA residues may become accessible to DMS when transcribed by RNA polymerase. In exponentially-growing B/r F_{26}, two cytosine-adenine (CA) pairs at position 109-110 and 209-210 within the IHF and FIS binding sites, respectively, were found to be methylated. Sensitivity of the IHF and FIS sites to DMS was examined in temperature-sensitive, initiation-defective, B/r F_{26} dnaA and dnaC mutant strains. At non-permissive temperature (40°C), the CA pair in the IHF site was not methylated in either strain. However, methylation of the FIS site was found to be enhanced at the non-permissive temperature in both strains. When cultures were shifted to permissive temperature (25°) after one hour at non-permissive temperature, methylation of the IHF site became enhanced immediately after the shift in both mutant strains while the FIS site was resistant. Twelve minutes after the shift to permissive temperature, just prior to a second initiation event, the IHF site was once again resistant, and the FIS site accessible to DMS. IHF and FIS site accessibility was also examined during the E. coli cell division cycle in synchronously-growing B/r cultures generated by the baby machine. Based on these observations, the binding or removal of IHF and FIS proteins at sites in oriC appears to be intimately related to the initiation event. A model linking transcription within oriC to IHF and FIS binding, DNA bending, and initiation loop formation will be presented.

V 212 SITE SPECIFIC RECOMBINATION AT *dif* IN THE *E.coli* TERMINUS REGION,
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Site-specific recombination occurs at the *dif* locus in the replication terminus region of the *E.coli* chromosome. This is believed to resolve chromosome dimers arising by homologous recombination, in order that nucleoid segregation and cell division may follow. XerC and XerD, two related lambda integrase family recombinases act upon the site. In strains mutant for *dif*, *xerC*, or *xerD*, a significant fraction of cells are filamentous with aberrant nucleoid distribution. A 33 base pair sequence from *dif* binds XerC and XerD, and is sufficient for both resolution and multimerisation of plasmids *in vivo*. Our work aims to determine the precise sequences and proteins required for wild type *dif* function *in situ*, as there is evidence to suggest that this minimal site is not sufficient. We are also trying to illuminate the biological role of recombination at *dif* by constructing strains in which *dif* has been replaced by other recombination sites.

References:

Blakely *et al.*, (1991), *New Biologist* **3**: 789-798
Kuempel *et al.*, (1991), *New Biologist* **3**: 799-811

V 213 A *bglJ* MUTATION ACTIVATES THE EXPRESSION OF THE CRYPTIC *bgl* OPERON IN *E. coli* K-12, Jane Lopilato, Nichole Napolitano, Katerina Michaels, Kathleen Ottina*, Department of Biology, Simmons College, Boston, MA 02115. *Science Department, Thayer Academy, Braintree, MA 02184

The *bgl* operon encodes proteins necessary for the utilization of β-glucosides such as salicin and arbutin. However, the *bgl* operon is a cryptic operon meaning that wild-type cells are not able to use β-glucosides as sole carbon sources for growth. A variety of mutations serve to activate the *bgl* operon including those that affect DNA structure. Mutations in *gyrA* and *gyrB* decrease DNA supercoiling and result in *bgl* expression. *bglY* (*osmZ*) encodes a DNA binding protein H-NS and mutations in *bglY* also result in *bgl* expression. A search for spontaneous Bgl^+ mutants yielded yet another locus, *bglJ*. *bglJ* has been mapped genetically and physically to the minute 99 region of the *E. coli* chromosome. The wild type allele was first cloned and then the *bglJ4* mutation was recombined onto the cloned gene *in vivo*. Complementation analysis showed that whenever the mutation is present in the cell, the *bgl* operon is activated. Efforts are in progress to further subclone and sequence the *bglJ* gene. Sequence analysis will determine whether *bglJ* is a member of the group of genes which encode histone-like proteins in *E. coli* or if *bglJ* encodes an unrelated protein that is involved in *bgl* expression.

V 214 THE HEMIMETHYLATION DEPENDENT SEQUESTRATION OF *E. COLI* REPLICATION ORIGIN, Min Lu, Joseph Campbell and Nancy Kleckner, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

DNA adenine methylation has been implicated in the functioning of the *E. coli* origin, *oriC*. Immediately after replication initation, the hemimethylated *oriC* is sequestered for 30-40% of the cell cycle and then released; the *dnaA* promoter region is sequestered coordinately. These events appear to be part of a mechanism to prevent secondary replication initiations. It has been suggested that they may also ensure proper segregation of newly replicated sister chromosomes to different daughter cells. To study the *in vivo* mechanism of hemimethylation-dependent sequestration of the replication origin in *E. coli*, we have isolated one mutant, *seqA*, which is defective in this process. Three properties of the *seqA* mutant suggest that the *seqA* mutation directly affects the cell's ability to sequester hemimethylated origins. First, the *seqA* mutant allows *dam*⁻ strains to be transformed with methylated minichromosomes nearly as efficiently as *dam*⁺ strains are, while it has no effect on the efficiency of transformation of *dam*⁻ strains by unmethylated minichromosomes. Second, flow cytometry analysis (courtesy of E. Boye') shows that *dam*⁺*seqA*⁻ strains contain random numbers of origins, as observed in *dam*⁻ strains. Third, DAPI staining experiments show that *dam*⁺*seqA*⁻ strains have aberrant nucleoid morphology identical to that observed in *dam*⁻*seqA*⁺ cells.

The *seqA* mutation has been mapped, and found to be linked to a Tn *10* insertion located at 16.25 min on the *E. coli* chromosome. We have screened the Kohara library, and identified two phages that complement the *seqA* mutation. The *seqA* gene has been identified by complementation analysis of appropriate subclones from the overlap region. The nucleotide sequence of the *seqA* gene has been determined; and it contains a 546-nucleotide open reading frame encoding a 182-amino acid protein. We are currently examining the phenotypic consequences of null mutation in the *seqA* gene, and investigating the biochemical properties of the seqA protein in order to test its ability to bind hemimethylated *oriC* DNA.

V 215 STUDIES ON LEFT-HANDED Z-DNA IN CHROMOSOMES AND PLASMIDS, Slawomir Lukomski and Robert D. Wells, Institute of Biosciences and Technology, Texas A&M University, Texas Medical Center, Houston, TX 77030

The existence and behavior of left-handed Z-DNA is being investigated in plasmids and the *E. coli* chromosome as a function of transcription and other processes that affect DNA topology. A family of plasmids has been constructed with different lengths of alternating -GC- tracts for these determinations. Prior investigations revealed that Z-DNA can be monitored *in vitro* and *in vivo* by the inability of certain enzymes (methylases and restriction enzymes) to act on the unusual conformation when the target sequences are located in or near the Z-helices.

We are exploiting the use of both the MHaeIII (GGCC) and NotI (GCGGCCGC) for these determinations. The NotI site was cloned in the center of alternating -GC- tracts so that GC doublets of NotI were part of the flanking pur-pyr stretch. *In vitro*, we observed a strong inhibition of MHaeIII methylation as revealed by digestion with NotI. This effect was found only with long -GC- inserts and was supercoil dependent. Hence, we believe that this inhibition was due to Z-DNA formation and this system is currently being exploited *in vivo*. These sequences were cloned on the Tn5 transposon and several mutants were mapped in different locations on the *E. coli* genome. For *in vivo* studies, the MHaeIII was expressed in a controlled manner inside *E. coli* bearing transposon insertions. The kinetics of methylation of a NotI site flanked by -GC- runs may be compared with the control NotI sites which are present within IS elements of Tn5.

V 216 THE NER/NLP/TMF FAMILY OF DNA ALLOSTERIC BINDING/REGULATORY PROTEINS, Georgina Macintyre, Jérôme Lemieux, and Michael S. DuBow, Department of Microbiology and Immunology, McGill University, Montréal, Québec, Canada, H3A 2B4.

Transcriptional regulatory proteins are classified into groups according to their structure, the specific sites they recognize, and their mechanism of action. Although they possess specific regulatory functions, these DNA-binding proteins may also perform more general roles in chromosome organization. This dual-function has been proposed for a number of proteins including IHF, FIS, Crp and Lrp. Recently, another family of dual-function, DNA-binding proteins has been characterized. These consist of the Ner (negative early regulation) proteins of the transposable coliphages Mu and D108, E. coli Nlp (Ner-like protein), and the human TMF protein (HIV-1 TATA-modulating factor; Garcia et al., 1992, P.N.A.S., 89,9372). These proteins share many common characteristics and are strikingly homologous.

The Ner proteins of Mu and D108 repress transcription from two overlapping and divergent promoters. However, they recognize different DNA binding sites. D108 Ner bends DNA in a manner similar to the cAMP receptor protein (Crp), and the D108 Ner and Crp DNA binding sites are highly homologous (8/11 base-pairs). TMF activates transcription at a site in the HIV-1 LTR, which consists of two imperfect direct repeats flanking an AT-rich region, in a manner similar to the Mu Ner binding site. Nlp is a positive activator of several sugar-metabolizing operons (e.g. mal) and is highly conserved throughout the Enterobacteriaceae (Autexier and DuBow, 1991, Gene, 114, 13-18). Bending of the mal operator regions by Mal T and Crp is required for full expression of the mal genes. We have purified the 10 kDa Nlp protein to homogeneity and are characterizing its DNA-binding properties. Although the Nlp and both Ner proteins can activate the maltose operons, Nlp cannot bind to the Mu Ner or D108 Ner binding sites. DNA footprinting analyses to identify the differences in the binding properties of Nlp and Ner are underway. As an aid to examine nlp regulation, we are characterizing two cloned E. coli DNA fragments which enhance nlp expression. Using a series of D108 Ner mutants, we are further defining the D108 Ner DNA binding domain, as well as the sequences for phage operon expression and mal operon activation.

V 217 A LOCUS THAT REGULATES COPY NUMBER IN THE REPLICATION ORIGIN OF PLASMID PSC101

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The pSC101 origin encodes a protein essential for replication, RepA. *The ori* DNA sequence revealed the presence of a cluster of direct repeated sequences RS1, RS2, and RS3 and two imperfect palindromes, IR2 and IR1, partially homologous to the direct repeats and overlaping the *repA* promoter. We found evidence suggesting that RepA binds to directly repeated sequences and initiate replication in monomeric form but that it binds to inversely repeated sequences and autoregulates its transcription in dimeric form (Manen *et al.* PNAS **89**: 8923-8927, 1992). In this work we study in more details the influence on the regulation of the plasmid copy number of a region which is at the junction of the *ori* and *repA loci*. This region comprises the IR1 inverted repeated sequences which straddle the -35 region of the RepA promoter. We show that the integrity of IR1 greatly influences pSC101 copy number. IR1 is separated from the nearest repeated sequence, RS3, by approximately four turns of the DNA helix. Copy number is preserved if this distance is increased by one whole turn but not if it is increased by a fraction of a turn. These results suggest interactions between RepA dimers binding at IR1 and RepA monomers binding at the RS sequences in the initiation of replication.

V 218 **ORDERED BINDING OF DnaA PROTEIN TO THE *ESCHERICHIA COLI* CHROMOSOMAL ORIGIN, *oriC*** Carla Margulies and J.M. Kaguni, Department of Biochemistry, Michigan State University, East Lansing, MI 48824

DnaA protein, essential for the initiation of *E. coli* chromosomal replication, binds to and induces a localized unwinding of the origin of replication, *oriC*. The unwound structure is then recognized by other replication proteins that act subsequently to promote DNA replication. Binding of DnaA protein to four DnaA boxes in *oriC* appears to involve 20-30 DnaA protein monomers, based on electron microscopic data (Funnell, B., Fuller, R. S., and Kornberg, A. 1988 *J. Biol. Chem.* **38**, 889-900). We have examined the binding of DnaA protein to each of the DnaA boxes in *oriC*. By gel mobility-shift assays, DnaA protein forms discrete complexes that are resolved from each other. Footprinting methods with 1, 10-phenanthroline-copper on isolated complexes suggest an ordered binding of DnaA protein to the four DnaA boxes in *oriC*. These results are compared to the pattern of protection observed with DNase I.

V 219 LACK OF CONSERVATION OF SOME *TER* REGION SEQUENCES, Millicent Masters and I.R. Oliver, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, EH9 3JR, Scotland

The terminus region of *E. coli* although deficient in essential or even identified genes does contain identifiable coding units (Moir *et al.*, *J. Bacteriol.* **174**:2102 (1992)). A possible explanation of these facts is that terminus DNA consists of sequences that vary considerably between strains and species which, otherwise, are closely related. To test this idea we compared, by hybridization, the conservation of DNA from the *trg* region with that of DNAs from conserved parts of the chromosome. Hybridization was to DNAs of several Enterobacterial species and to DNA from 10 selected strains of the ECOR collection. We found that terminus region DNA was indeed less conserved than control DNAs and contained sequences of several kb in length that were entirely missing both from several Enterobacterial species and also form some of the ECOR strains.

V 220 SEQUENCE HOMOLOGY IS NOT REQUIRED FOR INITIATION OF STRAND EXCHANGE *IN VITRO*,
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In vivo, a stretch of about 30 base pairs (bp) of perfect homology is required to initiate recombination in *E. coli* (Shen, P. & Huang, H. V. (1986) *Genetics* **112**, 441-457).

We show that *in vitro* RecA protein promotes an unexpectedly efficient strand exchange between circular ssDNA and duplex DNAs containing short (< 60 bp) heterologous sequences (HS) at the initiating or proximal end of the duplex. The transfer efficiency decreases sharply for HS between 70 and 120 bp and is completely blocked for HS > 150 bp. Controls show that the bypass of HS is not due to endo or exonucleolytic contaminating activities and is independent of sequence effects. The mechanism by which this topological barrier is bypassed might involve unwinding of proximal HS resulting from interactions occurring in the homologous regions beyond them.

The high tolerance of RecA for heterology at the initiating end *in vitro* contrasts with the high fidelity required *in vivo* for the initiation of recombination. The fact that RecA is not sufficient to ensure perfect homology at the initiation site suggests that one or several correction mechanisms are necessary.

We show that helicase II (UvrD) can discriminate between joint molecules according to the extent of heterology at the active end of the duplex DNA. This supports our hypothesis (P. Morel *et al.*, submitted for publication) that UvrD could participate in the search for homology as part of a proofreading mechanism operating at the synaptic step and involving RecA and possibly other proteins.

V 221 ROLE OF H-NS PROTEIN IN *oriC*-COMPLEX FORMATION OF *E. coli*
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The replication origin of *E. coli* chromosome (*oriC*) is known to be recovered in the outer-membrane fraction, forming an *oriC*-complex, after sucrose density gradient centrifugation of the cell lysates. Formation of the complex was observed periodically at the time of initiation of replication and only *oriC* and its flanking region were recovered from the complex. 35-, 55- and 75-kDa proteins, which were found in the *oriC*-complex fractions, were shown to sediment with glycogen particles and not to be the constituents of the complex. Using an anti-H-NS antibody, H-NS protein was detected in the *oriC*-complex fractions. The results prompted us to examine the cell growth and chromosome replication of *hns* mutants. DAPI-staining revealed that *hns* mutants produced a significant amount of annucleated cells. The mutant cells were more sensitive to UV irradiation than wild type cells. Decrease of ploidity, which might be responsible for UV sensitivity, was observed in the mutant cells by analysis using a flowcytometer. The results suggest that H-NS protein participates in the initiation event of chromosome replication in *E. coli*.

V 222 IDENTIFICATION OF *STAPHYLOCOCCUS WARNERI* BY OBSERVED AND PREDICTED PATTERNS OF *Eco*R1 FRAGMENTS CONTAINING RIBOSOMAL RNA SEQUENCES,
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A study of *S. warneri* was used to determine the general applicability of the method developed for the identification of *Listeria* species based on the detection and analysis of the genomic *Eco*R I fragments that contain portions of the rRNA genes. Analysis of the *Eco*R I patterns of 90 strains of *Staphylococcus warneri* has led to the generation of a data base used for identification. Further, through the analysis of fragment polymorphisms, a method has been developed that can predict diversity and therefore identify with certainty patterns that are not represented in the data base. A set of fragments with high frequencies of occurrence among strains of the species and additional sets that contain all possible combinations of polymorphic variants were used to describe a species. This permitted formation of taxonomic groups or types on the basis of both observed and predicted sets of characters. When these sets of fragments were compared to equivalent sets of fragments from other *Staphylococcus* species, *S. warneri* was distinguished from the other species. Additional strains of *S. warneri* were correctly identified with this species description. Use of this method allowed strains of *S. warneri* to be identified according to species and classified into types with a single, standard procedure.

V 223 A THETA-REPLICATING PLASMID AS A MODEL OF THE CHROMOSOME TO STUDY ILLEGITIMATE RECOMBINATION,
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Deletion formation, which is an ubiquitous phenomenon, is the most studied example among chromosomal rearrangements. Deletions are frequently generated by a recombination event between short (≤20bp) direct repeats that removes one of the repeats and the sequences in between (the insert). We developed in *Bacillus subtilis* a system allowing positive selection of the molecules that underwent deletion, by interrupting a chloramphenicol-resistance gene with 18 bp direct repeats. The deletion restores a functional gene. The direct repeats are carried by a plasmid, derived of pAMβ1, which replicates via a theta mechanism (1). This plasmid was chosen for two reasons; (i) deletion formation between short direct repeats occurs at the same frequency in theta-replicating plasmids and in the chromosome (2,3), (ii) plasmids are much easier to modify than the chromosome.

We have investigated the effect of the distance between the direct repeats on deletion formation. Deletion frequency decreases smoothly with the increase of the insert size and drops 5000-fold for inserts ranging from 15 to 2000bp. Our data suggest that two different mechanisms can generate deletions, one acting at a short range (≤400 bp) an the other acting at a longer range (400 - 2000bp). In order to recombine, the repeats must come into close contact. The energetic cost of bending the DNA duplex to bring the repeats together becomes very high if the distance is ≤400 bp (4). This implies an interaction "at distance" between the repeats. Our hypothesis is that this interaction could be due to a "slippage" of the DNA polymerase within the replication fork.

We are currently integrating into the chromosome the chloramphenicol-resistance gene carrying a series of inserts between the repeats. Preliminary results suggest that the same relation between insert size and deletion frequency holds in the plasmid and in the chromosome, at least for the long inserts (≥ 400bp). Thus, they indicate that a theta-replicating plasmid could be used as a model system of the chromosome to study the effect of structural parameters on deletion formation.

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V 224 THE DNA SUPERCOILING-SENSITIVE EXPRESSION OF THE *SALMONELLA TYPHIMURIUM his* OPERON IS MODULATED BY ANAEROBIOSIS AND BY HIGH OSMOLARITY, Conor P O'Byrne and Charles J Dorman. Molecular Genetics Laboratory, Department of Biochemistry, University of Dundee, Dundee DD1 4HN, Scotland, UK.

Growth under anaerobic conditions or at high osmolarity results in increased negative supercoiling of bacterial DNA. Enteric bacteria possess a group of genes whose expression is modulated by changes in anaerobiosis and osmolarity and whose promoters are sensitive to changes in DNA supercoiling. These genes code for products required by the cell for adaptation to environmental change. The histidine biosynthetic operon of *Salmonella typhimurium* is derepressed by mutations mapping to the gyrase subunit genes, *gyrA* and *gyrB*. This raised the question of whether the expression of this DNA supercoiling-sensitive system was also sensitive to anaerobic growth or growth at high osmolarity.

Data will be presented to show that DNA gyrase inactivation induces the *his* operon in a growth-phase-dependent manner; that the supercoiling response requires the *his* attenuator, indicating that the site for DNA supercoiling regulatory input is at *hisR* and not at *his P1;* that the induction of *his* by DNA gyrase inactivation is reversible by anaerobic growth or growth at high osmolarity; that the inputs from these environmental stimuli require the *his* attenuator and therefore act via the route as the DNA supercoiling regulatory inputs (as expected); that inactivation of *relA* does not influence the induction of *his* by DNA gyrase inactivation or the environmental reversal of this induction, showing that the *his* P1-dependent RelA response and the DNA supercoiling control are distinct. Taken together these data demonstrate that the *his* operon is a member of the family of bacterial genes whose expression is modulated by changes in environmental parameters known to affect DNA topology *in vivo*.

V 225 TRANSCRIPTION INVOLVED IN THE INITIATION OF REPLICATION OF *E. coli* CHROMOSOME

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The origin of replication of the *Escherichia coli* chromosome (*oriC*) is located in an intercistronic region between the *gidA* and the *mioC* genes. Transcription from the promoters of these two genes activates replication of a minichromosome. Both promoters (P*gid* and P*mioC*) direct leftward transcription and are required for maximal activation but P*gid* located at the left of *oriC* has a more significant effect on initiation. These two promoters on a *oriC*-pBR322 chimeric plasmid were replaced by a *lac* promoter inserted either into the left or into the right of *oriC* and transcription was induced by the addition of IPTG. Effect of the transcription on replication was measured by three methods: (i) measurement of transformation frequency of *polA* cells, (ii) Southern hybridization to estimate plasmid copy number and stability, and (iii) in vitro assay for replication. In all three assays, leftward transcription starting either from the left or from the right of *oriC* activated replication of the plasmid. In contrast, rightward transcription was ineffective in the activation. Leftward transcription might facilitate the opening of the strands at the 13-mer region in *oriC* by generating negative superhelicity behind RNA polymerase.

The amounts of transcripts from the chromosomal P*gid* and P*mioC* promoter were measured using a temperature-sensitive *dnaC* strain, PC2. Initiation of replication was synchronized by shifting the culture to 29°C after 60-min cultivation at 40°C to terminate replication. Upon the temperature down-shift, amounts of the P*gid* transcripts decreased to a low level and after a short period, synthesis of the RNA resumed. On the other hand, transcription from P*mioC* was inhibited before the initiation of replication and resumed at 29°C. Since no inhibition of the P*mioC* transcription was observed at 40°C in a temperature-sensitive *dnaA* strain, the inhibition could be due to the DnaA protein bound to the DnaA box located close to the promoter. Thus, transcription from these two promoters seems to be tightly regulated in a cell cycle-dependent manner.

V 226 GENETIC DETERMINANTS OF ANTIBIOTICS RESISTANCE AND PROPERTIES OF THE MEMBRANE-BOUND ATP-ase OF STAPHYLOCOCCI,Oryabinskaya L.B., Vinnikov A.I.,Ruban O.V.,Department of Microbiology,State University,Dniepropetrovsk,Ukraine, 320625

Properties of membrane-bound ATP-ase of Staphylococci in connection with mechanisms of cells resistance to antibiotics have been studied. We used the antibiotic resistant Staphylococcus aureus strain 8325-4,transductants which were obtained on its base and lysogen by phage 52A strains which carry out a resistance plasmid to penicillin,tetracycline,chloramphenicol, erythromycin. All the investigated strains posessed ATP-hydrolase activity with optimum pH 6,5 and at 45°C. Mg^{2+}-cations in contrast to K^{+},Na^{+},Ca^{2+} renderd the strong stimulated effect.However sensitive and resistant Staphylococci strains have different properties,which can be defined by character of antibiotics resistant mechanism. It was signed the sufficient intensification of velocity ATP hydrolysis by resistant strains.The presence of phage DNA lowered the activity of enzyme in comparison with plasmid strains. ATP-ase of resistant cells was more sensitive to inhibitors NaF, Na azide and N,N'-dicyclohexylcarbodiimide than ATP-ase of sensitive cells.The effect of inhibition was proportional to ATP-hydrolase reaction. On the base of the results we can suppose that forming of resistance to antibiotics is accompaind by intensification of energy dependent processes.

V 227 REGULATION OF Fis EXPRESSION IN *E. COLI* AND *SALMONELLA*, Robert Osuna, Jimin Xu, Catherine A. Ball, and Reid C. Johnson. Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.

Fis is a small DNA binding protein from *E. coli* and *Salmonella* that was initially identified for its role in stimulating site-specific DNA recombination reactions and subsequently shown to participate in essential cell functions such as rRNA and tRNA transcription and initiation of DNA replication at *oriC*. *fis* forms an operon with an upstream gene of unknown function (ORF1) in both *E. coli* and *Salmonella*. While the deduced amino acid sequences for ORF1 show a 95% conservation between the two species, the Fis amino acid sequences are fully conserved. Cellular Fis levels are virtually undetectable during stationary phase but rapidly increases upon subculturing cells in LB medium, reaching a peak of 50,000 to 100,000 molecules/cell within the period required for the first cell division. As cells enter exponential growth in batch cultures, nascent synthesis is largely shut off and intracellular Fis levels decrease as a function of cell division. A similar response is observed when exponentially growing cells are shifted to a richer medium. *fis* mRNA levels follow a similar expression pattern suggesting that regulation occurs largely at the transcriptional level. Fis represses its own mRNA levels 6-10 fold in *E. coli* but only about 2 fold in *Salmonella*. Purified Fis binds to six sites within the *fis* operon promoter region in *E. coli* and prevents stable complex formation of RNA polymerase in this region. The finding that two of these Fis sites are absent in *Salmonella*, can be correlated with a lower efficiency of Fis autoregulation in the latter. In addition to autoregulation, other regulatory mechanisms are implicated. For instance, sequences located between 63 and 163 bp upstream of *fis* in ORF1 are required for a ~10-fold increase in translation efficiency of Fis.

V 228 ISOLATION AND CHARACTERIZATION OF TRANS-ACTING MUTATIONS WHICH CAUSE OVEREXPRESSION OF A CELL CYCLE REGULATED OPERON, Kim C. Quon and Lucy Shapiro, Department of Developmental Biology, Stanford University School of Medicine, Stanford CA 94305-5427

The Gram-negative bacterium *Caulobacter crescentus* is an excellent model system for the study of the bacterial cell cycle, due to the ease with which cells are synchronized, and the presence of morphological and molecular landmarks which spatially and temporally mark the cells. One of these landmarks is the single polar flagellum, which is assembled at a specific time in the cell cycle, reflecting the temporal expression of the *fla* genes required for flagellar biogenesis. To address the question of how flagellar gene expression is regulated in the cell cycle, we are studying the transcriptional regulation of *fliQR*, an operon which resides at the top of the known flagellar regulatory hierarchy, and whose expression is sensitive to inhibition of DNA replication. We have isolated trans-acting mutations which cause overexpression of *fliQR*. As flagellar function is not required for viability, we have screened the mutants for temperature sensitive lethality in hopes of identifying those flagellar regulatory genes which interact with or are actually part of the machinery which drives the cell cycle.

Mutants which overexpress *fliQR* were selected on high levels of kanamycin from an EMS mutagenized strain containing a chromosomal *fliQR*::*neo* operon fusion. The overexpression phenotype was confirmed by assaying beta-galactosidase activity from a *fliQR*::*lacZ* operon fusion on a plasmid. 16 mutants have been identified and characterized further by direct observation and by assaying various *Caulobacter* promoter::*lacZ* fusions in the mutant backgrounds. Phenotypic analysis suggests that the mutations lie in 5 different genes, which we are in the process of confirming by complementation and mapping studies. In addition, we have isolated 6 mutants which are simultaneously temperature sensitive lethals. Preliminary results indicate that both the overexpression of *fliQR* and the temperature sensitive lethality phenotypes in these mutants are due to single mutations or tightly linked multiple mutations. The cause of lethality has been examined in one of these mutants, and appears to be due to a defect in the initiation of DNA replication.

Presenters R-Z

V 300 **REGULATION OF EXPRESSION OF THE DAM METHYLTRANSFERASE GENE (*dam*) FROM *Escherichia coli*.** Lene Juel Rasmussen[1], M. G. Marinus[1] and Anders Løbner-Olesen[2], [1]Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01655; 2Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347.

The level of Dam protein (DNA adenine methyltransferase) in *E. coli* is critical for the efficient action of Dam-directed mismatch repair and synchronous initiation of chromosome replication from *oriC*. A drastic increase or decrease from the normal level of 130 molecules per cell causes hypermutability and asynchronous initiation.

The Dam methyltransferase is encoded by the *dam* gene located at 74 min on the genetic map. The *dam* gene is part of a transcriptional unit which includes five promoters and at least four genes: *aroK* (shikimic acid kinase I), *aroB* (3-dehydroquinate synthase), *urf74.3* (an unidentified open reading frame) and *dam* (Dam methyltransferase).

As a first step to elucidate the mechanism of regulation we have found that the expression of the *dam* gene is growth rate regulated and coordinated with cell growth. In order to determine the region responsible for this regulation we have separated and characterized the promoters individually.

V 301 FUNCTIONS OF ALL CURRENTLY IDENTIFIED *E. COLI* GENE PRODUCTS, Monica Riley, Marine Biological Laboratory, Woods Hole, MA 02540

We now know a significant fraction of the reactions, structural components and regulatory functions of the *E. coli* cell. The gene products of over 1700 genes have been characterized well enough to allow one to assess the present state of knowledge in relation to the ultimate goal of complete knowledge of all of the genes, the gene products and the regulatory circuits that are necessary to make a free-living cell. A summary of all presently known *E. coli* gene products will be presented, derived both from the list compiled by B. Bachmann (Microbiol. Rev. 54, 130, 1990) and also from lists of more recently defined genes assembled and kindly shared by Kenn Rudd (National Library of Medicine, NIH, Bethesda). In categorizing the gene products, they have been grouped into five major categories, Intermediary Metabolism, Biosynthesis of Small Molecules, Metabolism of Macromolecules, Cellular Processes and Other Functions. These groups are further subdivided into altogether about 60 subgroups. Although classification was necessarily arbitrary, perhaps even idiosyncratic, especially for multifunctional gene products, still the classification provides an estimate of the relative sizes of groups of genes devoted to particular physiological ends, and some sense of how far we have come and how far we have yet to go. The data will be displayed with an invitation to scientists to correct errors and enter new information.

V 302 ACTIVATION OF TRANSCRIPTION IN E.COLI BY UPSTREAM SYNTHETIC CURVED SEQUENCES IS DEPENDENT ON THE EXPRESSION OF THE NUCLEOID-ASSOCIATED PROTEIN H-NS, Sylvie Rimsky, Florent Zuber, Denise Kotlarz, Henri Buc. Departement de biologie moléculaire, Institut Pasteur, Paris, FRANCE.

One of the most abundant proteins of the nucleoid is H-NS also called H1a. Although there is no strong specificity for DNA binding (Rimsky and Spassky 1990), H-NS protein is known to bind preferentially to curved DNA sequences (Bracco *et al* 1989, Yamada *et al* 1990). Our interest is to elucidate the detailed mechanism by which curved DNA activates transcription in E. Coli. We had previously constructed a set of promoters containing different upstream curved sequences (Bracco et al). We have studied the effects of H-NS protein *in vivo* on expression from these promoters. We show that two different curved sequences situated upstream of a promoter may have different effects on transcription in the presence or the absence of the protein H-NS. We also demonstrated that these effects can be modulated by changes in the spacing between the inserted curved sequences and the start site for transcription. Run off transcription assays show that *in vitro* H-NS appears to be a repressor for all promoters studied today.

V 303 **Residual activities of mutant RecF proteins.** Steven J. Sandler and Alvin J. Clark. Department of Molecular and Cell Biology, Division of Genetics, University of California at Berkeley, Berkeley, CA 94720.

The *recF* gene of *E. coli* K-12 plays a role in recombination, DNA repair, mutagenesis, and induction of the SOS and Adaptive Responses. The *recF* protein has been reported to bind azido-ATP (and by extention ATP), dsDNA in a ATP dependent manner and ssDNA in an ATP independent manner [Madiraju and Clark (1992) J. Bact. (in press)]. We have been studying the *recF* gene by (1) altering the translational regulation of *recF*, (2) looking for overproduction phenotypes using the wildtype *recF* gene, and (3) testing how different *recF* mutations affect the overproduction phenotypes. We report on these subjects below.

Analysis of the translational regulation of the *recF* gene has shown the region between 50 and 140 nt. is responsible for a 30 fold drop in expression at the level of translation using *recF-lacZ* translational fusions. Mutations have been found which eliminate most of the inhibition of overproduction and yield a 20 fold increase in the rate of *recF-lacZ* fusion proteins. The same mutations cause a 10 fold increase in the rate of translation *recF* mRNA.

Transient overproduction of wildtype RecF protein in sufficient quantity interferes with four normal cell functions: (1) UV induction of transcription from the LexA-protein-repressed *sulA* promoter, (2) UV resistance (3) formation of recombinants after Hfr mating and (4) cell viability at 42°C [previously reported Griffin and Kolodner (1990) J. Bact 172 pp. 6291-6299]. One mutation, *recF4101*, known to be in the putative ATP binding fold [Sandler et al. (1992) NAR 20 pp.839-845] produces a nul mutant phenotype for *recF* function when tested on the chromosome. Overproduction of this mutant protein however produces overproduction phenotypes 1 and 2 (see above) but not phenotypes 3 and 4. This suggests that the phenotypes can be grouped. The grouping may signify differential sensitivity to amounts of RecF protein or to a differential dependence on ATP in vivo. Investigations of the biochemical characteristics of RecF4101 may shed some light on this point. Correlation of in vitro properties with phenotypes (of *recF4101* and other mutants) will help establish a structure-function map of *recF*.

Others have previously suggested that RecF, RecO and RecR may form a complex to perform their role in recombination. To explore this idea, we have extended the above approach to overexpression of the *recO* and *recR* genes.

V 304 WHAT KEEPS THE PROMOTER OF THE *BGL* OPERON CRYPTIC? Karin Schnetz[1,2], Bodo Rak[1] and James C. Wang[2], [1]Institut für Biologie III, Universität, D-7800 Freiburg, FRG, [2]Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138.

Activity of the promoter of the *bgl* operon of *E.coli* is enhanced by various types of spontaneous mutations within the promoter region which results in activation of the normally inactive operon. These mutations include (i) insertion of mobile elements IS*1* or IS*5*, (ii) point mutations within its CAP binding site and (iii) deletions within a region upstream of the CAP binding site. The operon is also activated by mutations in *hns* or the gyrase genes.

Experiments aimed to understand the mechanisms by which mutations in cis activate the promoter gave a surprising result. When the *bgl* promoter and/or its CAP binding site were replaced by the corresponding *lac* promoter sequences or by the CAP-independent *lacUV5* promoter the normally active *lac* or *lacUV5* promoter was inactivated. When tested in the *lac* context the *bgl* promoter as well as its CAP binding site were perfectly active. Promoter inactivation is thus context-specific rather than promoter-specific. Since deletions within a region upstream of the CAP binding-site as well as replacement of the *bgl* sequences downstream of the promoter region can result in promoter activation these regions are presumably organized into a structure within which a promoter is inactive.

Additional *in vitro* studies on possible mechanisms of promoter inactivation and activation will also be discussed.

V 305 JUXTAPOSITION OF DNA MOLECULES: BIMOLECULAR COMPLEXES IN LAMBDA SITE-SPECIFIC RECOMBINATION, Anca M. Segall and Howard A. Nash, Lab of Molecular Biology National Institute of Mental Health, Bethesda MD 20892.

Bacteriophage lambda uses site specific recombination to insert and excise its genome into and out of the genome of its host, E. coli. The recombinase, Integrase (Int) protein, and several accessory proteins act on specific short DNA sequences, the attachment (att) sites. We have studied a simplified version of the lambda system, in which Int protein interacts with one kind of att site, attL. We find that Int can align and hold together two DNA molecules, each with an attachment site, to form noncovalent *bimolecular* complexes (*i. e.*, with 2 DNA molecules). Int protein has two different DNA binding domains, each of which binds a different DNA sequence, one known as a core site and the other as an arm site. Both types of sites must be present on each att-containing DNA molecule that participates in the bimolecular complexes. These very stable complexes require the interaction of Int with the arm sequences present in the attL and attP attachment sites; the arm sequences present in the attR site do not suffice.

We exploited several properties of bimolecular complex formation to address their relationship to recombination between two attL sites. 1) While bimolecular complexes form in the absence of spermidine, recombination absolutely requires spermidine. Preforming the complexes in the absence of spermidine (and subsequently adding spermidine) significantly reduces the time required for appearance of recombinant products. 2) The IHF protein prevents both formation of bimolecular complexes and depresses the level of recombination when it is added simultaneously with Int protein. However, preformed bimolecular complexes and recombination reactions started in the absence of IHF are more resistant to this protein. Taken together, our findings strongly suggest that the bimolecular complexes represent an intermediate in Int-mediated recombination between two attL sites.

V 306 *Abstract Withdrawn*

V 307 IMAGING OF DNA BASES AND OLIGONUCLEOTIDES USING SCANNING TUNNELING MICROSCOPY,

Douglas W. Smith[1], W. Blaine Stine[1], William F. Loomis[1], Morris F. Scharff[2], Steven E. Mills[2], and Robert L. Kelly[2], [1]Department of Biology - 0322, and Center for Molecular Genetics, University of California, San Diego (UCSD), La Jolla, CA 92093; [2]Science Applications International Corporation (SAIC), San Diego, CA 92121

The goal of this joint program between UCSD and SAIC is to develop a novel high-speed, low-cost method to sequence large DNA molecules using scanning microscopy. Results from imaging DNA bases and oligodeoxynucleotides, mainly using scanning tunneling microscopy (STM) on pyrolytic graphite (HOPG), are presented. Images of each of the four bases, obtained from islands of 2-D arrays of the individual bases on pyrolytic graphite, show considerable signal within the dimensions of each base. The signal due to each base is distinctly different from those of the other bases. Although the base signals will be different in DNA, and dependent on the STM substrate used, these results support the notion that reliable discrimination between the four bases will be possible. A variety of oligonucleotides, including short homooligomers, synthesized oligomers of desired sequence, e.g. $(A)_6T(A)_{12}$, oligomers of unknown sequence, as well as plasmid DNA and phage lambda DNA, are being used. Images of single-stranded oligonucleotides of length 54 nucleotides (54-mer) in water dried on pyrolytic graphite have two clear end points, with a length of 41 nm and a width of 1.2 nm. The images show a periodic repeat of 0.75 nm. These are the dimensions expected for a straightened 54-mer and argue strongly that this is an image of single-stranded DNA. Immobilization of the ssDNA to the pyrolytic graphite remains the critical problem, so we are pursuing a variety of physical and chemical approaches to substrate material selection and preparation. Images of a 9-mer of oligo(A) dried on pyrolytic graphite coated by Alcian blue have been obtained at resolution sufficient to show periodic structure within the regions where the bases are expected to be found. Advanced signal processing techniques for detection and classification of subtle signals in complex background scenes are being applied to discern base signal patterns. Results indicate significant progress toward obtaining the signal needed to identify bases in an ssDNA environment and to discriminate reliably the signals associated with each base. This project was supported by SAIC and by Beckman Instruments, Inc.

V 308 ANALYSIS OF SITE-SPECIFIC RECOMBINATION BETWEEN COLE1 *cer* SITES AND NPT16 *npr* SITES

George Szatmari, Nicolina Zakova-Pavlova and David J. Sherratt, Department de Microbiologie et Immunologie, Universite de Montreal, Montreal, Quebec, Canada, and Institute of Genetics, University of Glasgow, Glasgow, Scotland.

Bacterial plasmids and chromosomes utilise site-specific recombination to aid in their partitioning during cell division. Several multicopy plasmids utilise a site-recombination system which require a *cis*-acting DNA sequence and host-encoded *trans*-acting factors. In *Escherichia coli*, both the 280 base pair ColE1 *cer* site and the 300 base pair NTP16 *npr* site utilise the *argR, pepA, xerC* and *xerD* gene products to catalyse this site-specific recombination reaction. Both *cer* and *npr* sites contain a binding site for the arginine repressor (*argR* gene product) and binding sites for the xerC/D recombinases, separated by about 100 base pairs. These sites are functionally identical, display regions of homology within their recombination sites, and are capable of recombining with each other *in vivo*. The largest region of homology between the two sites occurs within their crossover regions:

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ColE1  GGTGCGTACAA TTAAGGGA TTATGGTAAAT
NTP16  GGTGCGCGTAA -TGAGACG TTATGGTAAAT
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One of the differences between these recombination sites occurs at the central "spacer" region separating the left and right arms of the crossover site. We have utilised this difference to analyse the outcomes of site-specific recombination between *cer* and *npr* and to precisely define the point of strand exchange. In addition, we have constructed variant recombination sites in which the highly conserved spacing between the arginine repressor binding site and the crossover region have been disrupted by 3, 8, 10 and 12 base pairs. The results of these studies and their relevance to the site-specific recombination mechanism will be presented.

V 309 GENES, PROTEINS, AND DNA STRUCTURES POTENTIALLY INVOLVED IN LINEAR PLASMID REPLICATION IN BORRELIA BURGDORFERI, THE LYME DISEASE AGENT,

K. Tilly[1], A.G. Barbour[2], and J. Hinnebusch[1], [1]Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT USA and [2]University of Texas Health Science Center, San Antonio, TX USA

The linear plasmids of *Borrelia burgdorferi* are unusual in that they have covalently closed ends and the distributions of ends vary from isolate to isolate. These characteristics suggest that the linear plasmids may have novel mechanisms for replication and that the ends are the sites of frequent recombination. We looked for evidence that the linear plasmids are found as circles or concatamers, which could be intermediates in replication or recombination. Using a PCR assay, we found that various *B. burgdorferi* isolates contain forms of their linear plasmids that have joined ends. The sequences of the junctions were those expected from simple joining of the ends. We have also cloned *B. burgdorferi* genes whose products may play roles in linear plasmid replication, in order to study them genetically and biochemically. Among these are the *grpE*, *dnaK*, and *dnaJ* homologs, whose products are involved in low copy number plasmid replication in *Escherichia coli*, the *him* gene homologs, whose products bend DNA and thereby facilitate many protein-DNA interactions in *E. coli*, and the *priA* gene homolog, whose product binds to DNA hairpins and is involved in primosome assembly in *E. coli*. We plan to assay the products of these genes for binding to specific sequences and structures found at the termini of the linear plasmids.

V 310 BACTERIOPHAGE MU REPRESSOR AND THE *E.COLI* ATP-DEPENDENT CLP PROTEASE, Ariane Toussaint[1][2], Amina Mhammedi-Alaoui[1], Jamal Laachouch[1], Vincent Geuskens[1] and Lucie Desmet[1].(1) Laboratoire de Génétique, Université Libre de Bruxelles, B1640 Rhode St Genèse, Belgium, (2) Laboratoire de Biochimie des Microorganismes, Université J. Fourier, 38041 Grenoble, France.

The importance of proteases in global gene regulation is well documented both in procaryotic and eucaryotic systems.
Virulent mutants of bacteriophage Mu, carry a frameshift mutation (*vir*) in their repressor gene that provoke a change of the carboxy-terminal end of the repressor protein. Mu*vir* successfully infect Mu lysogens and induce the resident Mu prophage. Mutant Vir repressors are abnormaly sensitive to the attack by the host ATP-dependent Clp protease and this susceptibility is communicated to the wild type repressor present in the same cell. This provides the Mu*vir* phages with their trans-dominant phenotype. It is the first example of a genetic regulation controlled by the *E.coli* Clp ATP-dependent serine protease and, to our knowledge, the first example of induction of degradation of a wild type protein by one of its mutant forms. It involves the catalytic subunit ClpP but not the ATPase subunits ClpA and ClpB.
In an attempt to further understand this proteolysis mechanism, we have looked for additional host genes required for degradation and analysed the effect of *vir* and wild type repressor on the expression of ClpP.
Since we hypothesized that induction of degradation of the wild type by the *vir* mutant repressors results from the formation of mixed repressor oligomers, we attempted to characterize the repressor domain required for multimer formation.

V 311 COMPARISON OF DECATENATION AND UNKNOTTING ACTIVITIES OF DNA GYRASE AND TOPOISOMERASE IV *iN VITRO* AND *IN VIVO*: . Christian Ullsperger, Arkady Khadursky, Eugene Shekhtman and Nicholas R. Cozzarelli, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

Unknotting and decatenation of covalently closed duplex DNA molecules are reactions specific to enzymes with type 2 topoisomerase activity; i.e., the topological changes require the breaking and rejoining of both strands of a DNA double helix. DNA gyrase has been shown to be an essential enzyme in prokaryotes with well-characterized type 2 topoisomerase activity *in vivo* and *in vitro*. Recently, another type 2 topoisomerase has been identified in prokaryotes, topoisomerase IV. Inhibition of topo IV leads to accumulation of catenanes as the major products of plasmid replication. Also, roughly 10% of the catenanes are knotted, and the isolated knots have a different topology from the knots seen in wild-type cells.

The discovery of topoisomerase IV and its effects on DNA topology opens the door to the possibility that some earlier results implicating DNA gyrase as the primary decatenating and unknotting enzyme in prokaryotes may have been misinterpreted. An important step in correctly establishing the roles of the enzymes *in vivo* is a characterization of their relative efficacy in catalyzing biologically relevant reactions *in vitro*. Decatenation, knotting, and unknotting activities of the enzymes have been examined *in vitro* using purified enzymes. Purified topoisomerase IV readily unlinks isolated replication catenanes formed *in vivo*. The implications of these experiments with respect to the division of labor between topoisomerases in the cell will be discussed.

V 312 CORRELATION BETWEEN ENERGY PROCESSES AND PLASMID DNA TRANSLOCATION OF STAPHYLOCOCCI, Vinnikov A.I.,Gavrilyuck V.G.,Department of Microbiology,State University,Dniepropetrovsk, Ukraine, 320625
The dependence of plasmid DNA transport in Staphylococcus aureus cells on protonmotive force upon cytoplasmic membrane has been studied, during the conjugation process.The process was carried out on Schleicher's and Schuell's membrane filters by the transport of pG873 conjugative plasmid,which posseses the marcer of resistance to gentamycin, from the donor cells (strain 8325-4) to the recipient cells (strain 1055). The generation of protonmotive force on the Staphylococci membrane was supressed by the protonophore uncoupler: m-carbonylcyanide-3-chlorophenylhydrazone (CCCP).As a result the conjugation frequency of treated donor and recipient cells has been decreased 5-20% as compare as control.The decreasing of transconjugant colonies 10% as compare as control has been found in the case of deenergization of cells when H^+-ATPase specific inhibitor N,N'-Dicyclohexylcarbodiimide (DCCD) was used. This effect was observed only when donor and recipient cells were incubated in the presence of DCCD before conjugation process. The preliminary processing of reacting cells by inhibitor of respiratory chain KCN caused insignificant lowering of conjugation frequency. While addition of KCN in the medium of incubation mixture of conjugative cells on membrane filters suppressed the process of conjugation 10% as compare as control.Our results testify that transfer of DNA macromolecules in the conjugation of Staphylococci is the energy dependent process. Both generators of protonmotive force:the respiratory chain and H^+-ATPase take part in the transmembrane transfer of DNA.

V 313 EPITOPE MAPPING OF CELL DIVISION PROTEIN FTS Z IN *ESCHERICHIA COLI* BY MONOCLONAL ANTIBODIES, Jan L. A. Voskuil, Carla A.M.Marres and Nanne Nanninga, Department of Molecular Cell Biology, University of Amsterdam, Plantage Muidergracht 14, 1018 TV Amsterdam, The Netherlands
Cell division protein Fts Z is essential for *E. coli* to initiate the cell wall constriction process, after DNA segregation. Fts Z has been detected both in the cytoplasm and in the membranes. To distinguish different domains that interact with either cytoplasmic or membrane bound elements, we have isolated monoclonal antibodies (Mabs) against Fts Z.
We obtained six Mabs, three of which recognize besides Fts Z an unknown cytoplasmic 50 kDa protein. Apparently, this protein shares an epitope with Fts Z. To characterize the Mab specific epitopes, a gene fusion between *lac Z* and *fts Z* was used to generate C terminal truncations in the fusion protein. Nine of these truncated fusion proteins were screened with the set of Mabs. In addition, purified Fts Z was cleaved chemically by CNBr. By Western blotting we determined which fragments were recognized by the Mabs.
Taken these results together, Mab4 recognizes Fts Z from amino acid 80 to 97, Mab8, Mab13 and Mab18 recognize Fts Z from amino acid 144 to 158, and Mab11 and Mab12 recognize Fts Z from amino acid 337 to 383. The former two epitopes directly flank either side of the hydrophobic region in which the G-box (GTP binding site; De Boer *et al* 1992 Nature **359**, 254-256) is situated, the latter epitope constitutes the carboxy terminus.
These antibodies will be used to study the functional significance of these epitopes. In addition, native immune precipitations may provide information on the domains involved in interactions with other cell division proteins.

V 314 ANALYSIS OF THE *E. coli mre* GENES WHICH REGULATE CELL DIVISION AND CELL ELONGATION
Masaaki Wachi and Kazuo Nagai, Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama, Kanagawa 227 JAPAN.
The *E. coli mre* genes, *mreB*, *mreC* and *mreD*, located at 71 min on the chromosome map, are responsible for formation of normal rod shape of the cells. Two mutants, *mreB129* and *Δmre678*, which show spherical cell shape, altered sensitivity to mecillinam and increase of amount of penicillin-binding protein (PBP)-3, have been reported. *mre-lacZ* fusion analysis showed that these three genes are transcribed as an operon. Moreover, complementation analysis of a *mreB* disruptant and a newly isolated *mreD* mutant indicated that normal expression of the *mreB* gene is required for expression of the downstream *mreC* and *mreD* genes. A stem-and-loop structure between *mreB* and *mreC*, covering a SD sequence of *mreC*, is likely to function as an attenuator. Overexpression of the *mreC* gene caused rounding of the cells and, on the contrary, that of *mreD* caused elongation of the cells. Decrease of amount of PBP-3 was observed in the cell membranes of the *mreC* overproducer. The results indicate that the ratio of expression of the *mreC* and *mreD* genes determines the balance of cell division and cell elongation.

V 315 GROUPING OF RecF PATHWAY RECOMBINATION GENES IN *Escherichia coli* K-12.
Yu-Chin Tseng, Jai-Li Hung and Tzu-Chien V. Wang, Department of Molecular Biology, Chang Gung Medical College, Kwei-San, Tao-Yuan, Taiwan.
Mutations affecting the RecF pathway of recombination (*recF*, *recG*, *recJ*, *recN*, *recO*, *recQ*, *ruvA*, *ruvC*) were systematically introduced into two sets of strains: (a) *uvrA* and *uvrA recA2020*, (b) *uvrA recBC sbcBC* and *uvrA recBC sbcBC recA2020*. We examined: (i) the effect of these mutations on the repair of daughter-strand gaps which are produced in the nascent DNA synthesized after UV irradiation, (ii) the ability of *recA2020* (a suppressor for *recF* mutation) to suppress the UV radiation sensitivity caused by these mutations. In the *uvrA* cells, mutations in *recF*, *recR* and *recO* produced a major deficiency in the repair of daughter-strand gaps, whereas mutations in *recJ*, *recG*, *recN*, *recQ*, *ruvA* or *ruvC* had little or no effect in the repair of daughter-strand gaps. In both *uvrA* and *uvrA recBC sbcBC* backgrounds, the UV radiation sensitivity caused by *recF*, *recR* or *recO* mutation was partially suppressed by *recA2020*, whereas the UV radiation sensitivity caused by *recJ*, *recN*, *recG*, *recQ*, *ruvA* or *ruvC* mutations was not significantly suppressed by *recA2020*. These results further support our previous proposition that the *recF*, *recR* and *recO* gene products (abbreviated as RecRFO) may function together as a complex. It also suggests that this putative RecFRO complex does not contain proteins encoded by other genes involved in the RecF pathway of recombination.

V 316 FLOW CYTOMETRY OF *CAULOBACTER CRESCENTUS*: IDENTIFICATION AND CHARACTERIZATION OF A CELL-CYCLE MUTANT,
Elizabeth A. Winzeler, Pil J. Kang and Lucy Shapiro, Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305
Other researchers have shown that flow cytometry is a powerful method for studying DNA replication in *E. Coli*. We have shown than it can also be an effective tool for the study of the cell cycle in *Caulobacter crescentus*. Like that of eukaryotic organisms, the *C. crescentus* cell cycle can be described in terms of G1, S and G2 phases. The proportions of cells in G1, S and G2 in a log phase culture can be easily determined from a single histogram generated by analyzing a culture of cells stained with the fluorescent DNA-binding dye, Chromomycin A3, on a flow cytometer. This method was used to rapidly analyze a collection of temperature sensitive mutants which had been grown at the nonpermissive temperature prior to analysis. Out of the 60 mutant strains tested, six were distinguished by having very few cells in S phase after the equivalent of two cell cycles at 37°C (the nonpermissive temperature). All cells from these cultures contained either one or two completed chromosomes, indicating an ability to complete, but not reinitiate DNA replication. The six strains were examined further by measuring the rate of incorporation of radio-labeled dGTP into DNA after a shift to the nonpermissive temperature. All six of the strains had a "slow stop" phenotype which is suggestive of initiation of DNA replication or chromosome partitioning mutants. Five of the six mutants also showed a defect in protein synthesis at the nonpermissive temperature. However, one mutant, ntg36, appeared normal with respect to protein synthesis and was examined further. Synchronous cultures of ntg36 do not appear to normally progress through the cell cycle at 37°C, as indicated by the appearance of cell cycle-dependent molecular markers. Furthermore, cell mass increases linearly in a liquid culture of ntg36 at 37°C in the absence of cell division suggesting an increase in cell size. A long filamentous appearance can be confirmed by microscopic analysis of cultures incubated for 10 hours at 37°C. Our results indicate that flow cytometry can be used to rapidly identify cell cycle mutants, defective in the initiation of DNA replication, or chromosome partitioning in *Caulobacter crescentus*.

V 317 ON THE ASYNCHRONY PHENOTYPE OF *fis* MUTANTS,
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When initiation of replication in rapidly growing, wild-type *E. coli* cells is inhibited by rifampicin, completion of ongoing rounds of replication leads to cells containing 2^n (n=0,1,2...) fully replicated chromosomes. Strains lacking Fis (factor for inversion stimulation) protein are shown to yield cells containing aberrant numbers (i.e. $\neq 2^n$) of chromosomes after such runout of replication (Boye et al., 43. Colloquium Mosbach 1992; von Freiesleben and Rasmussen, pers. comm.). This phenotype, termed the asynchrony phenotype, is in some mutants (*dnaA*(Ts), *dam*$^-$) due to defects in the timing of initiation. The phenotype can also arise from specific degradation of single chromosomes (*recA*$^-$; Skarstad and Boye, pers. comm.).

Employing flow cytometry, we have measured the DNA content after rifampicin treatment in different *fis* mutants and wild-type strains, and in a *dam*$^-$ strain. The relative increase in DNA content during runout replication was found to be significantly lower in the *fis* mutants than in the wild-type and *dam*$^-$ cells. Preliminary data indicate that the asynchrony phenotype of *fis* mutants, at least in part, may be explained by a degradation of individual chromosomes.

V 318 EFFECT OF NUCLEOID SEGREGATION ON CELL DIAMETER IN *Escherichia coli*: THE CONCEPT OF SEGREGATION AXES, Conrad L. Woldringh[1], Arieh Zaritsky[2], and Norman B. Grover[3]. [1]Department of Molecular Cell Biology, Section of Molecular Cytology, The University of Amsterdam, Plantage Muidergracht 14, 1018TV Amsterdam, The Netherlands. [2]Department of Life Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Be'er-Sheva, Israel 84105. [3]Hubert H. Humphrey Center for Experimental Medicine and Cancer Research, The Hebrew University Faculty of Medicine, P.O.Box 1172, Jerusalem 91010, Israel

Escherichia coli nucleoids were visualized by fluorescence microscopy of cells fixed with OsO_4, stained with DAPI (4',6-diamidino-2-phenylindole dihydrochloride hydrate), and not dehydrated at any step during the preparation. The various shapes of nucleoids both in rapidly-growing rod-shaped cells and in spheres, were considered to reflect different stages in the concomittant processes of DNA replication and segregation. In newborn cells, two-lobed nucleoids were seen positioned perpendicular to the major axis of the cell; they developed additional lobes, culminating in four-lobed structures at division. The two-lobed nucleoids occurred at angles other than 90° more frequently in longer rods than in spheres. These qualitative observations led to the concept that the chromosome segregates along an axis that is formed at initiation of replication perpendicular to the previous segregation axis. In rod-shaped cells, it is forced by the elongating cylindrical cell to rotate until, eventually, it lies along the major axis of the cell. Development of second-order segregation axes during multi-fork replication requires more space in the radial direction, forcing the cell to extend its diameter. These ideas are brought together in a formal model that succeeds in predicting cell diameter (and thus, cell shape) adequately over a broad range of growth rates.

V 319 INTERACTION OF THE StbB PROTEIN WITH THE CIS-ACTING PARTITION SITE OF THE STABILITY LOCUS OF IncFII PLASMID NR1, David D. Womble, You nong Min, Wei Liu and Robert H. Rownd, Center for Molecular Biology, Wayne State University, Detroit, MI 48202

The stability (*stb*) locus of plasmid NR1 is responsible for partitioning of plasmid molecules to daughter cells during cell division. It is composed of two genes, *stbA* and *stbB*, that encode trans-acting proteins that are essential for stable inheritance, and a cis-acting site that is located upstream from the genes and overlaps their transcription promoter, P_{AB}. Transcription of the *stb* operon is autoregulated by StbB protein. Using deletion analysis and trans complementation tests, the DNA sequence that comprises the cis-acting site was determined. DNA-protein binding studies indicated that StbB protein bound specifically to the sequences in the cis-acting site and protected them from attack by DNase I or hydroxyl radical. The concentration dependence of binding of StbB to the cis-acting site in gel retardation experiments suggested that binding was cooperative. StbB protein from an unstable mutant that contains a nonsense mutation near the 3'-end of the *stbB* gene bound the cis-acting site as well as the wild-type StbB protein. In similar experiments, no evidence for binding of StbA protein to the cis-acting site was obtained, nor did StbA appear to affect the binding of StbB protein.

V 320 THE ISOLATION OF PHAGE T4 MUTANTS ULTRASENSITIVE TO *m*-AMSA: DEFINING A PATHWAY FOR REPAIR OF PROTEIN-DNA CROSSLINKS, Denise L. Woodworth and Kenneth N. Kreuzer, Department of Microbiology, Duke University Medical Center, Durham, NC 27710

Several groups of antitumor drugs and the antibacterial quinolones damage DNA by stabilizing a cleavage complex consisting of topoisomerase covalently linked to cleaved DNA. Evidence from our lab and others suggest that a pathway of recombination can repair this damage and thereby allow survival. We are identifying the components of this repair pathway by isolating phage T4 mutants that are ultrasensitive to the antitumor agent *m*-AMSA. This compound inhibits both mammalian and phage T4 type II DNA topoisomerases, in each case stabilizing the cleavage complex.

In order to look for mutants which are ultrasensitive to the drug *m*-AMSA, we have developed the first transposon system of mutagenesis for T4 (Woodworth, D. L. and Kreuzer, K. N., *Mol. Microbiol.* **6:** 1289-1296 [1992]). Using this system, we isolated several transposon insertion mutants with increased drug sensitivity. One mutant contained an insertion in the gene *uvsX* (encodes DNA synaptase) which was already known to cause *m*-AMSA ultrasensitivity when mutated. A second mutation contained an insertion in *uvsW*, a gene known to be required for recombinational repair of UV damage; *uvsW* mutants had not previously been tested for *m*-AMSA sensitivity. Finally, we found a mutation in the *rnh* gene, which encodes the T4 RNase H, and another in the *αgt* gene, which encodes α-glucosyltransferase. These gene products had not previously been shown to be involved in any recombination or repair. One caution in using transposon mutagenesis is that the phenotype could be caused by a polar effect on downstream gene(s). Therefore, particularly in the cases of the *rnh* and *αgt* mutants, further analysis is needed to rule out polar effects before proceeding. Further experiments are also planned to identify the function of each gene product in the repair of the drug-induced protein-DNA complexes.

V 321 *IN VIVO* AND *IN VITRO* STUDIES ON A COPY NUMBER MUTATION OF THE REPA REPLICATION PROTEIN OF PLASMID PSC101.

Guixian XIA, Danielle MANEN, and Lucien CARO. Department of Molecular Biology, University of Geneva, 30 quai Ernest Ansermet, 1211 Geneva 4, Switzerland

The RepA replication protein of plasmid pSC101 binds as a monomer to three repeated sequences in the replication origin of the plasmid to initiate duplication, and binds as a dimer to two inversely repeated sequences in its promoter region to autoregulate its transcription. A mutation in the protein, *repA(cop)*, affecting a single amino acid, increases the plasmid copy number four-fold. *In vivo* experiments show that, when provided *in trans* under a foreign promoter, the RepA(cop) protein increases the replication of a fragment containing the origin of replication without *repA*, but decreases the repression of its own promoter. *In vitro* experiments show that the purified RepA(cop) protein binds more readily to the repeated sequences within the origin than does RepA and that it forms stable complexes with them while the RepA complexes are highly unstable. Binding to an inversely repeated sequence within the *repA* promoter gives opposite results: the wild type protein binds stably and efficiently to that sequence while the mutated protein binds less stably and less efficiently. The results, together with earlier work, show that RepA does not bind in the same manner to the two types of binding sites in the replication origin and that its affinity for the three repeated sequences in that origin plays an important role in the regulation of plasmid replication.

V 322 USE OF RANDOMLY AMPLIFIED POLYMORPHIC DNA(RAPD) FRAGMENT ANALYSIS FOR THE IDENTIFICATION OF EITHER GENUS OR SPECIES-SPECIFIC GENETIC MARKERS FOR BACTERIA, Ron Grosz, John A. Webster, Mark Barbour, Anna Bossler, Carl J. Noszek, Virginia Petty, George Tice, Heidi G. Zanecosky, and Mark A. Jensen, Central Research and Development Department, E. I. DuPont de Nemours & Company (Inc.), Wilmington, DE 19880 USA

A simple and rapid method has been developed for the identification of conserved regions of DNA that are either genus or species specific. The method consists of performing DNA amplification reactions on individual bacterial genomic DNAs with single 12-base primers of arbitrary sequence followed by the analysis of these reaction products. These amplification reactions produce multiple DNA fragments, collectively referred to as Randomly Amplified Polymorphic DNA (RAPD) markers which, when resolved by electrophoresis, generate product size profiles characteristic of the specific bacterial strain. The profiles are examined for specific products which are uniformly present in the positive panel (the panel of microorganisms to be characterized by a specific genetic marker) and completely absent in the negative panel (a test panel of related organisms which the marker must discriminate against). Once the marker is identified, it is isolated and sequenced. Sequence specific primers are then prepared and used in an amplification reaction to determine whether they are conserved among the target organisms at the desired level of genus or species. Equally important is that they do not result in the generation of amplification products in the non-target organisms. Satisfaction of the above criteria suggest that these primers can be used to carry out a PCR-based determination for the presence of that organism. Because of the extreme sensitivity and selectivity of the PCR process, the necessity for prior isolation and growth of the target organism is eliminated.

V 323 COMPARATIVE DNA TOPOLOGY & CYTOLOGY OF GYRASE AND TOPOISOMERASE IV MUTANTS IN ESCHERICHIA COLI. E. Lynn Zechiedrich and Nicholas R. Cozzarelli, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720

The lethality of mutations in the topoisomerase IV-encoding genes, *parC* or *parE*, has been linked to the accumulation of dimeric plasmid catenanes in bacteria (Adams et al. [1992], Cell *71*, 277-288). We have compared cell cytology and plasmid topology of E. coli topo IV mutants (Kato et al.[1990] Cell *63*, 393-404) to that of a gyrase mutant strain known to form doublet nucleoids (Steck & Drlica [1984] Cell 36, 1081-1088). Using ^{3}H-thymidine pulse labeling, we found that ~65% of the pBR322 plasmids that had replicated were catenated in the *parC* mutant. This level of catenation was dependent on the plasmid type. No plasmid catenanes accumulated in the gyrase mutant. In the gyrase mutants, the cells remained at wild type length and nucleoid size after an hour at non-permissive temperature although the nucleoid was never completely segregated. This suggests that DNA synthesis stops immediately in the gyrase strains and the partially replicated chromosomes are not able to segregate. In contrast, the topo IV mutants were up to ten-fold longer than the wild type or gyrase mutant strains. The nucleoids of the topo IV mutant were either very large, singular and localized to the middle of the cell (*parC*) or were very long and threadlike (*parE*). Since the nucleoid abnormalities and the plasmid catenation occurred at approximately the same time after shift to nonpermissive temperature, it seems that the mechanism(s) that leads to each event is the same. Thus, the plasmid system may be a true reflection of nucleoid segregation. These results are consistent with our previous observation that cytoplasmic doubling and DNA replication were unaffected in the topo IV mutants at nonpermissive temperature. Therefore, topo IV has no role in replication initiation or elongation, as gyrase does, but functions at the end of DNA replication, presumably by decatenating sibling DNA molecules. Supported by NIH grants GM14574 (ELZ) and GM31655 (NRC).

V 324 GENOMIC STRUCTURE, ORGANIZATION, AND REARRANGEMENT IN LEPTOSPIRA. Richard L. Zuerner, NADC, USDA, ARS, P.O. Box 70, Ames, IA 50010

Leptospira spp. are spirochetes which belong to one of the deepest evolutionary branches of eubacteria. Little is known about the genetics of these bacteria. Currently, the structure and organization of Leptospira genomes are being investigated. A physical map of the L. interrogans genome was recently constructed. The L. interrogans genome has two replicons: 4,700 and 350 kb in size. Although most essential genes mapped to date are on the larger replicon, the smaller replicon contains the asd gene, encoding aspartate β-semialdehyde dehydrogenase. This result suggests that the smaller replicon may function as a minichromosome.

Several different classes of repetitive DNA were identified in Leptospira spp. These repetitive DNA elements varied from 4 to over 60 copies per genome. Since repetitive DNA sequences are often sites for recombination, the stability of the Leptospira genome was analyzed. Several genetic rearrangements were detected, some involving large (>200 kb) segments of the genome. Hybridization analysis showed that many rearrangements are located within or near copies of repetitive DNA. The repetitive DNA sequences are being characterized. One of these elements is a 1.5 kb insertion element. Other elements are smaller and may be analogous to the REP or IRU elements described for other bacteria. DNA binding proteins interacting with repetitive DNA have been identified. These studies are aimed at developing an understanding of the mechanisms involved in generating these rearrangements and the consequences of these changes on gene expression.

Late Abstracts

THE POSITIVE REGULATOR CfaD OVERCOMES THE INHIBITORY EFFECT OF THE HISTONE-LIKE PROTEIN H-NS (H1) IN ESCHERICHIA COLI, Bart J.A.M Jordi, Bjorn Dagberg [1], Louise A.M. de Haan, Anja M. Hamers, Bernard A.M. van der Zeijst, Wim Gaastra and Bernt Eric Uhlin [1]. Institute of Infectious Diseases and Immunology, Department of Bacteriology, Faculty of Veterinary Medicine, University of Utrecht, Yalelaan 1, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands and [1]Department of Microbiology, University of Umea, S-901 87 Umea, Sweden

As demonstrated with other temperature regulated genes recently, a mutation in the gene coding for the nucleoid-associated H-NS (H1) protein resulted in derepression of CFA/I expression. CFA/I fimbriae were now expressed both at 20° C and 37°C. More strikingly the positive regulator CfaD was not needed for CFA/I expression in a H-NS negative strain. We also showed that in this H-NS negative strain CfaD still could enhance activity of the promoter of region 1. These data demonstrate two things. First the CfaD protein diminishes an inhibitory effect of the H-NS nucleoid-associated protein and secondly the CfaD protein still has a positive effect on the transcription in the absence of this H-NS protein.

PHYSICAL MAPPING OF BACILLUS CEREUS AND BACILLUS THURINGIENSIS GENOMES.

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Bacillus cereus (B.c.) and Bacillus thuringiensis (B.t.) are closely related, but whereas B.t. is considered a beneficial producer of insecticides and spread by tons over farmland in many countries, B.c. is a main dairy contaminant and several strains produce enterotoxins that causes diarrhoea in man.

To study the relationship among B.c. and B.t. strains, we have embarked upon a study involving establishing and comparing physical maps of their genomes. Complete physical maps based upon pulsed field gel electrophoresis (PFGE) of NotI fragments have been established for 5 B.c. strains and 1 B.t. strain. Genome sizes and NotI "fingerprints" have been established for another 28 strains (17 B.c. and 11 B.t.). Finally, we have analysed all 34 strains for the presence of

A. Genes hybridizing with a probe for the CryIA insecticide gene
B. The Tn4430 transposon previously reported only in B.t. strains
C. Enterotoxin (Oxoid latex agglutination test for B.c. enterotoxin)

Based on these data we conclude that

1. Most B.t. strains investigated carry cryIA-like genes. Also some B.c. strains carry insecticide genes.
2. Some B.c. strains carry the Tn4430 transposon.
3. Almost all B.t. strains produce enterotoxin.
4. Variation in genome size among strains fulfilling usual criteria for B.c./B.t. is more than 2 fold (2.7 - 6.2 Mb).
5. The relationship between B.c. and B.t. strains should be reevaluated.

GENE 5.5 PROTEINS OF COLIPHAGE T7 INHIBIT THE E. COLI NUCLEOID PROTEIN H-NS AND SUPPRESS REX-MEDIATED EXCLUSION BY λ LYSOGENS.

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Gene 5.5 of bacteriophage T7 encodes two proteins through frame shifting in a single transcript. Gene 5.5A protein is synthesized from the initial reading frame and consists of 98 amino acid residues. Gene 5.5B protein is made by shifting the reading frame of gene 5.5A protein to the -1 frame at codon 88 and then joining the gene 5.7 reading frame, resulting in a protein of 168 amino acid residues.

During the purification of gene 5.5A protein, we noticed that an *E. coli* protein purified together with gene 5.5A protein consistently. This 16-kDa protein was identified by protein sequencing as the nucleoid protein H-NS. Overexpression of gene 5.5A protein in *E. coli* leads to derepression of the *proU* promoter repressed by H-NS protein. In addition, a fusion protein of the maltose binding protein and gene 5.5A protein suppresses the inhibitory effect of H-NS protein on transcription by T7 and *E. coli* RNA polymerases. The fusion protein also binds to DNA/H-NS complexes. Furthermore, an *E. coli* strain with a mutation in the promoter region of the *hns* gene restricts the growth of T7 phages lacking gene 5.5A. This defect of T7 mutant phage can be complemented by a plasmid containing gene 5.5B but not by a plasmid containing only gene 5.5A, indicating the function of gene 5.5B is essential in this *hns* mutant of *E. coli*.

Another function of gene 5.5 is to suppress the rexAB-mediated exclusion of heterogous phages by λ lysogens. Similar to the much studied T4 rII mutants, a T7 mutant with a leucine to proline change at position 30 of gene 5.5A protein is unable to grow in λ rex$^+$ lysogens of *E. coli*. This T7 defect can be complemented *in trans* by wild type gene 5.5A but not by gene 5.5B. Furthermore, gene 5.5A can complement T4 rII mutants in growth in λ rex$^+$ lysogens. Plasmids expressing rexAB proteins can not co-exist with plasmids expressing gene 5.5A. Such incompatibility can be overcome by altering plasmid copy number or changing the level of gene expression, suggesting direct interactions between the two proteins. Implications of the dual functions of gene 5.5 will be further discussed.