BACTERIAL CHROMOSOMES

Organizers: Nancy Kleckner and John Roth

January 6-12, 1995; Santa Fe, New Mexico

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Genome Structure and Evolution

A2-001 CHROMOSOME REARRANGEMENTS -- FORBIDDEN INVERSIONS AND ADAPTIVE DUPLICATIONS

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The bacterial chromosome appears to be conserved on an evolutionary time-scale, suggesting that selection operates against rearrangements. Yet, on a short time-scale recombinational repair is highly active and duplications are formed and segregated at high frequency. We suggest that repair and reversible duplication formation are selectively valuable processes and that inversions destabilize the map. We suggest that the recombination system discriminates against inverse-order sequences and thereby minimizes inversion formatior while maintaining the advantageous ability to perform sister strand exchanges between direct repeats. Inverse order sequences, added to particular positions in the bacterial chromosome, are able to exchange information, bu do not generate an inversion (forbidden intervals). We have tested the idea that inversions require RecBCD and that some chromosomal sites lack sequences needed for RecBCD function (breaks, chi). We think this model is eliminated because forbidden intervals do not revert when breaks are introduced (X-rays) and chi sites are sidestepped (recD). Furthermore the dependence on RecBC is variable from one permissive interval to another, suggesting that there are RecBC-independent modes of inversion formaton. For permissive intervals, the combination of a recB and a recF mutation reduce inversion formation 100-fold while either individual mutation causes only a 2-4 fold effect. Thus both recombination pathways operate without the need for sbcBCD suppressors. Duplications (and deletions between large repeats) show a similar rec dependence.

Duplications are frequently selected in stressed cell populations. We have developed a model for adaptive mutation that involves amplification of the gene under selection. In principle, this model can explain how bacteria appear to direct mutability to particular (growth-limiting) sites. We have begun to test this model and have found evidence that much of the reported phenomenology of "adaptive mutation" may reflect complexities in the behavior of the F-factor. We avoid these problems by concentrating on analysis of chromosomal mutations.

Replication Initiation I

A2-002 REGULATION OF *ORIC* INITIATION IN *ESCHERICHIA COLI*, Erik Boye, Kirsten Skarstad, and Sture Wold, Department of Biophysics, Institute for Cancer Research, Montebello, 0310 Oslo, Norway.

Initiation of chromosomal replication is a tightly regulated event, normally occuring once per cell cycle at a defined cell age. Two aspects of initiation control must be considered: (i) Cell age at initiation varies with the growth conditions, reflecting the essential coupling between DNA replication and general cell growth. It is known that increasing the intracellular DnaA concentration causes initiation to occur at a lower cell age[1]. It has long been accepted that the initiation mass is constant and independent of the growth rate[2], but recent measurements show that the initiation mass is indeed varying with growth rate[3]. (ii) When two or more copies of chromosomal *oriC* are present in the same wild type cell they are initiated simultaneously[4]. This synchrony of initiation is absent in certain *dnaA*(Ts) mutants and in *dam, seqA, fis,* IHF $^-$, *rpoC*, and *dnaC*(Ts) mutant strains. Also, changing certain non-essential parts of *oriC* may produce asynchronous initiations. A prerequisite for synchronous initiations is that initiated origins are sequestered and protected from a second round of initiation in the same cell cycle. This sequestration is dependent upon Dam[5] and SeqA[6] functions.

To study the two aspects of replication control we have grown the different strains on poor carbon sources, yielding doubling times of 100 min or more. Under such conditions, all strains studied were born with one origin, which was initiated at a defined time in the cell cycle. Thus, some strains that displayed the asynchrony phenotype at higher growth rates were able to properly set the time and cell age for initiation of their single origin; they were virtually indistinguishable from their wild type parent. The ability to set the time for and perform initiation at a cell age corresponding with growth rate can therefore be distinguished from the ability to perform synchronous initiations at high growth rates. Interestingly, flow cytometry revealed that a large fraction of the slowly-growing cells containing a single chromosome contained two fully replicated chromosomes after run-out of replication in the presence of rifampicin. The data suggest that these cells have passed the rifampicin-sensitive stage of initiation, but if replication forks have been formed they have not travelled far from *oriC*.

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[3]Wold S, Skarstad K, Steen HB, Stokke T, and Boye E (1994) EMBO J. **13**, 2097-2102.
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[6]Lu M, Campbell JL, Boye E, and Kleckner N (1994) Cell **77**, 413-426.

A2-003 ROLE OF *E. COLI* SEQA PROTEIN IN DNA REPLICATION INITIATION. Steve Slater[1], Min Lu[1], Erik Boye[2], and Nancy Kleckner[1].
[1]Dept. of Molecular and Cellular Biology, Harvard University, Cambridge; [2]Dept. of Biophysics, Inst. for Cancer Research, Oslo, Norway.

Initiation of chromosomal replication in *E. coli* occurs at a unique site, *oriC*. The time between initiation events is regulated in response to growth rate and coordinated with other cell cycle events, such as chromosome partitioning and cell division. The precise control of initiation requires at least two processes. First, an initiation signal must occur at the correct point in the cell cycle. Second, the origin must be sequestered from initiating secondary rounds of replication. We have previously described the identification of *seqA*, a gene that is required for normal sequestration of *oriC* (1). Additional genetic observations suggest that *seqA* functions, directly or indirectly, as a negative regulator of replication initiation *per se*.

Purification of SeqA Protein. SeqA has been overproduced and purified essentially to homogeneity as judged by analysis on a silver stained polyacrylamide gel. The final preparation elutes from a gel filtration column as a higher order oligomer. Gel shift analysis demonstrates that purified SeqA protein binds specifically to hemimethylated *oriC* DNA, and with much lower affinity to fully methylated *oriC*. No binding to unmethylated *oriC* was detected. SeqA has also been purified by S. Austin and colleagues as a factor that binds to the phage P1 replication origin.

Membrane Binding of *oriC*. Binding of *oriC* fragments to partially-purified membrane fractions (2, 3) has been assayed using an unpublished gel shift protocol provided by L. Rothfield and colleagues. Membrane fractions isolated from a *seqA* null mutant are proficient for specific binding of hemimethylated *oriC*. We refer to the corresponding activity as OMB (Origin Membrane Binding). Thus, *E. coli* encodes at least two activities capable of interacting preferentially with hemimethylated *oriC*. Herrick et. al. (4) have recently described HobH protein, which specifically binds hemimethylated *oriC* and can inhibit DNA replication initiation. HobH is not SeqA, but it may be OMB.

Hypersequestration. Overproduction of SeqA from a *Pmac* fusion construct leads to the accumulation of elongated cells containing one or two centrally-positioned nucleoid masses; small cells lacking DNA also appear. The *oriC* regions in such cells are hemimethylated, suggesting that the overproduction of SeqA in this situation has blocked "de-sequestration". We suggest that, in wild type cells, SeqA-bound hemimethylated *oriC* is passed along to the OMB and that the presence of excess SeqA can block this transition. We have previously demonstrated that multicopy plasmids encoding a wild type *seqA* gene have no effect on growth of a wild type strain and specifically inhibit growth of mutant strains compromised for replication initiation (1). The effects of the *Pmac* fusion are qualitatively different: growth of both wild type and replication-compromised mutants are reduced similarly, to an intermediate level. The relationship between the "hypersequestration" and "initiation inhibition" phenotypes remains to be determined.

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4. Herrick, J., R. Kern, S. Guha, A. Landoulsi, O. Fayet, A. Malki and M. Kohiyama. 1994. EMBO J. *in press*.

A2-004 MECHANISM OF HEMIMETHYLATED oriC ATTACHMENT TO MEMBRANE, John Jerrick, Renée Kern, Sibajyoti Guha, Ahmed Landoulsi, Olivier Fayet*, Abderrahim Malki, Jean Meury and Masamichi Kohiyama. Inst. Jacques Monod - CNRS-Université Paris 7, Paris ; *CNRS, Toulouse.

The replication origin of E.coli chromosome (oriC) interacts with the outer membrane only in the hemimethylated state (Ogden et al., 1988). This interaction was analysed by footprinting experiments with the following results ; i) it occurs mainly at the left half moiety of oriC where Dam sites are concentrated ; ii) at minimum inhibitory concentrations of the membrane for in vitro oriC replication two regions flanking the IHF binding site are preferentially recognized, thus preventing bending of oriC ; iii) specific labelling of a defined oriC fragment either at the methylated (parental) strand or unmethylated (daughter) strand allowed us to demonstrate a higher affinity of the membrane for methylated strand ; iv) NEM reverses the interactions more easily at the unmethylated strand side suggesting a loose binding of unmethylated strand to membrane which facilitates access for Dam methylase ; v) neither 5' parental nor 3' parental specific binding site is clearly seen. We anticipated that this specific interactions would require specific proteins. Southwestern blotting analysis of membranes using a hemimethylated oriC probe reveals a major 24KD protein especially enriched in the outer membrane of Ogden et al. with minor 16KD and 50KD proteins. Using a filter binding assay, a clone has been selected from an expression genomic library. It contained the 3'-end of a new gene called hobH (hemimethylated oriC binding) whose entire nucleotide sequence revealed 175 amino acids (92 min). A purified lacZ-HobH fusion protein attaches to the two preferential binding regions of oriC for the membrane, demonstrating that the HobH protein itself has hemimethylated oriC binding activity. A hobH mutant has been obtained ; it manifests abnormal division behavior although the mutation is not lethal. The synchrony of initiation examined by flow cytometry (Boye et al., 1990) has been shown to be perturbed partially in the hobH mutant. The mutant has an increased frequency of spontaneous mutations yet less than a dam mutant. This suggests that hemimethylated Dam sites close to replication fork are protected transiently by the Hob protein.

Replication Initiation II

A2-005 BIOCHEMICAL AND MUTATIONAL ANALYSIS OF *Escherichia coli* DnaA PROTEIN, Jon M. Kaguni[1], Kevin Carr[1], Mark Sutton[1], Carla Margulies[1], and Jaroslaw Marszalek[1,2], [1]Department of Biochemistry, Michigan State University, East Lansing, MI 48824-1319, Department of Biophysics, University of Gdansk, Poland.

DnaA protein of *Escherichia coli* participates in the initiation of bacterial replication by binding to sequences, DnaA boxes, present in the chromosomal origin region. Subsequent to binding, it directs the binding of DnaB protein that acts as the replicative helicase to advance the replication fork. The requirement for ATP at 1-5 mM for replication activity correlates with a conserved a P-loop motif shared by homologs of DnaA protein, and the ability to bind ATP with high affinity.

In structure-function studies, the role of ATP in DnaA protein function has been examined by the study of mutants in the P-loop motif. These mutations render the protein temperature-sensitive in replication activity. Additionally, some P-loop mutations result in a dominant negative phenotype when the mutant alleles are present on a moderate copy number plasmid under control of their own promoter. Other results suggest that the N-terminal and C-terminal domains of the protein are involved in DNA binding.

The complex of DnaA protein bound to the chromosomal origin was previously estimated to contain 20-30 molecules in a nucleosome-like structure. We examined the nature of binding of monomeric DnaA protein to the chromosomal origin and found that it binds to the four DnaA boxes in an ordered and sequential manner. These results will be compared to the aggregate form of DnaA protein and a model of the initiation process.

A2-006 INITIATION OF DNA REPLICATION AT *oriC* AND ELSEWHERE, Tokio Kogoma, Departments of Cell Biology and Microbiology, University of New Mexico Health Sciences Center, Albuquerque, NM 87131.

Strand separation is a prerequisite to the events leading to the initiation of chromosome replication. There are at least three known mechanisms of strand separation (1): i) the initial melting by DnaA binding at *oriC*, ii) strand displacement by D-loop formation at *oriM*, and iii) strand displacement by R-loop formation at *oriK*. We will summarize our recent findings on these three types of initiation.

oriC: The minimal *oriC* sequence was defined by the *polA* assay, i.e., the ability of *oriC*-pBR322 chimeric plasmids to replicate in *polA* strains. Since the strand separation depends on the local supercoiling, and supercoiling can be significantly different in the chromosome and plasmid, we have undertaken the reexamination of the minimal *oriC* on the chromosome. Using a lambda phage system we have systematically introduced various modifications at the *oriC* site on the chromosome (2). We have found that the chromosomal *oriC* can tolerate a large insertion at the *Hin*dIII site between DnaA boxes R3 and R4, as well as a 41-bp deletion which removes R4. These *oriC* mutants grow well with slightly slower growth rates compared to $oriC^{+}$. The effects of inactivating the *gidA* promoter singly or in conjunction with the *mioC* promoter will also be discussed.

D-loop: Initiation at *oriMs* has been postulated to be triggered by a double-strand break (DSB). The end at DSB is processed by RecBC(D) and assimilated into an intact homologue by RecA, leading to formation of a D-loop, an intermediate in homologous recombination. We have demonstrated that artificially generated DSBs can trigger extensive DNA replication in a manner dependent on RecA and RecBC(D) (3). Furthermore, initiation at *oriM* is dependent on $PriA^{+}$ (4). Finally, *pri*A mutants are defective in homologous recombination and are very sensitive to γ-rays and mitomycin C, indicating a defect in DSB repair (5). We will discuss a model which postulates an involvement of extensive DNA replication in the processes of homologous recombination and DSB repair.

R-loop: Initiation has been proposed to occur at a certain specific type of persistent R-loops (*oriKs*), giving rise to cSDR. How R-loops are generated or what factors are involved in the process is unknown. We have found that the combination of an *rnhA* and a *recG* mutation is lethal to the cell and that *recG* single mutants exhibit cSDR as do *rnhA* mutants (6). We will entertain a model that persistent R-loops can be generated by assimilation of a nascent RNA transcript into the duplex by the action of RecA and that the RecG helicase activity opposes this RecA action. Initiation from R-loops i s also primed by a ΦX174-type priming system which requires $PriA^{+}$.

References:

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A2-007 REPLICATION INITIATION IN BACTERIOPHAGE T4, Kenneth Kreuzer, Kelly Carles-Kinch, Rita Schmidt, Laura Weislo, and Marion Saunders, Department of Microbiology, Duke University Medical Center, Durham, NC 27710.

Bacteriophage T4 utilizes two distinct strategies for the initiation of DNA replication. Origin-dependent replication requires discrete origin DNA sequences (but does not require recombination proteins), while recombination-dependent replication requires phage-encoded recombination proteins (but does not require origin sequences).

We have subdivided T4 origins into two components necessary for maximal replication: a middle-mode promoter and a region downstream of the promoter. The downstream region is a DNA-unwinding element, because it displays hypersensitivity to a ssDNA-specific nuclease when tested within supercoiled plasmid DNA in vitro. Further, the propensity to unwind is likely important for DNA replication in vivo, because the native downstream region can be replaced with a heterologous DNA-unwinding element (from pBR322) without loss of replication activity. Two important results have emerged from an analysis of DNA unwinding in vivo. First, we found that the non-template strand (but not the template strand) of the origin downstream region is hypersensitive to permanganate (ssDNA-specific reagent). We infer that a stable RNA-DNA hybrid exists at the 5' end of the origin transcript, and we presume that this stable hybrid is important for replication initiation. Second, neither the DNA-unwinding element from the T4 origin nor that from pBR322 displays permanganate hypersensitivity in constructs without the nearby origin promoter. Thus, stable unwinding in vivo requires either the act of transcription or the continued association of the transcript with the unwound DNA. We speculate that the presence of the DNA-unwinding element just downstream of the promoter interferes with the normal ejection of RNA from the transcription complex, leading to a stable hybrid. The role of the origin transcript in replication initiation is still unclear: it may function as a primer for leading-strand synthesis or it may activate replication by exposing the nontemplate strand for primase function.

We are also analyzing the mechanism of T4 recombination-dependent DNA replication. We have shown that a site-specific double-strand break triggers recombination-dependent replication of a homologous DNA molecule during a T4 infection. This result confirms an important prediction of the reigning model for T4 recombination-dependent replication (formulated by G. Mosig) and provides a site-specific version of the process for detailed molecular analysis. We are currently using a variety of 2-dimensional gel methods to analyze possible intermediates in the double-strand break-directed DNA replication.

A2-008 PROPERTIES OF DnaA PROTEIN AND ITS ROLE IN *E. coli* REPLICATION INITIATION, Walter Messer, Margret Krause, Uwe Langer, Angelika Roth, Sigrid Schaper, Christoph Weigel, Max-Planck-Institut für molekulare Genetik, Berlin-Dahlem, Germany.

DnaA protein binds non-palindromic 9 bp consensus sequences, DnaA boxes, which are present four times in the replication origin, *oriC*, as well as in many other, mostly regulatory, regions. In order to understand the interaction of DnaA with *oriC* in the initiation complex we quantified the binding of DnaA to DnaA boxes by gel retardation experiments, using purified DnaA protein and oligonucleotides containing different members of the consensus sequence. Dissociation constants for specific binding ranged between 3 nM and 60 nM. Both the actual sequence and the sequence context of the DnaA box determined the dissociation constant. K_D for unspecific binding was >200 nM. Oligonucleotides (21mers and 39mers) formed a single complex with DnaA protein. Oligomeric complexes with different numbers of DnaA protein monomers accumulated at single DnaA boxes on longer DNA fragments (320 bp).
DnaA protein does not contain one of the known DNA binding motifs. In order to determine the DNA binding domain we constructed protein fusions to β-galactosidase. These fusions were probed with *oriC* DNA in a sandwich containing in addition a biotinylated monoclonal anti-β-galactosidase antibody and streptavidin coated magnetic beads: solid-phase DNA binding assay (SPDB). DNA binding was found to reside in the 94 C-terminal amino acids of the *E. coli* DnaA protein. Fusions with DnaA proteins from mutants which map in this region (*dnaA*204, *dnaA*205, *dnaA*211) were inactive in DNA binding.
Mutations were introduced into the DnaA boxes in *oriC* by oligunucleotide-directed mutagenesis. DnaA binding to the different DnaA boxes was either partially or completely abolished. All these mutants have a functional origin. Combinations of mutations in two or three DnaA boxes impaired origin function. Mutations in all four boxes inactivated *oriC*. SPDB was used to quantify DnaA binding to mutated origins, and to relate it to their *in vivo* function. Mutations that alter the distance between DnaA boxes have a much greater effect on *oriC* function. The phenotypes of both types of mutants suggest that the *oriC*/DnaA complex has a compact and ordered structure.
The tight initial complex between *oriC* and DnaA results in the partial unwinding in the AT-rich left part of *oriC*: open complex. The binding of histone-like proteins HU, FIS and IHF assists in this process. These proteins are required at *oriC* in *cis*. Mutations in the specific binding sites for IHF and FIS inactivated *oriC* function on plasmids and is known to impair the control of chromosomal initiation.
The interplay between *oriC*, DnaA and assisting factors will be discussed.

Recombination and Repair

A2-009 *E coli* FUNCTIONS FOR HOMOLOGOUS RECOMBINATION, Robert G. Lloyd, Gary J. Sharples, Matthew C. Whitby, Lizanne Ryder, Akeel A. Mahdi, Simon D. Vincent, and Sau N. Chan, Genetics Department, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

Recombination serves continually to shape, and reshape, the genomes of all organisms and to promote repair of damaged DNA. The genetic control of this process has received particular attention in *Escherichia coli* where more than twenty genes have been identified, and where the activities associated with the products of these genes can be related to specific stages in recombination. These studies support models of recombination based on the formation and subsequent resolution of Holliday junctions. They have also identified additional recombination reactions involving annealing of complementary single-stranded DNA, or the priming of DNA replication forks following invasion of duplex DNA a by 3' single-stranded DNA end.

The activities of RecA protein have provided insights into the homologous pairing and strand exchange reactions that lead to the formation of Holliday junctions. Studies of RecBCD enzyme and of its interactions with Chi sequences have shown how exchanges may initiate at DNA ends through nuclease and DNA helicase activities that provide the ssDNA substrate for RecA to initiate a search for homology. RecJ nuclease, RecQ helicase and the RecFOR proteins have accessory roles that help RecA. Processing Holliday intermediates into mature products involves RuvAB, RuvC and RecG proteins. RuvA has a high affinity for Holliday junctions and targets the assembly of RuvB hexamer rings. These two proteins together drive branch migration of the junction to extend the heteroduplex joint formed by RecA. RuvC is a sequence specific endonuclease that is able to cleave junctions and resolve the Holliday intermediate into recombinant products. Genetic evidence suggests that resolution *in vivo* depends on RuvAB activity, which may reflect the need to position the junction at sequences recognised by RuvC.

Null mutations in the *ruv* genes confer sensitivity to DNA damage, but do not block recombination in conjugational crosses. We have shown that this is due to the activity of RecG. Like RuvAB, RecG binds to a Holliday junction and dissociates the structure by catalysing branch migration. Recent studies have shown that RecG is a junction specific DNA helicase. The unwinding of partial duplex helicase substrates proceeds with a distinct 3' to 5' polarity, which is the reverse of that catalysed by RuvAB. This difference may account for the observation that in strand exchange reactions catalysed by RecA *in vitro*, RuvAB helps to drive the reaction in the direction initiated by RecA, whereas RecG appears to hold up the reaction and dissociate intermediates to substrate molecules. This reverse branch migration activity could in theory remove junctions from DNA *in vivo*. It may explain the ability of RecG to promote repair of UV-damaged DNA in strains lacking any specific activity for junction cleavage. We have recently identified a new resolvase called Rus that cleaves Holliday junctions in much the same way as RuvC, though it has a different sequence specificity. Overexpression of Rus protein allows efficient recombination and DNA repair in the absence of both RuvAB and RuvC by a process that depends to some extent on RecG. However, Rus can also improve repair in strains that lack RuvAB, RuvC, and RecG. Conversely, overexpression of RecG improves repair in strain lacking RuvC and Rus. A model for the repair of UV-damaged DNA incorporating alternative pathways for the resolution of recombination intermediates will be presented.

A2-010 PHAGE λ RECOMBINATION IN VIVO, Stuart Hill[1], Andrei Kuzminov[2], Richard Myers[2], Mary M. Stahl[2], Trudee Tarkowski[2], Lynn C. Thomason[2], and Franklin W. Stahl[2], [1]National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Montana 59840, [2]Institute of Molecular Biology, University of Oregon, Eugene, OR 97403-1229.
Properties of genetic recombination by λ in its lytic cycle will be examined for each of several recombination pathways. These will include, but not be limited to, the roles of λ's double-chain cut site, *cos*, in recombination.

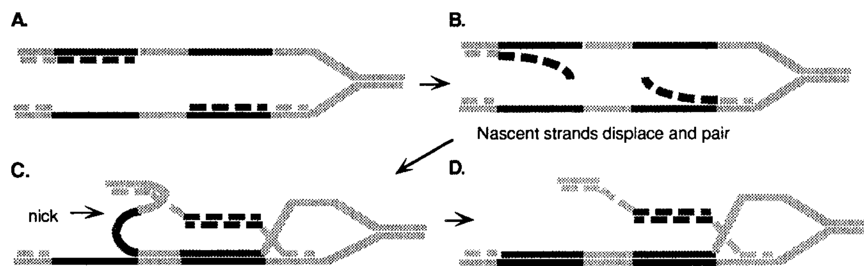
Elongation and Termination of Replication

A2-011 THE 3-DIMENSIONAL STRUCTURE AND MECHANISM OF ACTION OF A REPLICATION TERMINATOR PROTEIN, Deepak Bastia, Dirksen E. Bussiere, Bidyut Mohanty, Trilochan Sahoo, Sadashiva Pai, Sunil Kaul, and Stephen W. White, Department of Microbiology, Duke University Medical Center, Durham, NC 27710.

The crystal structure of the replication terminator protein (RTP) of B. subtilis has been solved to 2.5 Å. The protein crystallizes as a dimer and has an unordered N-terminal DNA-binding domain followed by three small α-helical regions, two β sheets connected by a loop and a longer C-terminal, antiparallel coiled-coil, dimerization domain. RTP functions in E. coli in vivo and in vitro and is a polar contrahelicase to DnaB replicative helicase but fails to antagonize the activities of helicase I and helicase II of E. coli, that are involved in chromosome transfer and DNA repair. Using a variety of techniques, we have established that the molecular basis of polar contrahelicase activity is not steric hindrance by RTP and the ter protein of E. coli, imposing a barrier to helicase movement by forming a nucleoprotein complex. The contrahelicase activity is potentiated by specific protein-protein interaction between DnaB and RTP (or ter). Interestingly, ter protein antagonizes the helicase activity of SV40 T antigen by directly binding to the viral protein. The contrahelicase domains have been mapped by using the M13 phage epitope display and in vivo biotinylation of the interacting proteins. The physiological significance of the helicase-contrahelicase interaction, especially with regard to SV40 T antigen, will be discussed.

A2-012 RECOMBINATION BETWEEN REPLICATING SISTER-CHROMOSOMES, Susan T. Lovett, Catherine J. Saveson, Paola T. Drapkin, Vincent A. Sutera, Jr., Tyler J. Gluckman and Peter J. Simon. Department of Biology and Rosenstiel Basic Medical Sciences Center, Brandeis University, Waltham, MA 02254-9110.

Deletion mutations in *E. coli* do not occur at random but between short repetitive sequences. Because deletions arise independently of the *recA* gene, recombinational mechanisms for deletion formation have generally been discounted. However, our studies of *recA*-independent deletion of repeated sequences support a recombinational model for deletion formation. We propose that aberrant "post-replication" repair events between replicating sister-chromosomes, with misalignment of strands at repetitive sequences, result in deletions or expansions of repetitive sequences. Such events, we propose, are triggered by blocked replication forks. The genetic basis for the deletion process is unknown and is presently under investigation.



Sister-strand exchange mechanism for deletion formation. Pairing of nascent strands in a blocked replication fork to yield deletion and crossing-over at the fork.

A2-013 TOPOISOMERASES AND DNA REPLICATION.
Hiroshi Hiasa, Hong Peng, and Kenneth J. Marians. Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Theta-type DNA replication, whether of a plasmid DNA or the bacterial chromosome, requires the action of topoisomerases at several stages in order to ensure the generation of two completed daughter molecules. During initiation, superhelicity is required. During chain elongation, the positive superhelicity that builds up must be removed. And at the terminal stages, the daughter chromosomes must be topologically resolved. We have used *oriC* plasmid DNA replication reconstituted with purified *E. coli* replication proteins to examine the ability of the four *E. coli* topoisomerases to act at each of these stages.

Only DNA gyrase is capable of generating the superhelicity needed for initiation. DNA gyrase can also support nascent chain synthesis. Both topoisomerases III and IV will support DNA chain elongation in the absence of DNA gyrase. Of the three enzymes, Topo III is actually the most efficient. Topo I is incapable of acting at this stage. Of the four topoisomerases, only Topos III and IV will decatenate the replicating daughter DNA molecules to yield monomer product. Both DNA gyrase and Topo I are incapable of this task.

A2-014 SUBUNIT DYNAMICS IN THE MULTIPROTEIN REPLICASE OF *E. COLI*, DNA POLYMERASE III HOLOENZYME
Mike O'Donnell, Linjua Fang, Jeff Finkelstein, Zvi Kelman, Vytautas Naktinis, Rene Onrust, P. Todd Stukenberg, Jennifer Turner, Hui Xiao, Nina Yao, Microbiology Department and Howard Hughes Medical Institute, Cornell University Medical College, 1300 York Avenue, NY, NY 10021

The 10 subunit replicase of *E. coli*, DNA polymerase III holoenzyme (Pol III), couples hydrolysis of ATP to a tight grip on primed DNA. The molecular basis underling the high processivity is the β subunit, a ring shaped dimer that completely encircles DNA and freely slides along the duplex. This β-sliding clamp acts to tether the Pol III machinery to DNA for highly processive synthesis. The β clamp does not assemble onto DNA by itself, for this it requires the 5 subunit γ complex, which functions as a "clamp loader" to couple ATP to assemble β around primed DNA. A protein ring surrounding DNA generalizes to the PCNA accessory factor of the eukaryotic replicase and the gene 45 protein (g45p) of the T4 phage. The clamps of all three systems have been compared. PCNA is very similar to *E. coli* β, it binds DNA tightly and the crystal structure shows it is a ring with the same folding pattern as β. The T4 g45p is unlike β and PCNA in being unstably bound to DNA. Comparison of the Kd for the ring structure shows their tightness in the order: β > PCNA > g45p, consistent with their stability on DNA.

How are these ring proteins assembled around DNA. No one subunit of the 5-subunit γ complex clamp loader can assemble the β ring around DNA. Studies using individual subunits of the clamp loader in the *E. coli* system were obtained through use of subunit genes. These studies using pure subunits (and mutant subuntis) identify those that are required for this process and the contribution of ATP to the mechanism.

This picture of a polymerase with a sliding clamp riding along behind fits nicely for continuous polymerase action on the leading strand. However, too tight a grip on DNA would hinder action on the lagging strand which is synthesized as a series of Okazaki fragments. Here, Pol III must be capable of hopping rapidly from one fragment to another. Study of this hopping reaction reveals a novel mechanism whereby Pol III "knows" when it has completed a DNA chain and then it rapidly separates from its sliding clamp and reassociates with a new sliding clamp on another primed template.

The C-terminal 5 residues of β protrude from one face of the ring and these residues are essential to activity, having their basis in interaction with the γ complex and with the polymerase. In fact, point mutants and a "protein footprinting" assay reveal that the same residues on the β ring that the clamp loader binds, are also used for polymerase interaction. Indeed, these two assemblies compete for binding the β ring. Such internal competition within this multiprotein assembly is needed on the lagging strand for the coordinated actions of assembling the ring onto DNA, followed by processive extension, and then finally removal of the polymerase and then dissassembly of the β ring from the DNA for reuse on future Okazaki fragments.

All ten subunits of Pol III have been overproduced individually and purified in quantity. These subunits have been used to learn how to assemble the Pol III particle. The assembly of Pol III requires a defined staged order of subunit addition. From the assembly process, combined with structural studies of the holoenzyme, the overall organization of subunits in Pol III can be deduced. The structure of Pol III, together with its actions in loading clamps, using clamps for processivity, hopping from one clamp to another upon completing DNA, and recycling the clamps, converge to explain how Pol III replicates both strands of a chromosome in a tightly coordinated fashion.

Site-Specific Recombination and Transposition

A2-015 Tn7: TARGET SELECTION & TRANSPOSITION IMMUNITY
Nancy L. Craig, Howard Hughes Medical Institute, Johns Hopkins School of Medicine, Baltimore, MD 21205

The bacterial transposon Tn7 is distinguished by its ability to participate in two distinct recombination pathways which promote element insertion into different classes of target sites. In one pathway, the Tn7-encoded transposition proteins TnsABC+D promote insertion into a specific site in the E. coli chromosome called attTn7; a distinct but overlapping set of Tns proteins, TnsABC+E promote insertion into other sites unrelated to attTn7. Thus TnsD and TnsE are alternative targeting proteins which act in conjunction with and modulate the core TnsABC machinery. A feature common to target selection in both the TnsD and TnsE pathways is that the frequency of Tn7 insertion into a target DNA molecule is much decreased by the presence of Tn7 in that molecule. Thus Tn7, like bacteriophage Mu and Tn3, displays target or transposition immunity. An intriguing feature of Tn7 target immunity is that it can act over considerable distance: the presence of Tn7 at a location in the E. coli chromosome more than 190kb from attTn7 can substantially decrease the frequency of subsequent Tn7 insertions into attTn7.

Global Chromosome Structure

A2-016 LONG RANGE INTERACTIONS IN PHAGE AND BACTERIAL CHROMOSOMES IN VIVO, N. Patrick Higgins[1], Katherine E. Kézdy[1], Xiuhua Wang[1], and Martin L. Pato[2], [1]Department of Biochemistry and Molecular Genetics, University of Alabama at Birmingham, Birmingham AL 35294, [2]Department of Microbiology, University of Colorado Medical School, Denver, CO 80262.

Efficient replicative transposition in bacteriophage Mu requires transposase binding sequences located near the left and right borders of viral DNA, a transposase activator site about 1 kb from the left end, and a high affinity DNA gyrase binding site located at the center of the virus (1). The role of gyrase in Mu transposition reactions has been examined to find out how crucial for transposition is the occupancy of the high affinity central site by gyrase. Replacement of the high affinity site with lower affinity gyrase sites did not allow efficient transposition. Movement of the strong gyrase site to positions away from the center toward either the right or left end of the phage chromosome caused dramatic decreases in Mu replication efficiency (2) One explanation for this position effect is that movement of the high-affinity site away from the normal position toward either end leads to impaired binding and subsequent poorer supercoiling by gyrase. However, gyrase occupancy of the high-affinity site was not influenced by its position in Mu as measured by an in vivo quinolone induced gyrase cleavage assay. Molecular analysis of the block in transposition when a central gyrase molecule is absent or out of position is consistent with gyrase facilitating the alignment of left and right transposase binding sites into an interwound "plectosome." One model is that Mu forms a supercoiled domain using a centrally anchored gyrase molecule to persistently supercoil the DNA. Waves of supercoiling induce sites equi-distant from the anchored gyrase molecule to be interwound together. In this model, the loops of interwound supercoils, like those pictured in unfolded nucleoids by Kavenoff & Bowen (3), could be organized from the loop ends rather than from the loop base. Tests of the long-range interwound structure of phage Mu and bacterial chromosomes will be discussed.

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A2-017 DNA BINDING OF PROTEINS IN BACTERIAL NUCLEOIDS. David Pettijohn, Vara Vissa, Vern Shellman and John Cann. Department of Biochem./Biophysics/Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262.

The effects of high DNA-packaging densities on the targeting of DNA-binding proteins are being investigated. The HU protein:DNA interaction is studied as a model system for targeting of proteins in the bacterial nucleoid. The theory of conditional probabilities is combined with transport-reaction equations to account for the dissociation of HU-DNA complexes in gel cages during electrophoresis gel-retardation experiments. Experimental patterns are closely simulated by the computed patterns. Related analysis of HU:DNA association-dissociation reactions indicate that the apparent off-rate of HU protein increases greatly at high DNA concentrations. These and other results suggest that in nucleoids the targeting of HU is greatly facilitated by dissociations induced by the high DNA concentration.

Global Regulation

A2-018 SIGNALS THAT REGULATE MYXOCOCCUS DEVELOPMENT, Mitchell Singer, Ingrid M. Keseler, and Dale Kaiser, Departments of Biochemistry and Developmental Biology, Stanford University, Stanford, CA 94305

When starved, 10^5 myxococcus cells assemble to form a fruiting body which has species-specific shape and within which individual cells differentiate into spores. At least thirty new proteins appear during fruiting body development, each one at a particular time. Fruiting body development is costly since cells must synthesize these new proteins despite starvation and, perhaps as a consequence, only a fraction of the cells survive as spores.

How do cells recognize starvation and decide to initiate the program of fruiting body development? Conditions that induce fruiting body development also induce a stringent response with synthesis of the regulatory nucleotides guanosine penta and tetraphosphate, (p)ppGpp. To test whether an elevation of (p)ppGpp is sufficient to induce development, the level of these nucleotides was raised without starvation. For this purpose the (p)ppGpp synthetase gene (*relA*) from *E. coli* was placed under control of an *M. xanthus* light inducible promoter, and introduced into *M. xanthus* for ectopic expression. Even in the presence of nutrients, the ectopic expression of *relA* induced accumulation of the synthetase protein and a six to eight fold increase in (p)ppGpp. This elevation was observed to induce the expression of three early developmentally regulated reporter genes.

One of the reporter genes (Ω4521) that responds to (p)ppGpp also requires an extracellular signal, called A-factor, for its expression. A-factor is a set of six amino acids that are released by one to two hours after initiation by starvation. A-factor is a cell density signal; Ω4521 is expressed only if the extracellular level of A-factor exceeds a threshold. Expression of this gene thus depends on at least two input signals: intracellular (p)ppGpp and extracellular A-factor. The Ω4521 gene is driven by a σ^{54}-like promoter which may integrate these two signals.

A2-019 CONTROL OF A GLOBAL REGULON IN *Escherichia coli* BY THE LEUCINE-RESPONSIVE REGULATORY PROTEIN,
Rowena G. Matthews[1], Deborah W. Borst[1], Don E. Wiese II[1], Brian R. Ernsting[1], Sha Huang[1], and Robert M. Blumenthal[2], [1]Biophysics Research Division and Department of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109, and [2]Department of Microbiology, Medical College of Ohio, Toledo, OH 43699.

The leucine-responsive regulatory protein (Lrp) displays unusual versatility in the patterns of regulation of its target genes. It can either activate or inhibit transcription of target genes, and can do either of these in a manner that is antagonized by leucine, unaffected by leucine, or stimulated by leucine. We have been interested in understanding the molecular basis for such diverse regulatory patterns. Our recent studies have focused on in vitro and in vivo regulation by Lrp of the *gltBDF* operon, which codes for glutamate synthase. DNase I footprinting has shown that Lrp protects three sites upstream of the *gltBDF* promoter, and phased hypersensitivity indicates that Lrp binding results in bending of more than 100 bp of DNA. The central protected region is a palindromic sequence which matches well a consensus sequence described by Wang and Calvo for Lrp binding to *ilvIH* (1). This central region exhibits the protection pattern characteristic of a dimeric protein binding to the major groove of the DNA. Surprisingly, the flanking sites show protection patterns characteristic of minor groove contacts. This suggests that Lrp is a bifunctional DNA-binding protein with the ability to make major and/or minor groove contacts in forming a large loop in the DNA.

We have also compared the regulation of a *gltBD::lacZ* fusion in vivo with the previously observed binding of Lrp to a DNA fragment containing the *gltBDF* promoter (2). These measurements have been made in an AAEC546 background [a strain constructed by Ian Blomfield as described in reference 3], in which *lrp* is transcribed from an inducible *lac*UV5 promoter. We have shown that the intracellular concentration of Lrp is linearly related to the IPTG concentration in the medium over a broad range of IPTG concentrations. Expression of the *gltBD::lacZ* fusion is measured both by Western blotting using an antibody to β-galactosidase and by measurements of β-galactosidase activity. Both in vivo and in vitro, the effect of leucine as an antagonist diminishes as the concentration of Lrp increases, supporting a model for regulation of Lrp-dependent genes by leucine previously proposed by our laboratories (2).

Funded by National Science Foundation Grant MCB-9203447.

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Chromosome Distribution, Cell Cycle and Cell Division

A2-020 P1 PLASMID MAINTENANCE: CONTROL OF REPLICATION AND PARTITION IN THE *E. COLI* CELL CYCLE.
Stuart Austin, Therese Brendler and Ann Abeles. ABL Basic Research Program, NCI Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201

The prophage of bacteriophage P1 is a plasmid that can be maintained essentially without loss at copy numbers as low as two per dividing cell. This is achieved by the coordinate activities of systems for the control of initiation of replication and the active partition of plasmids to daughter cells. Partition is promoted by a centromere-like site and two plasmid encoded proteins ParA and ParB. ParB binds to the site. ParA is an ATPase that recognizes ParB and may act as a motor to move the plasmid copies during partition. Replication control has two elements: a temporal control which helps limit each plasmid copy to a single initiation event in each cell cycle, and a copy-control system that limits the total number of copies that can accumulate in the cell. Temporal control is achieved by a system that recognizes the methylation state of four adenine methylation sites nested within heptamer repeats in the origin core. These are methylation-dependent binding sites for the host SeqA protein. SeqA is required for origin function in vivo. It appears to promote initiation of the fully methylated P1 origin and block initiation of the hemimethylated products of replication, causing an eclipse period during which reinitiation is prevented.

The maximum copy number of the plasmid is controlled by the plasmid *incA* locus, an orderly array of nine 19 bp. iterons that bind the P1 RepA initiator protein. A further five repeats of this sequence are present in the origin where they are essential for initiation. Interplasmid contacts between the *incA* locus of one plasmid and the origin of another block replication (plasmid handcuffing). This provides a direct feedback regulation of plasmid copy number. Using an in vitro system that reconstructs this regulation, we show that individual *incA* iterons behave as if they were freely diffusing nucleoprotein repressors that interact reversibly with the origin. Copy number is therefore dependent on cell volume, thus explaining why copy number is proportional to cell growth rate. Modeling of this control suggests that, in cells that have only two plasmids at the time of cell division (as is usually the case at slow growth rates), the initiation of the origin would be triggered once in each generation by plasmid partition and cell division. We will describe how the interplay of the two replication control systems, the active P1 plasmid partition system and the cell division cycle can achieve accurate plasmid maintenance at such low copy numbers.

Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with ABL.

A2-021 SITE-SELECTION IN BACTERIAL CELL DIVISION, Cynthia Hale, Dinesh Rao, and Piet de Boer, Department of Molecular Biology and Microbiology, Case Western Reserve University Medical School, Cleveland, OH 44106.

An early event in the cytokinetic process in bacteria is the assembly of the FtsZ protein into a ring structure at the cytoplasmic side of the inner membrane. Formation of the FtsZ ring precedes septal invagination at this site, and the ring shrinks in diameter as it remains associated with the leading edge of the ingrowing septum. The FtsZ protein is an atypical GTPase with properties that are reminiscent of eukaryotic cytoskeletal proteins, and studies of its behavior are consistent with the idea that the ring may be a large FtsZ polymer which guides or forces the growth of the cell envelope inwards. Furthermore, the interaction of the protein with GTP is likely to be important for formation and/or shrinkage of the ring.

Under normal conditions, the FtsZ ring, and hence the division septum, is placed accurately at the mid-point of the long axis of the mother cell. The selection of this site depends on the activities of the MinC, MinD and MinE proteins. MinC is an endogenous division inhibitor which prevents septum formation by blocking the formation of the FtsZ ring throughout the cell. The MinD ATPase is normally required for MinC-mediated division inhibition, and is thought to activate MinC function. MinD is a peripheral membrane protein which belongs to a class of ATPases that includes a number of proteins involved in the faithful partitioning of low copy number plasmids. The MinE protein suppresses MinC/MinD mediated division inhibition in a site-specific fashion such that FtsZ ring formation is allowed at midcell, but still blocked at potential division sites (PDS) that are present at the cell poles. In the absence of MinC or MinD, or when either MinE or FtsZ is overexpressed, FtsZ rings are formed efficiently at the cell poles, giving rise to a minicell phenotype where small anucleate cells are pinched off the cell ends.

It is not known how MinC blocks FtsZ ring formation. MinC could interact with FtsZ directly. Alternatively, the two proteins could compete for interaction with a third factor. One possibility, for instance, is that ring formation depends on the presence of a membrane-associated molecule or structure which acts as a nucleation point for ring formation, and that MinC inhibits ring formation by blocking access of FtsZ to this factor. It is also not known how MinD activates MinC function, how MinE suppresses this, nor how MinE function is confined to the PDS at midcell. A full understanding of septum formation and septum site-selection will require knowledge of the molecular interactions of the various proteins involved. We have taken several approaches in biased and unbiased searches for molecules that interact directly with Fts and Min proteins. Results of these studies will be discussed.

A2-022 MOLECULAR MECHANISM OF CHROMOSOME PARTITION IN *ESCHERICHIA COLI*, Abu Z. M. Saleh, Tadao Mitani, Makiko Kido, Makoto Hirano, Kunitoshi Yamanaka, Hironori Niki, Teru Ogura and Sota Hiraga, Kumamoto University School of Medicine, Kumamoto 862, Japan.

To analyze mechanism of chromosome partition in bacteria, we isolated *muk* mutants of *E. coli* which produce normal-sized anucleate cells upon cell division (Hiraga *et al.* J. Bacteriol., **171**:1496-1505, 1989). The MukB protein is essential for proper partitioning of sister chromosomes into the two daughter cells in *E. coli* (Niki *et al.* EMBO J., **10**:183-193, 1991). Regulation of initiation of chromosome replication is normal in *mukB* mutants, but normal-sized anucleate cells are produced at moderate frequency upon cell division. A *mukB* null mutant can grow and produces anucleate cells at 22°C, but colony forming ability markedly reduces at higher temperatures (Niki *et al.* EMBO J., **10**:183-193, 1991). Homologous recombination, mutation frequency, and UV sensitivity in *mukB* mutants are normal as well as those of the wild-type strain (Niki *et al.* EMBO J., **10**:183-193, 1991). A mini-F plasmid, which has its specific centromere (*sopC*), a centromere-recognition protein (SopB), and an ATPase (SopA), is stably maintained in the *mukB* null mutant at 22°C (Ezaki *et al.* J. Bacteriol. **173**:6643-6646, 1991). The MukB homodimer has a coiled-coil rod-and-hinge structure with globular domains at the amino- and carboxy-terminal regions (Niki *et al.* EMBO J., **11**:5101-5109, 1992). A nucleotide binding consensus sequence was found in the amino-terminal globular domain. Purified MukB protein has DNA-binding and ATP/GTP-binding activities (Niki *et al.* EMBO J., **11**:5101-5109, 1992). MukB is the first candidate of motor protein involving in bacterial chromosome partition. To determine the DNA-binding domain, plasmids carrying mutated *mukB* genes were isolated by *in vitro* chemical mutagenesis followed by amplification by PCR. These plasmids did not complement the temperature sensitivity and the anucleate cell production of the *mukB* null mutant. Mutant MukB proteins purified from cell extracts of a *mukB* null mutant harboring each of these mutant plasmids were assayed for the DNA-binding activity. Results suggested that the carboxyl-terminal region was critical for the DNA-binding activity. The temperature-sensitive *mukB106* gene has one amino acid substitution ($Ser_{33} \rightarrow Phe$) in the amino-terminal region. Extragenic suppressors, which allowed the *mukB106* mutant to grow at high temperature, were isolated. Some of them showed anucleate cell production at moderate frequencies in the background of the wild-type *mukB* gene. Some extragenic suppressors were mapped at 24-25 min on the *E. coli* chromosome by Hfr mating and P1 transduction. A cosmid which complemented one of the suppressor mutants was isolated from a cosmid library of the wild-type *E. coli* chromosome. Subcloning and properties of the suppressor gene will be reported.

A2-023 CHROMOSOME REPLICATION AND THE BACTERIAL CELL CYCLE. Kurt Nordström, Rolf Bernander, Andrzej Poplawski, Emilia Botello, and Thomas Åkerlund, Department of Microbiology, Uppsala University, The Biomedical Center, Box 581, S-751 23 Uppsala, Sweden

We are interested in how chromosome replication, nucleoid processing, and cell division are controlled to match growth in mass and how they are co-ordinated. Plasmid R1 was inserted into a small deletion in *oriC*; this inactivates *oriC* and uncouples chromosome replication from its normal control; instead, chromosome replication is controlled by plasmid R1[1]. Since plasmid R1 replicates unidirectionally, two sets of strains were constructed, $intR1_{CC}$ and $intR1_{CW}$. The DNA content and cell size distribution of $intR1_{CW}$ strains are similar to those of normal strains. Replication in $intR1_{CW}$. strains is bidirectional and random in time, whereas it is co-ordinated in normal strains. By using a runaway-replication derivative of R1, the copy number of the chromosome can be varied[2]. The growth rate of the bacteria is very sensitive to changes in the DNA/mass ratio. Under- as well as over-replication disturb cell division[3]. Our data suggest that cell division has its own control[4]. We are at present investigating whether also nucleoid processing ("mitosis") is independently controlled or is part of the replication cycle. By using various degrees of moderate under-replication, we are determining the latest time at which replication can occur and still allow normal cell division and what happens if replication is completed even later. Replication of plasmid R1 is determined by the rate of transcription of the *repA* gene (coding for the rate-limiting initiator protein) and of the *copA* gene (coding for an antisense RNA that regulates the synthesis of the RepA protein from the *repA* mRNA)[5]. Conditional expression of the *copA* gene makes it possible to reversibly inhibit initiation of replication and to follow the kinetics and localisation of cell divisions after resumption of replication. Induction of only one round of replication in most of the cells gives synchronous replication and a burst of cell divisions about one hour later. Completed replication is required but not enough for cell division to occur. The rules governing cell division are being studied.

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Evolution of Bacteria

A2-024 THE AMELIORATION OF BACTERIAL GENOMES, Howard Ochman[1] and Jeffrey G. Lawrence[2]. [1]Department of Biology, University of Rochester, Rochester, New York 14627 and [2]Department of Biology, University Univeristy of Utah, Salt Lake City, Utah 84112.

Analyses of the nucleotide (G+C) contents of within and among hundreds of species have resulted in several insights into the attributes, organization and evolution of bacterial chromosomes. These studies have established that: (1) base composition varies widely among species, ranging from 25% to 75% G+C; (2) base composition is related to phylogeny, *i.e.*, G+C contents of the chromosomes of closely-related organisms tend to be similar; (3) base composition is relatively uniform over the entire bacterial chromosome; and (4) within each species, the first, second and third positions of codons have characteristic base compositions. As a consequence, base composition has served as a guide to ancestry, whereby regions of anomalous base composition or codon usage patterns provide evidence of horizontal transfer. Differences in G+C content among species arise from biases in the mutation rate to each nucleotide and, therefore, sequences introduced by horizontal transfer should eventually manifest features common to genes ancestral to the chromosome. To monitor this process of amelioration, whereby a sequence adjusts to the characteristics of the genome, we have analyzed the base composition, codon usage patterns and phylogenetic distribution of sequenced genes from *Salmonella typhimurium*. By examining these properties for this large set of genes, we have been able to identify factors governing the compositional structure of bacterial genes and genomes, calculate the rate of change in G+C content on an evolutionary timescale and estimate the proportion of the genes in the *Salmonella* genome that arose through introgression.

Late Abstract

CONTROL OF CHROMOSOME REPLICATION IN *E. COLI*: THE INITIATOR TITRATION MODEL, Flemming. G. Hansen[1], Bjarke B. Christensen[1], and Tove Atlung[2], [1]Technical University of Denmark, Lyngby, [2]Roskilde University, Roskilde,Denmark.

Initiation of chromosome replication is one of the most important processes in living cells. In bacteria the DnaA protein is a key factor in this process. The DnaA protein prepares the origin of replication for other factors necessary for initiation. Several observations strongly suggests that the DnaA protein besides its role for the actual initiation reaction might also be involved as a main controlling molecule for initiation. Control of initiation of replication is often described by the initiation mass, i.e., the mass per origin at the time of intiation. A reduced DnaA protein activity, which can be obtained in DnaA(Ts) mutants, causes an increase in initiation mass. An increased DnaA protein activity, which can be obtained by inducing a *lacP* controlled *dnaA* gene, leads to a decrease in the initiation mass. Within certain limits there is a very good correlation between the DnaA protein concentration and the initiation mass. The DnaA protein binds to DnaA boxes located in *oriC* and at several other places on the chromosome. It has been shown that the introduction of extra DnaA boxes into cells titrates DnaA protein. The initiator titration model postulates that the control of initiation of chromosome replication is caused by cyclic changes in the balance between the initiator - DnaA protein - and the titrating devices - the DnaA boxes. During most of the cell cycle the DnaA protein concentration is lower than the DnaA box concentration (activity), but eventually all DnaA boxes are filled with DnaA protein allowing free molecules to start the formation of the initiation complex. Using a stochastic approach the initiator titration model has been simulated extensively. In principle all previous experiments addressing initiation control can be simulated, e.g., temperature shifts for *dnaA*(Ts) mutants; thymine starvation and readdition of thymine in thymine requiring mutants; modulation of DnaA protein activity and of Dam activity, etc. Also when we are asking new experimental questions we ask the initiator titration model for a prediction. The initiator titration model shows a remarkable ability to precisely match computer simulations and experimental results. This includes *E. coli*'s ability to handle the chromosome as well as minichromosomes present in a high copy number. Various aspects of the initiator titration model will be discussed.

Posters A-J

A2-100 DNA CONTENT AND CELL SIZE DISTRIBUTION IN EXPONENTIALLY GROWING AND STATIONARY PHASE CULTURES OF *Escherichia coli*, Thomas Åkerlund, Rolf Bernander, and Kurt Nordström, Department of Microbiology, Biomedical Center, Uppsala University, Box 581, S-751 23, Uppsala, Sweden.

Using high resolution flow cytometry it is possible to perform sensitive measurements of various growth parameters, such as DNA content and cell size, of individual bacterial cells. Previous flow cytometry experiments have indicated that these parameters vary during the exponential phase in batch cultures of *Escherichia coli*. These results therefore question whether it is possible to use batch cultures in experiments where balanced growth is required.

The amount of DNA in exponentially growing *E. coli* cells is dependent on the growth rate. Since one round of DNA replication takes roughly 40 min (at fast and moderate growth rates), rapidly growing cells contain several ongoing rounds of replication. When exponentially growing cells enter stationary phase, growth declines and the cells become smaller. We wanted to know if stationary phase cells contain one chromosome per cell, *i. e.* if all completed rounds of DNA replication lead to cell division. Therefore, flow cytometry was used to analyse DNA content and size distribution of exponentially growing as well as stationary phase *E. coli* cells over a wide range of cell concentrations in batch cultures.

The results showed that the average cell size started to decrease at cell concentrations of 10^7 cells per ml in rich media. This implies that when balanced growth is needed, samples should be collected at low cell densities. Cells growing in rich media often contained two, four or eight chromosomes per cell after entry into stationary phase, whereas after growth in minimal medium, approximately equal amounts of cells with one and two chromosomes were present. Thus, fast growing cells do not, as previously assumed, rapidly end up with a single chromosome upon entry into stationary phase.

A2-101 GROWTH INHIBITION OF THE HOST BACTERIA CAUSED BY THE *letB* (*sopB*) MUTATIONS OF THE F PLASMID DEPENDS ON THE PLASMID DNA LENGTH. Hisako Amino, Kazuhisa Sekimizu and Takeyoshi Miki, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812, Japan.

The F plasmid in *Escherichia coli* has its own partition mechanism controlled by the *sopA* and *sopB* genes and by the cis-acting *sopC* (*incD*) region; plasmids lacking these genes showed unstable inheritance without inhibiting the growth of the host bacteria (Ogura & Hiraga, 1983). In the course of genetic analysis of the F plasmid, we found a novel type of mutants, *letB*, which inhibited growth of the host bacteria under non-permissive conditions although they carried mutations in the *sopB* gene. Since these mutants showed a different phenotype from the *sopB* mutation described by Ogura and Hiraga, we elucidated the reason why the *letB* mutations inhibit the growth of the host bacteria.

The rationale for our analysis of the mutants is that if the F plasmid carries a putative gene that inhibits the host bacteria, and if the *letB* gene product suppresses the synthesis or activity of the putative gene product, we may expect that the *letB* mutants carrying mutations in this gene would no longer be inhibitory. Based upon this assumption, we tried to identify the DNA region essential for expression of the lethal activity by isolating a series of deletion mutants. But, unexpectedly, the results indicate that, 1) unless deletions were very large, mutants that had any region of the F plasmid deleted, except the 44.0-49.4 F region where replication and partition genes are located, did not lose inhibitory activity, and 2) when deletions were very large, the plasmid became less inhibitory as the length of the deleted segments in the mutants increased. Furthermore, the mini-F plasmid carrying the *letB* mutation, which did not show any growth inhibition, recovered the inhibitory activity when it was connected to large *E. coli* chromosomal DNA fragments. These results suggest that the growth inhibition of the host bacteria caused by the *letB* mutations of the F plasmid depends on the plasmid DNA length.

1) Ogura, T. and Hiraga, S. (1983). Cell 32; 351-360

A2-102 MULLER'S RATCHET IMPAIRS FITNESS OF BACTERIA. Dan I. Andersson[1] and Diarmaid Hughes[2]. 1) Department of Microbiology, Uppsala university, Biomedical Centre, Box 581, S-75123 Uppsala, Sweden. 2) Department of Molecular Biology, Uppsala university, Biomedical Centre, Box 590, S-75124 Uppsala, Sweden.

Muller proposed that an asexual line of descent will inevitably accumulate deleterious mutations, which result in an increase of the mutational load and an irreversible ratchet-like loss of the least mutated class. We have examined whether Muller's ratchet operates in *Salmonella typhimurium*. To examine this, cells were grown under asexual conditions at high genetic drift, and the increase in mutational load was determined. Our results show that *Salmonella typhimurium* is indeed accumulating mutations under these conditions: after 1700 generations, 3.6% of the lineages (16/444) had suffered a loss of fitness as measured by auxotrophy and slow growth. These results suggest that in the absence of sex and with high genetic drift, genetic mechanisms such as back- or compensatory mutations cannot prevent the accumulation of deleterious mutations. The appearance of auxotrophs allowed us to calculate a mean average spontaneous mutation rate of approximately $0.2\text{-}1\times10^{-9}$/ basepair and generation for the largest genetic target studied so far.

A2-103 EFFECTS OF *hns* MUTATIONS ON CHROMOSOME REPLI CATION AND CELL CYCLE PARAMETERS IN *Escherichia coli*. Tove Atlung, Department of Life Sciences and Chemistry, Roskilde University, DK-4000 Roskilde, Denmark.

The *hns* gene of *E. coli* encodes the abundant small nucleoid binding protein H-NS. Mutations in *hns* are very pleiotrophic and affect expression of a number of unrelated genes. H-NS is a non-sequence specific DNA binding protein, with a preference for curved DNA. Expression of the *hns* gene is induced approximately fourfold by cold shock and by entry into stationary phase. One model proposes that changes in the level of supercoiling are responsible for many of the phenotypes of *hns* mutants; another that the effects are due to direct interaction of H-NS with curved DNA in promoter regions.

The chromosomal origin and the *dnaA* promoter region both contains curved DNA. Flow-cytometric analysis showed that the *hns* mutations have effects on several cell cycle parameters. The mutants have fewer origins per cell (Fig. 1), indicating that the C or D time is reduced. Preliminary data from marker frequency analysis showed that there is a small reduction in C, and thus there must be a significant reduction in D. Analysis of the cell sizes showed that the mutants have an increased mass per origin. This is not due to a decreased DnaA protein concentration, on the contrary, the mutants have a slightly increased *dnaA* gene expression. In sharp contrast to the asynchrony phenotypes of both *himA himD* (IHF) and *fis* mutants as well as topoisomerase mutants (*gyrB*/*topA*) the *hns* mutants were as synchronous as the wild type (Fig. 1). Analysis of the topoisomers of a reporter plasmid showed that the mutants have the same degree of negative supercoiling as the wild type during exponential growth, irrespective of growth temperature. Addition of NaCl increased negative supercoiling to the same extent in wild type and mutants and had no effect on the cell cycle parameters I am presently investigating the effect of reducing negative supercoiling by addition of the gyrase inhibitor novobiocin.

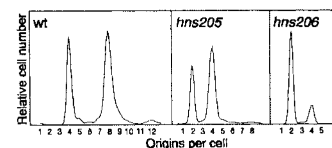

Fig.1 Strains carrying the indicated *hns* alleles were grown at 30°C in glucose + amino acids and samples incubated with rifampicin and cephalexin for 5 hrs were analysed by flow cytometri.

A2-104 COMPLETE TRANSPOSITION REQUIRES FOUR ACTIVE MONOMERS IN THE MU TRANSPOSASE TETRAMER

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A tetramer of Mu transposase (MuA) cleaves the Mu DNA and joins these ends to a target DNA to catalyze transposition. We have been probing regions of the transposase (MuA) involved in these reactions using deletion analysis, site-directed mutagenesis and chemical modification. Mutagenesis of acidic amino acids in the central domain of MuA has been especially informative. Substitution mutations at Asp269 or Glu392 destroy both the DNA cleavage and joining activities without blocking tetramer assembly, indicating that the mutations specifically affect catalysis. Amino acid sequence alignment and the similar effects of mutations suggests that D269 and E392 of MuA are analogs of the first D and final E of a conserved triad of acidic amino acids present in many transposases and the retroviral integrases (the D-D-35-E motif). Guided by sequence similarities, additional mutations have been made. Proteins with different amino acids in conserved positions and those with multiple changes were assayed using different concentrations of divalent cations to test the idea that the conserved acidic amino acids are involved in coordinating divalent metal ions in the active sites of transposases. Derivatives of MuA altered at the essential acidic amino acids were also used to analyze the contribution of individual monomers to the activity of the tetramer. The performance of different protein combinations demonstrates that not all monomers need to be catalytically competent for the complex to promote an individual cleavage or joining reaction. Furthermore, the results indicate that each pair of essential residues are probably donated to the active complex by a single monomer. Although stable, tetramers composed of a mixture of mutant and wild-type MuA generate products cleaved at only one end, and with only one end joined to the target DNA. The abundance of these abortive products and the ratios of the two proteins in complexes stalled at different steps indicate that the complete reaction requires the activity of all four monomers. Thus, each subunit of MuA appears to use the conserved acidic amino acids to promote one DNA cleavage or one DNA joining reaction.

A2-105 murI AND mbrC ARE IDENTICAL,

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The gene responsible for the synthesis of D-glutamic acid, a specific cell wall component, was identified as a previously sequenced open reading frame (ORF1) located at 89.5 min on the E. coli genetic map (P. Doublet, J. van Heijenoort, and D. Mengin-Lecreulx, J. Bacteriol. 174:5772-5779, 1992). Earlier we had constructed a conditional lethal mutant strain in order to learn the function of this gene (G. Baliko and P. Venetianer, J. Bacteriol. 175:6571-6577, 1993). The gene was inactivated on the chromosome by homologous recombination with a temperature sensitive plasmid (pHKO289) carrying ORF1 disrupted immediately after the start codon by a resistance marker gene. The reciprocal recombinant strain carried the intact ORF1 gene on the low copy number plasmid. Growing the mutant at a temperature allowing inefficient plasmid replication the cells became elongated with unseparated chromosomes in a ribbon-like structure. This phenotype is very unusual for a cell wall synthesizing mutant. However, it is similar to that of mbrC mutants, thought to be involved in chromosome separation and mapped to 89.5 min on the E. coli genetic map (N. J. Trun and S. Gottesman, Genes & Dev. 4:2036-2047, 1990).

We tried to decide, whether the mbrC mutation (dominant) is an allele of murI (ORF1) by a homologous recombination experiment using the pHKO289 plasmid. If the two genes were identical, and the relative positions of the mbrC mutation and the plasmid resistance marker would allow, the intact ORF could be restored by plasmid-chromosome cointegration. The mbrC mutant NT617 which was unable to grow on rich medium or at high temperature was transformed with pHKO289 and colonies were selected at 42 °C on rich medium. The predicted plasmid-integration structure was confirmed by Southern analysis of several clones. The growth and cell morphology of these clones at different temperatures in minimal or rich medium were indistinguishable from that of the isogenic NT597 strain, carrying the wild-type mbrC allele. From this recombinant structure the mutation can be rescued after the resolution of the plasmid at 30 °C.

A2-106 LARGE INSERTIONS AND DELETIONS IN THE ESCHERICHIA COLI MINIMAL *oriC* REGION ARE TOLERATED ON THE CHROMOSOME,

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Traditionally, *oriC* plasmids have been used as reliable models for chromosomal replication. However, origin activities in *oriC* plasmids vary depending on several factors including cloning vectors, fragment orientations, and host strains used. We have developed a phage transducing system by which *oriC* mutations in plasmids are transferred to the chromosome by a series of double crossover events. Using this system we have shown that the insertion of an omega fragment (2082-bp) at the HindIII (244) site between DnaA boxes R3 and R4 is tolerated on the chromosome. The presence of the modified *oriC* and the absence of an intact *oriC* were verified by Southern blot hybridization analysis of chromosomal DNA. The same mutation on a pBR322 plasmid could not transform a *polA* strain, indicating that this insertion on a plasmid renders *oriC* inactive. The *oriC* mutant strain showed a slowed doubling time, 27.3 min compared to 18.8 min of wild type. Flow cytometric analysis indicated unsynchronous initiations, and a lowered DNA content/cell. Furthermore, deletion of a 41-bp segment between HindIII (244) and AccI (285) which removes DnaA box R4 from the minimal *oriC*, also resulted in a viable chromosome. Conversely, a much larger deletion between BglII (38) and AccI (285) was shown to inactivate *oriC* activity on the chromosome. This *oriC* deletion could only only be introduced into an *rnhA* strain, in which an alternative mode of DNA replication is activated. These findings demonstrate that the *oriC* sequence is much more tolerant of alterations on the chromosome than on a plasmid.

A2-107 CHROMOSOMAL AND PLASMIDIC CONJUGATIVE ELEMENTS INVOLVED IN THE DISSEMINATION OF ANTIBIOTIC RESISTANCE IN CLINICAL ISOLATES OF *ESCHERICHIA COLI* ,

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Transposons have contributed to the evolution of resistance plasmids, providing genetic variation and allowing the dissemination of genes between plasmids and their incorporation into the chromosome .

Ec2418 a clinical isolate of *E.coli* , is a multiresistant strain that contains a conjugative plasmid (R2418) encoding resistance to β-lactam antibiotics and two transposons . The first one (Tn2418-1) confers resistance to streptomycin and can transfer during mating onto both R2418 plasmid or onto the chromosome of the recipient cell . The second transposon (Tn2418-2) carries tetracycline resistance and has been observed to transfer only onto R2418 plasmid .

The R2418 plasmid exhibits some similarities to the conjugative R46 plasmid in that it carries resistance to sulfonamide-trimethoprim and ampicillin . In addition, it is able to integrate tetracycline or streptomycin resistance genes . Whether the R2418 plasmid has some form of integron-related structure as shown in R46 plasmid is under investigation .

Cojugative transposons appear to have played a major role in the spread of multiple antibiotic resistance in gram-positive bacteria . More recently, a conjugative transposon has been identified in gram-negative bacteroides . In the present work, we also describe a chromosomal transposon (Tn1941) encoding ampicillin resistance found in clinical isolate of *E.coli* (Ec1941) . Tn1941 is able to conjugatively transfer in the absence of a mobilizing plasmid .

A2-108 THE ROLE OF F' TRANSFER IN ADAPTIVE MUTAGENESIS,

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Adaptive mutagenesis implicate environmental stress factors as a cause of specific advantageous mutations and not just a selective force that enriches for random beneficial mutations within the population. In nonlethal genetic selection systems used to study adaptive mutagenesis mutations occur without significant population growth and without the accumulation of on nonadvantageous mutations. An important question is how adaptive mutations occur during nonlethal selections in the apparent absence of chromosome replication. Many studies of adaptive mutagenesis use reversion of a *lac* frameshift mutation, *lacI ΩlacZ*, present on an F' genome. Our recent work shows that during extended selections on minimal media there is high rate of redundant transmission of F's occurring in the population due to loss of F mediated surface exclusion. This high rate of transfer provides a mechanism by which mutations can preferentially occur on the F' genome without replication of the host chromosome. We are testing if mutations that affect the adaptive mutagenesis of the F' Lac selection system do so by affecting redundant transmission of the F' plasmid. Mutations that reduce the frequency of late arising Lac$^+$ revertants concomitantly reduce the transmission of F's, suggesting a linkage between the two phenomena. Our preliminary results suggest that F transmission is an important contributor to adaptive mutagenesis in the F' Lac system.

A2-109 ISOLATION OF *lacZ* TRANSCRIPTIONAL FUSIONS TO Lrp - CONTROLLED GENES IN *Escherichia coli*: EFFECTS OF IN VIVO TITRATIONS WITH Lrp,

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Lrp, the leucine-responsive regulatory protein, affects the expression of at least 40 genes in *Escherichia coli*. Apparent Lrp homologs have been found by others in genera as distant as Pseudomonas and Rhizobium, suggesting that it plays a fundamental role in Gram-negative bacteria; and the abundance of this DNA-binding protein in *E. coli* suggests that it may affect chromosome organization. Surprisingly, the expression of many genes repressed or activated by Lrp is relatively insensitive to leucine. To understand the physiological rationale for this pattern of regulation, and to provide additional tests of a proposed mechanism for leucine effects on regulation by Lrp [Ernsting et al., J. Bacteriol. **175**:7160-7169 (1993)], we have made random fusions to *lacZ* in an *E. coli* strain inducible for Lrp. The λ*placMu* fusion library was generated in strain AAEC546 (I.C. Blomfield; *lrp* is under the control of the *lac*UV5 promoter and LacI). By screening the library on isoleucine- and valine-supplemented glucose minimal media containing or lacking IPTG and/or leucine, we have to date identified a group of 10 fusions that exhibit Lrp dependence for expression with varying degrees of leucine sensitivity. These fusions are being cloned and identified, in part to determine if their regulation by Lrp is direct or indirect. Among these fusions are two patterns of regulation by Lrp that have not been seen previously: stimulation by Lrp but inhibition of growth by leucine; and stimulation by low levels of Lrp but depression by higher levels of Lrp, with leucine increasing the stimulation and antagonizing the depression. The responses of these fusions to IPTG and leucine have been quantitated during steady-state growth in glucose minimal media, and the expression patterns of several were found to be consistent with the in vitro data of Ernsting et al. Specifically, the sensitivity of fusion expression to leucine is maximal at low levels of Lrp and then decreases hyperbolically as the in vivo concentration of Lrp is increased.

Funded by the National Science Foundation through grant MCB-9203447

A2-110 DISTANCE RELATIONSHIPS AMONG PHAGE GENOMES,

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We studied distance relationships among all 21 non-redundant complete genome sequences or large sequence collections of phages and of 3 related bacteria. Our method converts the aggregate sequences of each phage to 16-vectors of the dinucleotide symmetrized relative abundances. Let fX be the average frequency of a base and its inverted complement (eg. fX=(fA+fT)/2) and fXY similarly (eg. fXY=(fAC+fGT)/2).Then the symmetrized relative abundance is 2fXY/(fX*fY). We used three distance measures: (1) existence of extreme values of the symmetrized relative abundances of di, tri or tetranucleotides, (2) sums of the absolute differences, between two phages, of the 16 di relative abundances, (total ordering), (3) extreme counts of the number of times the absolute differences between the 16 relative abundances of phage X and an arbitray standard 16-vector exceeds the absolute differences between the the values of phage Y and the same standard, (partial ordering). The method has the folowing advantages over the conventional method of counting mismatches between pairs of aligned homologous gene sequences: (1) it does not depend on finding the same homologous gene in every phage in the set (such genes do not exist for the phages), (2) it does not require a prior alignment and is unaffected by the presence of gaps in the sequence, (3) it does not use noisy short homolgous segments which frequently lead to different distance relationships. Application of our method to comparison of 20000 bp sequences from the same phage yields for our three measures distances generally small compared to those between different phages. For total orders the maximum within phage average difference is 0.044+0.015 and the minimum between phage average difference is 0.045+0.017. Some results: phage MU is closer to E. coli than any other phage and the four other dsDNA temperate phage rank 2,3,5,7 out of 21, T7 is most distant generally from all other phages.

A2-111 CONSERVATION OF THE *dnaX* GENE AND ITS PROGRAMMED RIBOSOMAL FRAMESHIFT SIGNAL IN *SALMONELLA TYPHIMURIUM*,

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E. coli DNA polymerase III holoenzyme subunits τ and γ are products of one gene - *dnaX*. τ is the full-length translation product. γ is initiated at the same codon but is terminated within the *dnaX* reading frame by a programmed -1 ribosomal frameshift which results, after incorporation of one unique residue, in a stop codon. τ and γ, synthesized and incorporated into holoenzyme in equal amounts, participate in two major classes of reactions in vitro: τ dimerizes core, and presumably holoenzyme, and τ and γ participate in loading the processivity factor β onto primed templates. τ is essential in vivo but γ is dispensable, suggesting that the τ dimerization function is essential and that τ can also perform all the γ functions in vivo.

The significance of the production of both τ and γ from *dnaX* has been investigated by studying the conservation of the τ, γ pair. Extracts of several *Enterobacteriaceae* and the more distantly related *Aeromonas* and *Vibrio* were probed for τ and γ homologs by Western blots using anti-*E. coli* polyclonal antiserum. All the *Enterobacteriaceae* tested (*Shigella, Salmonella,* and *Enterobacter*) synthesized two proteins which had the same molecular weights as τ and γ (71 and 52 kDa) and which reacted with antibody to *E. coli* τ and γ. The *S. typhimurium dnaX* gene has been cloned (from a transducing λ derivative isolated by Russell Maurer) and sequenced. The *S. typhimurium dnaX* sequence is 83% identical to the *E. coli dnaX* sequence, except that the 42 nucleotides which form the known components of the ribosomal frameshift signal are 100% conserved. Conservation of the τ, γ pair and of the frameshift signal suggest that both τ and γ serve physiological roles. However, the more distantly related organisms *Aeromonas* and *Vibrio* synthesized only one protein recognized by the *E. coli* antibody.

A2-112 LRP REGULATION OF THE *gltBDF* OPERON OF *Escherichia coli* : IN VIVO RESULTS IN SUPPORT OF AN IN VITRO MODEL, Deborah W. Borst,[1] Robert M. Blumenthal[2] and Rowena G. Matthews,[1] Biophysics Research Division,[1] University of Michigan, Ann Arbor, MI 48109, and Department of Microbiology,[2] Medical College of Ohio, Toledo, OH 43699

The leucine-responsive regulatory protein (Lrp) of *E. coli* regulates the expression of over 40 genes either positively or negatively. Many of these genes are regulated in response to exogenous leucine. However, a number of the genes appear to be relatively leucine-insensitive. Previously, Ernsting et. al. [J. Bacteriol. **175:** 7160-7169 (1993)] have proposed a model for leucine sensitivity of Lrp-regulated genes based on differing affinities of Lrp for target promoters as determined by gel-shift experiments. This in vitro model predicts that the degree of sensitivity to leucine is correlated with the level of Lrp in the cell. We have manipulated the intracellular Lrp concentration by utilizing a strain, AAEC546 (constructed by Ian C. Blomfield), in which *lrp* is transcribed from an inducible *lac*UV5 promoter. The intracellular concentration of Lrp is varied within a linear response range by induction with a concentration ramp of IPTG. The effect on the expression of target genes is monitored by β-galactosidase assays of *lacZ* fusions of these genes. The actual concentration of Lrp is determined by Western immunoblot analysis using polyclonal anti-Lrp antibodies. Using this approach we show that the glutamate synthase operon (*gltBDF*), which is relatively leucine-insensitive in vitro and has a promoter with a high affinity for Lrp, becomes more leucine-sensitive in vivo at low Lrp concentrations. Our preliminary data also suggest that leucine decreases the expression of Lrp in AAEC546.

Funded by National Science Foundation Grant MCB-9203447.

A2-113 **EFFECTS OF THE NDD PROTEIN OF T4 BACTERIOPHAGE ON THE *E. coli* NUCLEOID.**

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Within a few minutes after T4 infection, the structure of the nucleoid changes dramatically. The bacterial DNA, initially condensed in the center of the cell, is dispersed to the periphery where it is distributed along the inner membrane. This phenomenon, called nuclear disruption, depends on the phage encoded *ndd* gene (nuclear disruption deficient). We have reactivated the analysis of the nuclear disruption phenomenon, which could eventually turned out as a tool for dissecting the structure and defining components of the bacterial nucleoid.

Identification of the *ndd* gene of T4 by marker rescue, gene interruption experiments and DNA sequencing has been already reported (Bouet *et al.*, 1994). This gene encode a very basic (pI = 10.5) 16.9 KDa protein which has not yet been purified.

Since *ndd* is toxic to *E. coli*, a mutated version of the gene carrying an *amber* mutation suppressible by *supF* has been constructed. In a *supF*(Ts) background, this allele is well accepted at 42°C, but kills rapidly the cells at 30°C, inducing a clear phenomenon of nuclear disruption: the nucleoid losses its central location in the cell and appears divided into small globules that look stuck to the inner membrane. Thus, Ndd protein alone is sufficient for nuclear disruption. We have shown that Ndd has no *in vivo* endo- or exo- nucleolytic activity on the chromosome. Induction of Ndd result in a shut-off of DNA synthesis, which seems to be due to a progressive arrest of replication forks and not to a defect in initiation at *oriC*.

A search for mutants resistant to Ndd and for chromosomal segments suppressing Ndd deleterious activity when present at high copy number is currently underway and will be presented.

A2-114 CHARACTERIZATION OF THE GTP-DEPENDENT POLYMERIZATION OF THE *ESCHERICHIA COLI* FTSZ PROTEIN.

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The FtsZ protein is a GTPase which is essential for cell division in *Escherichia coli.* During cytokinesis, FtsZ becomes localized to a ring adjoining the inner membrane at the leading edge of septum synthesis. Polymerization of purified FtsZ required GTP and could be monitored by sedimentation or by light scattering. Stopped flow measurements indicated that 50% of the FtsZ protein has polymerized within 500 milliseconds. FtsZ depolymerized upon removal of GTP, and re-polymerized following subsequent GTP addition. Like the unpolymerized, purified FtsZ protein, the depolymerized FtsZ protein behaved as a dimer on gel filtration, demonstrating that polymerization was completely reversible. Mutant FtsZ84 protein polymerized inefficiently, arguing that polymerization is important for the cellular role of FtsZ in division. Electron microscopy of polymerized FtsZ revealed structures including tubules 14-20 nm in diameter with longitudinal arrays resembling the protofilaments of microtubules. The possibility that tubules of FtsZ protein form a cytoskeleton involved in septum synthesis is consistent with our data.

A2-115 A PROTEIN THAT BINDS TO THE P1 ORIGIN CORE AND *oriC* 13-MER REGION IN A METHYLATION-SPECIFIC FASHION IS THE PRODUCT OF THE HOST *seqA* GENE, 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 plasmid replication origin, P1 *oriR*, is controlled by methylation of four GATC adenine methylation sites within heptamer repeats. A comparable (13-mer) region is present in the host origin, *oriC*. The two origins show parallel responses to methylation; negative control by recognition of hemimethylated DNA (sequestration) and a positive requirement for methylation for efficient origin function. We have isolated a host protein that recognizes the P1 origin region only when it is adenine methylated. The substantially purified 19 kilodalton protein also binds to the 13-mer region of *oriC* in a methylation-specific fashion. This DNA binding activity appears to be specific for origin sequences, since the protein fails to recognize a number of other DNA fragments, including a non-origin sequence with multiple GATC sites. It proved to be the product of the *seqA* gene that acts in the negative control of *oriC* by sequestration. We conclude that the role of the SeqA protein in sequestration is to recognize the methylation state of the origin by DNA binding.

The protein binds to and regulates both P1 *oriR* and host *oriC* origins despite the lack of any substantial homology between them. SeqA protein binds both fully methylated and hemimethylated origins with a preference for one of the strands in the duplex. Competition experiments show that the activity also binds to methylated single-stranded origin DNA, but duplex DNA is not converted to single-stranded products during binding. We present a model in which DNA binding is strand selective and promotes initiation from fully methylated origins while blocking initiation of the resulting hemimethylated products. This model is in agreement with *in vivo* results which show the failure of unit copy P1 plasmids to be established and maintained in Δ*seqA* strains.

Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with ABL.

A2-116 ISOLATION AND CHARACTERIZATION OF COLD SENSITIVE SUPPRESSORS OF *dnaG2903* AND *parB*. Robert A. Britton and James R. Lupski, Department of Molecular and Human Genetics and Cell and Molecular Biology Program, Baylor College of Medicine, Houston, TX 77030.

The *dnaG* gene encodes primase, which synthesizes the primer RNA necessary for the initiation of both leading and lagging strand synthesis of *Escherichia coli* chromosomal DNA. Conditional lethal mutations within *dnaG* appear to cluster into two regions which may represent distinct functional domains of the primase protein. The amino terminus contains the enzymatic function of primase while the carboxy terminus appears to be involved in protein:protein interactions. Two temperature sensitive alleles, *dnaG2903* and *parB*, are located in the 3' end of the gene, 9bp apart, and both give rise to Glu-to-Lys missense amino acid substitutions in the carboxy terminus of primase. These mutants are different from other *dnaG* alleles in that they are able to keep synthesizing DNA at the non-permissive temperature and display a chromosome partitioning defective phenotype. Determining how the carboxy terminus affects the interactions of primase with the replisome and/or the partitioning apparatus could answer what molecular interactions are involved in governing primase function during DNA replication and/or chromosome partitioning. We have isolated cold-sensitive suppressors of *dnaG2903* and *parB* in an effort to identify proteins that directly interact with primase during DNA replication or that may regulate the expression of *dnaG*. A total of 6000 suppressors of these two alleles were screened and 35 were found to be cold-sensitive. There are at least five distinct classes of suppressors as determined by genetic mapping. Combination fluorescence-phase microscopy has been performed on selected suppressors from each class and a variety of phenotypes are apparent. Efforts towards the cloning and characterization of the genes responsible for the suppression of *dnaG2903* and *parB* are now underway and will be presented.

A2-117 THE *E. COLI* GENOME PROJECT V. Burland, G. Plunkett III, and F.R. Blattner, Laboratory of Genetics, University of Wisconsin, Madison, WI 53706.

The complete genomic sequence of the wild type *E. coli* MG1655 is being determined at the University of Wisconsin Project. At the time of writing (September 1, 1994), the first contiguous megabase of sequence (76-100 minutes) is finished, analysed and released to GenBank. Three quarters of the data for the second megabase are collected and in the process of editing and finishing.

The sequencing strategy combines random and directed operations using the Janus vector. Our research efforts address balancing these in an optimal mix for adequate coverage and maximium efficiency. The switch from the random phase to the directed phase has been automated by a computer program that analyses the partially assembled contigs, selects clones for further sequencing and then retrieves them robotically. This allows an earlier switch from random to directed and gives better management control over the sequencing process.

Analysis of the first megabase revealed a total of 928 open reading frames, consisting of 378 previously known genes, 44 newly identified genes, and 506 remaining unidentified even after extensive database searches. The amount of sequence collected allows a global analysis of the genomic arrangement of both coding and non-coding features, and suggests methods of global analysis of other functions such as transcription.

A2-118 GAIN-OF-FUNCTION LETHAL MUTATIONS IN THE recA GENE, Michael Campbell and Ron Davis, Dept. of Biochemistry, Stanford Medical Center, Stanford, CA 94305-5307

The *recA* gene of E. coli is involved in homologous recombination, SOS induction, lesion bypass replication, and UV-induced mutagenesis, but is not required for viability in the absence of DNA damage. It was thought that mutations in the *recA* gene which kill the cell upon being synthesized would define an interaction between *recA* and an essential component of the cell. 8 gain-of-function mutations have been identified, 7 of which lie in the recA polymer interface. Lethality does not require SOS induction. However, they are all constitutive proteases, indicating that they are more highly activated than wild-type. 7 of the mutants are Rec^+ as shown in a λ *red gam* cross. Furthermore, the mutants are lethal in a *recB recC* strain, but are suppressed in a *recB recC sbcA* (RecE+) strain indicating that the lethality is in some way related to DNA metabolism. Experiments are underway to understand the biochemical nature of the mutant proteins and to isolate extragenic suppressors of the recA mutations.

A2-119 SUPPRESSION OF THE *recA polA* LETHALITY BY ACTIVATION OF A RecA- INDEPENDENT HOMOLOGOUS RECOMBINATION. Yang Cao and Tokio Kogoma, Departments of Cell Biology and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM 87131

Our hypothesis on the inviability of *recA polA* double mutants is that RecA protein plays an essential role in DNA replication in the absence of DNA polymerase I. RecA could protect gaps between Okazaki fragments during DNA replication, or could recruit other factors to carry out nick translation for defective DNA polymerase I, or might be involved in recombinational repair to fill the gaps. To shed more light on the mechanism of the *recA polA* lethality, we examined by complementation test the requirement of the activities of DNA polymerase I (or RecA) in the absence of RecA (or DNA polymerase I) for viability. Both the 5'->3' exonuclease and polymerase activities of DNA polymerase I are essential for viability in the absence of RecA protein, while the survival of *polA* mutant cells depends on the recombinase activity of RecA. DNA synthesis in Δ*recA polA12*(Ts) miniF-*recA428*(Rec^-) ceased while that in Δ*recA polA*(Ts) miniF-*recA730*(Rec^+) continued after 2-hour incubation at the nonpermissive temperature. Therefore, the recombinase activity of RecA is essential for DNA synthesis in the absence of DNA polymerase I. This suggests that a RecA-dependent recombination is involved in gap filling. We previously reported that a *lexA*::Tn*5*(Def) mutation can suppress the temperature-sensitive growth of *recA200*(Ts) *polA25*::*spc*. We further found that DNA synthesis rate in the *recA*(Ts) *polA25*::*spc lexA*(Def) cells is improved ~5 folds, and that the joining of Okazaki fragments is also enhanced over that in the *recA*(Ts) *polA25*::*spc* cells. Moreover, *recF*$^+$ is essential for this suppression pathway, and *recJ* or *recQ* mutations have partial effect on the suppression; *ruvA*$^+$ is dispensable, however. The *recA*(Ts) allele in the temperature-resistant strain *recA*(Ts) *polA25*::*spc lexA*(Def) can be replaced by Δ*recA*, indicating that *lexA*(Def)-induced suppression pathway is RecA-independent. P1 transduction experiments showed that the recombination activity in Δ*recA* cells increases by introduction of *lexA*(Def), and the P1 transduction frequency in the Δ*recA polA25*::*spc lexA*(Def) cells reaches ~7.3% of that in *recA*$^+$ *polA25*::*spc* cells. These results suggest that *lexA*(Def) activates a RecA-independent and RecF-dependent recombination pathway which alleviates the defect in DNA replication in *recA polA* double mutants.

A2-120 CIRCULARIZATION OF THE LINEAR CHROMOSOME OF *STREPTOMYCES LIVIDANS*.

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We have found linear chromosomes in at least eight *Streptomyces* species investigated, indicating the linearity of the chromosomes as characteristic for the genus. The telomeres appear to contain covalently-bound proteins, which presumably primer the replication of the terminal sequences. The replication of the internal majority of the chromosome is initiated at the centrally-located *oriC*. The *S. lividans* chromosome contains terminal inverted repeats of 32-35 kb, of which the extreme 16 kb is homologous to that on one end of the 50-kb linear plasmid SLP2.

The termini of the *S. lividans* and *S. coelicolor* chromosomes correspond to the 'silent region' of the genetic map, and the 'unstable region'. In these regions, few genes have been mapped genetically, and long stretches of DNA undergo frequent (ca. 10^{-3} per spores) spontaneous deletions, some accompanied by amplifications. This structural instability is ubiquitous among the streptomycetes. Genes located in the unstable region include those encoding argininosuccinate synthetase (*argG*), pigment biosynthesis, antibiotic biosynthesis and resistance (notably resistance to chloramphenicol in *S. lividans* and *S. coelicolor*). Examination of some previously found spontaneous chloramphenicol-sensitive mutants of *S. lividans* ZX7 containing large deletions at the termini revealed the circularization of the chromosomes. The morphology and growth of these mutants strains appeared normal.

The linear chromosome of *S. lividans* could also be circularized artificially by targeted recombination, which removed the telomeres and joined the two arms with a continuous DNA containing the *aphII* gene. Under kanamycin selection a 22-kb sequence containing part of the terminal inverted repeat and *aphII* was tandemly amplified on the circularized chromosomes. These cultures grew and sporulated poorly. On release of the kanamycin selection pressure, further rearrangements resulted in deletions of the amplified and neighboring DNA. The resulting cultures exhibited improved growth and sporulation. These results indicate the readiness of the linear chromosome to be circularized, and implicate the termini in the structural instability of the *Streptomyces* chromosomes.

A2-121 THE SbcCD NUCLEASE OF *ESCHERICHIA COLI*

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In *E. coli*, the *sbcC* and *sbcD* genes mediate the inviability of replicons carrying long DNA palindromes. The secondary structures that can be formed by such sequences are potentially mutagenic. We propose that SbcCD plays a role in avoiding mutagenesis by secondary structures that can be formed during DNA replication.

The *sbcC* and *sbcD* genes have been subcloned into a plasmid vector and expressed from a host carrying a deletion of the chromosomal genes. The SbcCD holoenzyme has been purified and shown to have nucleolytic activity on double-stranded linear DNA. This activity is dependent on the presence of ATP and manganese ions. Both SbcC and SbcD polypeptides are essential for this activity, which is inhibited by antibodies raised against a C-terminal peptide of SbcD. The product of the SbcCD nuclease activity is currently being characterised.

A2-122 *Dif* ACTIVITY IN THE CELL CYCLE. MAPPING THE REGION WHERE *dif* MUST LIE, AND COMPARISON WITH THE TERMINAL RECOMBINATION DOMAIN.

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The terminus of replication of the *E.coli* chromosome is the theatre of two types of recombination events that arise with very high frequencies: homologous recombination and specific recombination at the *dif* site. Terminal recombination was identified by a high frequency of recA-dependent deletions between direct repeats. Strains deficient for the *dif*-specific recombination display a characteristic phenotype in which a fraction of the population forms filaments with accumulation of unseparated nucleoids. It has been proposed that the role of *dif* is to resolve chromosome dimers that can arised by recombination events between two newly replicated chromosomes during replication. We have constructed tools to test both recombination activities at the very same positions on the chromosome, and have explored the limits of occurence of the two phenomena. Our data show that every position where insertion of the *dif* site suppresses a Dif⁻ phenotype is also prone to terminal recombination, and support the model that both phenomena are restricted by features of nucleoid organization to the same chromosomal domain.

A2-123 THE CCD KILLER SYSTEM OF PLASMID F.

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DNA topoisomerases perform essential roles in DNA replication, gene transcription and chromosome segregation. They are the targets of potent molecular therapeutic agents, including the quinolone antibiotics and antitumor drugs. Recently, we identified a new type of topoisomerase II poison: the CcdB protein of plasmid F. The *ccd* locus contains two genes *ccdA* and *ccdB* whose gene products are involved in autoregulation and in a poison-antipoison mechanism. When its action is not prevented by CcdA protein, the CcdB protein is a potent cytotoxin. Using purified CcdA, CcdB and gyrase, we show that CcdB efficiently traps gyrase in a cleavable complex. The mechanism by which the CcdB protein induces gyrase-mediated DNA strand breakage is closely related to the action of quinolone antibiotics. However, the nucleotide dependence of the CcdB cleavage process differentiates the CcdB mechanism from quinolone-dependent reactions.
The *ccd* locus contributes to the stability of plasmid F by postsegregational killing of plasmid-free bacteria. Using pulse-chase experiments, we have obtained direct evidence that the half-life of CcdA is shorter than that of CcdB. Using the same technique with protease mutant strains, we have shown that CcdA is stable in a Lon⁻ strain. Moreover, our results indicate that this specific proteolysis of CcdA by LON is required for *ccd*-mediated postsegregational killing. This allows the conclusion that Lon-dependent proteolysis of CcdA is the key control for activation of CcdB cytotoxin in plasmid-free segregant bacteria.
In collaboration with Michael Maurizi and Susan Gottesman (NCI/NIH, Bethesda, USA), we have analyzed the *in vitro* proteolysis of CcdA by Lon. These results will be presented in the poster.

A2-124 WHY RECA PROTEIN HYDROLYZES ATP, Michael M. Cox, Department of Biochemistry, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706

In 1978, Ogawa and colleagues (Cold Spring Harbor Symp. Quant. Biol. 43, 909-915) reported that RecA protein was a DNA-dependent ATPase. This report catalyzed a number of experiments within an embryonic effort in the Lehman lab, with Kevin McEntee and George Weinstock, to elucidate the function of RecA protein. In spite of over fifteen years of work by many labs, the role of the ATP hydrolytic activity of RecA remains controversial. Reports in the early 1990s demonstrated that (a) some RecA-mediated strand exchange proceeds without ATP hydrolysis, (b) some RecA mutants that do not hydrolyze ATP promote limited strand exchange, and (c) a variety of eukaryotic proteins that lack an ATPase activity can promote DNA strand exchange. This led to a widespread view that RecA-mediated ATP hydrolysis is largely irrelevant to DNA strand exchange. We have recently shown that ATP hydrolysis has substantial effect on RecA-mediated DNA strand exchange. When ATP is hydrolyzed, the reaction is rendered unidirectional, can bypass structural barriers in the DNA, and can accommodate four DNA strands. The temperature dependences of the ATP hydrolytic and strand exchange activities of RecA are identical, providing additional evidence that they are coupled. Together, the results reveal an intrinsic capacity of a RecA filament to facilitate DNA strand exchange without ATP hydrolysis. The ATP hydrolysis is nevertheless coupled to DNA strand exchange, serving as an augmentation of this process that confers properties essential to recombinational DNA repair. Mechanisms for coupling ATP hydrolysis to DNA strand exchange and new data addressing the mechanisms will be discussed.

A2-125 THE EFFECT OF TEMPERATURE ON DNA SUPERCOILING IN A *gyrB* MUTANT IN *Salmonella typhimurium*, John P. Davidson, Jiliang Chiu, Wileena Session and Vishnu Suppiramaniam, Erman Munir, Department of Biology, Tuskegee University, Tuskegee Institute, AL 36088

The level of plasmid supercoiling was examined in a *gyrB* mutant (*hisU1820*) of *Salmonella typhimurium*. The mutant and an isogenic normal strain were transformed with pBR325 and pBR322 by electroporation. The degree of supercoiling was determined by chloroquine-agarose gel electrophoresis. The *hisU1820* mutation is temperature sensitve; it shows no growth above 42°C in minimal medium but continues to grow in enriched medium at the non-permissive temperature. Plasmid was extracted from cells grown at 20°, 30°, 37°, 40° and after a 30°-42°C shift. The linking number decreases with temperature for reporter plasmid extracted from the normal strain when cells are grown in enriched medium. The linking number shows little change upon increase in temperature above 20°C for reporter plasmid extracted from the *gyrB* mutant strain when grown in enriched medium. When cells are grown in minimal medium, there is a proportional decrease in linking number as the temperature is raised from 20° to 30° to 40°C in the normal strain. The mutant exhibits a decrease in linking number at 30°C from that observed at 20°C, but as temperature approaches restrictive temperuature, the mutant shows progressive loss of bands migrating at the position corresponding to negatively supercoiled plasmid and progressive accumulation of a slow migrating band which apparently represents catenanes or some other form of condensation. A previous report which failed to show a quantitative difference between *hisU1820* and a normal strain, may reflect the effect of temperature. A quantitatively significant difference between the mutant and the normal strain is observed for plasmid extracted from cells grown at 37°C. Supported by NSF grant #PRM-8208510 and HHS-NIH-DRR-MBRS RR08091 to JPD.

A2-126 IN VIVO THE UMUD'C COMPLEX SWITCHES REPAIR FROM RECOMBINATION TO SOS MUTAGENESIS. Raymond Devoret, Adriana Bailone, Suzanne Sommer, Séverine Delacour, Institut Curie, Bât. 110, F-91405 ORSAY, France.

We postulate that the three SOS repair processes which take place in *E. coli* after DNA damage by UV-light are *sequentially* expressed:
(1) *Excision repair* removes 80-85% of the lesions within the first 20 min; (2) then, *homologous recombination* puts aside about 50% of the remaining lesions in the next 40 min. Substrates acted upon by recombination are 2 kb gaps produced downstream a lesion by a first round of replication; (3) *SOS mutagenesis* resolves half of the gaps left unrepaired by recombination during the next hour. This process involves a second round of replication that is *error-prone*.
This mechanism is supported by the *sequential* induction of proteins such as RecA, UmuD, and UmuC. First, RecA protein nucleates on the single-stranded DNA present at gaps giving rise to an SOS signal resulting into more RecA protein produced. Induction of RecA protein is immediate reaching a maximum at 30 min. A consequence of RecA induction is the formation of RecA filaments expanding 5' to 3' on the single-stranded DNA present at gaps.
The mutagenesis proteins UmuD and UmuC are induced late, slowly and parsimoniously. Furthermore, UmuD is matured by cleavage on a RecA filament to produce UmuD', which when bound to UmuC constitutes the active complex in mutagenesis. UmuD' peaks at 60 min when excision and recombinational repair are over. The ratio of UmuD'C complexes over RecA monomers is 30/73000 for 12 J/m^2. This ratio is compatible with the binding of one UmuD'C complex to the extremity of one RecA filament remaining at gaps unresolved by recombinational repair. Thus, the UmuD'C complex is positioned right at a lesion and in contact with the replisome, which then becomes able to by-pass a lesion.
RecA protein behaves as an accordion on single-stranded DNA generated by a lesion: it expands by forming a long filament that shrinks when the single-stranded DNA segment becomes replicated. Formation of UmuD'C switches repair from repair to SOS mutagenesis.
Supporting evidence for this scenario will be provided on the poster.

A2-127 STRUCTURE/FUNCTION ANALYSIS OF *ESCHERICHIA COLI* DNA TOPOISOMERASE III. Russell J. DiGate, Hongliang Zhang, and Swati Malpure, Medical Biotechnology Center, Cell and Molecular Biology Program, and Department of Pharmaceutical Science, University of Maryland at Baltimore, Baltimore, MD 21201.

A chimeric topoisomerase molecule was constructed by replacing the generalized binding domain of *Escherichia coli* DNA topoisomerase III (Topo III) with carboxyl-terminal amino acids of *E. coli* DNA topoisomerase I (Topo I). The addition of the Topo I C-terminus to Topo III restored the ability of the enzyme to efficiently relax DNA; however, the ability of the enzyme to catalyze segregation of replication intermediates, *in vitro*, was not substantially improved. This suggests that the generalized binding domains of both Topo I and Topo III are contained within their C-terminal amino acids and the inability of Topo I to segregate replication intermediates may at least be partially explained by the properties of its generalized binding domain.

Footprinting experiments were performed using an oligonucleotide containing a strong Topo III binding/cleavage site. These experiments revealed that Topo III binds asymmetrically to its substrate with respect to the cleavage site, protecting ~12 nucleotides 5' to the cleavage site and ~ 2 nucleotides 3' to the site. These results are consistent with the minimal substrate requirement of the enzyme, which has been determined to be 7 nucleotides, 6 nucleotides 5' to the cleavage site and 1 nucleotide 3' to the cleavage site.

A substrate containing a strong Topo III binding/cleavage site was compared to a substrate lacking a site for the ability to act as an inhibitor of Topo III-catalyzed relaxation of negatively supercoiled DNA and for the ability to be bound by the topoisomerase. The substrate containing the binding/cleavage site bound Topo III more efficiently and served as a better inhibitor of the enzyme.

A2-128 VARIATION AMONG ENTERIC BACTERIA IN GENES OF THE *MRR-HSDRMS-MCRBC* RESTRICTION CLUSTER

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Restriction systems regulate entry of foreign DNA into bacterial cells. Double-strand cleavage prepares the DNA for destruction or recombination; further processing induces SOS repair and recombination functions; and integration of remaining fragments may foster assortment of characters within a populations. These enzymes are an extremely diverse group. At least five different mechanistic classes of enzymes exist; individual strains may express five or more enzymes at the same time; and populations may express as many as 28 different specificities of a single mechanistic class. We are interested in how populations become differentiated for restriction spectrum, beginning with the systems of *E. coli* K-12.

K-12 expresses four chromosomally-encoded restriction functions. Three of these, Mrr, *Eco*K and McrBC, are encoded by six genes--*mrr, hsdRMS, mcrBC*--in an 11 kb cluster. Each of these systems is mechanistically distinct and unrelated by sequence analysis. Extensive work by the Murray laboratory has characterized variation in *hsd* genes. We have begun to ask how much variation exists at the flanking loci in order to understand what mechanisms might promote acquisition of new restriction specificities

Analysis of existing variation at this locus in 19 species from 6 genera of Enterobacteriaceae employed Southern blots and PCR examine 5 segments of the ICR: *mrr*; *hsdRM*; the intergenic region between *hsdS* and *mcrB* (IG); *mcrBC;* and 3 kb of material downstream of *mcrC*. The two nucleotide-dependent restriction systems were highly variable, with few hits and weak hybridization where hits were found. In contrast, *mrr* is present in all species of all genera tested. The IG was erratically distributed but gave stronger signals than those given by the flanking restriction systems.

PCR analysis allowed us to verify physical linkage of hits to the IG and *mcrBC*. We made use of two candidate conserved elements to design PCR primers: a unique primer based on conserved sequence in the IG; and a degenerate primer based on the sequence encoding element I (the P-loop) of the GTP binding site motif in McrB. Sequence analysis of these products should enable us to delineate the borders between variable and conserved elements of this region. Such knowledge will inform models for the evolutionary history of the different parts of the region.

A2-129 **OCCURRENCE OF HISTONE-LIKE PROTEINS IN GRAM-POSITIVE BACTERIA**, Sundiatu M. Dixon-Fyle, Pierre Prentki* and Lucien Caro, Department of Molecular Biology, University of Geneva, 1211 Geneva 4, Switzerland.

Bacterial histone-like proteins (HLPs) are small, basic, heat-stable DNA-binding proteins. In *E. coli* they include HU, which binds DNA non-specifically, and the closely related protein Integration Host Factor (IHF), which is sequence-specific and strongly bends DNA upon binding. These proteins are involved in several aspects of gene expression in *E. coli* and HU at least is likely to play a role in the organisation of bacterial chromatin. There is a degree of functional redundancy between the *E. coli* HLPs, but this is not the case in all bacteria; for example the *B. subtilis* HU is essential, suggesting that it may be the sole protein of its kind in this organism.

We sought to investigate the extent to which these proteins are conserved in bacteria. Using a combination of PCR and Southern blotting we have identified genes for HLPs in several gram-negative and gram-positive bacteria. Using reverse PCR we have cloned the HU homologue of the lactic acid fermenting bacterium *Streptococcus thermophilus*. The gene encodes a protein 57% identical to that of *E. coli*, and 71% identical to *B. subtilis* HU. There is a single copy of the gene, indicating that the HU protein is a homodimer. We have also identified a potential second gene bearing homology to the α subunit of IHF.

* deceased 24 July 1993

A2-130 SITE-SPECIFIC INVERSION OF THE *fimA* TRANSCRIPTIONAL SWITCH IS SUBJECT TO CONTROL BY DNA SUPERCOILING

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In *Escherichia coli K-12* expression of FimA, the major subunit protein of type 1 fimbriae, is dependent upon the orientation of a promoter-housing invertible region abutting the *fimA* structural gene. Ultimately the orientation of this transcriptional switch is controlled by a site-specific recombination system. We describe here the effects of perturbing cellular DNA supercoiling on inversion of the *fim* switch and subsequent expression of the *fimA* gene. Introduction of a *topA::Tn10* mutation into strain VL386 (harbouring a native chromosomal *fimA-lacZ* fusion) resulted in reduced switching of the invertible element in either orientation. Interestingly, this effect correlated with loss of topoisomerase I function and not the net level of DNA supercoiling. Inhibition of DNA gyrase however, introduced an orientational bias to the recombination event favouring the OFF to ON transition. Transcription of *fimA* itself was found to be relatively insensitive to DNA supercoiling in strains where the switch was locked in the ON position. We found transcription of the *fimB* gene (encoding the site-specific recombinase responsible for inversion of the *fim* switch) to be responsive to alterations in DNA supercoiling. More specifically, the *topA::Tn10* mutation reduced *fimB* transcription whereas inhibition of DNA gyrase increased it. The reduction in *fimB* transcription apparent in the *topA* mutants was not seen if a compensatory mutation was acquired. Thus the observed alterations in *fimB* transcription arising in *topA* mutants are insufficient to account for a topoisomerase I requirement in switching of the invertible element. Biasing of the switch resulting from gyrase inhibition again could not be accounted for by the observed effect on *fimB* transcription. Generation of localised supercoils within the *fim* switch arising through transcription from the *fimA* promoter lying therein may have consequences for inversion of this region which are born out in our experimental findings.

A2-131 VARIATION IN GENOME ORGANIZATION AMONG *VARIOVORAX PARADOXUS* CLONES ISOLATED FROM SOIL, John Dunbar, Igor Zlatkin, and Larry Forney, NSF Center for Microbial Ecology and Department of Microbiology, Michigan State University, E. Lansing, MI 48824

Genome organization in clones of a localized population of a soil bacterium was examined by using primers to amplify DNA between repeated sequences (REP-PCR) and by restriction analysis of genomic DNA. From a 2X3m fallow agricultural plot, 145 isolates of *Variovorax paradoxus* were collected. These isolates were taxonomically indistinguishable by colony morphology, substrate utilization profiles, and cellular fatty-acid profiles. The close phylogenetic relationship of these isolates was also indicated by identical 16S-rDNA restriction patterns. In contrast to the apparent similarity of these clones, REP-PCR fingerprints of the genomes showed there were at least 14 distinct strains. Extensive genetic differences between strains were equally evident in megabase genomic DNA restriction fragment length polymorphisms (RFLPs) obtained from a subset of isolates. The REP-PCR fingerprints and genomic DNA restriction patterns both suggest recombination events have contributed greatly to the divergence of the *V. paradoxus* strains. Among clones of the same strain, genetic diversity was revealed by minor differences in megabase RFLPs (such as the loss or appearance of one or two fragments) and isolation of plasmids that varied in size and homology. Genetic variation among clones was reflected phenotypically in the form of different growth rates on several different substrates. These data indicate that the *Variovorax paradoxus* population in the field site we examined consists of numerous strains with highly polymorphic genomes.

A2-132 SEQUENCE AND ANALYSIS OF THE 5' CHROMOSOMAL REGION ADJACENT TO gyrB GENE FROM A HALOPHILIC ARCHAEON, Christiane Elie, Marie-France Baucher and Patrick Forterre, Institut de Génétique et Microbiologie, Université Paris-Sud, 91405, Orsay cedex, France.
In bacteria, the genomic organization upstream of gyrB gene is conserved and two essential genes implicated in DNA replication are grouped in this chromosomal region : the dnaA gene encoding the protein involved in the specificity of initiation at the replication origin and the dnaN gene encoding the β subunit of the replicase, the DNA polymerase III holoenzyme (1). At the amino-acid level, DnaA proteins are well conserved among bacteria whereas DnaN proteins are quite divergent. Up to now, little is known about replicative proteins in archaea, and only one type of DNA polymerase whose function is unknown has been characterized from several species (2). We sequenced and analysed a 5 kb fragment adjacent to gyrB gene from the archaeon Haloferax volcanii and detect several ORFs. Whereas none of the putative encoded proteins is related to bacterial DnaA proteins or to any proteins of the data bank, one of them shows some similarities with bacterial DnaN proteins. This result suggests that a DnaN-like protein could be present in archaea and that the chromosomal organization is conserved to some extent between bacteria and archaea.

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2-Forterre P. and Elie C."The Biochemistry of Archaea " M. Kates, D.J. Kushner and A.T. Matheson - Ed. New Comprehensive Biochemistry. 1993, 26, 325-365.

A2-133 INITIATION OF HELICASE ACTIVITY FOR THE DNAB PROTEIN REQUIRES A 5' OVERHANG OF AT LEAST 8 BASES, Jessica Epstein, Dorina Trusca‡, Chris Thompsom‡, John Kozarich and David Bramhill‡, Department of Biochemistry, University of Maryland, College Park, MD 20742.
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The dnaB protein of *E. coli* is the primary DNA helicase which serves to propagate the replication fork. It processively migrates in the 5' to 3' direction, functions to unwind duplex DNA in advance of DNA synthesis and guides primase onto the lagging strand. Single stranded DNA binding, ATPase and helicase activities can be assayed independently. In this manner, the characteristics of dnaB may be dissected. Studies with short, synthetic oligonucleotides suggested that dnaB required at least 8 bases of single stranded DNA for binding as well as ATPase activity, although optimal activity was attained with slightly longer fragments. Furthermore, one hexamer of dnaB would bind to several 8-MERs, suggesting several binding sites per hexamer and a preference for a longer single stranded region. To compliment these findings, several partial duplex substrates with various length 5' overhangs were constructed. DnaB exhibited helicase activity for a 10 base overhang, but not a 5 base overhang. This was consistent with the requirement of at least 8 bases to produce single stranded binding and ATPase activities.

Experiments to determine the number of base pairs unwound per ATP hydrolyzed are currently in progress. Together with these results, we hope to gain detailed insight into the mechanism of dnaB at the replication fork.

A2-134 GENOME OF *RHODOBACTER CAPSULATUS*: FURTHER MAPPING AND SURVEYING BY HYBRIDIZATION
Michael Fonstein and Robert Haselkorn, MGCB Dept., University of Chicago, 920 E. 58th st., Chicago, Il 60637

In a previous work, one hundred ninety two cosmids from a gene library of *R. capsulatus* had been ordered in two contigs: one corresponding to the chromosome and one to the 134 kb plasmid (Fonstein M. & Haselkorn R. (1993) Chromosomal structure of *Rhodobacter capsulatus* strain SB1003: cosmid encyclopedia and high-resolution physical and genetic map. Proc Natl Acad Sci USA 90:2522-6). The map contained forty regions which had been connected only by colony hybridization having no common restriction fragments. We have verified this map by blot-hybridization and the actual sizes of the overlaps have been determined. Several corrections to the earlier map include single cosmid shifts and inversions. One additional gap in a cosmid contig (linked only by PFGE map) was found. About 2500 additional *Eco*RI, *Bam*HI and *Hin*dIII restriction sites (RSs) were added to the 560 *Eco*RV RSs previously mapped onto the *Rhodobacter* chromosome, increasing the resolution of the physical map to a gene size level. Twenty five new genetic markers were located on the genetic map. The 50 markers which have now been mapped represent nearly 300 genes and ORFs found in different species of *Rhodobacter*. The orientation of transcription of the four *rrn* operons, an important element of genome architecture, was established using 16S and 23S specific probes and digestion with the extremely rare cutting enzyme, *Ceu*I. Gel-blots of 192 cosmids of the miniset of *R. capsulatus* digested with *Eco*RV were prepared. Such a "hybridization template" represents the whole genome split into 560 DNA fragments varying in size from 0.4 to 25 kb. The application of this template for high resolution mapping of single genes, total genomic DNAs and differentially expressed RNAs in one step was demonstrated.

A2-135 TRANSCRIPTION OF THE *hns* GENE OF *E. coli* IS COUPLED TO DNA SYNTHESIS BY AUTOREGULATION
Andrew Free and Charles J. Dorman, Department of Microbiology, Moyne Institute, Trinity College, Dublin 2, Republic of Ireland.
The DNA curve-binding H-NS protein of enteric bacteria is a major component of bacterial chromatin which regulates the expression of a variety of genetic systems. It is possible that the expression of such systems may be modulated by variations in the level of H-NS protein. The *E. coli hns* gene, which encodes H-NS, is known to be transcriptionally autoregulated via high-affinity H-NS binding sites containing curved DNA located upstream of its promoter; loss of H-NS activates transcription of *hns*-reporter gene fusions *in vivo*, while H-NS inhibits *in vitro* transcription from the *hns* promoter.
It is predicted from these observations that *hns* transcription should be downregulated under conditions where new DNA targets for the H-NS protein are not being synthesised. Here, we show that *hns* transcription does indeed shut down when DNA synthesis is blocked by shifting a *dnaC* temperature-sensitive mutant to the non-permissive temperature or by nalidixic acid treatment. *hns* expression, measured by Northern analysis, recovers when the block to DNA synthesis is removed. This expression pattern is dependent upon continuing protein synthesis.
Our data are consistent with a model in which the *hns* promoter "monitors" the synthesis of new DNA targets for the protein and responds accordingly. Also, as predicted by this model and contrary to previous experiments using gene fusions, we find that transcription of the *hns* gene shuts down as cells enter stationary phase and is re-activated when logarithmic growth resumes. Therefore we propose that the cellular levels of H-NS remain relatively constant under normal growth conditions, although increased H-NS may be present under certain stress conditions (cold-shock is a known example). Variations in the expression of H-NS-regulated genes must therefore occur by means other than alterations in the concentration of this global regulator.

A2-136 PARTITION OF P1 PLASMID CHROMOSOMES IN *Escherichia coli*,
Barbara E. Funnell, Megan J. Davey, and Liane Gagnier, Department of Molecular and Medical Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Partition of the low-copy-number prophage/plasmid of bacteriophage P1 is an active process in *Escherichia coli*. The plasmid encodes two proteins, ParA and ParB, and contains a DNA site called *parS*, all of which are required for plasmid stability. First, we have investigated the role of ATP and other nucleotides in ParA activity. ParA is an unusual site-specific DNA binding protein, in that the latter activity is strongly affected by ATP. The *parA* and *parB* genes are cotranscribed from a promoter upstream of *parA*. Their expression is negatively regulated by ParA, presumably because the protein binds specifically to a site in the promoter region. Our data indicate that ATP affects the oligomerization of ParA, which in turn influences ParA's DNA binding affinity. Second, ParB and the *E. coli* IHF protein bind to *parS* to form the partition complex. This complex bears several biochemical similarities to the bacteriophage λ Int/IHF/*attP* complex which λ uses to capture and pair with a DNA site on the bacterial chromosome, *attB*. We tested whether this similarity extends to function; that is, whether P1 uses the partition complex to pair with, and thus co-partition with the bacterial chromosome. Our experiments indicate that P1 actually partitions independently of the bacterial chromosome, and support a model in which P1 plasmids pair with each other via their partition complexes. Proper cellular localization is presumably directed by host-plasmid protein-protein interactions. (Supported by the Medical Research Council of Canada.)

A2-137 PROTEOLYTIC MAPPING OF A MEMBRANE BINDING DOMAIN OF DnaA PROTEIN
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DnaA protein plays an important regulatory role as the initiator of *E. coli* chromosomal replication. The *in vitro* activity of DnaA protein is strongly influenced by the tight binding of ATP and ADP, with ATP-DnaA protein being the form active for initiating replication at *oriC*. Acidic phospholipids in a fluid bilayer promote the release of bound nucleotide *in vitro*, and in the presence of *oriC*, facilitate rejuvenation of DnaA protein by exchanging ATP for bound ADP. Using proteolytic mapping, we have identified a region of DnaA protein involved in phospholipid binding. ATP-DnaA protein was subjected to limited digestion by a variety of proteases. Digestion conditions were optimized for complete consumption of full length DnaA protein and generation of stable proteolytic fragments which retained bound nucleotide and responded to treatment with acidic phospholipids. Such fragments were isolated by standard chromatographic techniques and their amino terminal sequences obtained by Edman degradation. Their location within the full length DnaA protein will be presented.

A2-138 ENHANCEMENT OF DNA REPLICATION IN THE pT181 PLASMID,
Maria Laura Gennaro, Selma Soares de Oliveira, Qing Zhang, Natalie Bogdanos and Padmaja Paderu, Public Health Research Institute, New York, NY 10016.

cmp, the replication enhancer found in the staphylococcal plasmid pT181, stimulates the interaction of *ori* with the replication initiation protein, RepC, from a site located at a distance of 1,000 base pairs (bp) from *ori*. Deletion of *cmp* drastically lowers the plasmid's ability to compete with an incompatible cmp^+ plasmid for RepC. The observed competitive defect in the cmp^- mutant suggested that *cmp* stimulates utilization of RepC by *ori*. Indeed, second-site suppressor mutations of cmp^- map in *repC* and produce mutant RepC proteins that fail to discriminate between cmp^+ and cmp^- plasmids or even prefer cmp^- to cmp^+ plasmids.

The *cmp* locus is a 100-bp DNA region containing two loci of intrinsic DNA bending and hexameric sequences sharing a loose consensus sequence. Site-directed mutagenesis of the *cmp* sequence suggests that *cmp* may indeed contain discrete sequence motifs that require precise spacing to express *cmp* activity.

cmp is a binding site for a 65-70 Kda host-encoded, site-specific DNA-binding protein we call *cmp*-binding factor (CBF). We have cloned the gene encoding CBF by screening an *S. aureus* expression library in a lambda vector for binding to a 100-bp *cmp* DNA probe. Nucleotide sequence analysis of *cbf* and biochemical characterization of the CBF protein are in progress.

We tested the effect of *cmp* on plasmid DNA topology, and we found that plasmids carrying wild type *cmp* have a higher titratable negative supercoiling than plasmids carrying non-functional *cmp* variants. *cmp* raises supercoiling levels by a mechanism that does not appear to involve transcription or preferred binding of gyrase. We are currently testing whether *cmp*-associated supercoiling reflect changes in torsional tension or restrained supercoiling in vivo by using torsionally tuned Z-DNA probes to measure intracellular plasmid superhelical tension.

A2-139 IDENTIFICATION OF THE CATALYTIC CENTRE OF E.COLI PRIMASE USING SELECTIVE AFFINITY LABELLING.
G. Nigel Godson and Arkady Mustaev. Biochemistry Dept., NYU Medical Centre, N.Y., N.Y. 10016 and Public Health Research Institute, N.Y., N.Y., 10016, respectively.

E.coli primase is a 67 kDa (581 amino acids) protein that initiates DNA replication by synthesising a small pRNA on ss DNA. Little is known of the active sites of primase. Using the highly selective method of affinity labelling of catalytic centers developed for RNA and DNA polymerases (Grachev et al, Eur. J.Biochem., 163, 113-121 (1987)) we have identified the NTP binding site and catalytic centre of primase. In this method, ATP derivatives modified on the 5' side with groups that are reactive with Lys and His residues are cross linked to the protein. A second $[\alpha\text{-}^{32}P]$ NTP is then added and polymerisation on a template allowed to preceed. This adds a $[^{32}P]$ tag to the ATP derivatives that are cross linked to the catalytic centre. The cross linked $[^{32}P]$ labelled dinucleotide is mapped using chemical cleavage of the labelled protein. We have adapted this to the primase/SSB/G4oric pRNA synthesis system. Lys residues 211, 229 and 241 are part of the active centre. This is in an evolutionarily conserved region of primase. His 43, part of the putative zinc finger, is also close enough to the active centre to be cross linked. The cross linked ATP derivative can be elongated to a 5 nt pRNA, which implies significant stretching of the active site during pRNA synthesis. The architecture of the primase active centre closely ressembles that of RNA polymerase. Long arm ATP derivatives demonstrated that SSB is within 12 Angstroms of the primase catalytic centre during pRNA synthesis.

In addition, we have used this method of measuring pRNA synthesis to estimate the smallest DNA sequence that primase recognises on G4oric. Using the isolated 47 kDa N-terminal domain of primase that retains the pRNA synthesis activity (Sun et al, PNAS in press) and which utilises oligonucletides efficiently as a DNA substrate in the absence of SSB, we have shown that the smallest G4oric sequence that can be used for pRNA synthesis is a 13 nt (5' CCTACTGCAAAGC 3').

A2-140 STUDIES ON THE INTERACTION OF IHF WITH ITS RECOGNITION SITE.

Nora Goosen, Laurence Zulianello, Peter van Ulsen and Pieter van de Putte, Laboratory of Molecular Genetics, Department of Biochemistry, Leiden Institute of Chemistry, Leiden University, the Netherlands.

Integration Host Factor (IHF) of *E.coli* is a heterodimeric DNA binding protein, consisting of the subunits HimA and HimD. An important property of IHF is, that binding of a single dimer to its recognition site results in a sharp bending of the DNA (estimated around 140°).
We have constructed a set of HimA and HimD deletion mutants. The binding of IHF containing these mutated subunits to wild type and mutant *ihf* sites was studied with gelretardation and footprinting. The results show that the C-terminal part of HimD is important for the stability of the IHF-DNA complex, whereas the C-terminal part of HimA seems more involved in the specificity of the IHF-DNA complex.

A2-141 ARCHAEAL HISTONES: A NOVEL PERSPECTIVE ON PROKARYOTIC CHROMOSOME STRUCTURE AND GENE REGULATION.

Rowan A. Grayling, Kathleen Sandman, and John N. Reeve; Department of Microbiology, The Ohio State University, Columbus, OH 43210.

HMf is the archetype of a widely distributed family of archaeal histones. This structural DNA binding protein is from the hyperthermophilic archaeon *Methanothermus fervidus*. HMf is small (monomer MW 7.5 kDa), basic (pI ~9-10), abundant (≥ 1% soluble protein), and binds DNA with little sequence specificity. Preparations of HMf contain two polypeptides, HMfA and HMfB, that are 85% identical, and have 50% sequence similarity to the structured central regions of the eukaryal core histones H2A, H2B, H3, and H4. Computer modeling, CD spectroscopy, and NMR data indicate that members of the HMf protein family are prokaryotic homologs of the nucleosome core histones. *In vivo*, the HMf population exists as homo- and hetero-dimers of HMfA and HMfB, that form tetramers to bind and wrap DNA. HMfA/HMfB heterodimers can be reconstituted from preparations of homodimers *in vitro*, under conditions that mimic those found *in vivo*. The HMfA/HMfB ratio is growth-phase regulated *in vivo*, and different DNA binding and supercoiling properties have been established for the two homodimers, suggesting that this may be a means to regulate global changes in chromosome structure and gene expression. HMf forms nucleosome-like structures (NLS), observable as "beads on a string" in electron micrographs, and 60 bp ladders are protected from micrococcal nuclease digestion by NLS, in both *in vivo* chromatin, and in *in vitro* reconstituted complexes (*cf.* 146 bp ladders for nucleosomes). The HMf proteins represent a simple, experimentally tractable model for eukaryotic histone-DNA interactions, and also serve as an important bridge between prokaryotes and eukaryotes in understanding the roles structural chromosomal proteins may play in gene regulation.

A2-142 CRISS-CROSS REGULATION OF THE EXPRESSION OF GENES ENCODING NUCLEOID PROTEINS,

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Escherichia coli Fis protein stimulates transcription in vitro from the promoter of *hns*, the gene encoding the major nucleoid protein H-NS; H-NS, on the other hand, inhibits transcription from the same promoter. The stimulation by Fis and the inhibition by H-NS can cancel each other when the two proteins are added together, in appropriate amounts, to the in vitro transcription system. Gel shift and footprinting experiments show that Fis binds to multiple sites in the promoter regions of *hns*; the main Fis binding sites flank a bend in the DNA located at -150 from the transcriptional start and partly overlap the H-NS binding sites responsible for transcriptional auto-repression of *hns* by its product. The opposing effects of Fis and H-NS on *hns* transcription is explained by the different location of the additional binding sites occupied with lower affinity by these proteins after the main binding sites are filled-in: H-NS binds to a site overlapping the -35 element of the promoter while Fis binds further upstream. These results and the displacement of H-NS by Fis in band shift assays suggest that the two proteins compete for the same binding sites on the promoter of *hns*. In vivo the steady state level of *hns* mRNA was found to be two to four-fold higher in a wild-type compared to a *fis*⁻ strain, the maximum difference being seen within a narrow time window of the growth curve, presumably corresponding to a critical intracellular concentration of Fis. Very similar results were obtained for the *cspA* gene encoding the nucleic acid-binding protein CS7.4, the transcriptional activator of cold shock genes. The transcriptional activity of the *cspA* promoter is stimulated by Fis and repressed by H-NS both in vivo and in vitro. On the other hand, protein CS7.4, which stimulates *hns* transcription by binding to a DNA segment containing a CCAAT box downstream the transcription startpoint and favoring the interaction of the RNA polymerase with the promoter, was found to interact also with its own promoter which, like that of *hns*, contains two CCAAT motifs. In addition to these two CCAAT motifs, the promoter of *cspA* contains several other boxes with striking sequence homology to corresponding boxes found in *hns*.
We postulate the existence in the nucleoid of a complex mechanism of criss-cross regulation of gene expression based on changing intracellular levels of functionally interplaying DNA-binding proteins with loose and partly overlapping recognition and binding sites and synergistic or antagonistic effects on transcription.

Acknowledgement. This work was supported in part by a Human Capital and Mobility grant from the EC.

A2-143 RecBCD-χ INTERACTION: GENETIC DISSECTION OF EXONUCLEASE REGULATION AND RECOMBINATION STIMULATION WITH A VARIANT OF χ,

Naofomi Handa, Seishi Ohashi, Kohji Kusano and Ichizo Kobayashi, Department of Molecular Biology, Institute of Medical Science, University of Tokyo, Tokyo 108 JAPAN

χ sequence (5' GCTGGTGG), first identified as a sequence that increases production of some lambda strains, interacts with RecBCD enzyme, modulates its exonuclease activity and locally stimulates RecA-mediated recombination.

χ is not recognized by RecBCD* mutant enzyme. Hence lambda with χ make tiny plaques in recBCD* mutants. We cloned *E. coli* chromosomal fragments that allow lambda to form larger plaques in a recBCD* mutant. They had a consensus sequence, which turned out to be the active sequence when synthesized *in vitro* and was named χ*. χ* blocks exonuclease activity of RecBCD* enzyme *in vivo*, but it does not restore recombination hot spot activity.

Thus we were able to uncouple two steps of χ-RecBCD interaction: stop of exonuclease activity and recombination stimulation. These results also suggest that block of exonuclease, as opposed to recombination stimulation, is the primary determinant of lambda burst size. We discuss possible mechanisms of χ*-RecBCD* and χ-RecBCD interaction.

A2-144 TUS-CATALYZED TERMINATION OF BIDIRECTIONAL DNA REPLICATION FROM *oriC*. Hiroshi Hiasa and Kenneth J. Marians, Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Minichromosomes containing *oriC* and two *Ter* sites, oriented as they are on the *Escherichia coli* chromosome, have been used to study the role of Tus in termination of bidirectional DNA replication. Coupling of leading- and lagging-strand DNA synthesis in *oriC* system required high concentrations of primase. At high primase concentrations, initiation occurred exclusively at *oriC* and two coupled replication forks proceeded bidirectionally around the plasmid on each active template. In the presence of Tus, both replication forks terminated DNA synthesis at one or the other *Ter* site, in an apparent stepwise manner. First, the progress of one replication fork was arrested by a properly oriented Tus-*Ter* complex. Then, either because of steric hindrance resulting from the stalled replication machinery of the first fork, or because of the formation of a branched DNA structure, the progression of the second opposing fork was halted at the same site on the DNA template. In the absence of Tus, over-replication, which required DNA ligase, arose *via* a template strand switching mechanism. Thus, the role of Tus is likely to prevent over-replication, thereby ensuring that DNA replication ceases after the completion of one round.

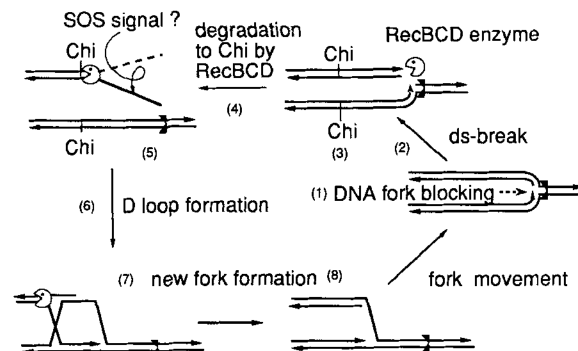
A2-145 PREMATURE ARREST OF DNA REPLICATION AT INVERTED Ter SITES IN THE CHROMOSOME OF *ESCHERICHIA COLI* INDUCES THE SOS RESPONSE, Thomas Hill and Bela Sharma, Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA 19104

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 (called the Inv*Ter* strain). We then introduced a functional *tus* gene by transforming the Inv*Ter* cells with a plasmid carrying a copy of the *tus* gene under control of an arabinose-inducible promoter. These cells grew normally in the absence of arabinose, when *tus* gene expression was repressed. However, when grown in rich medium containing 0.2% arabinose, the Inv*Ter* strain demonstrated a mixed cell morphology, including the formation of elongated cells and filaments. Filaments stained with DAPI showed fluorescence throughout most of the length of the cell, with only the ends of the filament free of DNA. Also, the generation time of the cells was increased to 41 minutes in arabinose, as compared to 28 minutes in the absence of arabinose. These results suggested that activation of the inverted *Ter* sites by induction of *tus* gene expression was arresting DNA replication and delaying the onset of cell division. Filamentation of the Inv*Ter* cells was shown to be a result of the SOS response, through the use of a *sfiA::lacZ* reporter gene and by the observation that *recA* or *lexA3* alleles suppressed filamentation in the Inv*Ter* strain. Interestingly, in both *recA* and *lexA3* derivatives, suppression of filamentation did not produce high numbers of anucleate cells, as would be expected if the partially replicated chromosomes were partitioned to a single cell. Instead, virtually all cells contained a nucleoid, suggesting that cell division was proceeding even though DNA replication was not complete. In addition, we observed a high loss of viability (>90%) in the *recA* and *lexA3* strains. We postulate that septation occurred in these cells even though the chromosome was incompletely replicated, partitioning the two chomosomes via a "guillotine" mechanism.

A2-146 RecA AND RecG PROTEINS IN R-LOOP FORMATION. Xiankang Hong, Gregory W. Cadwell, and Tokio Kogoma, Department of Cell Biology, University of New Mexico Health Sciences Center, Albuquerque, NM 87131.

Accumulating evidence points to the existence of persistent R-loops in *E. coli*. However, how the persistent R-loops are formed or what factors are involved in the process is unknown at present. We previously proposed that RecA protein catalyzes assimilation of a nascent transcript into the template duplex, giving rise to an R-loop. In *rnhA* mutants devoid of RNase HI, the R-loop is stabilized and becomes an origin of DNA replication (i.e. *oriK*s). R-loops, if not removed, are deleterious to the cell. In the absence of RNase HI, they must be removed by a mechanism that involves DNA polymerase I or by recombination repair. Thus, *rnhA::cat polA* and *rnhA::cat recB* double mutants are inviable. We have found that mutations in *recG* that inactivate the RecG helicase activity impart phenotypes similar to those exhibited by *rnhA* mutants: *recG* mutants exhibit cSDR as do *rnhA* mutants, and *recG polA* double mutants are inviable. The *recG* mutations do not inactivate the RNaseHI activity of *rnhA*$^+$ strains. These results suggest that R-loops are stabilized in *recG* mutants and imply that the RecG helicase has a function to prevent R-loop formation and/or to remove R-loops. This possible involvement of RecG in R-loop removal has been strongly supported by the observation that the combination of a *rnhA* and *recG* mutation is lethal. We will discuss a model that RecG protein either counteracts the RecA-catalyzed invasion of transcript into the duplex or removes RNA from R-loops or both.

A2-147 IS A DOUBLE STRANDED BREAK INTRODUCED AT THE STALLED DNA REPLICATION FORK? Takashi Horiuchi, National Institute for basic Biology, Okazaki, 444 Japan.
To examine the physiological effects of DNA replication arrested at terminus (*Ter*) site, we constructed a replication blocked *E. coli* strain so that both bi-directional replication forks would be impeded at two flanking *Ter* sites, one is artificial and other is natural. While the blocked strain grew slightly slower than a control strain, it had abnormal phenotypes similar to those of *E. coli dam*$^-$ mutants, i. e., hyper-rec phenotype, *recA*- and *recB(C)*-dependent growth, and constitutive SOS induction. The observation that these two apparently unrelated mutants cause similar phenotypes led us to the design of a model (see the below Figure). This model can a) explain the puzzling phenotype of *recA*$^-$ and *recB*$^-$(*C*$^-$) mutants and the SOS inducing phenotype of *polA*, *lig* and *dna* mutants under restrictive conditions b) provides an interpretation for the role of the Chi sequence, c) suggests a possible key role for the homologous recombination with regard to cell survival following the arrest of DNA replication.



A2-148 THE PUTATIVE IS2 TRANSPOSASE IS PRODUCED BY A TRANSLATIONAL FRAMESHIFT IN ITS REPRESSOR GENE, Shiau-Ting Hu and Lim-Chee Lee, Institute of Microbiology and Immunology, National Yang-Ming University, Taiwan, R.O.C.

One protein of 14-kDa (InsA) was detected when the expression of IS2 was examined in *E. coli* minicells. When the *insA* promoter was replaced by a T7 promoter, another protein of 46-kDa was observed. Since the size of the 46-kDa protein is greater than predicted from ORF on IS2 and a retroviral frameshift signal A_6G is near the C-terminus of *insA*, it was proposed that the 46-kDa protein was produced by a -1 frameshift mechanism. This was confirmed by changing the nucleotide sequence of the frameshift singal and examining the effects on their translational products. To investigate whether the 46-kDa protein of IS2 plays a role in IS2 transposition, the DNA binding ability of this protein was examined. Gel retardation assays indicated that this protein bound both inverted repeats of IS2, a characteristic of transposases. Therefore, we proposed that this 46-kDa protein is a transposase produced by translational frameshift in its repressor gene, as in the case of IS*1*.

A2-149 FUNCTIONAL MOTIFS AND THREE-DIMENSIONAL STRUCTURE OF THE HOLLIDAY JUNCTION RESOLVASE RUVC

Hiroshi Iwasaki[1], Atsushi Saito[1], Kenji Ichiyanagi[1], Takashi Hishida[1], Kosuke Morikawa[2], and Hideo Shinagawa[1], [1]Department of Molecular Microbiology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan, [2]Protein Engineering Research Institute, Suita Osaka 565, Japan

Escherichia coli RuvC protein (19 kD) is an endonuclease that resolves Holliday intermediates of homologous recombination. To elucidate reaction mechanisms of Holliday junction by RuvC, we initiated mutational and crystallographic studies on RuvC. A large number of *ruvC* mutants have been isolated by site-directed and random mutagenesis, and characterized. The three dimensional structure of RuvC has been determined at 2.5 Å resolution. The combined studies revealed the following three functional motifs of RuvC.

(1) Formation of a dimer is required for the RuvC function. The structural studies predicted that the subunits are related with a dyad axis and held together by hydrophobic contacts and hydrogen bonds between the second alpha helices. Consistent with this, several recessive mutants have alteration in the implicated residues.

(2) Some of the dominant negative mutants have alteration in one of the 8 basic residues which occupy the wall of the cleft that can accommodate a duplex DNA, suggesting these residues are involved in the interaction with the phosphate backbone of DNA.

(3) Each mutant in one of the 4 acidic residues that occupy the bottom of the putative DNA binding cleft shows strong dominant phenotype. These mutant RuvC proteins show drastically reduced junction specific endonuclease activity although they retain the activity to form the dimer and to bind specifically to the junction DNA. We conclude that these four acidic residues constitute the catalytic center.

A2-150 ROLE OF LYSOGENIC CONVERSION CAUSED BY PHAGE ø80 IN E.coli EVOLUTION, Dmitri P. Kozyrev, Department of Biophysics, St.Petersburg State Technical University, 29 Polytechnicheskaya Str., St.Petersburg, 195251, Russia

Lysogenic conversion caused by temperate phage ø80 is that E.coli cells lysogenised by this phage exhibit TonA phenotype (the partial resistance to superinfecting phages TI and ø80). The phage gene responsible to the phenomenon has been termined cor. It was found the cor gene product inhibits the function of the tonA gene of E.coli in ø80 lysogens. The number of the active receptors is 4-7-fold less on ø80cor$^+$ than on ø80cor$^-$ lysogenic cells. The cor gene expression in ø80 lysogens preserved in anaerobic conditions is the reason of accumulation of cells exhibiting the complete TonA phenotype which retains after ø80 prophage elimination. The mutations resulting in the complete TonA phenotype were mapped on the E. coli genetic map within the tonA gene. Evidently the TonA mutants accumulate in the lysogenic cultures in anaerobic conditions because of their preferable survival under these conditions. It is known the part of the cells in the lysogenic population contains ø80 genome in lytic cycle. This leads to appearance of the free phages including virulent. The cor gene prevents the adsorbtion of the free phages. But this protection is not complete therefore during prolonged preservation all the lysogenic cells could lyse because of the infection with the virulent phages. But insteade this the TonA mutants accumulate in these cultures. On the contrary the preservation of the ø80 cor$^-$ lysogens results in destruction of the cell population and in accumulation of virulent ø80 mutants. Thus the role of the cor gene as a selective factor for the lysogens is that its product represses the tonA function what gives necessary time for accumulation of the TonA mutants in the lysogenic population.

Posters K-Z

A2-200 BACTERIAL SIMILARITIES ASSESSED BY THE METHOD OF SIGNIFICANT SEGMENT PAIR ALIGNMENTS (SSPA VALUES) FOR SEVERAL PROTEIN FAMILIES, Samuel Karlin and Volker Brendel, Department of Mathematics, Stanford University, Stanford, CA 94305

Protein sequence of prokaryotic origin are compared using the recently developed SSPA method which relies upon Significant Segment Pair Alignments. The SSPA value assigned to a pair of sequences is the normalized sum of substitution scores added up only over consistently ordered, nonoverlapping, statistically significant matching segment pairs. Thus, while allowing for gaps between high scoring segment pairs, the method does not depend upon gap penalties which are often difficult to assign in other commonly used alignment methods. A wide range of protein sequences in prokaryotes are examined including RecA, glutamatine synthetase, glutamate dehydrogenase, superoxide dismutase, and heat shock protein 60 and 70 homologues. Comparisons are interpreted with respect to questions of relationships and coherence within and between gram-positive and gram-negative eubacteria, mono- or polyphletic origins of archaebacteria, and the placement of cyanobacteria, mycoplasms, as well as other as yet unclassified prokaryotes.

A2-201 SPECIFIC INACTIVATION OF DnaA PROTEIN BY A SOLUBLE FACTOR FOR INITIATION OF *IN VITRO* MINI-CHROMOSOME REPLICATION OF *E. COLI*.

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DnaA protein has high affinities to ATP and ADP (K_D at 0°C is 0.03 and 0.10 μM, respectively); the ATP-bound form is active in initiating *in vitro* replication of *oriC* plasmids but the ADP-form is not. The fact that a series of *dnaA* mutants can cause excessive initiation indicates an important role of DnaA protein for regulating the "on-off switch" of initiation *in vivo*. The *dnaAcos* mutant is cold sensitive in cell growth and causes overinitiation at the restrictive temperature (30°C) (Kellenberger-Gujer *et al.* 1987 MGG 162; 9). Inasmuch as the number of DnaA molecules in a *dnaAcos* cell is not excessive compared to that of the wild-type cell (900 and 1400 per cell, respectively), oversupply of the initiator is not the cause of this excessive initiation (Katayama and Kornberg, 1994 JBC 269; 12698). Purified DnaAcos protein is no more active than the ATP-form of wild-type DnaA protein in replication *in vitro* at 30°C, a temperature at which the mutant cells cause overinitiation. However, unlike wild-type DnaA protein, DnaAcos protein activity is insensitive to ADP: DnaAcos protein is defective in binding ADP or ATP. Thus, the negative effect by ADP on DnaA protein may be essential in regulating the initiation switch *in vivo* (Katayama, 1994 JBC *in press*). In addition, we report that a soluble protein fraction from a cell lysate inactivates wild-type DnaA protein at 30°C in a manner dependent on ATP and DNA, whereas DnaAcos protein is unaffected. A heat-labile factor in the extract, which has the suggested native molecular mass of 150kDa, seems to be responsible for this inactivation. DnaA protein can initiate replication of single-stranded DNA which has a hairpin-structure containing a DnaA-box. DnaA protein which has been inactivated for initiation at *oriC* is still capable for this single-stranded DNA initiation. Moreover, the inactivation seems specific for DnaA protein since all other replicative components are not inactivated (Katayama and Crooke, *submitted*). Purification of the factor is in progress.

A2-202 THE REPLICATION EFFICIENCY OF BACTERIOPHAGE MU IS DEPENDENT ON THE LOCATION OF THE STRONG GYRASE SITE (SGS) AND THE OCCUPANCY OF THE SGS IS LOCATION INDEPENDENT,

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Supercoiling is required for Mu transposition in vitro and wt Mu is unable to grow on gyrase host mutants in vivo. The involvement of supercoiling in Mu transposition and replication has been puzzling. The Mu nuB mutants are able to grow on a host gyrB mutant suggesting that these mutants have a gyrase site with increased affinity for gyrase binding. A centrally located gyrase binding site has been identified in Mu that is required for efficient replication. Sequencing of this site showed that the binding site corresponded with the nuB mutants and that single base pair changes were responsable for the increased gyrase affinity of the mutants. The site is now referred to as the strong gyrase site or SGS.
To investigate the effect that the positioning of the SGS might have on Mu replication, lysogens were constructed in which the SGS was deleted and reinserted at various positions. Growth profiles show that as the SGS is moved further away from the center of Mu that replication is impaired and that the decrease in replication is a function of the distance of the SGS from the center of Mu. In vivo gyrase assays show that the decrease in Mu replication is not due to differential occupancy of the SGS by gyrase depending on the distance from the center of Mu. Gyrase is found at the SGS with equal frequency regardless of the distance of the SGS from the center of Mu. We propose that the delay in the replication of the Mu lysogens is due to the inability of gyrase to properly organize the chromosome for replication from its new location in the molecule. We suggest that the unique SGS functions as a chromosomal orgainzing element in Mu.

A2-203 SELFISH GENES: RESTRICTION / MODIFICATION SYSTEMS FORCE CELLS TO MAINTAIN THEM

Ichizo Kobayashi, Taku Naito, Naofumi Handa and Kohji Kusano, *Department of Molecular Biology, Institute of Medical* Science, University of Tokyo, Tokyo 108 JAPAN

The combination of a restriction endonuclease and a modification methylase can defend cells from foreign DNA. But is that the only reason for their presence? We found that several restriction/ modification gene pairs (including *Pae*R7 and *Eco*RI) on a plasmid greatly reinforce its stable maintenance. Our results support the following "post-segregational killing" mechanism: The restriction/ modification system depends on efficient modification of all the recognition sites in the chromosome. When a cell loses the restriction/ modification enzyme gene pair, the two enzymes will get diluted out in the descendant cells after some rounds of division. Eventually they cannot modify all of the numerous sites. At this point, any restriction enzyme molecules left will cut the chromosome at one of these unmodified sites and kill the cell. The net result is stable maintenance of the restriction/ modification plasmids in the population of the viable cells. Thus these restriction/ modification gene pairs constitute a symbiotic unit or *selfish gene* entity that forces its maintenance through selfish-altruistic suicide or *apoptosis* strategy.

A2-204 GENOME ORGANIZATION IN *BACILLUS CEREUS* AND *BACILLUS THURINGIENSIS*.

Anne-Brit Kolstø, Cathrine Carlson, Toril Lindbäck and Kirsti Kvaløy. Biotechnology Centre of Oslo and Institute of Pharmacy, Department of Microbiology, University of Oslo, PB 1125, 0316 Oslo, Norway.

Bacillus cereus (Bc) and *Bacillus thuringiensis* (Bt) are common, sporeforming bacteria. The sole established difference between these bacteria is the presence of intracellular insecticidal crystals during sporulation of Bt. The gene(s) for these toxins are usually found on plasmids. In order to analyse strains, we have cut Bc and Bt DNA using rare-cutting enzymes like NotI, SfiI and AscI and pulsed field gel electrophoresis to separate the fragments. All strains were different from each other, giving rise to individual "fingerprint" patterns.

The size of the chromosome was calculated for 10 strains, and varied from 2.4 to 6.3 Mb.

Complete physical maps were constructed of the chromosomes of 6 strains - 5 large chromosomes and one small. When the maps of the large chromosomes were compared with each other, one region was well conserved between the strains, while another region appeared to vary. When the small chromosome was compared to the large ones, the entire chromosome corresponded to the conserved region of the large chromosomes.

The Bc strain F0837/76 with the small chromosome contained in addition to the chromosome several large extrachromosomal elements of sizes more than A. 960 kb, B. 700 kb and C. 400 kb, - in other words - more than 2 Mb extrachromosomal DNA. These "plasmids" all hybridize to gene probes localized to the variable region in the chromosome of 5.4 Mb from Bc ATCC 10987.

We conclude that the genome of Bc and Bt may be very complex, consisting of either large chromosomes or smaller chromosomes and mega-large plasmids. The mechanisms involved in the genome flexibility remain to be elucidated.

A2-205 TWO HOST COMPONENTS NECESSARY TO INITIATE BACTERIOPHAGE MU DNA SYNTHESIS *IN VITRO*,

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During the initial phases of bacteriophage Mu transposition, a template for Mu DNA synthesis is created by strand transfer of Mu ends to target DNA. The transposition protein that catalyzes strand transfer, the Mu A protein, remains very tightly bound to the strand transfer product in a nucleoprotein complex called the strand transfer complex (STC). Mu DNA replication on the STC to form the cointegrate was reconstituted *in vitro* with eight purified *E. coli* replication proteins (DnaB, DnaC, DnaG, DNA pol III holoenzyme, ligase, gyrase, SSB and DNA pol I) and a partially purified host enzyme fraction. This fraction supplied unspecified host factors with Mu A-releasing activity, which is needed for removal of the replication blockade imposed by the Mu A protein tightly bound to the Mu ends. Removal of the Mu A protein by proteinase K treatment and phenol extraction also relieved the replication blockade. However, replication of the deproteinized template did not necessarily require DnaB, DnaC, and DNA pol III holoenzyme, which are all essential for Mu DNA synthesis on the STC. The host enzyme fraction needed to replicate the STC was further resolved into two fractions (Component A and B). The partially purified fraction containing Component A removed the Mu A protein from the STC. Initiation of Mu DNA synthesis on the modified STC required Component B, DnaB, DnaC, and DNA pol III holoenzyme. The results suggest that the disassembly of the Mu A nucleoprotein complex in the presence of Components A and B leads to the assembly of specific replication proteins at the Mu replication fork.

A2-206 POLYADENYLATION IS A GLOBAL REGULATORY MECHANISM FOR CONTROLLING mRNA DECAY IN *ESCHERICHIA COLI*.

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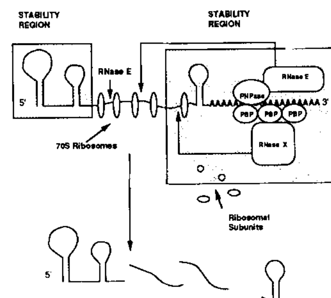
Polyadenylation of mRNAs in *E. coli* is primarily the result of the *pcnB* encoded poly(A) polymerase I. In wild type strains the average poly(A) tail ranges from 15 to 40 nt. In the absence of polynucleotide phosphorylase tail lengths increase to over 100 nt. It would appear that most mRNAs are polyadenylated. Of most importance is the observation that in the absence of polyadenylation, many mRNAs show very dramatic increases in their half-lives. We have examined the *lpp*, *ompA*, and *trxA* mRNAs using Northern analysis. Both the pattern and rate of decay is affected in *ΔpcnB* mutants. Based on these results we propose a new model for mRNA decay that involves specific poly(A) binding proteins that target molecules for nucleolytic decay. This model is outlined below. This work was supported by a grant from NIGMS (GM28760) to S.R.K.



A2-207 *IN VIVO* AND *IN VITRO* DIMETHYLSULFATE MODIFICATION OF THE *ESCHERICHIA COLI* REPLICATION ORIGIN,

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A primer extension assay was used to identify minichromosomal *oriC* nucleotides hyperreactive or protected from DMS modification. Unusually strong DMS hyperreactive sites were identified within the Fis and IHF protein binding sites of *oriC in vivo*, but not on naked DNA. The Fis and IHF hyperreactive sites and additional nearby suppressed/enhanced modifications measured within *oriC in vivo* were compared to the patterns observed for *oriC* DNA in the presence of purified IHF, Fis and DnaA proteins. A compilation of these sites will be presented.

In addition, accessibility of DMS to *oriC* was measured in temperature-shifted *dnaA*(Ts) and *dnaC*(Ts) mutant strains, as well as *fis*:kan, *himAΔ*Sma and isogenic *fis*$^+$ and *him*$^+$ strains. Differences in DMS modification patterns suggest that lack of Fis and IHF protein alter the DNA conformation and DnaA protein binding at *oriC*. A model is presented for the temporal bending of *oriC* into an initiation-competent complex by Fis, IHF and DnaA protein.

A2-208 HOW DOES *BACILLIS SUBTILIS* MAKE THE SWITCH FROM BINARY FISSION TO POLAR SEPTATION DURING SPORULATION?

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One of the first morphologically distinctive features of the sporulation process in *Bacillus subtilis* is the formation of an asymmetrically positioned septum that divides the cell into two unequally sized compartments each with a copy of the bacterial chromosome. In an attempt to identify genes that are involved in positioning the septum, we relied on the fact that the transcription factor, σ^F, is synthesized early in sporulation, but only becomes active following septation. Using a two reporter system (ß-glucuronidase and ß-galactosidase fusions to the promoter of the σ^F gene, *spoIIAC*, and to a promoter whose expression is controlled by σ^F, respectively) we identified a set of mutants that allow for transcription of the σ^F gene but prevent the sigma factor from becoming active. Further screening using fluorescence microscopy and DAPI to stain the nucleoids of the sporulating cells, allowed us to isolate a subset of these mutants in which formation of the asymmetrically positioned septum was prevented. We are currently in the process of further characterizing this subset in the hopes that these mutants will help us to understand the mechanisms guiding septum placement in *B. subtilis* during both vegetative growth and during sporulation.

A2-209 CLONING OF A PROKARYOTIC HOMOLOGUE TO THE EUKARYOTIC FAMILY OF SNF2 RELATED PROTEINS.

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Recently several eukaryotic regulatory proteins with similarity to the yeast transcription activator SNF2 have been discovered. Some of them are involved in DNA repair and recombination:

-RAD54 involved in both DNA repair and mitotic recombination in yeast
-RAD16 involved in exicion repair in yeast
-RAD5 involved in DNA repair in yeast
-ERCC6 involved in preferential repair of active genes in human.

Here we report cloning and analysing of a 4900 bp fragment from *Bacillus cereus* ATCC 10987. The complete nucleotide sequence have been determined. The sequence reveals three open reading frames of 177, 130 and 1054 amino acids. The 550 C-terminal aa of the largest open reading frame shows significent homology to the family of SNF2 related proteins.

The most conserved regions of this family correspond to the motifs defined for many helicases. The part mostly conserved in the SNF2 family includes one region where these motifs differ from the corresponding motifs in other helicase families. These differences suggest that this region is involved in specific DNA-binding functions.

A2-210 P1 PLASMID PARTITION: MUTATIONS IN *parB* THAT AFFECT INTERACTIONS OF ParB WITH *parS* AND ParA.

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Plasmid partition requires two plasmid proteins, ParA and ParB, and a cis-acting centromere analog, *parS*, to which ParB binds. Whereas some plasmids bearing *parS* are more than 100-fold stabilized by ParA and ParB proteins, others are actively destabilized. Destabilization does not require ParA. It depends on the context of *parS* and on the concentration of ParB. It can be so drastic that, even at sub-physiological concentrations, ParB can prevent the establishment of particular *parS* plasmids.

We report the isolation and characterization of ParB mutants selected, from among a PCR-generated random set, as unable to destabilize a ParB-sensitive *parS* plasmid. The mutations are widely distributed in *parB*. Many of them change amino acids conserved in ParB-related proteins of other plasmids. The mutant phenotypes were not due to protein instability or decreased synthesis. Most of the mutant proteins could not complement the partition defect of a mini-P1 *parB* plasmid. Mutations in three regions caused the failure of ParB to bind *parS*. Mutations in one of those regions, the C-terminal part of ParB, blocked dimerization of the protein, consistent with ParB binding as a dimer. Features of a mutant lacking this entire C-terminal region led us to propose that this part of ParB regulates dimerization. Putative regions of ParB that interact with *parS* are defined by the location of mutations that affect binding without influencing dimerization. Of mutant proteins that could bind to *parS*, a few exhibited a dependence of their destabilization-deficient phenotype on ParA. They were partition proficient, but only at low Par protein concentration. The mutations are clustered in the N-terminal moiety of the protein. Most likely this region of ParB is involved in its interaction with ParA in partition. The requirement of ParA for destabilization-deficient phenotype of certain mutant proteins suggests that the branch point between reactions leading to destabilization and partitioning occurs at a step of partition which requires ParA.

A2-211 FUNCTION OF RecO PROTEIN IN REPAIR AND RECOMBINATION IN *ESCHERICHIA COLI*,

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The *recO* gene product is required for the repair of DNA damage resulting from UV irradiation or mitomycin C exposure and for homologous recombination of plasmids in wild type *Escherichia coli* cells. In the absence of RecBCD protein, the *recO* gene product is also required for conjugation-mediated recombination. RecO protein has been previously purified and shown to bind to single- and double-stranded DNA, and promote the renaturation of complementary single-stranded DNA molecules in the absence of ATP. Results from this study show that purified RecO protein also catalyzes the assimilation of single-stranded DNA into homologous superhelical double-stranded DNA, an activity also associated with RecA protein. The RecO protein-promoted strand assimilation reaction requires Mg^{+2} and is ATP independent. Because of the biochemical similarities between RecO and RecA proteins, the ability of RecO protein to substitute for RecA protein in DNA repair *in vivo* was also assessed. The results show that overexpression of RecO protein suppresses the UV repair deficiency of a *recA* null mutant and support the hypothesis that RecO and RecA proteins are functionally similar. Analysis of double mutants suggest that RecO and RecA proteins function independently in the cell with respect to UV repair.

A2-212 TOPOLOGICAL CHARACTERIZATION OF THE CELL DIVISION PROTEIN FTSN,

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The *ftsN* gene is an essential cell division gene isolated as a multicopy suppressor of mutation in *ftsA*. Genetic and biochemical approaches were used to analyze a topological model for FtsN, a 36-kDa protein with a long stretch of hydrophobic amino acids near the N-terminus, and to ascertain the specificity of the cytoplasmic and membrane spanning domains of this protein. Analysis of FtsN-PhoA fusions revealed that the hydrophobic segment of FtsN could support the translocation of a fused PhoA to the periplasm consistent with the model. Protease accessibility studies of FtsN in spheroblasts and inverted membrane vesicles confirmed that FtsN had a simple bitopic topology with a short cytoplasmic amino terminus, a single membrane-spanning domain and a large periplasmic carboxy terminus. To ascertain the specificity of the N-terminal segments of FtsN various constructs were made. Deletion of the N-terminal cytoplasmic and membrane spanning domains led to intracellular localization of the carboxy domain, instability and loss of function. Replacement of the N-terminal cytoplasmic and membrane spanning domains with a membrane spanning domain from MalG restored subcellular localization and function of the carboxy domain. These N-terminal domains of FtsN could also be replaced by the cleavable MalE signal sequence with restoration of subcellular localization and function. It is concluded that the N-terminal, cytoplasmic and transmembrane domains of FtsN are not required for function of the carboxy domain other than to transport it to the periplasm.

A2-213 ISOLATION AND CHARACTERISATION OF MUTANTS RESISTANT TO RCD, A COLE1-ENCODED TRANSCRIPT WHICH INTERFERES WITH CELL DIVISION, Cindy Macpherson and David K. Summers, Department of Genetics, Downing Site, Cambridge, CB2 3EH, UK.

Multimerisation is a major cause of segregational instability in high copy number plasmids, as multimer-containing cells have fewer independently segregating units at cell division. Plasmid stability in the high copy number plasmid ColE1 is restored by the 240bp *cer* site, which acts as a substrate for site-specific recombination between directly repeated *cer* sites leading to the regeneration of plasmid monomers. Dimer resolution is assisted by the production, from within the *cer* site, of a 70 nucleotide transcript, Rcd (Regulator of Cell Division). It is proposed that in multimer-containing cells, transcription of Rcd causes a block in the cell division cycle prior to septation, allowing time for the *cer* -mediated resolution of multimers to monomers. In an attempt to elucidate the mechanism by which Rcd affects cell division, Rcd-resistant host mutants have been isolated and are currently being characterised in order to establish the target(s) and mechanism of action of Rcd.

A2-214 THE *dnaA* GENE OF *RHIZOBIUM MELILOTI* LIES WITHIN AN UNUSUAL GENE ARRANGEMENT, William Margolin*[1,2] and Sharon R. Long[1], [1]Department of Biological Sciences, Stanford University, Stanford, CA 94305 and [2]Department of Microbiology and Molecular Genetics, University of Texas Medical School, Houston, TX 77030

Rhizobium meliloti exists either as a free-living soil bacterium or as a differentiated endosymbiont "bacteroid" form within the nodules of its host plant, alfalfa (*Medicago sativa*), where it fixes atmospheric N_2. Differentiation is accompanied by major changes in DNA replication and cell division. In addition, *R. meliloti* harbors three unique large circular chromosome-like elements whose replication coordination may be complex. As part of a study of DNA replication control in *R. meliloti*, we isolated a *dnaA* homolog. The deduced open reading frame predicts a protein of 57 kDa that is 36% identical to the DnaA protein of *Esherichia coli*, and the protein was confirmed by immunoblot analysis. In a comparison with the other known DnaA proteins, this protein showed the highest similarity to that of *Caulobacter crescentus*, and was divergent in some domains that are highly conserved in other unrelated species. The *dnaA* genes of a diverse group of bacteria are adjacent to a common set of genes. Surprisingly, analysis of the DNA sequence flanking *dnaA* revealed none of these genes, except for an *rpsT* homolog upstream of *dnaA* also found only in *C. crescentus*. Instead, upstream of *rpsT* lie homologs of *fpg*, encoding a DNA glycosylase, and *fadB1*, encoding a enoyl-CoA hydratase. Downstream of *dnaA*, there are two open reading frames that are probably expressed but are not highly similar to any genes in the databases. The *R. meliloti dnaA* gene is therefore located within a novel gene arrangement. This finding suggests the interesting possibility that the regulation of DNA replication may be fundamentally different in this organism.

A2-215 SELECTION AND CLONING OF DNA THAT SPECIFICALLY BINDS THE FTS Q PROTEIN OF *E. COLIK12*. Alvin Markovitz. Dept. of Biochem. and Mol. Biol. Univ. of Chicago, Chicago, Il.60637.

The FtsQ protein is a transmembrane protein of 276 amino acids(AA) necessary for normal cell division. The N-terminal cytoplasmic 20 AA specify a putative helix-turn-helix motif and the next 30 AA are hydrophobic and are the membrane attachment region. The highly purified FtsQ protein binds nonspecifically to DNA in 50 but not in 150 mM KCl, as determined by nitrocellulose filter(NCF) binding assays. Oligodeoxynucleotides with the central 20 positions randomized (110 billion 65 mers), were chemically synthesized and made double stranded. Selection used 1) FtsQ protein in liquid with 150 mM KCl, 65 mer, competitor DNA (cDNA) and NCF. 2) FtsQ protein covalently linked to an agarose column with 150 mM KCl, 65 mer and cDNA. 3) Negative control- FtsQ protein in liquid with 50 mM KCl, 65 mer,cDNA and NCF. Bound DNA was eluted in all three with 0.5 to 2 M KCl and amplified by PCR with appropriate primers. After 5 rounds of selection and PCR amplification the central initially randomized 20 mers were cloned into pBluescript (3kb). Plasmid clones were sequenced: sel.1, 12; sel. 2, 16; sel 3, 10. Two clones from sel. 2 contained identical 20 mers (Clones173 and 1712). Clone 1511 from sel.1 matched 173 at 15 of 17 consecutive bases. PCR of plasmids produced 300 mers with the selected 20 in the middle. Kinase and [^{32}P-γ]ATP were used to label the 300 mers. FtsQ protein (as little as 10 ng in 15 ul) produced a gel retardation product(GRP) with ^{32}P-300(1712) that was not competed by 1000 ng polydI-polydC nor 100 ng of any plasmid except p1712/p173. Thus the 1712 20 mer is specifically bound by FtsQ protein in a 300bp linear as well as in a 3000bp plasmid context. FtsQ protein produced a GRP with ^{32}P-300(1511) that was competed only by p1511 and p173/p1712. Similar protein concentrations yielded 10 times more GRP with ^{32}P-300(173) as compared to ^{32}P-300(1511). Similar selection and PCR amplification experiments were done with DNA from the *E. coli* chromosome. One selected *E. coli* fragment was obtained 7 times out of the 25 clones sequenced and is part of the coding sequence of a known gene. A 145bp fragment of that gene produced a GRP with FtsQ protein with affinity similar to that of ^{32}P-300(1511). The 145bp and the 20 mer from 1511 have a region of sequence homology. The FtsQ protein, by tethering membrane and specific DNA, may initiate the events of cell division and/or may be directly involved in nucleoid segregation in a manner similar to that suggested by Jacob et al. in 1963.

A2-216 DNAA AS A TRANSCRIPTIONAL REGULATOR: AUTO-REGULATION AND REGULATION OF *FTSZ* TRANSCRIPTION REASSESSED, Millicent Masters and Richard W. P. Smith, Institute of Cell and Molecular Biology, Edinburgh University, Edinburgh, Scotland

DnaA: DnaA has been thought to autoregulate its own transcription (Braun et al Cell 40:159, Atlung et al, MGG 200:442) because in vivo inactivation of the protein leads to increased, and overproduction to decreased reporter synthesis. Deletion of a part of the *dnaAp* region containing a DnaA binding site abolishes these effects. *dnaA* messenger level is similarly affected (Kücherer et al MGG 205:115). We found (Masters et al MGG 216:475), surprisingly, that when DnaA is inactivated in integratively or extragenically suppressed strains (in which replication initiation continues in the absence of DnaA), *dnaA* expression (assayed using a single-copy *lac* reporter) is not increased. Furthermore, mutation of the DnaA binding site in the *dnaAp* region (TTATCCACA to ATATCGAGA) to a sequence shown by footprinting as unable to bind DnaA, did not alter promoter response to inducing or repressing conditions. These results together suggest that DnaA synthesis is not regulated by simple binding of DnaA at the binding site identified in the promoter region, rather than that, as suggested by Polaczek and Wright (New Biologist 2:574) abolition of DnaA binding increases transcription from one and concomitantly decreases transcription from the other of the two *dnaA* promoters, so as to leave protein synthesis unaffected. We are now replacing the binding site on the chromosome with the mutated site to determine whether any effects on initiation will result.

FtsZ: The region upstream of this key cell division gene contains three possible DnaA binding sites and earlier studies indicated that reporter synthesis is derepressed by DnaA inactivation. We now find that mutation of all three of the binding sites to sequences which should not bind DnaA does not alter the expression of the downstream *ftsZ* under any conditions examined. DnaA has also been implicated in termination in the vicinity of binding sites; we also failed to find any differences between transcription through the normal or mutated binding sites. We conclude that DnaA binding at these sites is unlikely to regulate transcription.

A2-217 LETHALITY OF DOUBLE MUTATIONS *rep-recB* AND *rep-recC* IN *Escherichia coli*.

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We report that *Escherichia coli* lacking both Rep and RecBC proteins are inviable. This conclusion is based on the following observations: i) *rep-recB* or *rep-recC* double mutant cannot be constructed, althought *rep-recB* clones could be obtained in the presence of a *recB* carrying plasmid; ii) induction of the λ Gam protein, which inhibits RecBC, is lethal in a *rep* mutant; iii) cells harboring *recBC* thermosensitive allele and a *rep* mutation are thermosensitive for growth.

This result implies a common role for these two proteins. The cellular role of the *E. coli* Rep helicase is unknown, althought the reduced replication rate in *rep* mutants suggests a role in chromosomal replication. The RecBCD protein complex plays a major role in the primary pathway of recombination in the cell. It is involved in the repair of double-stranded DNA breaks and several other types of lesions. It possesses four distinct activities including a helicase activity.

We investigated the reasons for *rep-recBC* inviability. The *rep-recD* double mutants, which lack only the exoV activity of the recBCD multicomplex, are viable. This indicates that it is not the loss of exonuclease activity which is responsible for the *rep-recB* or *rep-recC* lethality. The *rep-recA* double mutants are viable, which shows that recombination deficiency is not incompatible with a *rep* mutation. This is further confirmed by the observation that *sbcA* or *sbcB* mutations which revert the recombination deficiency of *recBC* mutants do not revert the killing of *rep* cells by induction of the λ Gam protein. We propose that the helicase activities of Rep and RecBC have an overlapping function. This function is essential for cell viability.

A2-218 DOES χ ACTIVITY DECAY AS A FUNCTION OF GENETIC OR PHYSICAL DISTANCE?

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Special sites involved in homologous recombination have been identified in a variety of organisms. In many cases, these sites are associated with higher levels of genetic exchange in their vicinity and are referred to as recombination hotspots. In *E. coli*, χ is a recombination hotspot that stimulates RecBCD-dependent exchange at, and to one side of, χ.Two general classes of Models can account for χ hotspot behavior:

Model I: The decay of χ-stimulated recombination reflects the decreased activity of the hotspot as a function of physical distance. (eg. The tendency of χ-activated RecBCD to dissociate from DNA may limit the potential for exchange to sites close to χ.)

Model II: The decay of χ-stimulated recombination reflects the decreased activity of the hotspot as a function of genetic distance. (eg. χ-activated RecBCD may act at the first opportunity for homologous interaction. The probability of exchange may be high near χ because opportunities to recombine frequently exist there. Once an opportunity is taken, χ-activated RecBCD may be unable to catalyze additional "downstream" exchanges.)

To test the Models, we buried χ in DNA sequence heterologies of known and varied length, examined χ-stimulated exchange in a fixed adjacent interval, and took the ratio of exchange to the left of the heterology to exchange in a χ-free control interval to the right of the heterology. Model I predicts the ratio will decrease as the size of the DNA sequence heterology to the left of χ increases. Model II predicts that the ratio will not change when the size of the heterology is changed.

We will interpret our results in light of our recent demonstration that χ is able to activate RecBCD recombination in *trans* without directly participating in genetic exchange.

A2-219 ILLEGITIMATE DNA RECOMBINATION AS A TOOL TO PROBE CHROMOSOME STRUCTURE,

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Deletion formation is one of the best-studied examples of genome rearrangements. Deletions are frequently generated by a recombination event between short (≤20bp) direct repeats, removing one of the repeats and the sequences in between. We developed in *Bacillus subtilis* a system allowing positive selection of the molecules that underwent deletion. An 18 bp sequence, located within the chloramphenicol-resistance gene (*cat*), was duplicated by insertion of a linker, thus interrupting the gene. A deletion between these repeats restored a functional *cat* gene.

Recently, we have examined the effect of distance between the repeats on the frequency of deletion formation, in the *B. subtilis* chromosome. We showed that the deletion frequency decreased exponentially more than 1000-fold as the distance increased from 33 to 2313 bp. We also showed that deletion formation was an intramolecular, *recA*-independent process. Since the 18 bp sequence duplicated is not known to be recognised by a specific protein, we proposed that the limiting factor in the recombination reaction could be the frequency of contact between the repeats (1).

By inserting fragments of lambda DNA between the repeats, we have extended this analysis beyond 2300 bp up to 9000 bp. Deletion frequency did not decrease significantly within this interval, thus suggesting that the frequency of contact between the repeats is now independent of distance. This observation is not in agreement with the behaviour predicted for DNA duplex in solution (2) and may indicate that the chromosome is organised in such a way that the repeats cannot be taken further apart.

We are currently testing whether these results are also valid in different regions of the chromosome. If so, recombination between short repeats could represent a useful tool to investigate the chromosome structure over long distances.

References

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A2-220 CafA PROTEIN FORMS CYTOSKELETAL FIBER BUNDLE IN *E. coli* CELL, WHEN OVERPRODUCED,

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Expression of the *cafA* gene at 71 min on the *E. coli* chromosme map by using a high copy number expression plasmid caused [1] overproduction of 51 kDa CafA protein, [2] formation of chains of normal cells and shorter anucleated cells, and [3] formation of "cytoplasmic axial filaments", and the gene was designated *cafA*. The structures were observed in spheroplasted chained cells by light microscopy, and also in sections of cell chains by electron microscopy. The structure was severl micrometers long and 0.1-0.2 μm in diameter, extending through the entire cell at the center of the cytoplasm. Cross sections of the cell revealed a regular equilateral hexagonal structure(Ref. 1).

Immunoaffinity purified anti-CafA antibody shows speciffic binding to the cytoplasmic axial structures in the CafA overproducing chained cells, indicating that the structures are formed by CafA protein itself. Amino acid sequences of the CafA protein displayed similarity to members of eucaryotic myosin and kinesin family, suggesting cytoskeletal nature of CafA protein. Moreover, purified CafA protein, when negatively stained, shows large sheet formation under electron microscopy. Immunoblotting analysis showed that the CafA protein is produced in normal cell. Staining of the normal growing cell by anti-CafA antibody to identify novel cytoplasmic structure is now in progress. Biochemical analysis using purified CafA protein is also in progress.

From these results, we suspect that the cytoplasmic axial filaments do exist in the normally growing cell, albeit only in a very small number, thereby constructing an invisibly thin structure. This structure could be a strong candidate for one of the procaryotic cytoskeletal/cytokinetic elements functioning in cllular extention and division, chromosomal segregation and other important mechanisms involved in the procaryotic cell proliferation.

Ref. 1. Okada et.al. *J. Bact.* **176**:917-922

A2-221 PURIFICATION AND CHARACTERIZATION OF MYCOBACTERIAL INTEGRATION HOST FACTOR (mIHF), Marisa L. Pedulla, Mong Hong Lee, and Graham F. Hatfull, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

Mycobacteria are the causative agents of several human diseases, including tuberculosis and leprosy. Relative to the well-studied *E. coli*, very little is known regarding mechanisms of mycobacterial gene expression. An understanding of these processes in mycobacteria would be a valuable tool for developing strategies of disease control. Given the broad range of bacterial processes in which Integration Host Factor (IHF) is involved, the characterization of its homolog in mycobacteria will be informative. The purification and initial characterization of the mycobacterial homolog of IHF (mIHF) are described below.

Mycobacteriophage L5 is a temperate phage with a broad host range whose entire genome has been sequenced. The L5 integrase gene has been cloned and the protein has been overexpressed and purified. The integrase, phage attachment site (*attP*), bacterial attachment site (*attB*), and mycobacterial cell extract are components of an *in vitro* recombination assay which results in recombination of radiolabelled *attB* into a supercoiled plasmid containing *attP*. This assay was utilized to test protein fractions of *Mycobacteria smegmatis* for the presence of a recombination-stimulating factor (mIHF). Ammonium sulfate precipitation and ion exchange chromatography were utilized to purify mIHF to homogeneity. This protein migrates as a single band in PAGE and has an apparent molecular weight of 13.5 kilodaltons.

Amino terminal sequence determination of purified mIHF allowed for the design of a degenerate oligonucleotide probe which was used to screen shuttle cosmid libraries from *Mycobacteria leprae, Mycobacteria smegmatis,* and BCG by colony hybridization of transformed *E. coli.* Segments of cosmid DNA hybridizing to the probe for mIHF were identified by Southern blots and cloned into pUC119 for DNA sequence analysis.

Initial biochemical characterization by DNase I footprinting and band shift assays has demonstrated mIHF to be a nonspecific DNA binding protein which is capable of forming a specific complex on *attP* DNA in the presence of L5 integrase. Purified mIHF stimulates D29 as well as L5 recombination *in vitro.*

A2-222 ANALYSIS OF THE TOPOLOGICAL SPECIFICITY FACTOR FOR CELL DIVISION, MINE. Sébastien Pichoff, Benedikt Vollrath, Christian Touriol and Jean-Pierre Bouché, Laboratoire de Microbiologie et de Génétique Moléculaires CNRS, 118 Route de Narbonne, 31062 Toulouse, France.

Mutants of Escherichia coli lacking a functional min locus generate abnormal divisions at the poles, resulting in a mixed phenotype of DNA-less spherical minicells and of cells of heterogeneous length. The min locus encodes proteins MinC, MinD and MinE, whose coordinate action is required for correct placement of the division septum. The current model (1) proposes that MinC and MinD act together to block septation at all division sites. MinE, at physiological levels, relieves the inhibition at the internal site. At high concentrations, it relieves inhibition at all possible sites, causing a minicell-forming (Min^-) phenotype.

We have analyzed the functions associated with MinE (88 aa.). Deletions of minE, placed on a high copy number plasmid under Plac control, were constructed using restriction sites or site-directed mutagenesis. These constructions were expressed and tested for their ability to i) permit the maintenance of a $minCD^+$ plasmid in a Δmin background, and ii) cause a Min^- phenotype in a min^+ background. The ability of MinE to dimerize was also tested using the two-hybrid system with vectors pAS2 and pACT2 (2).

Constructions having the 6-32 aminoacids region in common suppressed MinCD-dependent division inhibition. $MinE^{22-88}$ did not suppress MinCD lethality, but on the contrary caused a division inhibition phenotype in the Min^+ background, suggesting that it competes with $MinE^{1-88}$ for MinCD interaction but is unable to suppress the septation block.

Proteins carrying the N-terminal region only did not efficiently promote a Min^- phenotype in Min^+ cells. In contrast, polar divisions were abundant with $MinE^{36-88}$, although this peptide did not suppress MinCD lethality. This indicates that a domain of MinE presumably responsible for the recognition of a site-specific protein can be physically separated from the one responsible for the interaction with MinCD. Possible models that may explain the different phenotypes observed, taking into account the ability of MinE to form dimers, will be presented.

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A2-223 ANALYSIS OF TRANSCRIPTIONAL REPRESSION BY *ESCHERICHIA COLI* H-NS PROTEIN

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The H-NS protein constitutes a significant portion of the proteins present in the bacterial nucleoid. Our interest is to elucidate how H-NS interacts with curved DNA sequences, and the parameters that influence this binding. We have constructed a series of promoters containing different upstream curved sequences. We have examined the effects of the H-NS protein both *in vitro* and *in vivo* on the expression of these promoters. In addition, we have characterised the preferred sites on the curved DNA fragment by DNA footprinting. We show that the repression observed is correlated with the relative affinity of the upstream region of the promoter for the H-NS protein. In an attempt to further analyse the mechanism by which H-NS recognises these curved DNA sequences we have selected a number of dominant negative H-NS mutants.

A2-224 TRANSCRIPTION OF THE ESSENTIAL, CELL CYCLE REGULATED *dnaN* GENE OF *Caulobacter crescentus* IS HEAT SHOCK INDUCIBLE. Richard C. Roberts and Lucy Shapiro, Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305-5427

Caulobacter crescentus is a dimorphic bacterium that proceeds through a simple, well defined life cycle: a motile swarmer cell differentiates to a sessile stalked cell, which upon asymmetric division produces distinct swarmer and stalked cell progeny. Chromosomal replication is inhibited in the swarmer cell, but initiates soon after making the transition to the stalked cell. Our goal is to understand the regulatory mechanisms responsible for the tight coupling of chromosomal replication to cell cycle progression. One approach we have taken is to identify and characterize the regulation of genes encoding essential components of the replication machinery; we have recently identified and isolated the *Caulobacter dnaN* gene, encoding the beta subunit of DNA polymerase III. Sequence analysis revealed that *recF* and *gyrB* are located downstream of *dnaN* (Rizzo et al., (1993) J. Bacteriol. 175:6970-6981). Upstream are two heat shock genes, *grpE* and *orfA*, whose transcription proceeds toward *dnaN*. As expected, recombination experiments indicated that *dnaN* is an essential gene. Genetic and biochemical analysis of the *dnaN* regulatory region demonstrated the presence of two cell cycle regulated promoters whose expression is induced several fold at the transition from swarmer to stalked cell, corresponding to the time of initiation of DNA replication. A heat-inducible promoter for *dnaN* was also identified; heat shock regulation has not been previously observed among bacterial *dnaN* genes. The roles played by these different promoters in the regulation of *dnaN* transcription, and how this may be related to temporal regulation of chromosomal replication will be addressed.

A2-225 DNA RESOLUTION AND DNA INVERSION MEDIATED BY THE β RECOMBINASE OF PLASMID pSM19035 REQUIRES A CHROMATIN-ASSOCIATED NON-SPECIFIC DNA-BINDING AND DNA-BENDING PROTEIN.
Fernando Rojo and Juan C. Alonso, Centro Nacional de Biotecnología, C.S.I.C., Campus Universidad Autónoma de Madrid, Cantoblanco, 28049-Madrid, Spain.

The β protein encoded by plasmid pSM19035 from Gram positive bacteria is involved both in the resolution of plasmid multimers into monomers and in DNA inversion. The recombinase binds to an 85-bp region (crossing over site, *six* site) that contains two discrete subsites (I and II); one β dimer binds to each subsite. The highly purified β recombinase is unable to mediate *in vitro* DNA recombination unless a host factor is provided. In the presence of a *Bacillus subtilis* host factor, DNA resolution is obtained when the two *six* sites are directly oriented, whereas DNA inversion is the product when the *six* sites are in inverse orientation. The factor has been identified as the *B. subtilis* Hbsu protein, a non-specific DNA-binding and DNA-bending protein homologous to the *Escherichia coli* HU protein. In the presence of highly purified *B. subtilis* Hbsu, *E. coli* HU or mammalian HMG-1 protein, the β recombinase is able to catalyze *in vitro* intramolecular recombination between two specific recombination sites on a supercoiled molecule. *In vitro*, the *E. coli* IHF protein could partially substitute for Hbsu, while Fis and H-NS did not. The data presented indicate that the Hbsu protein is required for the assembly of the synaptic complex, but not for β protein binding to DNA. The β protein shares a significant homology with the Tn3 family of site-specific recombinases. The chromatin-associated protein could work as an accessory effector in β mediated recombination to compensate for the absence of the third binding site (DNA resolvases), or of the enhancer (DNA invertases), required by other enzymes of the Tn3 family.

A2-226 DEPENDENCE OF THE SEDIMENTATION COEFFICIENT OF CIRCULAR DNA ON SUPERCOILING AND IONIC CONDITIONS, Valentin V. Rybenkov, Alexander V. Vologodskii and Nicholas R. Cozzarelli, Depapartment of Molecular and Cell Biology, University of California-Berkeley, Berkeley, CA 94720, Department of Chemistry, New York University, New York, NY 10003

Recent data suggest that the properties of superhelical DNA should depend strongly on counterion composition and concentration. These conclusions are based mainly upon cryoelectron microscopy data and computer simulations. What is lacking is an experimental analysis in true solution. We have undertaken a systematic analysis of the biophysical properties of DNA as a function of superhelical density, σ, and ionic conditions. We have completed the determination of $S_{20,w}$ as a function of these parameters. The value of $S_{20,w}$ reflects the global size of DNA conformations. We found that for all ionic conditions studied, $S_{20,w}$ rises strongly with the value of $-\sigma$. The data imply that the conformations of supercoiled DNA do not change substantially in the range of sodium concentration between 0.1 and 3 M. A strong change was observed for low concentrations of sodium. We found that at high concentration of magnesium, supercoiled DNA adopts an extended conformation which may correspond to a collapsed interwound superhelix. We did not observe such conformations at any concentration of sodium ions. We conclude that there is no collapse of supercoiled DNA in NaCl solutions.

A2-227 CHROMOSOMAL ORGANIZATION NEAR THE ORIGIN OF DNA REPLICATION IN Mycobacterium tuberculosis(Mtb), M.smegmatis(M.smeg) and M.leprae(M.lep). Leiria Salazar[1], Hafida Fsihi,[2] Carmen Rios,[1] Stewart Cole[2], and Howard Takiff[1] IVIC, Caracas,Venezuela[1], and Institut Pasteur, Paris, France[2].
Mycobacterial (Mycobact) species grow slower than most other eubacteria. Colonies of the "rapid grower" M.smeg appear in three days, while M.tb colonies require at least three weeks. As DNA replication of the bacterial chromosome is linked to growth rate, replication must be strictly controlled during the long interreplication periods. In other bacteria control is exerted by regulation of replication initiation, and involves the initiation protein, DnaA, and other origin binding proteins that modulate its expression or function. To begin to study the mechanisms that control DNA replication in mycobact. we have cloned and sequenced the origin regions from mycobact. with differing growth rates. Streptomyces dnaA and gyrB genes were used as probes to select cosmids carrying the origin region from libraries of M.tb, M.lep, M.smeg which were subcloned and shotgun sequenced. Thus far we have determined the DNA sequence of the M.tb chromosome from gyrA through rnpA, M.lep from gyrA through dnaA, and 4 kb in the origin region of M.smeg. The DNA sequences show that the mycobact. share the gene organization previously reported for Streptomyces [1](rnpA-rpmH-dnaA-dnaN-recF-orf-gyrB-gyrA). The amino acid sequences of DnaA, DnaN and RecF are highly conserved amongst the mycobact., with M.tb and M.lep always the most alike. However, there is no apparent sequence similarity in the two intergenic regions flanking dnaA. These two regions are about the same size in any one chromosome, but vary amongst species in length and in the number of putative dnaA boxes. The dnaA-dnaN intergenic regions range from 518 to 590pb, are relatively AT-rich and contain two DnaA boxes in M.tb, five in M.lep and seven in M.smeg. The regions upstream of dnaA contain 6-8 dnaA boxes. The first four bases of the dnaA box consensus found in other organisms, TT(A/G)TCCACA, are poorly conserved, so we define a dnaA box as CCACA. The positions of some these boxes appear to be conserved.

[1] M.J.Calcutt and F.J. Schmidt, J. Bacteriol. (1992). 174:3220.

A2-228 THE CHROMOSOMES OF SALMONELLA TYPHI AND S. PARATYPHI C ARE REARRANGED IN COMPARISON WITH THOSE OF E. COLI K-12, S. TYPHIMURIUM LT2 AND OTHER SALMONELLA SPP. Kenneth E. Sanderson, Andrew Hessel, and Shu-Lin Liu. Salmonella Genetic Stock Centre, Dept of Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Using techniques of pulsed-field gel electrophoresis (PFGE), we have determined the genomic cleavage maps of several Salmonella species. For example, the genomic cleavage map of S. typhi with about 60 genes localized was established in strain H251.1, an Aro-deficient derivative of the strain Ty2, based on the separation of endonuclease-digested DNA by pulsed-field gel electrophoresis. Digestion with XbaI, BlnI (=AvrII), or I-CeuI produced 33, 28, and 7 fragments, respectively; the sum of the fragments for each enzyme indicated a genome size of 4.8 megabases (Mb). Tn10 insertions in specific genes, which add XbaI and BlnI sites to the chromosome, were transduced from S. typhimurium LT2 to S. typhi by the phage P22 to locate the fragments and to position the genes on the circular chromosome. The genomic maps of all these species and of E. coli K-12 show much conservation of gene location, but three types of rearrangements were detected. (1) All these species have insertions or deletions of unique DNA at numerous locations; for example, S. typhi has insertions in at least three sites relative to S. typhimurium. (2) Inversions, which are usually rare, occur commonly over the region for termination of replication. (3) Rearrangements (inversions or transpositions) due to homologous recombination in the rrn genes for ribosomal RNA are infrequent in most species, but are common in S. typhi and S. paratyphi C; different strains of the species differ from S. typhimurium and from each other. Detection of rrn gene rearrangements was through partial digestion by the intron-encoded enzyme I-CeuI, which cleaves only in rrn genes. Though chromosomal rearrangements (duplications, deletions, some inversions) occur in culture in enteric bacteria, they are not commonly observed in strains in nature, indicating that there are contraints on gene order. Apparently some of these constraints are relaxed in the species S. typhi and S. paratyphi C.

A2-229 Indirect suppressor studies of *priA2::kan* mutations suggest a required role for primosome assembly in homologous recombination in *E. coli*. Steven J. Sandler and Alvin J. Clark. Department of Molecular and Cell Biology, Division of Genetics, University of California at Berkeley, Berkeley, CA 94720.

We comfirm previous results that *priA* insertion mutants are recombination deficient (1), and UV sensitive (2). The *priA* gene product first identified as an essential component for primosome assembly in the ΦX174 in vitro DNA replication system has both a DNA translocase/helicase activity and a primosome assembly activity (3). A lysine to arginine mutation at codon 230 (*priA3*) in the ATP binding hole motif removes the translocase/helicase activity, but not the primosome assembly activity (3). Other work has shown that a *priA* insertion mutation leads to very high uninduced levels of SOS expression (4).

We tested the hypothesis that only the primosome assembly activity is needed in vivo for recombination and UV resistance by testing *priA3*'s ability to complement recombination and DNA repair deficiencies. We found that *priA3* on a high multicopy plasmid complements the UV sensitivity caused by *priA2::kan* and will test recombination before the meeting. We will also show evidence for an indirect suppressor of the UV sensitivity of *priA2::kan* mutants. This suppressor, called *pas-1*, restores normal levels of homologous recombination, basal level of SOS expression, and the ability to replicate plasmids to *priA2::kan* cells. The fact that this suppressor maps near the *dnaTC* operon and that DnaT and DnaC are required for ΦX174 primosome assembly leads us to hope that this will support the hypothesis that primasome assembly is required for recombination and DNA repair.

Based on these results, we suggest that primosome assembly has a required role in homologous recombination and DNA repair in *E. coli*. Molecular models for *priA*'s role in recombination and DNA repair will be presented.

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A2-230 FUNCTIONS NECESSARY FOR PARTITION OF PLASMID P1, James A. Sawitzke, Lyndsay Radnedge, Michael A. Davis and Stuart J. Austin, Cell Cycle Regulation Section, ABL-Basic Research Program, NCI-FCRDC, P.O. Box B, Frederick, MD 21702-1201.

The prophage form of phage P1 is stably maintained in *E. coli* even though at modest growth rates dividing cells contain only two copies of the plasmid. Stability is dependent on an active partition mechanism that apparently places daughter plasmids into daughter cells in a fashion analogous to mitosis in eukaryotes. The *par* region contains all the plasmid-encoded functions necessary for partition: the ParA and ParB proteins which are produced from an autoregulated operon, and *parS*, a *cis*-acting site. ParB and the *E. coli* protein, IHF, form a specific complex with *parS*. ParA contains ATPase activity (stimulated by ParB) essential for partition. The role of this ATPase activity is unknown but a motor function is an attractive hypothesis. The putative motor protein for the *E. coli* chromosome, MukB, is not required for P1 partition.

The closely related phage P7 has a similar *par* system. The P1 and P7 components are homologs, are similarly arranged and exhibit similar activities. However, the P1 and P7 components are not interchangeable and thus key interactions between the proteins and sites are species specific. Previous results from our lab have defined the specificity determinants of the P1 and P7 *parS* site (Hayes and Austin, 1993, PNAS, **90**:9228). We have been using these specificities to map regions of the Par proteins necessary for various interactions of these proteins. One approach involves the use of hybrid ParB proteins to determine the domains involved in ParB interaction with *parS*. P1::P7 hybrid proteins have shown that the C-terminal 54 amino acids include the principal determinant of specificity in recognition of *parS* by ParB, and presumably define a contact point with the DNA.

Another approach has utilized hybrid ParB proteins to map the domain responsible for contacting ParA protein. These experiments have defined the N-terminal end of ParB as essential for interaction with ParA both *in vitro* and *in vivo*.

Partition requires that daughter plasmids are able to "sense" their positions in the dividing cell and move accordingly. It is likely that this is through contact with unknown host structure(s). We are attempting to identify *E. coli* functions involved in partition by isolating second-site suppressors of defined mutations in the P1 *par* locus using both color-based screens and selection schemes.

Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with ABL.

A2-231 **REPLICATION ELONGATION RATE MEASURED BY FLOW CYTOMETRY: EFFECT OF VARYING THE RATIO OF DnaC TO DnaB,**
Kirsten Skarstad and Sture Wold, Department of Biophysics, Institute for Cancer Research, Montebello, 0310 Oslo, Norway.

Initiation of replication of the *E. coli* chromosome occurs when the initiator protein DnaA separates the double helix at *oriC* and recruits DnaB helicase. DnaB then starts unwinding, allowing primase to lay down primers and subsequently polymerase III to start elongating these. The recruitment of DnaB is dependent on DnaC. In solution DnaB forms a hexamer complexed with six DnaC molecules. After DnaB is loaded at *oriC*, DnaC protein leaves the complex. The level of DnaC is critical. In vitro, an excess of DnaC inhibits replication by slowing DnaB helicase at the replication fork (1).

The effects of varying the levels of DnaC protein in vivo were investigated with flow cytometry (2, 3). Initiation of replication was inhibited with rifampicin, while ongoing forks were allowed to continue. Analysis of samples taken during run-out of replication showed a substantial reduction in speed of fork movement in cells containing excess amounts of DnaC protein. The speed of fork movement was inversely proportional to the amount of excess DnaC protein. Co-overexpression of DnaB protein alleviated the slowing of fork progression caused by the excess DnaC. The results show that DnaC interacts with replication forks during elongation. Probably, it binds to DnaB and inhibits helicase activity. Thus, the ratio of DnaC to DnaB and the affinity of DnaC for a helicase hexamer at an established replication fork are of great importance for the rate of replication fork movement.

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A2-232 **MUTATIONS IN THE SUPPOSED ATP-BINDING REGION OF THE *E. coli* DNAA PROTEIN,** **Ole Skovgaard,** Institute of Life Science and Chemistry, University of Roskilde, Denmark and **Andrew Wright,** Department of Molecular Biology and Microbiology, Tufts Medical School, Boston, MA, USA.

The DnaA protein is essential for and has a regulatory role in *oriC* specific initiation of DNA replication in *E. coli*. The DnaA protein recognizes a specific DNA sequence, the DnaA-box, four copies of which are present in the minimally *oriC*. Binding of DnaA protein to these boxes probably melts an AT rich region in *oriC*, allowing the entry of DnaB helicase and subsequent assembly of the replication complex. Some "small" replicons, like P1 and mini-F, and some transposons also depend on the DnaA protein for replication and transposition, respectively.

A consensus ATP binding site was found in the DnaA protein by a database search [1]. Purified DnaA protein binds ATP and ADP with a high affinity [2]. DnaA protein free of nucleotide binds to DnaA-boxes, whereas the ATP form is required for complete initiation at *oriC* [3].

Three *dnaA* mutants with single nucleotide changes in the consensus sequence specifying the ATP binding site have been constructed by oligonucleotide mutagenesis. These mutant genes were tested for their ability to complement a chromosomal *dnaA46*(Ts) mutation, perform autorepression of *dnaA* gene expression and to support in vivo replication at the P1 origin *oriR*, at the mini-F origin *ori2* and at *oriC*.

Two of the mutant proteins were overexpressed, partially purified and tested for their ability to initiate in vitro replication at *oriC* and *oriR*.

Even though the three mutants behaves different, it can be concluded that the requirements to the DnaA protein for replication at *oriR* are less stringent than for replication at *ori2* and *oriC*. Whether this difference is due to ATP binding or other changes caused by the mutations must await characterization of purified mutant proteins.

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A2-233 **CLONING AND SEQUENCE ANALYSIS OF METHANOPYRUS KANDLERI *TOP5*, THE GENE ENCODING TOPOISOMERASE V.**

Alexei I.. Slesarev*†, J. A. Lake†, Sergei A. Kozyavkin‡, †University of California, Los Angeles, CA 90024, ‡National Institutes of Health, Bethesda, MD 20892

DNA topoisomerase V is a novel prokaryotic enzyme related to eukaryotic topoisomerase I. Although topoisomerase V has been purified as a robust nicking-closing enzyme [1,2], its role in DNA metabolism in hyperthermophiles is unclear. In order to address this issue as well as to establish topo V homology with other topoisomerases, the gene encoding topo V from *M. kandleri* and portions of flanking genes have been cloned and sequenced. The cloned DNA fragment contains an ORF that codes for a protein of 937 amino acids with molecular mass of 106.5 kDa. This value is close to that of topo V purified from *M. kandleri* (110 kDa). The amino acid sequences obtained from topo V peptides (100 amino acids total) confirm the data obtained from DNA sequencing. The sequence and implied secondary structure of topo V will be presented and compared with various type-1 group B DNA topoisomerases.

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A2-234 INTRINSIC AND PROTEIN-INDUCED DNA CURVATURE IN REGULATION OF FIMBRIAL ADHESIN GENES IN E. COLI.

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Expression of pyelonephritis-associated pili by uropathogenic *E. coli* is regulated in response to the environmental conditions. We have characterised the *pap* regulatory region and studied the regulatory features that controls the synthesis of Pap pili. The transcriptional activation of pili expression depends on a UAS region shared by two dirvergently orientated promoters (*papI*) and (*papB*).Genetic analysis and *in vitro* studies has shown that the UAS region contains binding sites for a number of activator proteins (e.g. PapB, CRP-cAMP, LRP). Expression of Pap pili is also subject to transcriptional silencing by the histone-like protein H-NS.

We have studied the effect of protein-induced and sequence directed bending on the activation of the *papB* promoter. Protein-induced and intrinsic DNA curvature probably facilitates the forming of a multiprotein regulatory complex needed for efficient transcription from the *papIB* promoters. We discuss the role of DNA bending in the regulation of fimbrial adhesin genes.

A2-235 OSMOREGULATION OF *PROU* IN *SALMONELLA TYPHIMURIUM* - IDENTIFICATION OF A REGULATORY MUTANT WITH A NOVEL PHENOTYPE,

Robert Stephen and Chris Higgins, Imperial Cancer Research Fund Laboratories, Institute of Molecular Medicine, Oxford.

The expression of the *proU* operon of *Salmonella typhimurium* is osmotically regulated and sensitive to the degree of DNA supercoiling. The expression of this operon is repressed by the bacterial histone-like protein H-NS. Many H-NS regulated genes require activator proteins, but no such protein has yet been found for *proU*.

A random mini-Mu mutagenesis was carried out, screening for mutants that had reduced *proU* expression under inducing conditions. One mutant was isolated with this phenotype, which is consistent with a mutation in an "activating" gene.

The mutation has pleiotropic effects, causing cellular elongation, auxotrophy and a loss of motility. The mini-Mu disrupts the *Salmonella* homologue of the *E.coli. phoM* gene, found at 0 minutes in *E.coli.* Insertion of the mini-Mu also appears to have caused a gross chromosomal alteration, most likely a deletion. This may explain the pleiotropic nature of the mutation.

Work is in progress to determine the nature of the chromosomal rearrangement, and to identify the gene(s) that affect *proU* expression.

A2-236 **THE MECHANISM OF ACTION OF THE McrBC RESTRICTION ENZYME OF E. coli K-12.**

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As more restriction sytems are characterised, their properties are being found to be more diverse and the traditional three classes of restriction enzymes (Types I, II and III) are becoming inadequate for classification. McrBC is one enzyme which fails to fit neatly into any of the three classes. Like enzymes of classes I and III it is a multi-subunit enzyme (consisting of 2 proteins, McrB and McrC) which requires two "half-sites" on the DNA for cleavage. However, unlike the enzymes so far described in these classes, McrBC recognises only methylated DNA, requiring at least one methylated C in each half-site, which is of the form 5'-R^{m}C-3'. Unusually, the half-sites can be symmetric or asymmetric as the DNA will be cleaved irrespective of which strand(s) the methylated Cs are on. Qualitatively, then, McrBC can recognise the half-sites in an orientation-independent manner. However, using synthetic oligos with the methylated bases in various configurations, we have shown that the efficiency of cleavage varies with the configuration of the methylated Cs.

Spacing requirements for the half-sites were further investigated using a series of plasmids which contain only two McrBC half-sites, flanking a polylinker into which were inserted DNA fragments of various sizes, we have also found that cleavage efficiency depends on the spacing between the half-sites. Maximal cleavage occurs with a spacing of approximately 40-80bp, but cleavage can occur, less efficiently, with spacing of up to and including 1.2kb but not 3kb. The DNA is cleaved neither at a single position close to the recognition site like Type III enzymes, nor at a site very distant from the recognition site as with Type I enzymes, but rather at multiple positions close to only one half-site in each molecule, with no apparent preference for one half-site over the other.

As McrBC shows no sequence similarity to other restriction enzymes (this is the case for many restriction enzymes) a clue as to its evolution may be obtained by elucidation of its mechanism of action. It is again similar to enzymes of Types I and III in requiring a nucleotide for cleavage but differs in its requirement for GTP rather than ATP. By gel retardation assays using synthetic oligos containing two appropriately-methylated and appropriately-spaced McrBC half-sites, we have shown that GTP is required for the initial binding of McrBC to DNA but it remains to be determined whether GTP is further required to allow communication between half-sites by McrBC as is true for ATP in the case of Type I and Type III enzymes.

A2-237 SEMI-RAPID GENOMIC MAPPING OF VIRULENCE GENES IN *BORDETELLA PERTUSSIS*: A COMPARISON OF SEVERAL STRAINS.

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We have developed a simple and relatively rapid method for mapping the genomic locations of bacterial genes in relationship to each other. The method uses two suicide vectors which are mostly non-homologous to each other and which are marked with different antibiotic resistance genes. Strains are constructed which have these two vectors inserted into the bacterial genome at two different locations. Insertion is mediated by homologous recombination within cloned sequences corresponding to genes to be mapped. As a result of the presence within each vector of a cleavage site for the intron encoded restriction enzyme I-*SceI*, which should not cleave anywhere else in the bacterial genome, a I-*SceI* restriction fragment is generated, the size of which can be measured by PFGE and which represents the physical distance between the two genes. By performing such an analysis with several pairs of genes, enough information is gathered to determine the relative genomic locations of these genes.

We have used this technique to analyze the genomic organization of virulence genes in the human respiratory pathogen *Bordetella pertussis*. We examined five strains of *B. pertussis* as well as the type strain of *Bordetella parapertussis*, a closely related species. It was found that the organization of these genes in *B. pertussis* Tohama I, *B. pertussis* 18323, and *B. parapertussis* ATCC15311 bore no obvious relationship to each other. However, *B. pertussis* strains 165 and W28 appeared to differ from *B. pertussis* Tohama I by a large inversion. *B. pertussis* Tohama III differed from Tohama I by a different large inversion. In addition, in the course of normal laboratory manipulations, spontaneous rearrangements have been observed in *B. pertussis* Tohama I. Taken together these results suggest that the genomic structure of *B. pertussis* strains is quite fluid.

A2-238 INTERACTION OF E. COLI PRIMASE WITH ITS SUBSTRATE IN THE PRIMASE/SSB/G4oric pRNA SYNTHESIS SYSTEM.

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E. coli primase/SSB /G4oric ss DNA is a simple system for studying the interaction of primase with its DNA template and associated proteins during initiation of DNA replication. In a previous study (Sun and Godson, J. Biol. Chem. 268, 8026-8039), we demonstrated that a 278 nt G4oric ss DNA fragment containing three stem-loops (100nt) bound four SSB tetramers and that two SSB tetramers were bound in a fixed position on the hairpin structure, leaving part of stem loop I and the 5' CTG 3' pRNA synthesis initiation site as free DNA between the fixed SSB tetramers and the adjacent downstream tetramer. We have now shown that this complex synthesizes pRNA and that binding of primase to this SSB/G4oric complex can be assayed by gel shift and nuclease footprinting. The results demonstrated that primase interacts with two areas of G4oric in the SSB/G4oric complex, one at the base of stem loop I covering the 5' CTG 3' pRNA initiation site on the 3' side and another at the 5' base of stem loop III. We have prepared G4oric substrates with different 5' and 3' extensions from the basic stem loop region and have measured the number of SSB tetramers bound, their position on the structure, the binding and location of primase, and the ability of the complex to support pRNA synthesis.

The results demonstrate that mutant oric's with no sequence 5' to the base of stem loop III do not give a gel shift after adding primase but still retain weak pRNA synthesis activity. In these complexes, although the stem loop I region is protected by primase there is no protection of the stem loop III. This suggests that the interaction of primase on the stem-loop I and the stem-loop III can be separated, which predicts that more than one, primase molecules function on the SSB/G4oric complex. Deletion of the 3' side of stem loop I demonstrates that in addition to the 5' CTG 3' sequence, a downstream SSB tetramer is required so that the 5' CTG 3' pRNA initiation site is flanked by an SSB tetramer (or octamer). These results suggest that a specific nucleosome structure is required for primase to synthesize pRNA on the SSB/G4oric complex.

A2-239 ISOLATION AND PRELIMINARY CHARACTERIZATION OF NOVEL MUTANTS OF THE *ESCHERICHIA COLI dnaA* GENE DEFECTIVE IN pSC101 REPLICATION,

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DnaA protein, encoded by the *dnaA* gene, is essential for the initiation of *Escherichia coli* chromosomal replication as well as a number of bacterial phage and plasmids. A selection method for mutants of *dnaA* unable to sustain replication of the DnaA-dependent pSC101 replicon was developed. A large collection of *dnaA* mutants defective in pSC101 replication have been obtained. These *dnaA* mutants have been characterized by polypeptide size, phenotype with respect to replication from the pSC101 origin, dominance over *dnaA+*, and ability to regulate expression of a *dnaA'-'lacZ* fusion *in vivo*. One class containing mutations in the putative ATP-binding domain (P-loop motif) displays a dominant negative phenotype to *dnaA+* when the mutant alleles are present on a moderate copy number plasmid under control of their own promoter. Other results suggest that the N-terminal and C-terminal domains of the protein are involved in DNA binding and autoregulation. Consistent with these findings, genetic characterization of *dnaA* nonsense mutants suggests that the C-terminal domain is required for autoregulation and possibly DNA binding.

A2-240 BACTERIOPHAGE MU REPRESSOR: A TARGET PROTEIN FOR THE *E.COLI* ATP-DEPENDENT CLP PROTEASE.

A. Toussaint(1)(2), J. Laachouch(1), L.Desmet(1), V. Geuskens(1) and R. Grimaud(1)(2); [1]Laboratoire de Génétique des procaryotes, Université Libre de Bruxelles, B1640 Rhode St Genèse, Belgium; [2]Laboratoire de Biochimie des Mircoorganismes, Université Joseph Fourier, F38041 Grenoble cedex, France.

Bacteriophage Mu repressor is a196aa protein which forms dimers and higher molecular weight oligomers in solution. Two distinct frameshift mutations located in the 3'-end of the repressor gene and which respectively replace as little as the 11 and 26 C-terminal residues of the protein by 6 (FRNHRR) and13 (FCQSMGFMNRK) new ones have been isolated. They make the protein very sensitive to the host ClpP-X protease and the phage virulent on Clp^+ hosts. In addition, the mutated *vir* repressors target the wild type protein towards degradation, most probably through the formation of mixed oligomers.

We used Mu repressor to further define some of the features necessary for the recognition by the ClpP-X protease. Revertants of Mu*vir* phages which recovred the capacity to lysogenise, have been isolated. Most of them carried a secondary framshift mutation in the *c* gene which truncated the C-terminal end by around 50 residues and hence removed the *vir* abnormal C-terminus. These proteins had retained some instability, especially at high temperature (42°C) and phages with those mutations were somewhat thermoinducible. One mutant had a point mutation in the FRNHRR C-terminus (N->D) and had regained a half life close to that of the wild type protein. A similar mutation (N->D) was introduced by *in vitro* targeted mutagenesis in the FMNRK C-terminal end and proved to also increase stability showing that the N residue is important for proteolysis. The FRNHRR sequence was hooked on the C-terminal end of the stable CcdB protein encoded by plasmid F and did not detectably change its stability. These results comfort the general view that even if primary sequence features might be important for proteolytic degradation, more general conformational properties of the protein are important for recognition by the protease.

A2-241 *IN VITRO* DECATENATION OF SINGLY-LINKED CATENANES CATALYZED BY DNA GYRASE AND TOPOISOMERASE IV, Christian J. Ullsperger, Alex Vologodskii*, and Nicholas R. Cozzarelli, Department of Molecular and Cellular Biology, University of California-Berkeley, Berkeley, CA 94720, *Department of Chemistry, New York University, New York, NY 10003

Topo IV is an essential topoisomerase in *E. coli.*, required for unlinking replicated DNA prior to chromosome segregation. DNA gyrase, another essential topoisomerase, has also been implicated as a decatenating enzyme *in vivo*, but it can not compensate for the loss of unlinking activity in topo IV mutants. We compared the decatenating activities of the two enzymes *in vitro,* using singly-linked catenanes as substrates, to better understand the biochemical basis for the division of labor observed *in vivo.* We show that the decatenating activity of both enzymes is enhanced by the superhelicity of the catenated rings, but that DNA gyrase is more sensitive to the absence of supercoiling than topo IV. We also show that unlinking of supercoiled singly-linked catenanes by topo IV is surprisingly efficient. Evidently, the plectonemic crossings in supercoiled DNA do not compete with catenane linkages for removal by topo IV. Monte Carlo simulations of DNA catenanes show that supercoiling can decrease the equilibrium constant for catenation by 30-fold. This effect, achievable through the action of DNA gyrase, may direct the intermolecular strand-passage activity of topo IV towards chromosome unlinking *in vivo.*

A2-242 ROLE OF CALCIUM IN *E. coli* CELL CYCLE
Masaaki Wachi, Sota Hiraga[1] and Kazuo Nagai
Department of Bioengineering, Tokyo Institute of Technology and [1]Kumamoto University, School of Medicine
In eukaryotes, calcium plays a fundamental role in the regulation of the cell cycle, but its role in the prokaryotic cell cycle has yet to be elucidated. In order to approach this problem genetically, we have screened for *E. coli* mutants which show altered behaviours in cell growth by the presence of high concentrations of calcium in the medium. Two kinds of mutants have been isolated. One was a calcium-sensitive mutant CS2-29, the growth of which was inhibited by $CaCl_2$. The other was a calcium-dependent mutant SH3450 with its temperature sensitive growth suppressed by $CaCl_2$. The CS2-29 strain showed cell filamentation by the addition of 5-10 mM $CaCl_2$ in the medium and became spherical giant cells by 20 - 100 mM $CaCl_2$. Viability of the cells decreased to less than 0.01 at 10 mM $CaCl_2$. Fragmentation of the chromosomal DNA was also observed in the mutant cells treated with 50 mM $CaCl_2$. The SH3450 strain showed temperature sensitive growth and cell filamentation at 42 ^{o}C. Anucleate cell production from ends of filamentous cells was also observed. Temperature sensitivity of the growth was suppressed by the presence of 50 - 100 mM $CaCl_2$. Further analysis of these mutants and cloning of the genes will reveal the role of calcium in the regulation of the *E. coli* cell cycle.

A2-243 HOW CAN A SYMMETRICAL DIMER OF A REPLICATION TERMINATOR PROTEIN BOUND TO A CHROMOSOMAL TERMINATOR CAUSE POLAR ARREST OF A REPLICATION FORK? R.G. Wake, M.T. Smith, D.B. Langley, A.H. Franks, P.A. Young and A. Griffiths, Department of Biochemistry, University of Sydney, NSW, 2006, Australia.
The major DNA replication terminator, IRI, of *Bacillus subtilis* was originally identified as a 47-bp sequence in the terminus region. It binds the replication terminator protein, RTP, to arrest a fork in a polar manner, its action in the chromosome being directed at the clockwise fork. IRI contains two binding sites, A and B, for RTP, each of which binds a symmetrical dimer of this protein. Protein-nucleoside contacts are restricted to the bp 1-30 segment of IRI; the two binding sites slightly overlap and the contacts over the two sites are very different. RTP binds first to the B site, which is proximal to the approaching fork to be arrested, and this enables cooperative filling of the A site. The B site of IRI is partially symmetrical, but there is little symmetry in the A site which is quite different in sequence from the B site. New experimental findings to be described relate to (i) the effect of sequential deletions into each end of IRI on RTP binding and fork arrest, (ii) the identification, characterisation and arrangement of additional DNA terminators in the *B. subtilis* chromosome, and (iii) the construction of new novel DNA terminators (based on the recent unpublished discovery of a naturally-occurring bidirectional terminator related to IRI by W.J.J. Meijer and S. Bron). The new findings have allowed the development of a model for the functioning of the RTP-terminator complex based on specific RTP-DNA and RTP dimer-dimer interactions. It explains how a symmetrical dimer of RTP when complexed to a chromosomal DNA terminator can arrest a fork in a polar manner.

A2-244 EXTRAGENIC SUPPRESSORS OF A TEMPERATURE-SENSITIVE *dnaX* POLYMERIZATION MUTANT OF *E. coli* ARE LOCATED IN THE INITIATOR GENE *dnaA* BUT BEHAVE AS A *cis*-ACTING SITE, James R. Walker, Edwin Gines-Candelaria and Alexandra Blinkova, Department of Microbiology, The University of Texas, Austin, TX 78712
Extragenic suppressor mutants which gained the ability to suppress a *dnaX2016(Ts)* DNA polymerization defect and which concomitantly became cold-sensitive have been characterized within the *dnaA* initiation gene. When moved into *dnaX+* strains, the new mutants retained cold-sensitivity and phenotypically were initiation defective at 20°C [Walker, J. R., J. A. Ramsey and W. G. Haldenwang (1982) Proc. Natl. Acad. Sci. U.S.A. 79:3340-3344]. Detailed localization by marker rescue and DNA sequencing have been completed. One mutation changed codon 213 from Ala to Asp; the second changed Arg 432 to Leu and the third changed codon 435 from Thr to Lys. It is striking that two of the three spontaneous mutations occurred in codons 432 and 435; these codons are within a very highly conserved, twelve residue region [Skarstad and Boye (1994) Biochim. Biophys. Acta 1217:111-130; Messer and Weigel, personal communication] which must be critical for one of the DnaA activities. To explore the mechanism of suppression, dominance of wild-type and suppressor alleles was examined. The wild-type phenotype was dominant over cold-sensitivity regardless of the chromosomal or plasmid location. However, suppression was dominant when the suppressor allele was chromosomal and recessive when it was plasmid-borne. Furthermore, suppression was not observed when the suppressor allele was present on a plasmid and the chromosomal *dnaA* was a null allele as the result of transposon insertion. These facts suggest that the suppressor allele must be integrated into the chromosome, perhaps at the normal *dnaA* location. Suppression was observed when initiation occurred at secondary origins and is, therefore, independent of *oriC*.

A2-245 EXPRESSION STUDIES OF *Escherichia coli RecR* GENE. Tzu-Chien V. Wang, Yi-Cheng Su, and Ying-Hsiu Liu, Department of Molecular Biology, Chang Gung College of Medicine and Technology, Kwei-San, Tao-Yuan, Taiwan.
The structural portion of *Escherichia coli recR* gene was synthesized by polymerase chain reaction and cloned onto the ATG-fusion cloning site of several expression vectors. The ability of the constructed *recR* plasmids to complement chromosomal *recR* mutation and to overexpress RecR protein was examined. A mutation at the second codon from CAA to GAA had little effect on the *in vivo* function of RecR. Fusion of the His·Tag oligohistidine to RecR at the N-terminus produced a fusion protein which is fully functional *in vivo*. With regards to the ability to overexpress RecR, we observed that the plasmids carrying a T7 Ø10 promoter overexpressed RecR protein in large quantity while the plasmids carrying a P_{tac} promoter overexpressed RecR poorly. Overexpresseon of RecR did not affect cell viability nor did it affect DNA repair in *uvrA recBC sbcBC recR* cells. The His·Tag fused RecR protein, which may be easily purified to homogenity within hours, does not appear to have any of the following activities *in vitro* by itself: binding to DNA; promote renaturation; nuclease activity.

A2-246 CHARACTERIZATION OF THE BINDING OF LRP TO THE UPSTREAM REGION OF *gltBDF*, Don E. Wiese II,[1] Brian R. Ernsting,[1] Robert M. Blumenthal,[2] and Rowena G. Matthews,[1] Biophysics Research Division, University of Michigan, Ann Arbor, MI, 48109,[1] and Department of Microbiology, Medical College of Ohio, Toledo, OH 43699.[2]
Lrp (Leucine-responsive regulatory protein) is important in the regulation of over 40 operons in *E. coli*. Two-dimensional gel electrophoretic analyses previously done in this laboratory identified the *gltBDF* operon as a member of the Lrp regulon, and mobility shift assays with a fragment containing the *gltBDF* promoter have shown that Lrp binds to one or more sites in a cooperative manner [Ernsting *et al.*, J. Bacteriol. **175**: 7160-7169 (1993)]. To define the nature of this interaction, DNase I footprinting experiments were performed. Our results indicate that Lrp binds at three sites, spanning the region from -140 to -260 nucleotides relative to the start site of transcription. Extensive phased hypersensitivity is observed throughout the entire binding region, suggesting that Lrp bends the DNA. The mode of binding of Lrp does not appear to be the same for these three sites. The middle site (centered at -215 bp), which matches at 11 out of 13 nucleotides with the Lrp consensus for *ilvIH* [Wang and Calvo, J. Mol. Biol. **229**: 306-318 (1993)], covers 15 base pairs and has a 5' overhang of 6 nucleotides on both strands. This 5' overhang protection pattern is characteristic of dimeric proteins binding in the major groove. The flanking sites (centered at -149 and -251 bp), with just 4 and 5 nucleotides in agreement with the *ilvIH* consensus, cover 6 and 14 base pairs respectively. Both have short 3' overhangs, a protection pattern characteristic of proteins binding in the minor groove. Our working model is that the Lrp dimer is a bifunctional DNA binding protein, capable of contacting a central palindromic site in the major groove and flanking minor groove sites to form a nucleoprotein regulatory complex.

Funded by grant MCB-9203447 from the National Science Foundation.

A2-247 IDENTIFICATION OF A NOVEL PROTEIN OR PROTEIN DOMAIN INVOLVED IN INITIATION OF DNA REPLICATION IN *CAULOBACTER*, Elizabeth Winzeler and Lucy Shapiro, Department of Developmental Biology, Stanford University School of Medicine, Stanford CA 94305.
Cell division in the bacterium *Caulobacter crescentus* gives rise to two morphologically distinct progeny cells, a stalked form and a flagellated, swarmer form. These two cell types also differ in their potential for DNA replication. The stalked cell is competent to initiate chromosomal replication immediately following division while the swarmer cell must first differentiate into the stalked form before it is able to initiate replication. To identify proteins which play a role in the regulation of the asymmetric onset of DNA replication in *Caulobacter* we have screened a collection of temperature sensitive mutants for ones unable to initiate DNA replication. One such mutant, LS439, was identified and characterized using flow cytometry. At the non-permissive temperature LS439 completes ongoing rounds of DNA replication but cannot initiate new rounds. LS439 forms long filaments after extended incubation at the non-permissive temperature. We have cloned and sequenced a 0.4 kB fragment of DNA which is able to completely rescue this temperature-sensitive phenotype. Translations of the sequence in all six reading frames contain no significant homology to any protein in the Swiss-Prot protein sequence data bank. The complementing fragment is located immediately upstream of the *Caulobacter* homologue of the *E. coli dnaZX* gene, which encodes the gamma-tau subunit of DNA polymerase III. The 0.4 kB fragment has promoter activity and we believe that it encodes a small protein because: 1. Truncated versions of the fragment which lack promoter activity are able to complement a *rec*+ but not a *rec*- derivative of the LS439. 2. A fragment containing a stop codon in the reading frame most likely to encode a protein is unable to complement the *ts* phenotype. The fragment may either encode a unique N-terminal domain of the gamma-tau subunit which is able to complement in *trans* or it may encode a separate protein. In either case we believe that we have identified a novel protein or domain involved in the initiation of DNA replication.

A2-248 NUCLEOID PARTITIONING BY SEGREGATION AXES IN *ESCHERICHIA COLI*, Conrad L. Woldringh[1], A. Zaritsky[2], and N. B. Grover[3]. [1]Dept. Mol. Cell Biol., University of Amsterdam, The Netherlands. [2]Dept. Life Sci., Ben-Gurion University, Be'er-Sheva, Israel. [3]Hubert H. Humphrey Center for Exptl. Med. and Cancer Res., The Hebrew Univ., Med.Faculty, Jerusalem , Israel.

The *E. coli* nucleoid expands gradually and continuously along with the elongating cell (1). Inhibiting DNA synthesis does not affect this movement, while the presence of a nucleoid, actively involved in transcription/translation inhibits the surrounding cell wall synthesis (2), modifying cell shape and division plane (3). To explain these observations, we propose that "Nucleoid Segregation Axes" (NSA) play a role in the mechanism relating direction of nucleoid movement to positioning of the division plane and cell shape. We envisage the formation of two microcompartments around the duplicated *oriC* by expression of four nearby *rrn* operons (A, B, C, E). Expansion of these "Ribosomal Assembly Centers" (RAC's) is the primary force separating the newly-replicated *oriC*'s; these nucleolus-like compartments would thus play a centromeric role. The transient attachment of DNA loops to the membrane through cotranslational transport of membrane proteins subsequently pulls newly replicated DNA loops into their respective cell halves. An axis for segregation (NSA) is assumed to develop between the two RAC's, which elongates at a rate proportional to the rate of protein translocation but independent of DNA synthesis (3). In rod-shaped cells, the NSA is forced to lie eventually along the long axis; it then induces a perpendicular division plane (2). In spherical cells, consecutive division planes occur at perpendicular or tilted angles (3), because their NSAs are free to float in the cytoplasm.

(1).Van Helvoort, J.M.E.M. and C.L.Woldringh, 1994. Mol. Microbiol.13:577-583. (2) Woldringh, C.L. *et al.* 1990. Res. Microbiol. **141**: 39-49. (3) Woldringh, C.L., A. Zaritsky and N.B. Grover. 1994. J.Bacteriol.176(19):001-009.

A2-249 ISOLATION AND CHARACTERIZATION OF MULTICOPY SUPPRESSORS OF mukB MUTATIONS IN ESCHERICHIA COLI. Kunitoshi Yamanaka, Tadao Mitani, Teru Ogura, Hironori Niki and Sota Hiraga. Department of Molecular Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862, Japan.

The *mukB* gene encodes a 170 kDa protein, which might be a candidate for a force-generating enzyme in chromosome positioning in *Escherichia coli*. The *mukB* mutants (*mukB33* and *mukB106*) produce normal-sized, anucleate cells and show a temperature-sensitive colony formation. To identify proteins interacting with the MukB protein, we isolated multicopy suppressors (*cspC* and *cspE*). The *cspC* and *cspE* genes suppressed the production of anucleate cells as well as the temperature-sensitive colony formation. CspC and CspE are small, basic proteins consisted of 69 amino acid residues and are highly homologous to each other (83% identical). Homology search revealed that they possessed high similarity to proteins containing the cold shock domain, such as CspA of *E. coli* and Y-box binding proteins of eukaryote, suggesting that CspC and CspE might be DNA binding proteins which recognize a CCAAT sequence. CspC and CspE may, either directly or indirectly, interact with the partitioning apparatus including MukB, because the multicopy suppression with CspC and CspE was observed in the *mukB* mutants, but not in the *mukB* null mutant.

A2-250 ROLE OF Mu ENHANCER AND Mu B PROTEIN IN ASSEMBLY OF AN ACTIVE Mu TETRAMER: PARTIAL-ACTIVE-SITE MODEL FOR CATALYSIS. Jinying Yang, Keetae Kim and R.M.Harshey, Department of Microbiology, University of Texas at Ausitn, Austin, TX 78712

Physical assembly of a chemically functional tetrameric configuration of Mu transposase (A protein) occurs through cooperation of multiple reaction components. The distant Mu enhancer promotes the cleavage of Mu ends, while the Mu B protein promotes strand transfer of the cleaved Mu ends into target DNA. To understand the role of the enhancer and dissect the role of the individual Mu A subunits within the assembled tetramer, we have carried out in vitro complementation experiments between MuA mutants carrying specific defects in various transposition functions. We interpret our results to suggest that each Mu A subunit carries only a partial active site and that two trans-complementing pairs of subunits are required to assemble two active sites for cleavage. The Mu enhancer functions in building the first pair of active sites. This model immediately suggests how the subsequent strand transfer step of transposition is promoted by Mu B protein. We propose that Mu B protein, in a manner analogous to the enhancer, is responsible for building the second pair of active sites for strand transfer. Thus all four subunits are likely required for cleavage and strand transfer. Predictions of this model have been successfully tested.

A2-251 CHROMOSOMAL REARRANGEMENTS IN *LEPTOSPIRA INTERROGANS*. Richard L. Zuerner and Gabriel A. Treuba, National Animal Disease Center, ARS, USDA, Ames, IA 50010.

Physical and genetic mapping of the genome of the spirochete *Leptospira interrogans* provides evidence for two chromosomal replicons. While little is known about the smaller (C2) chromosomal replicon, several genetic markers have been mapped on the larger (C1) chromosomal replicon. Comparison of the genomes of different members of *L. interrogans* showed that genetic organization in the C1 replicon is fluid. *L. interrogans* is an antigenically diverse species and antigenically distinct isolates can be differentiated by chromosomal rearrangements. Previous studies have shown that the *L. interrogans* genome contains several different species of repetitive DNA, including insertion sequence-like elements. Copies of these IS-like elements are found only on the C1 replicon, are often on polymorphic fragments, and usually are located near rearranged regions of the chromosome. The potential role of these elements in generating chromosomal rearrangements and their affect on phenotype was investigated. Comparison of two isogenic but antigenically distinct strains of *L. interrogans* serovar pomona (strains RZ11 and GT210) shows the presence of rearranged DNA involving an IS*3*-like element, IS*1500*. Comparison of RZ11 and GT210 shows the rearrangement is in the CI replicon, and this rearrangement was localized on the physical map. Amplified DNA was also detected using a probe containing part of IS*1500* and other genomic DNA. These data suggest that the rearrangement and/or the DNA amplification altered antigenic expression in GT210. Current studies are aimed at characterizing sequences flanking copies of IS*1500* to better understand how this IS may promote chromosomal rearrangements, and the role of this element on phenotypic expression in *L. interrogans*.

A2-252 A GENETIC SWICH CAUSED BY THE COR GENE OF PHAGE ø80 IN LYSOGENIC CELLS, Natalia Vassinova, Department of Biophysics, St.Petersburg State Technical University, 29 Polytechnicheskaya Str., St.Petersburg, I9525I, Russia

An alteration of properties of DNA binding proteins caused by interaction of those with other proteins counts for evolution of regulation of genes expression. The lysogeny of phage ø80 is one of example of this alteration. It was found the repression of phage ø80 depends on more than one gene of this phage. The cor gene participates in this process becides the cI gene. Indeed, I. phage ø80 with a mutation in the cor gene establishes lysogeny with low frequency. 2.The lysogenic cells containing prophage ø80 with a mutation in the cor gene and a leaky mutation in the cI gene simultaneously are unstable. Ones become more stable after introduction of the intact cor gene. 3.The survival of ø80 lysogens with a mutation in the cor gene exceeds the survival of the non-mutated lysogens after UV-irradiation. This difference in survival of the cor+ and cor- lysogens may reflect the distinction in accessibility of their CI proteins for cleavage with RecA protease. In all cases it may be suppose the CI protein put in touch with an another protein - with the cor gene product. The data obtained from determination of the relative number of the active receptors on the cells from the collection of the TonA mutant strains with the cor gene and without one allow to suppose the cor gene product not only participates in repression of the ø80 operons but does in repression of the tonA operon of E. coli cells lysogenised by phage ø80. Genes similar to the cor gene of phage ø80 are important for evolution of E.coli since they can swich on or swich off several operons.

This work was supported by Grant of Fundamental Natural History N°94-I0.6-I39 from State Committee of Russia Higher Educational Establishments.

Late Abstracts

MOLECULAR ARCHITECTURE OF THE ColE1 cer SYNAPTIC COMPLEX

Elizabeth R Andrews Ian S Viney
Anjan Guhathakurta and David K Summers

Multimer formation is a major cause of plasmid instability in bacteria. In E. Coli multimers of ColE1 are resolved to monomers by site specific recombination between 240 bp cer sites. Recombination is constrained between two sites in direct repeat and requires four proteins ArgR PepA XerD and XerC. There is evidence that only ArgR, XerC XerD bind to DNA and that the topological constraint is provided by ArgR and PepA. Mutants of the four proteins have been isolated to determine the sites of interaction between the DNA and protein and between proteins. Mutants of ArgR and PepA are being screened to find compensating mutations in the recombination and resolution.of the cer sites.

GENE ORGANIZATION IN THE *oriC* REGION OF THE *STREPTOMYCES COELICOLOR* CHROMOSOME, Michael J. Calcutt, Department of Biochemistry, University of Missouri, Columbia MO. 65212 and the Cancer Research Center, 3501 Berrywood Drive, Columbia, MO 65201.

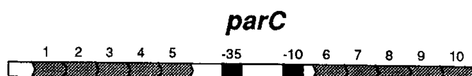
Members of the *Streptomyces* genus undergo a complex life cycle of mycelial growth followed by sporulation. To initiate an investigation of how the 8 Mb linear chromosome of *S. coelicolor* is replicated and partitioned during such development, we have isolated the origin of DNA replication. Nucleotide sequence analysis has indicated that the origin is much more complex than those previously isolated from other prokaryotes in that there are at least 18 recognition sites for the DNA replication initiator protein DnaA. The relative spacing of these motifs (DnaA boxes) appears to be important since it is strongly conserved in other *Streptomyces* origins that we have isolated.

We have also investigated the organization of genes and potential open reading frames in a 20 kb region of the chromosome spanning the origin. As in most other bacterial genomes investigated, the genes for DnaA, DnaN, RecF, ribosomal protein L34, RNaseP protein, the DNA gyrase A and B subunits are clustered in the *S. coelicolor* chromosome. In addition, the genes for thioredoxin and thioredoxin reductase are also present in the origin region, a situation that has not been observed in other bacterial genomes. Between the genes for RNAse P protein and thioredoxin are open reading frames with homology to the *spoOJ* and *soj* genes which have been shown recently to be involved in chromosome partitioning and the initiation of sporulation in *Bacillus subtilis*. In the *S. coelicolor* chromosome, both the *spoOJ* and *soj* homologs are flanked by a 14 base palindromic sequence that may be a target for the proteins that these genes encode.

PARTITIONING OF PLASMID R1: GENETIC DISSECTION OF THE CENTROMERE-LIKE *parC* SITE.
Kenn Gerdes, Anne Breüner, Rasmus Bugge Jensen and Mette Dam. Dept. of Molecular Biology, Odense University, Denmark. E-mail: gerdes@biobase.aau.dk

The *parA* system of plasmid R1 confers a c. 100-fold stabilization on R1- and F-derived replicons. The *parA* system consists of three components: The *trans*-acting proteins ParM and ParR, and the *cis*-acting *parC* site. The centromere-like *parC* site is located upstream of *parM* and *parR* and contains the *parA* promoter, that transcribes *parM* and *parR* as an operon. The *parC* site consists of 10 direct repeats to which the ParR protein binds efficiently and cooperatively *in vitro*. The *parA* promoter is located in the middle of the 10 direct repeats and is thus flanked by 5 direct repeats on either side. The structure of *parC* is shown in the figure below.

Here we present a detailed analysis of the phenotypes associated with the *parC* site: **(i) Centromere-like activity:** R1 plasmids carrying *parC* are stabilized when ParM and ParR are donated *in trans*. **(ii) Incompatibility:** *parC* present on pBR322 interferes with the stable inheritance of R1 plasmids stabilized by *parA*. **(iii) Operon regulation:** The *parA* promoter in the center of *parC* is highly repressed by ParR donated *in trans* (more than 500-fold). **(iv) Titration of ParR**. Surprisingly, our results indicate that sequences in the *parC* region not related to the direct repeats are also essential for *parC* function.



THE INTERACTION OF THE HISTONE-LIKE PROTEIN H-NS AND RNA-POLYMERASE AT THE PROMOTER OF *proU*, Bart J.A.M. Jordi, Thomas A. Owen-Hughes, Christopher S.J. Hulton and Christopher F. Higgins, Imperial Cancer Research Fund, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, OX3 9DU, England.

The *proU* locus of *Salmonella typhimurium* encodes a high-affinity glycine-betaine transport system which plays an important role in survival in high osmolarity environments. The expression of the *proU* promoter is upregulated (± 100-fold) when cells are grown in media of high osmolarity. The mechanism by which *proU* is osmoregulated is still not fully understood. However it's clear that the histone-like protein H-NS is involved in down-regulation of the *proU* promoter at low osmolarity. H-NS maintains repression of the *proU* promoter by interaction with curved DNA downstream of the *proU* promoter.
Using strains carrying chromosomal *proU::lacZ* fusions, promoter-mutations were isolated which expressed *proU* at both high and low osmolarity environments. Further mutations were made using site-directed mutagenesis. The expression of these promoter-mutations in low- and high-osmolarity was assayed both in wild-type and *hns*⁻*Salmonella* strains. In order to understand the expression of these promoter-mutations the interaction of RNA-polymerase and H-NS with these promoters was studied using footprinting techniques.