TRANSPOSITION AND SITE-SPECIFIC RECOMBINATION: MECHANISM & BIOLOGY

Organizers: Nancy Craig and Jef Boeke

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Transposition and Site-Specific Recombination: Mechanism & Biology

Keynote Address

F 001 ARCHITECTURE AND MECHANISM IN INTEGRATION AND EXCISION OF BACTERIOPHAGE LAMBDA, Howard A Nash[1], Alex B. Burgin[1], Anca M. Segall[1], Milton H. Werner[2], and Shuwei Yang[1], [1]Laboratory of Molecular Biology, NIMH, Bethesda, MD 20892-0036, [2]Laboratory of Chemical Physics, NIDDK, Bethesda, MD 20892-0005.

Lambda integration requires two proteins that act on defined segments of DNA called attachment sites: the phage-encoded recombinase, Int, and the host-encoded accessory, IHF. IHF protein binds to specific targets and severely distorts them. The mode of target recognition is unusual in that, although IHF contacts 30 bp of DNA, its principal recognition elements are clustered at one end of this region. In addition, footprinting data suggest that IHF principally contacts the minor groove of DNA. Recent work on target recognition by individual subunits of this heterodimeric protein and on binding of IHF to sites in which IC base pairs replace AT base pairs have solidified our understanding of both of these features. Moreover, specific cross-linking between the protein and its target has been used to test the predictions of our model for the way in which the domains of the protein contact DNA.

In the past, we have replaced IHF protein by other elements that bend DNA. The success of these replacements suggests that one source of bending can directly substitute for another. This hypothesis has now been confirmed through use of an electrophoretic band shift assay to examine protein-DNA complexes that are formed between attachment sites, Int and proteins that can replace IHF (like HU and HMG-2). Since the replacement proteins lack specific binding to naked attachment site DNA and none of them appears to interact directly with Int, we are led to propose that they become specifically entrapped at the attachment site through cooperativity in building a higher order structure.

A successful recombination event must achieve binding of recombinase to target sites, juxtaposition of targets, breakage of DNA strands, exchange of partners, religation of DNA strands to a recombinant configuration and disassembly of the recombination apparatus. To assist an enzymological analysis of this pathway, we are devising recombination targets that have altered capacities for various enzymatic steps. Our first venture has been to synthesize a novel suicide substrate. By replacing the 5'-bridging oxygen of a phosphodiester bond with sulphur, we have created an attachment site in which Int-promoted cleavage generates a fragment that is terminated by a 5'sulfhydryl and is incapable of religation. Unlike earlier suicide substrates, this 5'-phosphorothiolate is prepared as a continuous DNA and therefore can be included on supercoiled templates and on both strands of an attachment site simultaneously. We expect that this and related analogs will help in dissecting the elementary steps in the integration/excision pathways.

DNA Slicing and Splicing I

F 002 ASSEMBLY AND ORGANIZATION OF THE ACTIVE MuA TETRAMER, Tania A. Baker and Li Luo, Department of Biology MIT, 16-520, 77 Massachusetts Avenue, Cambridge, MA 02139

A tetramer of Mu transposase (MuA) cleaves the two ends of the donor DNA, and joins these cleaved ends to a target DNA by strand transfer. Formation of this tetrameric protein-DNA complex is a prerequisite for the chemical steps and may activate the protein for catalysis. Several strategies have been employed to search for regions in MuA protein important in tetramer formation, donor DNA cleavage, and DNA strand transfer. In one approach, eleven different positions within MuA were subjected to site-directed mutagenesis. An asparate was changed to an asparagine or a glutamate was changed to a glutamine at each position. While most mutant proteins retained substantial activity, substitution of either Asp269 or Glu392 rendered MuA unable to promote either DNA cleavage or strand transfer. MuA carrying the D269N substitution assembled into the tetramer normally, indicating that this protein is specifically defective in chemical catalysis. The amino acid sequence surrounding Asp269 and Glu392 shows similarity to several other transposition proteins, including the D,D-35-E motif of the retroviral integrases, indicating that this family of recombination proteins probably share a common type of active site.

To start to address how the four Asp269 residues in the normal MuA tetramer contribute to the activity of the complex, the impact of the D269N mutant protein on reactions containing wild-type MuA was investigated. Mixtures of MuAD269N and limiting amounts of wild-type MuA promoted donor cleavage or strand transfer efficiently. However, the protein mixtures often generated incomplete transposition products. For example, under certain conditions the strand transfer products made by MuA and MuAD269N reflected almost exclusively joining of only one end of the Mu DNA to the target site, a rare reaction with the wild-type protein alone. These data indicate that, while each monomer within the MuA tetramer need not carry Asp269 for the complex to catalyze individual cleavage or strand transfer reactions, more than one monomer must contribute Asp269 for pair-wise strand transfer of the two Mu DNA ends. Thus, the effects of the D269N mutation support models in which each monomer contributes one set of active site residues to the tetramer, enabling it to carry out a pair of DNA cleavages and a pair of DNA strand transfers, the four chemical steps central to transposition.

F 003 EARLY EVENTS IN THE PATHWAY OF RETROVIRAL INTEGRATION, Viola Ellison[1], Brian Scottoline[1], Samson Chow[1], Patrick Brown[1,2,3]. Departments of [1]Biochemistry and [2]Pediatrics, Stanford University Medical Center, Stanford, CA 94305-5428. [3]Howard Hughes Medical Institute.

We have identified and investigated several steps in the HIV-1 integration process that precede the first chemical step. Upon incubation with a model DNA substrate that mimics the end of a viral DNA molecule, in the presence of a metal ion, integrase binds to form a stable functional complex that processively mediates the two chemical steps in integration - endonucleolytic processing of the viral DNA 3' ends, followed by joining of the viral DNA 3' end to a target DNA molecule. The 5' terminal two bases of the viral DNA, whose essential function has been obscure, are required for maintaining the stability of the complex after 3' end processing. This role for these two bases is likely to be critical *in vivo*, where the end-processing and integration steps are often separated by hours. Formation of the initial stable functional complex with a viral DNA end depends upon an asymmetric, metal-ion dependent interaction between two distinct domains of integrase. One site, a zinc-finger-like N-terminal domain, appears to make direct contact with a second, distinct site recognized by its sensitivity to modification by N-ethyl maleimide. That intermolecular interaction is essential for assembly of a higher-order multimer of integrase dimers. Reconstitution of an active integrase multimer *in vitro*, using complementing pairs of defective forms of the enzyme, provides evidence that the HHCC domain functions in *trans* to the enzyme active site, while the NEM sensitive site acts in *cis* to the active site. After binding to its viral DNA substrate, integrase disrupts the terminal three base pairs. This "fraying" step appears to be essential to allow the 3'-end processing reaction to occur, and under some experimental conditions, this step can be rate-limiting. Integrase recognizes the attachment sites for integration of the provirus on the basis of both their sequence and their position near the ends of the linear viral DNA precursor. The requirement that the viral DNA attachment site be capable of fraying appears to provide the basis by which integrase can recognize that this site is at the end of a DNA molecule.

F 004 CONTROL OF GENE EXPRESSION AND TRANSPOSITION IN THE IS3 FAMILY OF BACTERIAL INSERTION SEQUENCES
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The talk will describe the IS*3*-family of bacterial insertion sequences, and present our current understanding of their expression and transposition with particular emphasis on one of the members, IS*911*. The IS*3* family of bacterial insertion sequences is composed of over thirty known members from a wide range of bacterial species. This represents nearly one quarter of all characterized bacterial elements. Members of the family exhibit similar terminal inverted repeats which terminate with 5'-TG.....CA-3'. They also generally carry two consecutive and partially overlapping open reading frames. The amino acid sequences of the predicted products of the downstream frame are highly conserved although the nucleic acid sequences show significantly less similarity. Moreover, a region of the downstream frame exhibits marked similarities with the retroviral integrases. Experiments with one member of the family, IS*911*, designed to examine the mode and control of transposase expression have shown that three proteins are generated from the two consecutive reading frames, *orf*A and *orf*B. The transposase, OrfAB, is synthesised from both frames as a fusion protein by a programmed -1 translational frameshift in the overlap region of the two frames. We have investigated the role of the three IS*911*-specified proteins in transposition in vivo: the products of the upstream (OrfA) and downstream (OrfB) open reading frames, and the transframe protein, OrfAB. The production of OrfAB alone leads to both excision and circularization of the element. It is sufficient for intermolecular transposition into a plasmid target. Simultaneous and independent production of OrfA stimulates OrfAB-mediated intermolecular transposition while greatly reducing the appearance of transposon circles. No role has yet been detected for OrfB. The use of IS*911* derivatives carrying mutations in the terminal 2bp suggests that circle formation represents a site-specific intramolecular transposition event. It appears unlikely that the transposon circles represent transposition intermediates, however, since they can be detected after long exposure to transposase and are relatively stable. Moreover, if OrfAB is induced for short periods of time other, less stable, transposon species can be detected. These include linear and open-circular molecules as well as additional forms whose structure is under examination. It is likely that true transposition intermediates will be included among these forms. Although we do not yet understand the exact roles of OrfA and OrfAB, recent studies with partially purified proteins indicate that both are capable of binding specifically to the terminal inverted repeats of IS*911*.

F 005 BIOCHEMICAL ANALYSIS OF RETROVIRAL DNA INTEGRATION BY NUCLEOPROTEIN COMPLEXES ISOLATED FROM VIRUS-INFECTED CELLS: AN ATTEMPT TO ANSWER SOME BASIC UNSOLVED QUESTIONS,
Robert Craigie and Myung Soo Lee, Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892

Integration of a DNA copy of the retroviral genome into the host genome is a necessary step in the replication cycle of retroviruses. The viral DNA, made by reverse transcription after infection of a sensitive cell, forms part of a nucleoprotein complex derived from the viral core. These complexes can be isolated from cells after infection and efficiently integrate their DNA *in vitro* when presented with a target DNA. Although a single viral protein - integrase - can carry out the key enzymatic steps of integration *in vitro* , differences between the reactions promoted by integration complexes made *in vivo* and reactions catalyzed by purified integrase protein and synthetic DNA substrates suggest that additional factors are required to reproduce a reaction that more closely parallels the integration process *in vivo*. Some of the pertinent differences are noted below.

Integration Complexes	Integration of pairs of DNA ends	DNA-protein complex is resistant to challenge by competitor DNA	Autointegration is blocked
Purified integrase and DNA	Integration of single DNA ends (mostly)	DNA-protein complex is sensitive to challenge by competitor DNA	Autointegration predominates

Although considerable progress has been made in understanding the enzymology of integrase protein, this work has not yet illuminated the mechanistic basis of the features of integration complexes noted above, all of which are necessary for a viable retrovirus. It seems likely that additional factors are required to form a nucleoprotein complex with the above properties, either as an integral part of the structure or as a catalyst for its formation.

We are attempting to solve these problems by studying authentic integration complexes isolated from cells infected with Moloney murine leukemia virus. These complexes possess all the properties listed above for *in vitro* integration of the viral DNA, including the barrier to autointegration. However, complexes that have been treated with high salt and separated from free proteins by sedimentation in sucrose lose the barrier to autointegration. We find that the barrier to autointegration can be restored by incubating these "autointegration complexes" with a sucrose gradient fraction that contains mainly free proteins. Presumably, a factor that prevents autointegration is stripped from the complexes by high salt treatment and separated in the sucrose gradient. We are in the process of determining the identity of this factor. We are also analyzing the functional role of viral proteins in the complex by studying complexes isolated from cells infected with radiolabeled virus.

Directed Conversion

F 006 THE MECHANISM OF SURFACE COAT GENE TRANSPOSITION IN AFRICAN TRYPANOSOMES,
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African trypanosomes are unicellular eukaryotic parasites transmitted by tse-tse flies. In their mammalian host trypanosomes are entirely covered by a dense surface coat. By drastically changing the composition of the coat - i.e. antigenic variation - a sub-fraction of the trypanosomes escapes immune lysis. The coat of bloodstream trypanosomes consists of a single protein species, the Variant-specific Surface Glycoprotein (VSG). A trypanosome has some 10^3 different genes for VSGs, but usually only one of these is expressed at any particular time. The expressed gene is invariably located near the end of a chromosome. The trypanosome can change its coat in three ways (see 1):

a. The VSG gene in the active telomeric expression site (ES) is replaced by a copy of a different VSG gene. Replacement may be partial and limited to gene segments encoding exposed VSG epitopes.

b. Another VSG gene ES is turned on and the previously active one turned off. There at least 6 and possibly 20 ES's.

c. When a trypanosome enters the tse-tse fly, it shuts off VSG gene transcription all together and replaces the VSG coat with a procyclin (PARP) coat.

In my talk I shall focus on process 1, the duplicative transposition of VSG genes. The analysis of the putative substrates and products of this process has indicated that transposition occurs by gene conversion mediated by short blocks of incomplete sequence homology between donor and target genes. The low rate of transposition (10^{-4} per trypanosome division) in most trypanosome lab strains and the large numbers of VSG genes available initially suggested that transposition might result from the repair of rare accidental breaks in DNA, i.e. genetic background noise (2). Recent experiments are not readily reconciled with this simple model. The rate of coat switching in some trypanosome strains can be as high as 10^{-3} per division (3); there are indications for bursts of DNA transposition in bloodstream trypanosomes (4); and DNA introduced into trypansomes is only inserted into the genome by precise homologous recombination (5). Possible transposition models that can account for these results will be discussed.

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F 007 MECHANISM OF TRANSPOSITION OF Tc1 AND Tc3 OF C.ELEGANS, Ronald H.A. Plasterk, Henri G.A.M. van Luenen, Jan C. Vos, Sean D. Colloms, Division of Molecular Biology, The Netherlands Cancer Instite, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

The Tc1/*mariner* transposons are the most widespread transposons in the animal kingdom known to date. They all consist of a single gene, encoding the transposase, flanked by terminal inverted repeats of up to a few hundred base pairs. They integrate into the target sequence TA. The best studied example of this family of elements is Tc1 of the nematode *Caenorhabditis elegans*. We study the mechanism of jumping of Tc1 and the related element Tc3. We previously showed that excision of the element is followed by DSB repair, suggesting that the element excises by breaks at both ends.

The tranposases of both elements were identified. Forced expression of these in a nematode strain in which Tc1 and Tc3 are normally inactive, activates jumping of the corresponding element. At the same time a linear extrachromosomal transposon is detected, presumably an intermediate in jumping. We determined the ends of the excised element. It turns out the element is excised by a stagerred cut: the cut at the 3' ends is between the transposon and the target TA, the 5' end is cut 2 nucleotides inside of the element. These remaining nucleotides provide a nice explanation for the most common 4 base pair footprint found after somatic Tc1 and Tc3 excision.

We started analyzing the *cis* requirements for jumping. It was found that the TA flanking the transposon can be changed without loss of activity. As described above the TA sequence is not co-excised with the transposon, and therefore one would predict that the requirement for the sequence TA at a target is not dependent on the presence of TA sequences at the donor tranposon ends. Indeed the element with altered flanks continues to integrate into TA targets.

The tranposases of Tc1 and Tc3 were purified from a recombinant *E.coli* strain; they specifically bind transposon DNA ends. Thus far we could detect transposase dependent nicking at the transposon ends, but not complete excision or integration.

Several hundreds of integration sites of both elements in a 1.1 kbp area of the genome were sequenced. Tc1 and Tc3 have strong, but clearly different, target preferences. Hot sites are not clustered into hot regions; no clear consensus sequence for a hot site has been found.

Jumping Backwards: LTR Retrotransposons

F 008 CONTROL OF RETROTRANSPOSONS AND RETROVIRUSES IN *DROSOPHILA*, Alain Bucheton, Fabienne Chalvet, Alexander Kim, Alain Pélisson, Nicole Prud'homme, Pedro Santamaria, and Christophe Terzian, Centre de Génétique Moléculaire, C.N.R.S., 91198 Gif-sur-Yvette, France.

The genome of *Drosophila melanogaster* contains many different retrotransposons that belong to two classes according to their structures: LINEs or non-viral retrotransposons that are devoid of long terminal repeats (LTRs), and viral or LTR-retrotransposons that contain typical LTRs like integrated proviruses of retroviruses. Apparently most LTR-retrotransposons, like *copia*, are similar to Ty1 elements in yeast. They contain two large open reading frames, the potential products of which show similarities with *gag* and *pol* polypeptides of retroviruses. They are not infectious because they are devoid of a third open reading frame coding for a product similar to *env* which is responsible for the infective properties of retroviruses. However, *gypsy* and a few other LTR-retrotransposons of *Drosophila* contain three open reading frames (1). The putative product of *gypsy* ORF3 shows some caracteristics of retroviral *env* proteins, but the function of this ORF is still unknown. Although their structure is more similar to that of retroviruses than to that of *copia*-like elements, they are considered as transposable elements because retroviruses are usually thought to be restricted to vertebrates.

It seems that most *Drosophila melanogaster* strains contain only a few active *gypsy* elements located in euchromatic regions of the chromosomes. Usually they are repressed by host genes and do not transpose with detectable frequencies. We have identified a *Drosophila* gene, *flamenco* (*flam*) that controls *gypsy* activity. Transposition occurs at high frequency in the progeny of females homozygous for mutations of *flam* and is associated with an increase of the transcripts of *gypsy* in homozygous females. In particular a 2 kb spliced RNA containing ORF3, similar to the transcript from which the *env* gene of retroviruses is translated, is synthesized in these females.

Stocks carrying *flam* mutations contain many copies of actively transposing *gypsys*. One of them is called *MS* (2). *SS* is an isogenic strain devoid of functional *gypsys* (3). It can be converted into an *MS*-like stock (*MSN*) by introducing an active *gypsy* in its genome by transformation. We have microinjected egg plasm from *MSN* into *SS* embryos and recovered in this way typical active *gypsys* integrated in the genome of their progeny. We have obtained similar results by growing *SS* individuals on a medium containing homogenized *MSN* pupae. These results indicate that *gypsy* is infectious and is in fact the first retrovirus described so far in invertebrates.

We are now studying the mechanisms by which the gene *flamenco* controls transposition and/or infective properties of *gypsy*.

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Site-Specific Integration

F 009 TRANSPOSITION OF THE BACTERIAL TRANSPOSON Tn7, Nancy L. Craig, Howard Hughes Medical Institute, Department of Molecular Biology & Genetics, Johns Hopkins School of Medicine, Baltimore, MD 21205.

The bacterial transposon Tn7 is distinguished by its ability to transpose to two distinct classes of target sites: Tn7 transposes at high frequency to a specific site (an attachment site, attTn7) in the chromosome of E. coli and of many other bacteria and can also transpose at low frequency to other target sites which are unrelated to attTn7 and are apparently random in sequence. Tn7 transposition to these different target sites is mediated by distinct but overlapping sets of the Tn7-encoded transposition genes, tnsABCDE. Transposition to attTn7 requires tnsABC + tnsD whereas transposition to random sites is mediated by tnsABC + tnsE. Thus, tnsABC form a common core machinery which can be alternatively activated by either tnsD or tnsE which also determine the class of target site used.

We have now established that TnsA and TnsB proteins mediate the DNA strand breakage and joining activities which underlie the translocation of Tn7 from place to place. The other component of the core Tn7 transposition machinery, TnsC, is apparently a regulator of Tn7 transposition. TnsC, an ATP-dependent DNA binding protein, is involved in modulating the interaction of the ends of Tn7 (presumably bound to TnsA and TnsB) to the target DNA and plays a central role in Tn7 target immunity (see abstract by Stellwagen and Craig). We have now also established that Tn7 target immunity can act over long, i.e. > 60 kb, distances in the E. coli chromosome (see abstract by DeBoy and Craig).

How do TnsA and TnsB mediate recognition of the ends of Tn7 and execute DNA breakage and joining? TnsB is a sequence-specific DNA binding protein that recognizes multiple repeated sites in the essential cis-acting sequences at the ends of Tn7. We have also established that another key sequence feature of the ends of Tn7 are the extreme 3' end terminal nucleotides. Alteration of these nucleotides blocks both the double-strand breaks that release the transposon ends from the flanking donor backbone and also blocks joining of these ends to the target DNA. We are using site-directed mutagenesis to identify the regions of TnsA and TnsB that mediate specific DNA recognition and DNA strand breakage and joining. It should also be noted that we have established that breakage and joining by these proteins alone is independent of ATP; thus ATP's only role in transposition is as a regulatory cofactor of TnsC.

F 010 DISSECTING THE RESOLUTION REACTION OF λ INTEGRASE USING SUICIDE HOLLIDAY JUNCTIONS, Simone Nunes-Duby, Sok Hong Kho and Arthur Landy, Division of Biology and Medicine, Brown University, Providence, RI 02912.

The site-specific recombination reaction of bacteriophage λ proceeds via two sequential and ordered single strand exchanges that are executed by the phage-encoded Int protein. Int cleavage at a pair of "partner" core-type Int binding sites initiates the first reciprocal strand exchange to generate a Holliday junction, or X-form, in which the two partner DNA duplexes are joined by a pair of bridging strands. Resolution of the *att* site Holliday junction is accomplished by Int cleavages at a second pair of partner Int sites located 7 base pairs from the first pair, to yield recombinant DNA duplexes. "Synthetic" Holliday junctions containing four core-type Int binding sites and the 7 base pair "overlap region" can be constructed by annealing the appropriate four DNA strands. They are efficiently resolved by purified Int protein cleaving at either pair of "partner" core-type sites, i.e., on analogous DNA strands. Two perturbations of the normal Holliday junction will be described.

The first is designed to study single cleavage events that are not normally observed because the covalent Int-DNA cleavage intermediate rapidly religates. To circumvent the rapid back reaction we generated Holliday junctions containing a nick within the overlap region. Single Int cleavages of this "suicide" Holliday junction are trapped as covalent intermediates because the 5'OH required for religation is lost by diffusion of either a small oligonucleotide or one or more arms of the Holliday junction. We have shown that these suicide substrates are a useful model of the intact Holliday junction and can provide some insights about the Int-dependent resolution reaction. The results indicate the usefulness of these and other suicide Holliday junctions; they ar discussed in terms of models for the Int resolution reaction and the potential for exploring Holliday junction conformation during this reaction.

The second type of Holliday junction is intended to study the effect of immobilizing the crossed strands at different positions within the seven base pair overlap region. Experiments with these substrates have led to a new view of the mechanisms and dynamics of strand exchange during the Int-dependent resolution (and presumably formation) of Holliday junction recombination intermediates. These results will be discussed in relation to current models of branch migration and DNA strand cleavage.

F 011 TEMPORAL AND POSITIONAL CONTROL OF THE RETROVIRUSLIKE ELEMENT TY3, Suzanne Sandmeyer, Jacqueline Kirchner, Tom Menees, Phil Kinsey, and Charles Connolly, University of California, Irvine.

The *Saccharomyces cerevisiae* element Ty3 resembles animal retroviruses in the proteins it encodes and in its sequence organization. Ty3 is 5.4 kbp in length and is composed of an internal domain flanked by long terminal repeats of 340 bp. It is transcribed into a genomic RNA which encodes the structural proteins capsid and nucleocapsid and the catalytic proteins protease, reverse transcriptase and integrase. High level expression of the element results in formation of intracellular viruslike particles (VLPs) about 156S in diameter which resemble the nucleoprotein core complexes of retroviruses. Reverse transcription of the genomic RNA into DNA is associated with these particles. The DNA is subsequently processed at its 3' ends by integrase and is integrated into the genomic target.

Both the expression of Ty3 and its reverse transcription are temporally regulated. Transcription of the wt Ty3 element is induced by mating pheromones and Ty3 elements transpose naturally in mating populations of cells. Examination of the time during which transposition occurs showed, however, that transposition does not occur during the G1 arrest caused by pheromone treatment during which transcription of the element is induced. Instead the transposition arrest during G1 is reversible. If transcription of a Ty3 under control of a *GAL1* UAS is induced in cells blocked in G1, but repressed by glucose before their release from the block, integration occurs efficiently. Thus particles synthesized during G1 arrest can become competent for integration. Analysis of VLPs from arrested cells showed that transposition is blocked at the point of reverse transcription.

Ty3 integration is also positionally restricted. Ty3 integrates into the transcription initiation region of genes transcribed by polymerase III. Mutagenesis of promoter elements has shown that they are important for efficient transposition *in vivo* and that factors that bind upstream of the initiation site, such as TBP and TFIIIB, are probably present when integration occurs. Recently an *in vitro* system has been developed in order to identify the proteins required for targeting. Using VLPs as the Ty3 DNA donor, integration can be detected by a PCR assay into a chromatin DNA target tRNA gene and in the presence of pol III transcription extracts, into a target plasmid containing a tRNA gene. Integration occurs at a lower level into a mutant tRNA gene or target DNA in the absence of chromatin or extracts.

Throwing Out DNA: Site-Specific Deletion

F 012 BROKEN DNA MOLECULES IN V(D)J RECOMBINATION, AND A POSSIBLE ROLE OF DNA SYNTHESIS
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We have previously reported the presence of broken DNA molecules associated with V(D)J recombination at the TCRδ locus in mouse thymocytes. Only broken signal ends could be seen in normal mice, but in mice with the *scid* mutation, which blocks joining of coding ends, broken DNA with these ends was also found, and the coding ends were sealed into hairpin structures. We believe hairpin DNA is a general intermediate in V(D)J recombination, because later nicking of this structure would explain the frequent short self-complementary (P nucleotide) insertions in coding joints.

More recently, the fine structure of broken signal ends has been studied. By direct ligation and ligation-mediated PCR, we find most of them to be full length, blunt, and 5'-phosphorylated. A few are recessed, usually on the 3' end. It seems likely, though hard to prove in DNA isolated from cells, that these blunt ends are the primary cleavage products.

In an extension of the use of plasmid substrates, we have detected recombined molecules directly in DNA reisolated from transfected pre-B cell lines. The plasmid resulting from a deletion, which retains its replication origin, can be detected on Southern blots. The excised circle (detected only by PCR) is unable to replicate after recombination, and thus reveals the replication state of the DNA at the time of the reaction. This circle is resistant to both MboI and DpnI, so it is hemimethylated, showing that most of the DNA has replicated no more than once before recombining. After extensive replication, the plasmid appears no longer to be available for recombination. But even with a non-replicatable substrate, the signal region of the excised circle has undergone some (repair?) DNA synthesis, indicating that local synthesis may be coupled to recombination.

F 013 DISSECTION OF RESOLVASE RECOMBINATION BY GENETIC AND STRUCTURAL ANALYSIS, Nigel D.F. Grindley and Martin R. Boocock, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520.

The DNA-protein complex within which γδ resolvase carries out site-specific recombination contains at least 12 protomers of resolvase and two copies of the 120 bp DNA substrate, *res* (each with 3 binding sites for a resolvase dimer). The resolvase protomers play unequal roles in the recombination process. In order to understand the structure of the macromolecular complex and the function of the individual protomers, we are attempting to address the question "what is each resolvase monomer doing and where is it doing it"? The strategy employed to address this question is to place specific mutants of resolvase — for example those defective in chemical catalysis or in a defined set of protein-protein interactions — at particular subsites within each *res*. The outcome of a resolution assay then indicates whether the resolvase bound at a defined site makes use of specific protein-protein interactions during assembly of the synaptic complex, or plays an active role in the chemical steps of strand exchange. Variant resolvases can be directed to defined subsites by means of an amino acid substitution in the C-terminal DNA binding domain that results in altered DNA binding specificity. The mutant R172L binds prefentially to sites in which the G at position 2 of the consensus half site (TGTCCGATAATT) is changed to a T. To date, our results indicate that:

- The resolvase dimers that initially bind to site I are the only ones that require the catalytically essential serine-10 residue.
- The resolvase side chains that appear to be important for chemical catalysis of DNA cleavage act in *cis* on the site I at which the resolvase is bound by its C-terminal domain.
- Although cleavage of the 4 DNA strands normally appears to be concerted, the cleavage reactions at the two crossover sites are not tightly coupled.
- The resolvase dimers bound at site I do not participate in the 2-3' interaction — an essential inter-dimer interaction that is implicated in synapsis of the *res* sites.

F 014 GENOME REMODELING IN THE CILIATE *EUPLOTES CRASSUS*, Lawrence A. Klobutcher, Leah R. Turner, Janice LaPlante, Cynthia Hale, and Ling Zhong, Department of Biochemistry, University of Connecticut Health Center, Farmington, CT 06030.

On the order of 100,000 DNA breakage and rejoining events occur during the development of a new macronucleus in the hypotrichous ciliate *Euplotes crassus*. Two types of sequences that are eliminated as a result of this process have been defined: 1) internal eliminated sequences (IESs), which are short segments of unique DNA, and 2) Tec elements, which are large, repetitive DNA elements that strongly resemble transposons. We have previously demonstrated that both IESs and Tecs are precisely excised during development such that one copy of the 5'TA3' direct repeat that defines the ends of both of these elements is retained in the resulting macronuclear DNA molecule. We have now used polymerase chain reaction (PCR)-based procedures to examine the free, circular IESs that are generated during the DNA breakage and rejoining process. The junction of the circular IESs contains two copies of the 5'TA3' direct repeat separated by 10 bp that are derived from the regions flanking the integrated IES. Using a modified form of PCR that we have termed "strand-biased PCR", we have obtained evidence that the central 6 bp of the circle junction are in the form of a heteroduplex, with one strand of the DNA being derived from left-flanking sequences and the other strand from right-flanking sequences. The results suggest a model for the excision process that differs from those suggested for site-specific recombination events in other organisms. In additional studies, we have begun to isolate monoclonal antibodies directed against proteins that are specifically expressed in developing macronuclei as a means of isolating genes and proteins involved in the DNA rearrangement process.

F 015 GENES INVOLVED IN V(D)J RECOMBINATION, Christina Cuomo, Susan Kirch and Marjorie Oettinger, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

Developing B and T cells assemble their antigen receptor molecules from component gene segments by a series of genomic rearrangement events collectively termed V(D)J recombination. Two genes involved in this assembly process, *RAG-1* and *RAG-2*, were identified based on their being the only genes that need be introduced into fibroblasts to induce recombinase activity. A targeted disruption of either gene results in the complete absence of V(D)J recombination, confirming the critical role of each gene in the reaction. Coexpression of RAG-1 and RAG-2 is restricted to immature cells of the lymphoid lineage, thereby explaining why V(D)J recombination is limited to pre-B and pre-T cells. However, it is also clear that other factors must be involved in the recombination reaction. Such factors need not be specific for V(D)J joining, but could instead play multiple roles in the cell and be recruited by the V(D)J recombination machinery. Examples of such factors include those defined by the *scid* mutation and by mutations in CHO cells which have defects in V(D)J recombination and in some aspect of general DNA repair.

We have used the yeast two-hybrid assay to identify genes whose products interact with the RAG proteins. One of these, *Rch1* (Rag Cohort), is a gene that encodes a 61 kd protein that interacts specifically with RAG-1. Rch1 is expressed at similar levels in lymphoid and non-lymphoid cells. The predicted Rch1 protein sequence is 47% identical to the yeast protein SRP1. *SRP1* was isolated as a suppressor of an RNA Pol I mutation and encodes a protein associated with the nuclear envelope. We tested the effect of Rch1 over-expression on V(D)J recombinase activity induced in fibroblasts by transient transfection of RAG-1 and RAG-2. Expression of a nearly full-length Rch1 clone had little effect; recombination frequencies were either slightly higher or the same as in controls. This result might have been expected given that HeLa cells already express Rch1 mRNA. However, we found that a truncated Rch1 derivative that interacts with RAG-1 reproducibly decreased the recombination frequency two- to three-fold when compared to the vector-only control. Thus, the specific interaction between Rch1 and RAG-1 appears likely to be involved in the induction of V(D)J recombination.

F 016 XER SITE-SPECIFIC RECOMBINATION AND CHROMOSOME SEGREGATION, David J. Sherratt, Lidia K. Arciszewska, Garry W. Blakely, Mary Burke, Nick Leslie, Richard McCulloch, Gerhard May and Jennifer Roberts, Institute of Genetics, Glasgow University, Glasgow G11 5JS, U.K.

Two related recombinases, XerC and XerD, catalyse Xer site-specific recombination in *Escherichia coli*. Recombination at sites naturally present in plasmids (e.g. *cer* in ColE1) converts multimers, that arise by homologous recombination, to monomers. Only monomers are stably inherited. Recombination at *cer* requires two accessory proteins, ArgR and PepA, in addition to XerC and XerD. ArgR, PepA and *cer* accessory sequences are required to ensure that recombination at *cer* is intramolecular and not intermolecular. Recombination at *cer* has been reconstituted in an *in vitro* system that uses supercoiled DNA containing two directly repeated *cer* sites as a substrate and requires all four proteins that have been implicated genetically in the process. Other *in vitro* experiments, in which partial recombination reactions have been studied, along with the binding characteristics of the two recombinases to a range of variant recombination sites, have provided insight into how recombinational synapses form at *cer* and *dif* and how strand exchange occurs. The use of mutants of each of the four proteins in these experiments has revealed further mechanistic details of the recombination reaction. The replication terminus region of the *E.coli* chromosome contains a site, *dif*, that is also a substrate for Xer recombination. Recombination at *dif* is required for normal chromosome segregation and cell division. A 32bp *dif* sequence is sufficient for function in chromosome segregation. When *dif* is reinserted into an *E.coli* strain deleted for the *dif* region, normal chromosome segregation is restored when *dif* is repositioned in the terminus region but not when it is placed close to the replication origin (within *phoS*) or when inserted in *lacZ*.

DNA Transposons in Flies and Plants

F 017 MECHANISM AND REGULATION OF DROSOPHILA P ELEMENT TRANSPOSITION, Donald Rio, Paul Kaufman, Sima Misra, Yvonne Mul, Charles Lee, Eileen Beall, Siobhan Roche, Christian Siebel, Roland Kanaar, Melissa Adams, David Rudner, Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, California 94720.

Our lab is interested in understanding the mechanism and regulation of P element transposition in Drosophila. Work in the lab is divided into three areas: biochemical analysis of P element transposition, genetic and biochemical studies of how transposition is regulated (cytotype), and biochemical studies of tissue-specific pre-mRNA splicing of the P element third intron. Studies on the biochemical mechanism of transposition have shown that the purified 87kD P element transposase is a site-specific DNA binding protein that recognizes internal sites near each P element end. Transposase protein was used to develop an in vitro reaction system for P element transposition using a genetic assay in *E. coli* to detect transposition events. These studies showed the guanosine triphosphate (GTP) was a cofactor for the reaction and that transposition occurred via a nonreplicative cut-and-paste mechanism. A host protein was identified that recognizes the P element 31bp terminal inverted repeats (termed IRBP for inverted repeat binding protein). Isolation of cDNA clones encoding IRBP revealed significant amino acid homology to the Ku mammalian autoimmune antigen. The mammalian Ku protein is known to bind DNA terminii in vitro and has been implicated in DNA repair and transposition. Cytogenetic mapping indicated that the IRBP cDNA maps to a region of the Drosophila genome containing a mutagen-sensitive (mus) mutation called mus 309. Experiments are underway to determine if IRBP corresponds to mus 309 and if P element transposition is affected in a mus 309 mutant background. P element transposition is controlled in P strains by a maternally inherited regulatory state termed "P cytotype". Our previous studies have shown that a shortened form of transposase (the 66kD protein) acts as a repressor of transposase activity in genetic tests; however, the observed repression was zygotic, not maternal like true P cytotype. Several experiments aimed at testing models of P cytotype have led to the finding that genomic position of the repressor element may influence its ability to exhibit maternal versus zygotic repression. The tissue specificity of P element transposition results from germline-specific removal of the third P element intron (IVS3) to produce transposase protein. At least one aspect of this control involves repression of IVS3 splicing in somatic cells and our biochemical studies have led to the identification of Drosophila RNA binding proteins that bind to a somatic splicing repression regulatory element in the 5' exon RNA adjacent to IVS3. Biochemical and gene cloning studies have identified two RNA binding proteins (97kD and 50kD) whose binding to this RNA element correlates with inhibition of IVS3 splicing in vitro. The 50kD protein corresponds to hrp48, a Drosophila hnRNP protein containing two N-terminal RNA binding domains (RNP-CS) and a C-terminal glycine-rich region. The 97kD protein contains three reiterated RNA binding domains (half of each repeat is similar to the yeast splicing regulator MER1) as well as a nucleic acid annealing domain and a glutamine-rich C terminus. Both of these RNA binding proteins bind specifically to the P element IVS3 5' exon. The hrp48 protein is expressed in both the germline and soma. However, the 97kD protein is expressed only in somatic cells and not in the germline, consistent with its putative role in somatic repression of IVS3 splicing.

F 018 MECHANISM OF TRANSPOSITION OF THE EN/SPM SYSTEM OF ZEA MAYS, Heinz Saedler, MPI für Züchtungsforschung, 50829 Köln, Carl-von-Linné-Weg 10, FRG

The En/Spm transposable element of Zea mays encodes two protein TNPA and TNPD, which both are required for transposition. TNPA is a multipurpose protein which is required for its own synthesis, shielding of the element from transcription initiated outside of the element and for the excision process itself. In the latter reaction it was proposed that TNPA serves as a "glue" for the association of the distant (8,3 kb) ends of the element. This process is initiated by TNPA binding to 12 bp DNA motifs scattered throughout terminal 200 bp ends. The complex thus formed apparently is recognized by TNPD which then promoters excision of En/Spm. In such excision events predominantly mutant DNA sequences are generated. The significance of this for evolutionary process will be discussed.

DNA Slicing and Splicing II

F 019 IN VITRO TRANSPOSITION OF Mu DNA, George Chaconas, Brigitte Lavoie and Zhen-guo Wu, Department of Biochemistry, University of Western Ontario, London, Ontario, N6A 5C1, Canada.

The strand transfer reaction, an early step in the *in vitro* transpostion of Mu DNA, is mediated by both Mu encoded (A and B) and host encoded proteins. The *E.coli* HU protein is a small, basic, sequence independent DNA binding protein capable of engineering DNA deformations required for the formation of higher order nucleoprotein structures. One such complex is the Mu Type 1 transpososome, where the ends of Mu are stably synapsed by a tetramer of Mu A and cleaved at their 3′ ends. Previously HU has been shown to bind much more strongly to the Mu transpososome core than to supercoiled DNA by immunoelectron microscopy, although its molecular target has remained elusive (Lavoie and Chaconas, 1990, J. Biol. Chem. **265**, 1623-1627). To directly probe HU interactions with the transpososome DNA, we have coupled HU to a DNA cleavage reagent, iron-EDTA, and report here the first HU "footprint". HU-nuclease cleavage was detectable at specific sites within an 83 base pair spacer DNA separating the left end-most L1 site from its neighbouring L2 site. We postulate that HU promotes the formation of a tight DNA loop in this region which increases its affinity for this DNA. This HU binding was specific since it could not be competed with 10-fold excess supercoiled DNA. Immunoelectron microscopic evidence for a second high affinity site which is refractory to cleavage by the HU-nuclease is also presented. Multiple roles of HU in transpososome formation will be discussed.

We have also further characterized the role of the flanking host sequences in the strand cleavage reaction by constructing mini-Mu libraries with random sequences at positions 1-5 or 6-10 in the flanking host DNA. Both inhibitory and stimulatory flanking sequences were enriched. DNA sequencing has indicated the existence of preferred nucleotides at positions 1-5 which are very similar to consensus Mu integration sites (M. Mizuuchi and K. Mizuuchi, personal communication). This suggests that the same active site in the Mu A protein is involved in both the strand cleavage and strand transfer reactions.

F 020 CONJUGATIVE TRANSPOSONS, Gordon Churchward and Fang Lu, Emory University, Atlanta, GA 30322

Conjugative transposons are genetic elements that during transposition promote conjugation, so that they transfer themselves from the genome of one bacterium to a new place in the genome of a different bacterium. Found in Gram-positive bacteria, they are remarkably promiscuous and can conjugate between widely different species. The best studied examples of conjugative transposons are the closely related elements Tn*916* and Tn*1545*. These transposons differ from more familiar transposons in that transposition is not accompanied by duplication of target sequences on either side of an integrated transposon. Rather, transposition can result in the introduction of a short DNA sequence (termed a coupling sequence), carried over from the donor, at either end of the integrated transposon. Excision of the transposon can either restore the original target sequence, or replace a short segment with the coupling sequence. A variety of genetic approaches have identified one transposon gene required for transposition. This gene is homologous to members of the phage lambda integrase family of site-specific recombinases, and thus has been named *int*. We purified fusion proteins carrying different regions of Tn*916* Int protein joined to the carboxy-terminal end of maltose binding protein and determined how Int binds to Tn*916* DNA. We found that Int protein bound to two distinct regions of DNA at each end of the transposon. One region overlapped the end of the transposon and extended into adjacent bacterial sequences. The other region lay within the transposon DNA. The terminal and internal Int binding sites appeared to be quite distinct, and the internal sites were not bound by a fusion protein bearing a carboxy-terminal fragment of Int. These results suggested that Tn*916* Int protein contains two independent DNA binding domains. Consistent with this hypothesis we found that DNA fragments containing either a terminal Int-binding site or an internal binding site did not compete with each other for binding Int protein. We also investigated if Tn*916* Int protein would bind to a preferred target site. We found that both intact Int as well as the carboxy-terminal fragment bound specifically to the target site. These results suggest that transposition of conjugative transposons proceeds by a mechanism similar to phage lambda site-specific recombination. Similarities and differences between the two systems will be discussed.

F 021 MECHANISM AND REGULATION OF Tn10 TRANSPOSITION. N. Kleckner, S. Bolland, R. Chalmers and J. Sakai. Department of Biochemistry and Molecular Biology. Harvard University. Cambridge, MA. 02138.

The bacterial transposon Tn10 is a composite transposon composed of two IS10 elements flanking non-repeated sequences. Tn10 moves by a non-replicative mechanism. The element is excised from its donor site by a pair of flush double strand breaks at the transposon ends. The 3'OH termini of the excised transposon fragment become joined to the two strands of target DNA, probably by single step nucleophilic attack as for bacteriophage Mu. All of these events take place within a stable synaptic complex that forms between the two ends of the element as one of the earliest events in the transposition process. Analysis of the reaction *in vitro* on short linear DNA fragment substrates demonstrates formation of the pre-cleavage synaptic complex, uncoupled cleavages at the two ends of the element, non-covalent capture of supercoiled target DNA by cleaved complexes and, finally, strand transfer.

The transposition mechanisms of bacteriophage Mu (and other non-composite transposons such as Tn7 and gamma-delta/Tn3) includes a transposition target immunity feature that prevents the element from using itself as a target; this feature is essential, as self-integration would be suicidal. In contrast, Tn10/IS10 and other IS element transposons do not exhibit target immunity *in vivo* and Tn10/IS10 can undergo efficient intra-transposon attack both *in vivo* and *in vitro*. For elements of this e the types of intramolecular rearrangements by which these elements generate new types of composite transposons are, in essence, "intra-transposon" integration events. This difference between Mu and Tn10/IS10 is reflected mechanistically in the time and manner with which target DNA enters the transposition reaction. For Mu, target DNA comes into the reaction at a very early stage, prior to cleavage or strand transfer, thus permitting establishment of transposition immunity before the donor molecule is at risk. In contrast, for IS10, target DNA only comes into the reaction subsequent to excision of the element from the donor site.

Tn10/IS10 transposition is already known to be regulated at many levels. Biochemical analysis suggests that transposition is additionally subject to both positive and negative control act at the level of the reaction mechanism *per se*. As appropriate for a small modular element, this regulation is mediated by accessory host proteins, most notably IHF. *In vitro*, IHF is required for formation of a pre-cleavage synaptic complex if reaction conditions are not optimal and/or the donor DNA is not supercoiled. However, high levels of IHF affect the type(s) of synaptic complexes formed prior to target capture, block intermolecular target capture, inhibit intermolecular transposition, and channel the reaction into an alternative mode which produces intra-transposon events of constrained topology. The *in vivo* effects of IHF- mutations on transposition are complex, but support the existence of both positive and negative control.

F 022 COORDINATION OF THE REACTION STEPS IN THE TRANSPOSITIONAL RECOMBINATION OF BACTERIOHAGE MU DNA. Kiyoshi Mizuuchi, Michiyo Mizuuchi, Harri Savilahti, and Phoebe Rice, Laboratory of Molecular Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892.

Bacteriophage Mu, like other transposons studied in detail, generates a critical transposition intermediate by a process involving two chemical steps. The first of these steps is a site-specific endonucleolytic cleavage at the two ends of the Mu sequence. In the second step, the target DNA is cleaved and covalently joined to the donor DNA 3'-ends (DNA strand transfer) in a concerted reaction. This step generates the strand transfer product containing a pair of forked DNA junctions between the donor and target DNAs.

These two chemical steps take place within a context of higher order protein-DNA complexes. We have identified a number of protein-DNA complexes as intermediates in Mu transposition. Structural and functional characterization of these complexes has revealed an overall picture as to how the physiologically critical reaction selectivities are achieved. The architecture of these complexes not only achieves temporal coordination of the events at the two ends of the element, but also provides mechanisms for selectivity that serve to eliminate physiologically detrimental side reactions. Special interactions between the Mu transposase (MuA) and the accessory MuA binding sites on the Mu donor DNA are required in order to achieve a structural transition of MuA to form a stable synaptic complex containing the two Mu ends bound by a tetrameric form of the MuA protein. The assembly and the activities of the transposase tetramer-donor DNA complexes are also controlled by MuB protein, an ATPase, which binds to DNA and activates it to be the transposition target, thereby controlling the location of the target sites.

We began to dissect the architecture of the MuA tetramer-donor DNA complex to further understand the cooperativities involved in the assembly and activation of the complexes. Complexes were formed in the presence of a mixture of the intact MuA protein and mutant MuA proteins with missing domains, and the activities and the protein composition of the resulting MuA tetramer were analyzed. The result obtained indicates the existence of multiple pathways for the complex assembly process as well as for the chemical steps of the reaction.

Efforts are being made to obtain structural information on the MuA transposase protein as well as the MuA tetramer- donor DNA complex. The structures of individual domains of the MuA protein are currently being studied by NMR and crystallographic methods as collaborative efforts. The minimum DNA size requirements for the formation of the stable MuA-DNA complex have been determined. This information will be used as a guide for the further structural and functional characterization of the complex.

Endless Retrotransposition: polyA Elements

F 023 RNA-MEDIATED RECOMBINATION IN YEAST *SACCHAROMYCES CEREVISIAE*, Leslie K. Derr[1], David J. Garfinkel[2], and Jeffrey N. Strathern[2], [1]Laboratory of Molecular Microbiology, NIH-NIAID, Bethesda, MD 20892, [2]Laboratory of Eukaryotic Gene Expression, NCI-FCRDC, Frederick, MD 21702.

Greater than 10% of the mammalian genome consists of sequences whose origin can be traced to the reverse transcription of RNA. These sequences include elements that encode mechanisms for their reverse transcription and integration, such as retroviruses and LINE elements, and elements, such as pseudogenes where the processes of reverse transcription and integration must be provided in *trans*. Until recently, pseudogene formation (an example of nonhomologous RNA-mediated recombination), occurring on an evolutionary time frame, has been difficult to characterize. We have shown that RNA-mediated recombination exists in yeast, *Saccharomyces cerevisiae* and have described an assay for distinguishing these rare RNA-mediated events from a background of events not requiring an RNA intermediate. This assay allows us to detect both pseudogene formation and homologous, RNA-mediated gene conversion. The reverse transcriptase activity for these processes is provided by the yeast retrotransposon Ty. Structural analysis revealed an RNA that had been reverse transcribed and inserted into the chromosome: the 5' end corresponded to the transcription start site and the 3' end was polyadenylated. Additionally, these cDNAs were found embedded in Ty sequences. This structure suggested that Ty may also be involved in priming reverse transcription of the cellular transcript and in insertion of the sequences into the chromosome. Studies aimed at understanding priming of the cellular transcript for reverse transcription revealed that priming involved a template switch. That is, priming initiated on the Ty transcript, requiring complementarity between the primer binding site and the initiator tRNA, and then a template switch occurred, with reverse transcription extending on the cellular transcript. Because we would like to make our system less dependent on Ty biology and extend our assay to mammalian systems, we have designed a self-priming template. This template has provided us with an *in vivo* genetic assay for reverse transcriptase.

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F 024 RETROTRANSPOSITION MECHANISM OF THE INSECT rDNA INSERTION ELEMENT R2, Thomas H. Eickbush, Dongmei D. Luan, William D. Burke, Danna G. Eickbush, Janet A. George, Warren C. Lathe III, and Jin Yang, University of Rochester, Department of Biology, Hutchison Hall, Rochester NY 14627.

The non-LTR retrotransposable elements R1 and R2 are inserted at unique sites in the 28S ribosomal RNA genes of most insect species. These elements have been stable, active components of some insect genomes for over 75 million years. We have expressed the single open-reading frame of an R2 element in *E. coli* and shown that it encodes a 120 kilodalton protein containing both endonuclease and reverse transcriptase activities. The initial steps in the integration of R2 elements can be reproduced in vitro utilizing highly purified preparations of this R2 protein. Cleavage at the insertion site involves two steps. The endonuclease activity of the protein first generates a specific nick in the coding DNA strand of the 28S gene at the insertion site. The reverse transcriptase activity utilizes the 3' hydroxyl group exposed by this nick to prime reverse transcription of the R2 RNA transcript. Cleavage of the second DNA strand is absolutely dependent upon the presence of RNA and occurs after reverse transcription is initiated. These initial steps in the integration of the R2 elements require specific recognition of the R2 transcript. This recognition involves the 250 bp 3' untranslated region of the RNA transcript. This region of the transcript can be folded into a secondary structure that is conserved in R2 elements from different insects species. Various additions, deletions and substitutions of the nucleotide sequence near the 3' end of the R2 transcript have been tested to determine the mechanism utilized by the polymerase to correctly position the template for reverse transcription. Reverse transcription can be directed to start either at the extreme 3' end of the RNA transcript or at internal sites near this end. The most efficient and accurate initiations occur on R2 RNA templates that contain a short sequence of 28S rRNA sequence at the 3' end. Polyadenylated transcripts are not utilized efficiently for integration. In some instances the reverse transcriptase adds non-templated nucleotide sequences until utilization of the RNA template begins. Other non-LTR retrotransposable elements (LINE-like elements) may utilize this R2 retrotransposition mechanism, but most would presumably involve less specific endonucleases. The R2 mechanism of integration also has similarity to various mitochondrial retroelements, particularly in the ability of the reverse transcriptase to recognize the 3' end of its transcript.

F 025 IDENTIFICATION OF A NEW HUMAN ACTIVE TRANSPOSABLE ELEMENT. S.E.Holmes, B.A.Dombroski, C.M.Krebs, C.D.Boehm, and H.H.Kazazian, Jr. Center For Medical Genetics, Johns Hopkins Hospital, Baltimore, MD.

In a patient with muscular dystrophy we found a 2.1 kb insertion of a rearranged L1 element plus unique flanking sequence in exon 48 of the dystrophin gene and we used oligonucleotides from the flanking sequence to clone the precursor of this element, the second known active human L1. We screened a human genomic placental library, sequenced the clones identified on a DNA sequencer, used PCR to characterize several polymorphic sites within the precursor element, and mapped the element using somatic cell hybrid panels. The locus LRE-2 (L1 Retrotransposable Element-2) maps to chromosome 1q. Two alleles of the precursor element were identified among the clones, and subsequent analysis of various individuals has shown the element to be polymorphic in at least 6 locations, including the polyA tail. In composite, an allele matching the insertion is seen, which is presumably present in the individual whose element gave rise to the insertion. LRE-2 is definitely the precursor, as the unique sequence was transposed next to it and serves as an identifying 'tag'. The precursor has an unusual 21 base pair truncation of the 5' end, inside a perfect 13-15 base pair target site duplication, which presumably occurred during its own transposition. No individuals so far examined by PCR and blotting show an allele containing L1 nucleotides 1 to 21. LRE-2 has 2 ORFs, so it appears likely that a slightly truncated element can still be functional. It belongs to a different subfamily than LRE-1, the first active element previously identified, differing in sequence by about 0.7%. The element is polymorphic as to presence in human genomes, suggesting that it is 1-2 million years old. Blotting revealed empty sites on about 25% of chromosomes, with a slightly higher presence of the L1 element in the patient's ethnic group. 20 chimps and gorillas analyzed were all lacking the element. Identification of LRE-2 provides further evidence that previously transposed L1 elements can themselves transpose, and indicates that read-through transcription can carry along flanking single-copy sequence in a retrotransposition event. It also suggests that priming of reverse transcription does not require an L1 sequence itself.

Invasive Introns and Transposon Invasion

F 026 MOBILITY OF GROUP I INTRONS: MECHANISM AND EVOLUTION, Marlene Belfort, Molecular Genetics Program, Wadsworth Laboratories, New York State Department of Health, Albany, NY 12201.

Group I introns are remarkable both as ribozymes that catalyze their own splicing, and as mobile genetic elements. The mobility or "homing" of group I introns, transfer from intron-containing to intronless alleles of the same gene, is dependent upon intron-encoded site-specific DNA endonucleases. Each endonuclease cleaves the corresponding intronless allele but not the intron-containing allele. Cleavage at or near the intron insertion site creates a double-strand break, with the DNA ends initiating recombination events that result in intron inheritance. After cleavage, the endonucleases appear to have no role in intron inheritance. Recombination has, however, been shown to be dependent on 5'-3' exonuclease and recombinase functions. Additionally, intron transfer requires the full complement of T4 replication functions and replication accessories. These results suggest that intron mobility and phage replication are tightly coupled.

The mobility endonucleases encoded by the group I introns of phage T4 are unique among this class of enzymes. First, unlike their eukaryotic counterparts they cleave at a distance from the intron insertion site. Second, although the enzymes manifest both sequence and distance preferences for cleavage, they are extremely tolerant of base substitutions within their recognition sequence. Finally, they contact the target helix across the minor groove of the DNA. Minor-groove recognition would effectively extend the range of available natural substrates, many of which contain modifications in the major groove, thereby increasing the potential for dissemination of the intron.

Homing-type endonucleases occur in group I introns in the bacterial and eukaryotic kingdoms and in the distinctive introns of the archaeal kingdom. They also occur in protein-coding sequences in all three kingdoms. Their distribution argues strongly that endonuclease coding sequences are themselves mobile genetic elements. Indeed, one of the phage endonucleases has recently been shown to recognize and efficiently cleave intron sequences from which the endonuclease open reading frame (ORF) has been deleted. These results strongly support the mobile endonuclease-ORF hypothesis. They further suggest that the endonuclease ORF invaded a preexisting intron via a self-induced double-strand break.

F 027 THE YEAST *VMA1* GENE PRODUCT INVOLVED IN PROTEIN INTRON HOMING AND PROTEIN SPLICING, Frederick S. Gimble, Center for Macromolecular Design, Institute of Biosciences and Technology, Texas A&M University, 2121 W. Holcombe Blvd., Houston, TX 77030.

A novel class of mobile genetic elements occur as in-frame insertions within coding sequences. The polypeptides encoded by these elements are flanked by adjoining protein sequences within a larger precursor protein. The internal protein sequences (or "inteins") self-excise from the precursor protein during a rearrangement process termed "protein splicing", and a second protein is generated by joining of the N-terminal and C-terminal segments. Protein splicing of the 119 kDa *VMA1* gene product from *Saccharomyces cerevisiae* yields an internal 50 kDa polypeptide and the 69 kDa subunit of the vacuolar membrane-associated H^+-ATPase, which is generated by ligation of two polypeptides. The 50 kDa protein is a site-specific DNA endonuclease that initiates the mobilization of its gene from donor strains to recipient strains by a gene conversion process. We have purified to near homogeneity the authentic *VMA1*-derived endonuclease (or VDE) from yeast that is generated by protein splicing and a recombinant form of VDE made in bacteria. A detailed characterization of the two enzymes reveals that they are indistinguishable as judged by their purification behavior, their apparent native molecular weight, and their catalytic properties (specific activity, cleavage site recognition, and optima for pH, temperature and ionic strength). The minimal site required for VDE-mediated cleavage has been delimited to a 30-base pair sequence. The VDE protein is a member of a larger family of related DNA endonucleases and mRNA maturases that each contain two conserved dodecapeptide motifs within their primary sequences. To elucidate the role that these motifs play in the cleavage mechanism, we have engineered amino acid changes within the motif and have characterized the mutant proteins.

F 028 MOBILE GROUP II INTRONS OF YEAST MITOCHONDRIAL DNA ARE SITE-SPECIFIC RETROELEMENTS, Philip S. Perlman[1], John V. Moran[1], Ronald A. Butow[1], Steven Zimmerley[2], John C. Kennell[2] and Alan M. Lambowitz[2], [1]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235-9038, [2]Departments of Molecular Genetics and Biochemistry, The Ohio State University, Columbus, Ohio 43210.

The group II introns aI1 and aI2 of the COXI gene of yeast mtDNA contain reading frames with homology to reverse transcriptase (RT). These reading frames also contain a domain resembling a Zn^{2+} finger, several domains resembling portions of known retroelements and a domain X, which we have found to be associated with the maturase function of aI2. We previously characterized an RT activity in RNP particles from yeast mitochondria which is highly specific for both introns and their flanking exons. Here we present genetic studies defining group II intron mobility and analyze the effects of various intron mutations on mobility and RT activity. Crosses between donor strains containing both introns (1^+2^+) and recipient strains lacking both introns (1^02^0), show that aI1 and aI2 are efficient, site-specific mobile elements. Intron mobility results in the efficient co-conversion of flanking exon sequences, but it is limited to just a few dozen nucleotides on either side. Studies of crosses using wild-type and mutant strains of the types 1^+2^0 and 1^02^+ show that both intron reading frames encode functions required for mobility. Intron 2 in our standard 1^02^+ donor strain is mobile in a cross with 1^02^0; however, aI1 in our standard 1^+2^0 strain is not mobile in the same test cross. In the cross of 1^+2^0 by 1^02^+, both introns are mobile showing that aI2 complements the aI1 mobility defect. A 1^02^+ strain containing a missense mutation in the Zn^{2+} finger-like domain is immobile in crosses with 1^02^0 even though that mutant strain retains RT activity and efficiently splices aI2. AI2 of the 1^02^+ Zn^{2+} finger mutant remains immobile in a cross with 1^+2^0; however, it complements the aI1 mobility defect. This shows that the Zn^{2+}-finger-like domain of aI2 is an aI2-specific mobility function that is distinct from RT and maturase. Using mitochondrial transformation, we then tested the role of the RT domain in mobility and splicing. A mutant in the highly conserved YXDD motif of the RT domain of aI2 does not block aI2 splicing, but blocks RT activity and inhibits aI2 mobility. This shows that the RT encoded by aI2 is needed for its mobility. YXDD mutations of aI1 in a 1^+2^0 strain block aI1 mobility in the trans-complementation assay, but do not affect its splicing; this indicates that aI1 encodes at least one mobility function even though it borrows at least one other from aI2. Overall, we conclude that there are at least two mobility functions encoded by the aI2 reading frame and that each function can be blocked without loss of maturase activity.

Flipping and Flopping: DNA Inversion

F 029 A UNIFIED MECHANISM FOR STRAND CLEAVAGE AND STRAND EXCHANGE IN FLP SITE-SPECIFIC RECOMBINATION, Jehee Lee, Sang-Hwa Yang, Jonsoo Lee, Ilson Whang, and Makkuni Jayaram, Department of Microbiology, U. T. Austin, Austin, TX 78712.

The model for site-specific recombination by the Flp protein has the following features:
1. A Flp monomer bound to its binding element within the recombination site activates the exchange site phosphodiester in cis for nucleophilic attack. 2. The nucleophile provided by the active site tyrosine (Tyr-343) of a second Flp monomer cleaves the activated phosphodiester (cleavage in trans). 3. The phosphodiester between DNA and tyrosine formed during cleavage is then activated in cis. In the simplest form of the model, the same Flp monomer is responsible for each of the two 'phosphate-activation' steps required for the recombination of one DNA strand. 4. The 5'-hydroxyl of the nicked partner DNA provides the nucleophile that attacks the activated DNA-tyrosyl phosphodiester (strand joining in trans).

The model unifies the chemistry of strand cleavage and strand union into a "cis-activation/trans-nucleophilic attack" mechanism.

The following experimental evidence supports the model:1. Each Flp monomer harbors a partial active site. A functional active site is assembled only when Tyr-343 is contributed by a second Flp monomer. 2. Exogenous nucleophiles, for example H_2O_2, can truly mimic Tyr-343; mutations of Flp that abolish cleavage by Tyr-343 also abolish cleavage by H_2O_2. 3. With appropriate substrates, recombination can be achieved by Flp(Y343F) together with a tyrosine analog supplied in solution. 4. Reaction between two 'half-site' substrates require two Flp monomers; that between two 'full-site' substrates require four Flp monomers. 5. The monomeric complex between Flp(Y343F) and a half-site (a half-site bound by a protein monomer) can mediate both cleavage and strand transfer when provided with a tyrosine analog.

F 030 GENETIC DISSECTION OF THE GIN-FIS MEDIATED DNA INVERSION REACTION, Petra Merker, Georgi Muskhelishvili, Annette Deufel, Katharina Rusch and Regine Kahmann; Institut für Genetik und Mikrobiologie der Universität München, Maria-Ward-Str. 1a, 80638 München, Germany.

The Gin invertase catalyzes site-specific DNA inversion in phage Mu. The inversion reaction requires the *E.coli* encoded FIS protein bound to a recombinational enhancer sequence. Only negatively supercoiled DNA substrates with recombination sites in inverted repeat configuration yield recombination products with high efficiency. Binding of Gin and FIS to their specific sites on the negatively supercoiled DNA substrate promotes the assembly of a topologically unique synaptic complex. Consequently the DNA inversion results in unknotted products and proceeds with a defined change in linking number. Although the crucial role of negative DNA supercoiling in the assembly of the synaptic complex has been unambiguously proved, little is known about the arrangement of the Gin protomers in the complex and how they interact with each other and with FIS protein. One of the questions we address is whether Gin and FIS molecules contact each other specifically. Since random mutagenesis of Gin yields Gin mutants that display a FIS independent phenotype with high frequency, we are using an in vivo test system to find *fis* mutants capable of suppressing this FIS-independent phenotype to demonstrate a direct interaction between FIS and Gin.

The specificity of the synapse is governed by two kinds of contacts between Gin molecules: The intermonomeric interaction within the dimer bound to the recombination site and the interdimeric interaction between the two DNA bound dimers. The analysis of FIS-independent Gin mutants has indicated that the respective mutations alter the interaction between the monomers within the DNA-bound dimer. Intragenic mutations suppressing the FIS-independent phenotype cluster in a domain, which is likely to be involved in the interaction between dimers of Gin. Suppression is also observed when the respective mutations are localized on separate Gin molecules. To learn more about these Gin-Gin interactions we have developed a system for the isolation of *gin* mutants that have an increased affinity between the Gin dimers within the synaptic complex. We hope that such mutants will enable us to study the structural organisation of the synapsed sites and the exchange of subunits during recombination.

Chromosome Scrambling and Restructuring

F 031 PARAMUTATION IN MAIZE: ALLELIC INTERACTIONS ASSOCIATED WITH HERITABLE CHANGES IN TRANSCRIPTION, Vicki L. Chandler, Garth I. Patterson, and Kenneth M. Kubo, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

The *b* gene of maize encodes a transcriptional activator of anthocyanin pigment biosynthetic genes. Certain *b* alleles undergo paramutation: a unidirectional, heritable alteration of one allele caused by the presence of another allele. *B-I* (intensely pigmented plant) is always changed to *B'* (weakly pigmented plant) in the *B'/B-I* heterozygote, such that all progeny receive the *B'* allele. The "new" *B'*, which was *B-I* in the previous generation, is weakly pigmented and fully capable of changing another *B-I* allele into *B'*. Our results show that *B'* acts in trans to alter the transcription of *B-I*, with transcription remaining low in subsequent generations, even when the original *B'* allele segregates away. Genetic experiments demonstrate that *B'* is extremely stable, as no *B-I* individuals were observed in 70,000 *B-I/B'* plants. In contrast, *B-I* is unstable, as it can spontaneously change to *B'* at high frequencies (1-10%). The products of *B-I* and *B'* are equally capable of activating the transcription of their target genes, indicating they are functionally equivalent. In addition, a functional B protein is not required for paramutation to occur. The region of *B'* required for paramutation maps to the 5' flanking region. Genomic restriction maps, DNA sequence, and methylation of *B'* and *B-I* have been compared. Despite dramatic differences in phenotype and transcription of *B'* and *B-I*, no evidence for rearrangements, changes in sequence, or changes in methylation was found. These results provide no support for models involving "dominant negative" proteins, gene conversion or transposable element interactions. A model in which *b* paramutation involves a physical interaction between the alleles that alters transcription and promotes a heritable change in chromatin structure will be presented.

F 032 GENOME PLASTICITY DURING DROSOPHILA OOGENESIS, Ping Zhang[1], Linda Keyes[1], Mary Lilly[1], and Allan Spradling[1], [1]Howard Hughes Medical Institute, Carnegie Institution of Washington, Baltimore, MD 21210

Many *Drosophila melanogaster* somatic cells become polyploid/polytene during development. Highly repetitive heterochromatic DNAs in such cells frequently become underrepresented relative to euchromatic DNAs, but the mechanisms causing these losses remain uncertain. We have utilized a Drosophila minichromosome, Dp1187, and new methods for obtaining single P element insertions in heterochromatic regions, to investigate copy number changes during development at several specific sites within telomeric and centromeric heterochromatin. Our results demonstrated that centromeric heterochromatin is structurally and behaviorally more complex that previous models postulating zones of α and β heterochromatin. Nurse cells become highly polyploid during oogenesis, and export large quantities of ribosomes and other materials to the developing oocyte. We have examined whether specific repetitive sequences are lost as part of this process. Furthermore, we have identified and analyzed several mutations, including fs(2)4506, that disrupt nurse cell nucleolar morphogenesis, chromatin structure, and their ability to support egg formation. Nurse cells provide a model system for analyzing whether heterochromatic sequence losses may be functionally important.

Spradling, A.C., Karpen, G., Glaser, R. and Zhang, P. (1992). Evolutionary conservation of developmental mechanisms: DNA elimination in Drosophila. 50th Symp. Society Dev. Biol., A. Spradling, ed. (Wiley-Liss: New York)pp. 39-53.

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Spradling, A.C. (1993). Position-effect variegation and somatic instability. Cold Spring Harbor Symp. Quant. Biol. 50:, in press.

Presenters A-L

F 100 A NOVEL SITE-SPECIFIC RECOMBINASE, β, ENCODED BY THE BROAD-HOST-RANGE *Streptococcus pyogenes* PLASMID pSM19035, Juan C. Alonso[1, 2] and Fernando Rojo[2], Max-Planck-Institut für molekulare Genetik[1], D14195 Berlin, Germany and Centro Nacional de Biotecnología[2], 28049-Madrid, Spain.

The broad-host-range low-copy-number, 27-kb, *Streptococcus pyogenes* plasmid pSM19035 is stably inherited in Gram-positive bacteria. pSM19035 has extraordinarily long inverted repeated sequences that comprise 80 % of the molecule.

Genetic evidence suggests that the pSM19035 gene product β is involved in DNA inversion and in the resolution of plasmid multimers into monomers. In the presence of a host factor the purified β protein is able to catalyse DNA rearrangements (deletions and inversions). The protein binding target has been narrowed down by DNaseI footprinting to an 85 bp region that can be divided into two discrete sites (sites I and II). These sites are about 34 bp in length, and they are separated from each other by about 16 bp. Protein β binds co-operatively to its specific target sequence with an apparent binding constant of 3.0×10^{-8} M, the dissociation rate being 8.2×10^{-4} sec^{-1} at 20° C. Protein β is a dimer in solution. Between 3.6 to 4.2 β promoters are required to saturate the DNA substrate.

The β protein binding sites can be divided into two discrete subsites. Each of the subsites (half sites) contains two imperfectly conserved sequences of 12 to 13 bp each, related by a dyad axis. These results, together with gel retardation assays, suggest that protein β binds to DNA as a dimer, one to each discrete site. Binding of protein β to its target sequence bends or loops out the DNA. The β protein binding site, though it resembles that of other DNA resolvases, differs in that only two adjacent sites are found (sites I and II). In addition, the purified β protein requires a host factor for function. The functional consequences of these characteristics are discussed.

F 101 SELF-ASSOCIATION PROPERTIES OF RETROVIRAL INTEGRASES AND INTEGRASE SUBDOMAINS

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The integration of viral DNA into the genome of the host cell is a unique and vital step in the normal life cycle of retroviruses. The viral integrase (abbreviated IN) is necessary and sufficient for the integration of a linear DNA with viral ends into the host target DNA. Our laboratory has shown that IN must multimerize to perform its normal catalytic activities (Jones *et al.*, (1992) J. Biol. Chem. 267:16037-40). Recently others have inferred multimerization from the restoration of enzymatic activity by complementation between different IN mutants (Engelman, *et al.*, and van Gent, *et al.*, (1993) EMBO J. 12(8):3261-3275). While a mutational analysis of conserved residues has highlighted the role of several residues in the catalytic activity of IN, a detailed mapping of the regions of IN that are responsible for multimerization is yet to be completed.

We have used a combination of gel filtration, crosslinking, protein blotting, and genetic assays to identify RSV and HIV IN subdomains that are competent or deficient in self-association. Results from these analyses reveal that C-terminal truncations of IN are severely deficient in multimerization, and correspondingly, an isolated C-terminal domain is capable of association with full-length protein. Mutational analyses of the regions defined by these subdomains is currently underway, and the effects of these mutations on the multimeric state and enzymatic activity of IN will be presented.

F 102 THE XER SITE-SPECIFIC RECOMBINATION. Lidia K. Arciszewska, Garry Blakely, Gerhard May, and David J. Sherratt. Department of Genetics, Glasgow University, Church Street, Glasgow, G11 5JS.

The Xer site-specific recombination system functions in normal segregation of circular chromosomes at cell division. Recombination occurs at differrent but related sites that have been found on natural plasmids (e.g. *cer* on ColE1) as well as in the terminus region of the *E.coli* chromosome (*dif*). Some of the sites are relatively simple and consist of about 30 bp *core* sequnce - two half sites flanking a central region; others contain additional accessory sequences. Two related recombinases XerC and XerD which belong to the lambda integrase family enzymes are necessary for recombination at all the sites. In addition accessory proteins e.g. Arg R and PepA are required for recombination at sites that need accessory sequnces.*In vivo* experiments have suggested that both recombinases are involved in catalysis of the recombination (Blakely et al.,1993, Cell, in press). In order to understand more about the roles of the two recombinases in Xer recombination we studied DNA binding and recombination of the wild type and mutant proteins *in vitro*. *Core* recombination sites that do not need accessory sequences and accsessory proteins were used in these studies. XerC and XerD bind to separate halves of the recombination site, binding being highly cooperative.XerD binding induces a substantial band in the DNA while XerC produces only a small deformation. *In vitro* strand exchange reactions using either Holliday junction-containing molecules as substrates or nicked linear 'suicide' substrates require the presence of both XerC and XerD, though catalysis of one pair of strand exchanges can occur when XerD is mutant in either of the *putative active site residues*. The relevance of this observation to the overall recombination mechanism will be discussed.

F 103 HETEROLOGOUS EXPRESSION OF YEAST RETROTRANSPOSON TY1 TO STUDY EARLY EVENTS IN TY1 LIFE CYCLE

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We are expressing reading frames of the yeast retrotransposon Ty1 in the plant *Arabidopsis thaliana* and in *E. coli*. The gag homologous protein encoded by the TyA reading frame is a phosphoprotein which apparently forms virus-like particles in both heterologous hosts. We are currently studying whether these particles contain RNA molecules. In particular, we want to find out whether the gag homologue of Ty1 is sufficient to package Ty1 mRNA, or whether additional Ty1 proteins such as reverse transcriptase are necessary, as well.

A gene made by fusion of ORFs A and B of Ty1 undergoes self-cleavage in *E. coli*. Deletion derivatives of this fusion gene are used to delimit the functional domain of the retroelement protease. Current data indicate that extending reading frame A by 100 amino acids of frame B gives rise to active protease and results in cleavage of the ensuing protein.

F 104 PROTEINS WHICH INTERACT WITH TYA, THE STRUCTURAL PROTEIN OF THE YEAST RETROTRANSPOSON Ty1,
Carrie L. Baker-Brachmann and Jef D. Boeke, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205

The yeast retrotransposon, Ty1, is a genetic element structurally and functionally related to the retroviruses. The life cycle of Ty1 requires that a genomic copy is transcribed and translated, that the structural protein TYA assembles into a virus-like-particle (VLP) in which the mRNA is reverse transcribed, and that the full-length DNA is transported back into the nucleus and integrated. Aside from its obvious structural role, TYA's functional role in transposition is unknown. In an effort to explore this, we used the two-hybrid system developed in the laboratory of S. Fields to identify *S. cerevisae* proteins which interact with TYA. We reasoned that we might expect several classes of proteins: proteins which may aid in the assembly of the VLP, proteins which may be involved in the nuclear targetting and transport of the VLP, or proteins which may be involved in the integration of the Ty1 genome. We identified three genes, *TIP1-3* (TYA Interacting Proteins) whose protein products appear to interact with TYA. TIP1p is identical to RIF1p, a protein identified in the laboratory of D. Shore by its interaction with RAP1p. A deletion of *RIF1* supports normal levels of transposition, indicating that *RIF1* is not essential for the process of transpostion. *TIP2* is *TSM1*, an essential gene of unknown function. We are currently analyzing the effect of a temperature sensitive allele of *TSM1* on transposition. *TIP3* encodes a previously unidentified gene, which we are in the process of characterizing.

F 105 TRANSPOSITION OF THE I FACTOR, A LINE-LIKE TRANSPOSABLE ELEMENT OF *DROSOPHILA MELANOGASTER*, Maria Balakireva, Isabelle Busseau, Marie-Christine Chaboissier, Danielle Teninges and Alain Bucheton, Centre de Génétique Moléculaire, CNRS, 91198 Gif-sur-Yvette, France

I factors are LINE elements that are the genetic determinants of the I-R system of hybrid dysgenesis in *Drosophila melanogaster*. There are two categories of strains in this species with respect to I-R hybrid dysgenesis: inducer which contain active I factors and reactive which do not. Crosses between reactive females and inducer males produce F1 females, called SF females, showing reduced fertility. Various genetic abnormalities occur in the germline of these females such as mutations and chromosomal rearrangements. I factors are stable in inducer strains but transpose at high frequency in the germline of SF females (1).

Active I factors are 5.4 kb long. Their organization is similar to that of other LINEs: they have no terminal repeats, they terminate at their 3' end by several repeats of the sequence TAA, and they contain two large open reading frames, one of which encodes a polypeptide showing similarities to reverse transcriptases and RNAseH (1).

For a long time, the ability of LINEs to transpose by reverse transcription of an RNA intermediate was only inferred from structural analysis. In order to get direct evidence for retrotransposition, introns have been introduced into I elements. Their transposition resulted in accurate removal of the intron, indicating that I factors transpose by reverse transcription of an RNA intermediate (2, 3).

Northern blot analysis of RNAs from inducer and reactive strains and from SF females have been carried out in order to characterize this RNA. A 5.4 kb long transcript is specifically synthesized in the ovaries of SF, in amounts correlating with the frequency of transposition. S1 mapping experiments indicate that it is a full-length RNA, starting precisely at the 5' end of the I factor and ending within its TAA repeats. We think that it is the transposition intermediate and that it is used as well to synthesize the polypeptides required for transposition (4). We are studying the transposition process.

(1) Bucheton A. (1990), TIG 6, 16-21.
(2) Pélisson *et al.* (1991), PNAS 88, 4907-4910.
(3) Jensen S. and Heidmann T. (1991), EMBO J. 10, 1927-1937.
(4) Chaboissier *et al.* (1990), EMBO J. 9, 3557-3563.

F 106 THE DROSOPHILA HET-A RETROPOSON: SPECIFIC TRANSPOSITION S TO BROKEN CHROMOSOME ENDS AND FUNCTION IN TELOMERE ELONGATION,
Harald Biessmann[1] and James M. Mason[2], [1]Univ. of California, Irvine, CA 92717, and [2]NIEHS, Research Triangle Park, NC 27709.
We have identified a novel *Drosophila melanogaster* retroposon, the HeT-A element. It is a 6 kb non-LTR, polyadenylated LINE-like element which carries a single ORF of 2.8 kb. This ORF encodes a gag-like polypeptide with three zinc finger nucleic acid binding motifs. A second ORF that could encode a reverse transcriptase is conspicuously absent. HeT-A elements reside solely in heterochromatic regions at the tips of all chromosomes, in the pericentric region, and on the heterochromatic Y. We have observed some 20 HeT-A transpositions to the broken end of a terminally deleted X chromosome end within 17 fly generations. All HeT-A element transpositions were highly polar, and the elements always became attached with their oligo(A) tails to the break. Since *Drosophila* and other dipterans apparently lack typical telomeric repeats at the ends of their chromosomes, we propose a telomere elongation model that does not require such repeats nor telomerase. We postulate that frequent transpositions of members of the *Drosophila* HeT-A family to natural chromosome ends counter-balance progressive nucleotide loss that occurs at chromosome ends due to incomplete DNA replication. This model of telomere length maintenance predicts a predominant orientation of HeT-A elements with their oligo(A) tails facing proximally, as well as the existence of irregular tandem arrays of HeT-A elements at chromosome ends. Sequence analyses of HeT-A fragments from directional libraries enriched in terminal DNA fragments are consistent with these two predictions and support our model. We also found such oriented tandem arrays of HeT-A elements at the natural end of chromosome 2L by walking distally from the distal-most single copy gene on this chromosome. The proposed role of HeT-A retroposons in chromosome healing and telomere elongation would constitute a genuine cellular function for this unique retroposon.

F 107 REQUIREMENTS FOR SUPERCOILING IN Tn3/γδ RESOLVASE REACTIONS: ROLES IN SYNAPSIS AND POSTSYNAPSIS, Kirsten R. Benjamin, A. Pia Abola, Roland Kanaar, and Nicholas R. Cozzarelli, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720

We investigate why substrates of Tn3/γδ resolvase must be supercoiled and, in particular, how supercoiling requirements differ for synapsis (pairing of *res* sites) and postsynapsis. In our first approach, catenated substrates with one recombination site on each ring are used. Although supercoiled catenanes react as well as standard circular substrates, catenanes with both rings nicked synapse but fail to recombine. Synapsis of catenanes where one ring is nicked is the same as synapsis of supercoiled substrates, but recombination is decreased four-fold. These results, along with the failure of nicked circular DNA to synapse, demonstrate that supercoiling plays critical roles in both synapsis and postsynapsis. A second approach involves circular substrates with various superhelical densities, σ. As σ decreases from 0 to -0.065, synapsis increases gradually and plateaus at a value near -0.03. In contrast, the dependence of recombination on σ is more sigmoidal; with decreasing σ, a lag is observed before recombination increases. We conclude that (1) supercoiling is required both synaptically and postsynaptically; (2) an important but not exclusive role of supercoiling in synapsis is providing the correct interwinding of sites; and (3) postsynapsis requires a higher level and a different role of negative supercoiling.

F 108 GENETIC DISSECTION OF THE γδ RESOLVASE SYNAPTOSOME USING A MUTANT RESOLVASE WITH ALTERED DNA BINDING SPECIFICITY, Martin Boocock, Raluca Verona and Nigel Grindley, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT 06520.

The DNA-protein complex within which γδ resolvase carries out site-specific recombination contains at least 12 protomers of resolvase and two copies of the 120 bp DNA substrate, *res* (each with 3 binding sites for a resolvase dimer). The resolvase protomers play unequal roles in the recombination process. In order to understand the structure of the macromolecular complex and the function of the individual protomers, we are attempting to address the question "what is each resolvase monomer doing and where is it doing it"? The strategy employed to address this question is to place specific mutants of resolvase — for example those defective in chemical catalysis or in a defined set of protein-protein interactions — at particular subsites within each *res*. The outcome of a resolution assay then indicates whether the resolvase bound at a defined site makes use of specific protein-protein interactions during assembly of the synaptic complex, or plays an active role in the chemical steps of strand exchange. Variant resolvases can be directed to defined subsites by means of an amino acid substitution in the C-terminal DNA binding domain that results in altered DNA binding specificity. The mutant R172L binds prefentially to sites in which the G at position 2 of the consensus half site (TGTCCGAT-AATT) is changed to a T. To date, our results indicate that:

- The resolvase dimers that initially bind to site I are the only ones that require the catalytically essential serine-10 residue.
- The resolvase side chains that appear to be important for chemical catalysis of DNA cleavage act in *cis* on the site I at which the resolvase is bound by its C-terminal domain.
- Although cleavage of the 4 DNA strands normally appears to be concerted, the cleavage reactions at the two crossover sites are not tightly coupled.
- The resolvase dimers bound at site I do not participate in the 2-3' interaction — an essential inter-dimer interaction that is implicated in synapsis of the *res* sites.

F 109 *P* ELEMENT CHARACTERISTICS IN AUSTRALIAN POPULATIONS OF *DROSOPHILA MELANOGASTER*, Ian A. Boussy, Anthony E. Romanelli, Jennifer Soriano, Amanda C. Kracen, and Ronny C. Woodruff, Dept. of Biology, Loyola University, 6525 N. Sheridan Rd., Chicago, IL 60626

Using collections made in 1991, we are reassessing the dramatic latitudinally clinal pattern of *P* element-associated characteristics that was described from collections of *D. melanogaster* made in 1983-1986. The original pattern defined three regions from north to south along the eastern coast of Australia, with P (with active *P* elements and strong *P* regulatory ability), Q (with no active elements but with strong regulation) and M (with no active elements and little regulatory ability) characteristics, respectively. The pattern was quite different from the usual observation of relative uniformity over broad geographic regions. Flies from all regions were found to have many *P* elements per genome, with a weak correlation between numbers of full-size *P* elements and the characteristics and with an inverse correlation between numbers of the internally-deleted *KP* elements and the characteristics. Flies from the northernmost P region virtually lacked *KP* elements.

Our preliminary data using 1991 lines indicate that the clinal pattern is still present, but that the Q-to-M boundary may have moved. In the northernmost P lines, *KP* elements are still few or none per genome. The results are consistent with a two-introductions hypothesis of the establishment of the cline, but the stability of the cline seems remarkable.

In addition to sterility tests and Southern blots, we also have evaluated the type of inheritance (maternal or chromosomal) of repressor function found in the lines. The inheritance type varies between lines within populations, and does not seem to show a clear latitudinal pattern, contrary to expectations based on the genomic *P* element complements and models of bases of repressor function.

F 110 A DIRECT PHYSICAL ASSAY FOR Ty1 INTEGRATION: EFFECTS OF *TRANS* FACTORS AND *CIS* SEQUENCES, Lelita T. Braiterman and Jef D. Boeke, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205

In order to understand the biochemical conditions, *trans* factors and *cis* sequences required for integration by Ty1 integrase (IN), a novel in vitro integration assay was developed enabling the direct physical examination of the products formed. Ty1 virus-like particles (VLPs) were isolated, used as the source of native IN and incubated with blunt-ended artificial radioactive substrate bearing Ty1 termini and target DNA. Two classes of radioactive products were identified; the majority resulted from single-end joins of substrate DNA to target DNA, termed half-reactions, and the other fraction, identified as simple insertions of substrate DNA into target DNA resulted from concerted reactions. The effect of a variety of target DNA alterations on the IN reaction was examined. A genetic assay was then used to recover the simple insertions; both the expected target 5 bp site duplication and a target site degenerate consensus sequence typical of in vivo integration events were observed. Mutations within the *TYA* and *TYB* genes which correspond to the retroviral *gag* and *pol* genes were initially analyzed for their effects upon transposition in vivo and VLP structure. Seven transposition-inactivating mutations, mapped to each of the functional domains of the *TYB* gene, were examined for in vitro IN activity. Two different mutations within protease (PR) exhibited some IN activity. One mutation within reverse transcriptase (RT) exhibited near WT levels of IN activity and two mutations within the variable C-terminal domain of IN retained a significant level of IN activity. In contrast, three different mutations within the conserved amino-terminal domain of IN completely abolish in vitro IN activity. Thus, the catalytic activity is abolished by mutations within the highly conserved N-terminal half of IN. Lastly, the impact of *cis* sequences were examined using IN substrates with a variety of alterations to their ends. Use of these substrates in the physical assay suggest that an adenosine residue at or near the 3' terminus stimulate integration.

F 111 SITE-SPECIFIC RECOMBINASE FROM *CHLAMYDIA TRACHOMATIS*: OVERPRODUCTION AND PURIFICATION. Jean-Pierre Buisson and Paul H. Roy. Département de Biochimie FSG et Infectiologie, Centre de recherche du CHUL, Québec, Canada.

Chlamydia trachomatis has been recognized as a cause of *lymphogranuloma venereum* and of ocular disease. The 7.5 kb plasmid of this organism is believed to have a role in modulating virulence, and is universally present in clinical isolates. The plasmid encodes eight open reading frames (ORFs), one of which codes for a homolog of DnaB and another of which codes for a potential site-specific recombinase of the phage integrase family. The latter protein may be a dimer resolvase functionally similar to the D protein coded by the F plasmid of *E. coli*. To characterize the role of the *C. trachomatis* protein in site-specific recombination, one needs to overproduce it in sufficient amounts, and then purify it from the crude extract. Using PCR cloning, we cloned the recombinase gene and overproduced the protein to approximately 20% of total protein in pET-3d. The protein is purified from inclusion bodies by using 2% sarkosyl; however, use of denaturants must be avoided if eventual crystallization and structural studies are to be carry out. In an attempt to circumvent this problem, we are cloning the recombinase gene so as to produce a fusion protein in pMAL-c2 expression vector. To determine the site of action of the recombinase on the *C. trachomatis* plasmid DNA, we are using gel retardation and footprinting.

F 112 RSV INTEGRASE PROTEIN: MAPPING FUNCTIONS FOR CATALYSIS AND SUBSTRATE BINDING, Frederic D. Bushman and Beibei Wang, Infectious Disease Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037

Rous sarcoma virus, like all retroviruses, encodes an integrase protein that is responsible for attaching the reverse transcribed viral DNA to host DNA. We have probed the organization of functions within RSV integrase by constructing mutant derivatives and assaying their activities in vitro. We find that deletion derivatives lacking the amino terminl 48 amino acids, which contain the conserved H-$X_{(3-7)}$-H-$S_{(23-32)}$-C-$X_{(2)}$-C motif, are unable to carry out efficiently two reactions characteristic of integrase proteins: specific cleavage of the viral DNA termini and DNA strand transfer. Deletions lacking the COOH terminal 69 amino acids are also unable to carry out these reactions. However, all deletions containing a central domain, 49-217, are capable of carrying out disintegration, a reversal of the normal DNA strand transfer reaction, indicating the catalytic center probably lies within this region. Another conserved motif, D-$X_{(39-58)}$-D-$X_{(35)}$-E, is found in this central domain. These findings with RSV integrase closely parallel previous findings with HIV integrase, indicating that an independently active catalytic domain is a general feature of this family of proteins.

Surprisingly, and unlike results with HIV integrase, strand transfer in the presence of Mn^{2+} can be restored to a mutant RSV integrase lacking residues 1-48 by fusion to various short peptides. Furthermore, these fusion proteins retain the substrate specificity of RSV integrase. These data support a model in which the integrase activities required for full function in vitro, including substrate recognition, multimerization, and catalysis, all lie primarily in residues 49-286.

F 113 ANALYSIS OF THE EXPRESSION OF THE TOBACCO Tntl RETROTRANSPOSON

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The tobacco retrotransposon Tntl is one of the very few plant retrotransposons that have been shown to be transcriptionally active. Previous experiments from our laboratory have shown that Tntl RNA is not detectable in most tissues of the adult plant, but strongly induced when preparing leaf-derived protoplasts.

We have shown that LTR sequences upstream of the TATA box are sufficient to confer protoplast-associated expression to an heterologous promoter and are probably involved in the protoplast-associated induction of Tntl transcription. These LTR upstream sequences contain two short promoter elements, BI and BII, that are able to increase the activity of an heterologous promoter. Furthermore, BII interacts with tobacco nuclear factors that are specifically induced in leaf-derived protoplasts. We think thus that the BII element is probably responsible, at least in part, for the induction of Tntl expression during protoplast isolation.

The tobacco genome contains more that 100 copies of Tntl. In order to analyse the involvement of BI and BII on the control of Tntl expression "in vivo", we have compared the PCR-amplified U3 region of transcribed or total genomic Tntl elements. The Tntl RNA found in tobacco protoplasts is not a unique sequence but a population of different but closely related sequences. Most of these U3 sequences contain four tandemly repeated BII sequences and there are no sequences with less that 3 BII elements. In contrast, only a small subset of U3 sequences amplified from total genomic DNA is well conserved and contains intact BI and BII elements. Most of the sequences contain deletions that coincide with one or two BII elements. Our results point to a good correlation between mutations or deletions in the BII element and transcriptional inactivation. In order to confirm this hypothesis, we are actually cloning a reporter gene downstream of these different U3 mutant sequences to test their transcriptional activity.

F 114 A MODEL LINKING LINE-1 AMPLIFICATION TO GENE FLOW AMONG MOUSE POPULATIONS,

N. Carol Casavant and Stephen C. Hardies, Department of Biochemistry, Univ. of Tx. Health Science Center, San Antonio, Tx 78284

We have examined the dynamics of subfamily expansion of the mouse LINE-1 transposable element in Mus spretus within the last 0.2 Myr. The majority of LINE-1 elements inserting within this time period belong to two subfamilies, indicating the existence of two propagating master sequences. These two families have spread thousands of copies per haploid genome while apparently excluding the spread of other subfamilies. This conflicts with an earlier study which suggested that there should be multiple subfamilies in existence. This conflict is resolved by the following model. We propose that other subfamilies are expanding in mice from other geographical locations. We propose that master sequences quickly spread thousands of progeny within separate mouse populations before both master sequences and their progeny are mixed by gene flow between populations. When different master sequences encounter each other, there is a competition among them to determine which will continue to propagate. Over the next 1 Myr, all but one of the masters cease to propagate. Subsequently, the failed subfamilies are diluted by chromosomal segregation, with a residual number surviving by virtue of becoming fixed in the global mouse population. [Supported by NIH HG00190].

F 115 FLANKING SEQUENCES SITUATED AT A DISTANCE CONTROL PROGRAMMED DNA DELETION IN *TETRAHYMENA*, Douglas L. Chalker, Allison K. Wilson, Antonella LaTerza, and Meng-Chao Yao, Department of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

The chromosomal DNA of the ciliated protozoa *Tetrahymena thermophila* undergoes extensive diminuation during macronuclear development. The five micronuclear chromosomes are broken into ~200 minichromosomes. In addition, approximately 15% of the genome is eliminated in about 6800 site-specific deletion events. Two of these eliminated elements, called M and R, have been further characterized *in vivo* by Yao and colleagues. M, a 0.9 kbp element, and R, a 1.1 kbp element, are found adjacent to each other, separated by a few kbp, in the micronuclear genome. Short direct repeats (5-8 bp) are present at each deletion boundary, one copy of which remains in the macronuclear sequence. Examination of the M region has identified a homopurine tract, A_5G_5, located 40-50 bp outside each end of the eliminated region, that is sufficient to define the deletion boundaries (Godiska and Yao, 1990 Cell *61*: 1237-46). To determine whether flanking sequences play a role in the elimination of other DNA regions in *Tetrahymena*, we examined the requirements for programmed DNA deletion of the R element. R-region constructs with external or internal deletions were transformed by microinjection or electroporation into developing cells and analyzed for their ability to undergo specific rearrangement. Approximately 100 bp flanking each deletion boundary is necessary to promote site-specific deletion of R. Insertion of ~300 bp from the left flanking sequences of R into the eliminated region directs the use of a novel left deletion boundary specified by the introduced sequences. Removal of small DNA segments, ~30-100 bp, immediately upstream of and, in some cases, spanning the left boundary result in a downstream shift of the boundary utilized, conserving the distance between the upstream sequences and the deletion junction. From these data, we conclude that sequences flanking the R element contain the necessary information to determine the boundaries of the eliminated region. Therefore, the deletion boundaries of both the M and R elements are controlled by external sequences situated at a distance. We propose that the use of external sequences to direct programmed DNA deletion is a general property of eliminated regions. The requirement for sequences outside the eliminated regions suggests that the signaling of DNA deletion in *Tetrahymena* occurs by a novel mechanism distinct from most other known deletion events, such as transposon excision, that utilize signals internal to the eliminated regions.

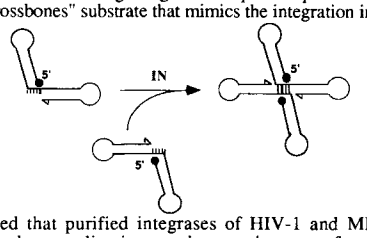
F 116 ISOLATION AND CHARACTERIZATION OF INSERTION SEQUENCES IN XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS, Jiann-Hwa Chen, Yi-Hsiung Tseng, Chih-Hong Wang, Institute of Molecular Biology, National Chung Hsiung University, Taichung 402, Taiwan, R.O.C.

Xanthomonas campestris pv. campestris is a gram-negative phytopathogenic bacteria that causes black rot of crucifers. It also produces the exopolysaccharide xanthan gum with many industrial uses. Strains isolated in Taiwan are genetically unstable in respect to their phage susceptibility, xanthan gum production and pathogenecity. We suspect that insertion sequences (ISs) play a role in the genetic instability. This initial study involves isolation of the ISs in *Xanthomonas campestris* pv. campestris.

Plasmid pUCD800 is a broad-host range, biphasic plasmid carrying the *sac*R and *sac*B genes of *Bacillus subtilis*. The *sac*B gene product catalyzes transfructorylation from sucrose to various acceptors, causing cell lysis. Cells carrying pUCD800 are lethal in sucrose medium unless a mutation occurred that would abolish expression of the *sac*B gene. We transferred pUCD800 into two locally isolated strains of *Xanthomonas campestris* pv. campestris by conjugation. The cells were grown in glucose broth and plated on sucrose plates. Plasmid DNA of all surviving colonies from one sucrose plate was extracted, restricted and analyzed by agarose gel electrophoresis. For each strain, we analyzed five independent cultures. Results from analyzing about total 300 mutant colonies indicated that mutant plasmids involved insertions or deletions in the *sac*B gene, or mutations without any sizable change of the plasmid. Deletions were observed in one independent culture, whereas insertions and mutations without any sizable change were observed in each of the ten independent cultures. Based on the size and the restriction sites contained in the inserted fragments, totally thirteen insertion fragments were isolated. The insertion junction sequences of four randomly chosen insertion mutant plasmids were determined and all showed the typical features of insertion sequences, i.e., both target site duplication and inverted repeats at the two termini. While all but one insertion sequence appeared in less than four independent cultures, there was one insertion sequence that appeared in nine of the ten independent cultures. The sequence of this insertion sequence is presented.

F 117 A NOVEL ASSAY FOR STUDYING MULTIMERIZATION OF INTEGRASES OF HIV-1 AND MLV, Samson A. Chow and Patrick O. Brown, Departments of Pediatrics, Biochemistry, and HHMI, Stanford University Medical Center, Stanford, CA 94305

Integration of retroviral DNA requires a viral protein, integrase, and specific DNA sequences at the ends of the viral LTR. *In vitro*, integrase alone can carry out major steps of retroviral integration, namely 3' processing and strand transfer. Integrase also mediates disintegration, a reversal of the strand transfer reaction. Recent complementation experiments using mutant proteins showed that HIV-1 integrase functions as a multimer. The exact nature of the multimer and the domains involved in multimerization are not known. In this study, we designed novel DNA substrates, termed "crossbones", for studying multimerization of integrase. The prototypic substrate is an oligonucleotide that folds to form two hairpin domains, each corresponding to "viral" and "target" DNA, respectively. The two hairpin domains are linked by a single-stranded region whose length equals the size of the staggered cut that is characteristic of a particular retroviral integrase. The assay requires that two separate oligonucleotides be brought together via protein-protein interactions to form a "crossbones" substrate that mimics the integration intermediate.



We showed that purified integrases of HIV-1 and MLV mediated concerted cleavage-ligation on the crossbones to form single- and double-disintegrated products that resulted from disintegration at one or both viral joining sites. Another major product, termed foldback, resulted from disintegration of an unannealed crossbones. The activity of integrase on the crossbones substrate was dependent on the presence of viral sequences and the length of target DNA between the viral sequences. Mutant integrases that were otherwise inactive with the crossbones substrate could complement one another. We are currently using the assay to identify functional domains of integrase that are involved in multimerization, catalysis, and DNA binding.

F 118 CHARACTERIZATION OF EUKARYOTIC DNA TOPOISOMERASE I CATALYSIS. Kent Christiansen, Birgitta R. Knudsen, and Ole Westergaard, Department of Molecular Biology, University of Aarhus, C. F. Møllers Alle', Building 130, 8000 Århus C, Denmark.

Eukaryotic type I DNA topoisomerases comprise a class of monomeric enzymes, which catalyzes changes in the topological state of negatively or positively supercoiled DNA by coordinated cleavage and religation of one strand of the DNA. The existence of a covalent intermediate in topoisomerase I catalysis allows uncoupling of the cleavage and ligation half-reactions on partially single-stranded DNA substrates containing a highly preferred interaction site. Utilizing this DNA substrate system we have found that the cleavage reaction requires bipartite interaction with two distinct DNA duplex regions located around the cleavage site (region A) and on the side holding the 5'-OH end generated by cleavage (region B)(1). Covalent activated intermediates are able to catalyze intramolecular ligation to a DNA strand with a minimal length of 2 nucleotides, within region A in the absence of interaction with region B. Topoisomerase I covalently attached at a DNA blunt-end is able to catalyze intermolecular ligation of the cleaved strand to heterologous duplex DNA regardless of sequence, while ligation does not proceed with single-stranded DNA (2). The obtained results suggest that bipartite DNA interaction is essential for the catalytic functions of topoisomerase I.

In continuation of these studies we have found that topoisomerase I-DNA intermediates are able to catalyze pH dependent hydrolysis and alcoholysis of the 3'-phosphotyrosyl bond by accepting water or alcohol compounds as substitutes for 5'-OH DNA ends. Topoisomerase I-mediated DNA cleavage, DNA ligation, and non-DNA utilization during hydrolysis and alcoholysis are inhibited to the same degree by modification of the enzyme with the sulfhydryl-specific agent NEM (N-ethylmaleimide). These observations suggest a role for cysteine residue(s) in catalysis rather than DNA binding activity.

1. Christiansen, K., Svejstrup, A. B. D., Andersen, A. H., and Westergaard, O. (1993). *J. Biol. Chem.* **268**, 9690-9701
2. Christiansen, K. and Westergaard, O. (1993) *J. Biol. Chem.*, in press

F 119 CONTROL OF *P* ELEMENT TRANSPOSITION IN *Drosophila melanogaster*: MATERNAL REPRESSION OF THE P PROMOTER IN THE GERMLINE, Dario Coen[1,2], Marion Delattre[1], Bruno Lemaitre,[1,2] Stéphane Ronsseray[1. 1]) Dept. "Dynamique du Génome et Evolution", Institut Jacques Monod, CNRS, Tour 43, 2 place Jussieu F-75251 Paris cedex 05, France [2]) Université Paris 6.

In Drosophila, *P* element transposition is under the control of a cellular state referred to as cytotype. The P cytotype represses whereas its absence (M cytotype) allows it: *P* transposition and associated phenomena occurs in the germline of F1 progeny of M female x P male crosses but the reciprocal cross (P female x M male) is normal. The P cytotype is long-term determined by regulatory chromosomal *P* elements but short-term maternally inherited. The genetic and molecular mechanisms of this regulation are complex. In addition, *P* transposition is restricted to the germline by the alternative splice of the IVS3, necessary to the production of the 87kDa transposase. Unspliced mRNA leads to the production of a 66kDa protein which has repression properties.

We have used *P-lacZ* fusion genes ("enhancer-trap" constructs) as reporter genes to study *P* regulation *in vivo*. We have shown that *P* element regulatory products are able to inhibit the activity of the *P* promoter in all tissues, in the germline as well as in the soma. In the soma, the same level of repression is observed when regulatory P element are derived paternally or maternally. The maternal effect characteristic of the P cytotype appears restricted to the germline, where a measurable repression is only seen when the mother is P. We have shown that P mothers transmit to the eggs they lay an extra-chromosomal component that we call *"pre-P cytotype"* and that we think is the 66kDa repressor. P cytotype determination necessitates the inheritance of both the pre-P cytotype (maternally) and chromosomal regulatory *P* elements (paternally or maternally). Depending on the pre-P cytotype of the embryo, an autonomous complete P element is able to specify either the 87kDa transposase, which has no repression capacities *in vivo*, or the 66kDa repressor. This positive feed back of the repressor on its own production will be explained in terms of a positive feedback of *P* pre-mRNA on its own splicing. This model is currently under test.

F 120 *Ascot-1*, A NOVEL FUNGAL TRANSPOSON LEAVING *Ac*-LIKE DNA FOOTPRINTS. Vincent Colot, Vicki Haedens and Jean-Luc Rossignol, Institut de Génétique et Microbiologie, Bât. 400, Université Paris-Sud, 91405 Orsay cedex, France

Several systems of genetic instability have been described in the heterothallic ascomycete *Ascobolus immersus* which are probably caused by the activity of transposable elements (Decaris *et al.*, 1981, CSH Symp. Quant. Biol. 45, 509; Nicolas *et al.*, 1987, Genetics 116, 33). We have recently shown by molecular analysis that this is the case, at least for the so-called *G* system of instability which affects the extensively characterized spore colour gene *b2*. The original unstable allele *G0* of *b2* corresponds to the insertion of a 409 bp element within the gene. This element produced an 8 bp duplication of the target site upon insertion and has features of a non-autonomous DNA-intermediate transposon, with near perfect inverted terminal repeats of 25 bp. Furthermore, sequence analysis of 25 excision products generated by the 409 bp element indicates that it corresponds to a novel type of fungal transposon, with striking similarities to plant transposons of the *Ac*-type, but not with transposons of the *Drosophila P*-type. We are currently searching for the autonomous element in an attempt to study the precisely timed activity of the *G* system during the life cycle of *A. immersus* and the possible involvement of the epigenetic phenomenon of Methylation Induced Premeiotically (MIP, Goyon and Faugeron, 1989, Mol. Cell. Biol. 9, 2819) in regulating the activity of that system.

F 121 DNA BINDING PROTEINS ENCODED BY THE *I* FACTOR AND *MARINER* TRANSPOSABLE ELEMENTS IN *DROSOPHILA*. Angela Dawson, Trevor Paterson, Ivan Clarke, Eve Hartswood and David J. Finnegan, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland.

I factors are 5.4kb LINE-like transposable elements of *Drosophila melanogaster*(1). They are responsible for I-R hybrid dysgenesis and transpose at high frequency in the germ-line of females from a dysgenis cross. *I* factors have two long open reading frames (2). ORF1 encodes a protein with some similarity to the product of a retroviral *gag* gene. We have purified this protein after expression in *E. coli* and have shown that it binds both RNA and DNA. At least two regions of the protein are responsible for nucleic acid binding and we are investigating whether or not this is sequence specific.

The transposable element *mariner* was originally found in *D. mauritiana* but related elements have been detected in a wide range of diptera and in other insects. It is similar in structure to *P* (3). It is 1286 bp in length with 28 bp terminal inverted repeats and has one long open reading frame coding for a polypeptide of 345 amino acids (4). This polyppetide is believed to be the transposase required, either on its own or in conjunction with host proteins, to mediate transposition by an excision-insertion mechanism.

We have expressed the *mariner* transposase in *E. coli* and have shown that it binds to the ends of the element in a sequence specific manner. We are investigating the consequences of transposase binding *in vitro*.

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(4) Medhora, M., Maruyama, K. and Hartl, D.L. (1991) Molecular and functional analysis of the *marnier* mutator element *Mos*1 in Drosophila. Genetics 128:311-318

F 122 Tn7 TRANSPOSITION AS A PROBE OF LONG-RANGE INTERACTIONS BETWEEN DISTANT SITES IN CHROMOSOMAL DNA, Robert T. DeBoy and Nancy L. Craig, Department of Molecular Biology and Genetics, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205

We are using transposition as a tool to probe the ability of widely separated (190 kb) DNA sites in the E. coli chromosome to interact. We are evaluating the effect of a pre-existing, distal Tn7 copy on a subsequent, site-specific Tn7 insertion into the chromosome.

The bacterial transposon Tn7 is profoundly influenced by a long-range feature of the target DNA, target immunity. Target immunity is the ability of a pre-existing transposon in a DNA molecule to substantially reduce the frequency of subsequent insertions into that DNA. Previous studies using plasmids showed that Tn7 insertion into an immune plasmid target is inhibited 100 fold. These plasmid studies revealed two features of this phenomenon: 1) A copy of Tn7 inhibits subsequent insertions into a plasmid target only when the copy is located in the plasmid, i.e. in cis to the insertion site, and not from any other location in the bacterial cell. 2) In one of its two transposition pathways, Tn7 can insert randomly anywhere in a plasmid target. However, a pre-existing copy of Tn7 in an F plasmid target (≥100 kb), appears to inhibit subsequent insertions anywhere in the F plasmid. Therefore, target immunity seems to act over large distances.

We have examined the effects of distal copies of Tn7 on subsequent insertion into the unique attTn7 site in the E. coli chromosome to determine: 1) if target immunity is active in the chromosome, and 2) over what distance target immunity can exert its effect. We have found that a copy of Tn7 located 64 kb away from the attTn7 site decreases by 12 fold the frequency of subsequent Tn7 insertion into the attTn7 site, and a copy of Tn7 located 190 kb away decreases 7 fold subsequent Tn7 insertion.

We are determining whether target immunity is effective over larger distances in the chromosome by increasing the spacing between the pre-existing Tn7 copy and the attTn7 insertion site.

F 123 FUNCTIONAL CHARACTERIZATION OF A TY1 INTEGRATION HOTSPOT LOCATED ON CHROMOSOME III OF *Saccharomyces cerevisiae*. S. Devine and J. Boeke, Dept. of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Ty1 is a yeast retrotransposon that resembles mammalian retroviruses: i) it transposes via an RNA intermediate, ii) produces virus-like particles (VLPs), and iii) encodes several retrovirus-like proteins, including Ty1 integrase which effects the insertion of new DNA copies of Ty1 into the host genome. A recent study of unselected Ty1 insertions into yeast chromosome III (chr III) revealed that preferred integration targets exist within the yeast genome (Ji et al., *Cell* **73**, 1007-1018, 1993). These chr III "hotspots" of integration were found near tRNA genes and/or Ty LTRs, implying a relationship between such sequences and hotspot activity. Hence, to further examine the properties of such hotspots, we have cloned an 8 kb region of chr III containing the most preferred site of Ty1 integration on the chromosome. This clone (76-2) spans nucleotides 136,155 to 144,333 of chr III, and includes both the *PGK1* gene, as well as this hotspot site of integration; the hotspot comprises the *SUF16* tRNA locus and its upstream region, and also includes a solo Ty1 delta (or LTR). Two approaches are underway to dissect *cis* and *trans* factors responsible for conferring hotspot activity upon this site. First, an *in vivo* transposition assay employing donor and target plasmids has been developed which will facilitate the identification of sequences within the *SUF16* region that are responsible for hotspot activity. Second, an *in vitro* assay employing a dominant-selectable *dhfr* gene has been developed to facilitate the reconstitution of this hotspot activity *in vitro*, by supplementing the basic *in vitro* reaction with various host extracts. Together, these assays will be exploited to identify factors that influence Ty1 integration specificity.

F 124 **IMMUNOLOCALIZATION AND CHARACTERIZATION OF THE MUT B PROTEIN ENCODED BY THE MAIZE TRANSPOSON MUTATOR.** Maureen J. Donlin and Michael Freeling
Department of Plant Biology UC Berkeley, Berkeley CA 94720

Transposition activity of the highly active transposable element Mutator in Maize is dependent on the presence of a 4.9 kb autonomous element which encodes two transcripts. The 2.8kb transcript, A, has a single open reading frame encoding a protein with a predicted size of 87 kDa. The 0.9 kb transcript, B, has a single open reading frame encoding a protein with a predicted size of 23 kDa. Comparison of the amino acid sequences of the Mutator encoded polypeptides to sequences in the Swiss-Prot and PIR data banks reveals no significant homology to any sequences presently in those data bases.

In this first study of the proteins ecoded by Mutator, the protein encoded by the B transcript (MUT-B) was overexpressed as a fusion with glutathione-S-transferase in *E. coli* and antibodies were raised to the fusion protein. Following affinity purification on an antigen column, the antibody recognized a protein of ~30 kDa specific to soluble protein extracts from plants with autonomous Mutator elements. This 30 kDa protein has been identified in root, seedling leaf, mature leaf, tassel and ear tissue from Mutator active plants. Several members of the *Gramineae* family were examined for crossreacting proteins.

Experiements are underway to use this antibody to localize the 30 kD protein in meristem, ear and tassel primordia of active Mutator plants, and the results of these immunlocalizations will be discussed. A comparison of the RNA and protein expression patterns in developing tassel tissue by *in situ* localizations is also in progress. Work has been initiated to study of the properties of the MUT-B protein in nuclear extracts.

F 125 ENHANCER-BLOCKING BY A RETROTRANSPOSON AND *su(Hw)* PROTEIN OF *DROSOPHILA*, Dale Dorsett, Bin Shen, Patrick Morcillo, Jaeseob Kim and Christina Rosen, Molecular Biology Program, Sloan-Kettering Institute for Cancer Research and Cornell University Medical College Graduate School of Medical Sciences, New York, NY 10021

Many mutations in *Drosophila* are insertions of the gypsy retrotransposon. The phenotypes of virtually all gypsy insertion alleles are suppressed by mutations in the *suppressor of Hairy-wing* [*su(Hw)*] host gene. The *su(Hw)* gene encodes a zinc finger protein (SUHW) that binds to a series of direct repeats in gypsy. When gypsy inserts into a gene control region, SUHW prevents enhancers that are promoter distal to gypsy from activating gene transcription, while enhancers that are promoter proximal to gypsy still function. The effects of SUHW occur over very long distances, and SUHW blocks a wing margin-specific enhancer in the *cut* gene from virtually any position in the 90 kilobase interval between the enhancer and promoter. Temperature-shift experiments indicate that the effects of SUHW are immediate and reversible, and that SUHW is only required when an enhancer is active to block the enhancer. Multiple domains of the SUHW protein are involved in blocking, and the sequences of the most critical domains suggest they are involved in protein-protein interactions. Although there are as yet no examples of a transcription control element in *Drosophila* that SUHW fails to block, SUHW does not block activation in yeast by a yeast activator that functions in *Drosophila*. Because yeast lack long distance activation, our working hypothesis is that SUHW does not interact or interfere with enhancer-binding proteins or the transcription activation reaction, but rather with a specialized apparatus specific to higher eukaryotes that brings distant enhancers and promoters into physical proximity of each other. Preliminary results of attempts to identify components of this apparatus will be presented.

F 126 CHARACTERIZATION OF A STABLE HIV INTEGRASE-VIRAL DNA COMPLEX: AN EARLY INTERMEDIATE IN THE INTEGRATION PATHWAY. Viola Ellison, Karen A. Vincent, and Patrick O. Brown

Retroviral integration consists of two independent reactions: 3' end processing, an endonuclease reaction resulting in the loss of the two terminal bases at the viral DNA ends, and strand transfer, a concerted cleavage and ligation reaction in which viral DNA is joined to target DNA. In vivo, these steps, catalyzed by retroviral enzyme integrase, are orchestrated by a large nucleoprotein complex. We have shown that several biochemical properties of the in vivo nucleoprotein complex, critical for efficient integration, are demonstrated in vitro with purified integrase. Similar to the in vivo integration complex, purified integrase formed a very stable complex with our model viral DNA substrates, in which the enzyme catalyzed processively the 3' end processing and strand transfer reactions and efficiently used non-viral DNA molecules as integration targets. The formation of an active integrase-viral DNA complex was shown to be an initial step in the integration pathway, and stable complex assembly required a divalent cation.

Within the stable integrase-viral DNA complex, the active form of integrase is a multimer, and integrase multimerization was shown to involve at least two distinct regions of the enzyme. The first, located in the N-terminus and referred to as the HHCC region, contains a Zinc finger like motif which is highly conserved among all retroviruses and retrotransposons. The second is sensitive to the alkylating agent N-ethylmaleimide. These two regions, as well as the evolutionarily conserved D-D-35-E, motif define functional domains of integrase as demonstrated by complementation studies. The interaction involving the HHCC and NEM sensitive domains between subunits of the active integrase multimer required a divalent cation, and defects in either region of the integrase rendered the protein incapable of forming a stable complex with viral DNA, suggesting multimerization of integrase is necessary for the formation of a stable integrase viral DNA complex. Thus, an initial step in the in vitro integration pathway is the formation of a stable, higher order integrase-viral DNA complex involving multimerization of integrase. After assembly, facilitated by the HHCC and NEM sensitive domains, integrase catalyzes processively the 3' end processing and strand transfer steps of the reaction.

F 127 IDENTIFICATION OF A DNA BINDING DOMAIN OF HIV-1 INTEGRASE BY UV-CROSSLINKING, Alan Engelman, Alison B. Hickman and Robert Craigie, LMB, NIDDK, Bethesda, MD 20892

Purified HIV-1 integrase possesses the polynucleotidyl transfer activities required to integrate HIV DNA into a host chromosome. Integrase cleaves viral DNA substrates 3' of a conserved CA in the 3' processing reaction and joins the processed CA_{OH}-3' ends to a target DNA in the DNA strand transfer reaction. Integrase can also separate viral DNA from target DNA by a process termed "disintegration".

The central core domain of HIV-1 integrase (amino acids 50-186 of the 288 residue protein) is sufficient for disintegration activity, demonstrating that this region contains an active site capable of polynucleotidyl transfer. However, both the N- and C-termini of the protein are required for 3' processing and DNA strand transfer. We recently described trans complementation of these functional domains within the multimeric complex that catalyzes 3' processing and strand transfer, but the role(s) of the terminal regions in the reactions is unknown.

In order to probe the roles of the terminal regions, we investigated the DNA binding activity of wild type and various mutant HIV-1 integrase proteins. The wild type protein was found to crosslink to duplex DNA following exposure to UV light. The reaction did not display any DNA sequence specificity. Crosslinking was maximum at low concentrations of NaCl, which is similar to the salt requirements for polynucleotidyl transfer. However, unlike polynucleotidyl transfer, crosslinking did not require any metal ion. In addition, the point mutant D116N, which does not possess any polynucleotidyl transfer activity, displayed crosslinking activity indistinguishable from that of the wild type protein.

We next examined the crosslinking of several deletion mutants of integrase. Both 50-288 and 107-288 displayed activity similar to that of wild type integrase, indicating that the C-terminal half of the protein is important for non-specific DNA binding. Similar results have been described by others using southwestern blotting. Deletion mutant 1-234 was capable of crosslinking to DNA, but 1-212 was not. This maps the DNA binding region of the protein in between residues 107-234. Results of others map the DNA binding region in between residues 200-263.

Our trans complementation studies identified two regions C-terminal to the core domain necessary for 3' processing and strand transfer: the C-terminal domain (residues 235-288) functioned either in cis or trans to the active core domain, while residues 187-234 were required in cis. Our current results suggest that the non-specific DNA binding function that is necessary for 3' processing and strand transfer, but dispensible for disintegration, functions in cis to the active core domain.

F 128 **MOLECULAR ANALYSIS OF IMMUNOGLOBULIN GENE RECOMBINATION,** Uthayashanker Ezekiel, Peter Engler, Andy Minn and Ursula Storb, Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637

V(D)J recombination is directed by a pair of recognition signal sequences (RSS) containing conserved heptamers and nonamers. Site specific recombination occurs between one RSS with a 12bp and one with a 23bp spacer region. The DNA is cleaved at the heptamers. The two RSSs are joined forming a signal joint and the adjacent coding ends are joined forming a coding joint. Before joining most coding ends undergo addition of nucleotides by TdT and/or deletion of nucleotides by the action of endo- or exonuclease. This imprecise joining of coding ends creates diversity in the variable regions of Igs and T cell receptors.
To understand the mechanism of coding end processing by TdT or nucleases we have constructed plasmid substrates containing coding ends with different base composition. Plasmid substrates are allowed to recombine in a pre B cell line. The plasmids are recovered from the pre B cells and transformed into E.Coli. Recombined plasmids confer resistance to chloramphenicol and kanamycin. The nature of the coding joints in the recombined plasmids is further analysed by sequencing. Additions or deletions at a coding end with a particular base composition are indirectly suggesting the nucleotide preference of the enzymes involved in the processing of coding ends.
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F 129 CELL-FREE RECOMBINATION AT SELECTIVE SEQUENCES BETWEEN ADENOVIRUS DNA AND A HAMSTER PREINSERTION SEQUENCE;
Katja Fechteler, Jörg Tatzelt, and Walter Doerfler; Institut für Genetik, Universität zu Köln, Köln, Germany; J.T.'s present address: Department of Neurology, University of California, San Fransisco, CA, USA

We have explored the mechanism of adenovirus type 12 (Ad12) DNA integration because of its importance for viral oncogenesis and as an example of insertional recombination. We have used a fractionated cell-free system from nuclear extracts of hamster cells and have partly purified nuclear proteins that could catalyze *in vitro* recombination. As recombination partners, the 20.880- to 24.049-nucleotide Pst I D fragment of Ad12 DNA and the hamster preinsertion sequence p7 from the Ad12-induced tumor CLAC 1 have proven to recombine at higher frequencies than randomly selected adenoviral or cellular sequences. A preinsertion sequence might carry elements essential in eliciting recombination. Patch homologies between the recombination partners seem to play a role in the selection of sites for recombination *in vivo* and in the cell-free system. Nuclear extracts from BHK21 cells were prepared by incubating the nuclei in 0.42 M $(NH_4)_2SO_4$ and fractionated by Sephacryl S-300 gel filtration, followed by chromatography on Mono S and Mono Q columns and on a Heparin Sepharose column. The purified products active in recombination contained a limited number of protein bands, as determined by polyacrylamide gel electrophoresis and silver staining. We used two different methods to assess the *in vitro* generation of hamster DNA-Ad12 DNA recombinants upon incubation with the purified protein fractions: (i) transfection of the recombination products into rec A$^-$ strains of Escherichia coli and (ii) the polymerase chain reaction by using amplification primers unique for each of the two recombination partners. In p7 hamster DNA, the nucleotide sequence 5'-CCTCTCCG-3' or similar sequences served repeatedly as a preferred recombination target for Ad12 DNA integration in the tumor CLAC 1 and in independent cell-free recombination experiments.

F 130 **AN IN VIVO ASSAY FOR L1 REVERSE TRANSCRIPTASE ACTIVITY IN YEAST**
Q. Feng[1], B. Dombroski[2], S. Mathias[2], A. Scott[2], H., Kazazian[2], J.D. Boeke[1] Departments of [1]Molecular Biology and Genetics, [2]Pediatrics and Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205.
L1 elements are a highly repetitive family of sequences which account for approximately 5% of the human genome. ORF2 of an active, full-length L1 element, L1.2A encodes a reverse transcriptase (RT) in an in vitro assay system (Mathias et al. Science (1991) 254:1808-1810). In order to test whether L1 RT can support reverse transcription in vivo, we took the advantage of the well characterized Ty1 retrotransposon of *Saccharomyces cerevisiae*. A construct replacing the RT of Ty1 with L1-ORF2 was cotransfected into yeast along with a plasmid containing an antisense *HIS3* gene interrupted by an artificial intron (Derr et al. Cell (1991) 67:355-364). His3$^+$ prototrophs can only arise in the progeny of those cells that underwent transcription of the antisense *HIS3* gene, splicing of the intron, reverse transcription into cDNA and integration into the yeast genome. We have shown that L1 RT is able to support this "retrotranscription" process as well as Ty1 RT. Also, a point mutation in the highly conserved DD box of RT as well as an in frame deletion of ORF2 completely eliminated the formation of His3$^+$ colonies. The genomic *HIS3* insertions are complex in structure and are always flanked by Ty1 or Ty1-L1ORF2 sequences. Reverse transcription of Ty1 construct transcripts and strand switching to HIS3 RNA may result in the formation of chimeric cDNAs. Although the normal L1 RT primer is unknown, in yeast, L1 RT probably uses the tRNA primer also used by Ty1 RT. This is the first biological demonstration of human L1 RT activity.

F 131 **THE EFFECT OF MUTATIONS IN *topI* *mei9* AND *mei41* ON P ELEMENT-INDUCED GENE CONVERSION.**

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When P elements transpose, the double strand breaks that remain are repaired by a gene conversion process. The template for this conversion is usually the newly replicated sister chromatid but can also be the homologue or an ectopic homologous sequence. The conversion appears not to be associated with crossing over which conflicts with the standard 'Double-Strand Break Repair' model. Moreover some of the products of repair seem to have copied from more than one template, (e.g. part of the sequence from the sister strand and part from an ectopic template), suggesting that repair synthesis can abort and switch templates before the single stranded tails finally anneal together.

We are searching for genes who's products are involved in the repair process. We report the effects of mutations in *topI mei9* and *mei41*.

We have also begun to characterize a Drosophila mismatch repair gene: a homologue of the *mutS* gene family.

F 132 IDENTIFICATION AND CHARACTERIZATION OF A DNA TRANSPOSON IN *ARABIDOPSIS THALIANA*, Mary J. Frank, Yi-Fang Tsay, Caroline Dean* and Nigel M.Crawford, Department of Biology, University of California, San Diego, La Jolla, CA 92093-0116 and *Department of Molecular Genetics, Cambridge Laboratory, John Innes Centre, Norwich, NR47UJ UK

Transposable elements are useful tools for tagging genes and studying developmental regulation in plants. Using a line of *Arabidopsis* containing the maize transposon *Ac*, a new allele of a nitrate transporter gene CHL1 was isolated. This mutant contained a 3.3 kb fragment inserted into the fourth intron of CHL1. Further characterization revealed that the insert was not *Ac* but was a new transposable element which was named *Tag1*. *Tag1* has 22 bp inverted terminal repeats and upon insertion duplicates 8 bp of genomic sequence. *Tag1* excises from the *chl1* gene and produces many revertants (25-30% of the total progeny). Investigation of the revertants reveals that a small "footprint" remains after *Tag1* excision. Studies are in progress to determine if *Tag1* is an autonomous or defective element. Sequence analysis of *Tag1* has shown that a region near the 3'end is similar to sequences found in the C-terminal region of the transposase of *Ac*, *Hobo* of *Drosophila*, and *Tam3* of snapdragon. Thus *Tag1* is a member of the Ac family of transposable elements.

F 133 INSERTION AND EXCISION OF TTAA-SPECIFIC LEPIDOPTERAN TRANSPOSABLE ELEMENTS. Malcolm J. Fraser, Teresa Elick, and Christopher Bauser. Laboratory of Molecular Virology, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556.

We are studying two representatives of a unique group of transposable elements isolated as insertions in the Baculovirus genome that are characterized by extreme specificity for the tetranucleotide target sequence TTAA. The larger of these elements, IFP2 (2.7 kb), when tagged with a marker gene in the second of it's two ORFs, is capable of movement from plasmid DNA into the Baculovirus genome via transposition in the presence of an untagged IFP2 element, but not in the absence of the untagged copy. The target site in all cases of transposition remains TTAA, with no apparent consensus outside of this target site. Precise excision of the tagged IFP2 element from it's position of insertion within the Baculovirus genome can occur in cells lacking a functional IFP2 element, suggesting that transposition and excision are not coupled events. Tagged representatives of the smaller (750 bp) TFP3 element, which also exhibits specificity for TTAA target sites upon insertion, also excise precisely in cells lacking resident copies of either element. The excision event for TFP3 is independent of orientation within the same TTAA target site. We conclude that a common mechanism exists in lepidopteran cells which mediates precise excision of the TTAA-specific group of lepidopteran transposons. Our analyses to date indicate that both TFP3 and IFP2 elements utilize a common mechanism for transposition and excision. In addition, the IFP2 encodes some function that facilitates it's own transposition. We have expressing fusion proteins utilizing the IFP2 element ORF1 and ORF2 sequences in both bacterial and Baculoviral expression systems for analysis of their activity, and are attempting to identify sequence-specific DNA binding proteins in the lepidopteran cells that mediate excision of these elements. We believe these elements will be useful for genetic engineering in lepidopteran species.

F 134 BACTERIOPHAGE MU AND THE *E.COLI* ATP-DEPENDENT CLP PROTEASE, Marie-José Gama[1], Amina Mhammedi-Alaoui[1], Martin Pato[2], Ariane Toussaint[1] and Lucie Desmet[1], (1) Laboratoire de Génétique, Université Libre de Bruxelles, B1640 Rhode St Genèse, Belgium.

The importance of proteases in global gene regulation is well documented both in procaryotic and eucaryotic systems.
Virulence in phage Mu requires the activity of the ClpP-ClpX form of the host ATP-dependent Clp protease. Mu*vir* mutants which successfully infect Mu lysogens and induce the resident Mu prophage synthesize mutant repressors which are abnormaly sensitive to the attack by that protease and this susceptibility is communicated to the wild type repressor present in the same cell.
In addition, contrary to ClpP which is dispensable for Mu lytic development, ClpX is essential for Mu replication. In the absence of ClpX, Mu replicative transposition is blocked at a stage beyond strand transfer, suggesting that ClpX may play an essential role during the transition of the strand transfer complex into replication. Preliminary results indicate that this role of ClpX in transposition is restricted to Mu.
Recent results aiming at a further characterization of the molecular mechanism of action of ClpX will be discussed.

F 135 **CONSTITUTIVE EXPRESSION OF HIV-1 REVERSE TRANSCRIPTASE HETERODIMER IN A HUMAN CELL LINE**, Richard A. Gibbs, and M. Ali Ansari-Lari, Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030

In order to determine the role of retroviral reverse transcriptase in LTR and non-LTR mediated retrotransposition, we have established a human cell line that constitutively expresses human immunodeficiency virus type-1 (HIV-1) reverse transcriptase (RT). HIV-1 RT is a heterodimer composed of a 51 kDa subunit (p51) and a 66 kDa subunit (p66). To generate a stable human HIV RT cell line with high level of RT activity, the entire 66 kDa RT region was cloned in a mammalian expression vector (pCMV66). Transient transfection of pCMV66 into the HT-1080 human fibrosarcoma cell line led to the expression of p66 without the generation of p51, and only a low level of RT activity could be detected. Stable pCMV66 cell lines also only expressed the 66 kDa subunit; thus, it appears that endogenous cellular proteases are not capable of cleaving p66 to generate the 51 kDa subunit. Attempts to establish a stable cell line expressing protease-RT region of HIV-1 were hampered by an apparent intolerance of HT-1080 cells to the HIV protease expression. Hence, to generate p51 independent of the HIV protease expression, the 51 kDa subunit was cloned separately (pSV51). Cotransfection of pSV51 and pCMV66 led to coexpression of the p51 and p66 subunits, with a dramatic increase in the RT activity. Stable HT-1080 cell lines expressing both the 51 kDa and the 66 kDa subunits exhibited on average a 17 fold increase in RT activity compared to the HT-1080 parental cell line.This high level of HIV RT activity in the HT-1080 cell line is stable for at least 30 cell generations.

F 136 SITES AND FUNCTIONS INVOLVED IN PIV-MEDIATED SITE-SPECIFIC DNA INVERSION IN *MORAXELLA LACUNATA* AND *MORAXELLA BOVIS*, Anna C. Glasgow, Anne Lenich, and Debbie Tobiason, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA 30329

Inversion of a 2.1 kb chromosomal DNA segment regulates type 4 pilin expression in *M. lacunata* and *M. bovis*. A subcloned ~6 kb DNA segment containing the invertible segment from *M. lacunata* exhibits site-specific DNA inversion in *E. coli*. Using a deletion analysis, we have confirmed that the only Moraxella-encoded gene product that is required for inversion of the subcloned chromosomal segment in *E. coli* is Piv. The *piv* gene is located immediately adjacent to the invertible DNA segment in both *M. lacunata* and *M. bovis* and its sequence is highly conserved between the two strains. We have analyzed the DNA sequence-derived amino acid sequence of Piv using a BLAST search of Genbank and BESTFIT comparison on GCG but could find no significant similarity to other site-specific recombinases or their accessory factors. However, Piv does exhibit strong homology with the proposed transposase/integrases of a group of atypical IS elements found primarily in Actinomycetes, suggesting that Piv is a novel site-specific DNA invertase.

Our initial characterization of Piv-directed site-specific DNA inversion has included mapping the *piv* promoter by primer extension and subcloning *piv* for overexpression and purification in order to identify Piv-DNA binding activity. We are presently characterizing the control of both *piv* expression and DNA inversion through mutational analyses in the *E. coli* host.

F 137 RECOMBINATION BETWEEN THE TWO IS*1* PRESENT IN PLASMIDS OF THE FII INCOMPATIBILITY GROUP, M. Carmen Gómez-Eichelmann and Martín I. García-Castro, Department of Molecular Biology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, 04510 México City, México.

Plasmids of the FII incompatibility group are composed of two regions: the RTF and the r-det region which contains most of the resistance genes and is flanked by two directly repeated copies of IS*1*. In *E.coli* and *Shigella* these plasmids are stable, whereas in *Salmonella typhimurium*, the IS*1* recombine at high frequency, inducing the dissociation of the RTF and r-det regions. *E.coli* and *S.typhimurium* strains have a similar general recombination level, and both bacteria are affected in recombination in a similar way by the repressor coded by the plasmid (1). *S.typhimurium* strains seem to have, in addition to a general recombination pathway, a more specific pathway involved in IS*1* recombination. This pathway may be the one determined by the gene *dor* (2). IS*1* recombination, cointegration formation mediated by IS*1* and transposition of Tn*9* occurred at a higher frequency in *S. typhimurium* than in *E.coli* or *S.sonnei* (3). In this work we studied the effect of chromosomal IS*1* copies, of multiple copies of IS*1*, and of the putative IS*1*-coded resolvase in the dissociation of FII plasmids such as R100 and R1-19. The function of gene *dor* in plasmid recombination was also studied. The plasmids is similar in strains carrying zero or several IS*1* copies; b) IS*1* probably does not code for a resolvase; c) recombination of large and small plasmids in similar in strains *dor*$^+$ and *dor*$^-$.

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F 138 ISOLATION AND PROPERTIES OF THE SEPARATE SUBUNITS OF IHF, Nora Goosen, Laurence Zulianello, Peter van Ulsen, and Pieter van de Putte, Department of Molecular Genetics, Leiden Institute of Chemistry, Leiden University., 2300 RA Leiden, The Netherlands.

Integration host factor (IHF) is an *E.coli* protein which, besides being essential for the integration of lambda DNA in the chromosome, is involved in a variety of different processes in the cell. IHF is a site-specific DNA-binding protein and consists of two homologous subunits HimA and HimD. Bacteriophage Mu contains an IHF binding site in its regulatory region. This site plays a role in the transcription activation of the gene encoding Mu transposase.

To get more insight in the interaction of IHF with the DNA we have purified and studied the individual subunits of IHF. For this purpose we constructed strains which overexpress the *himA* or *himD* gene separately. Overexpression of *himA* led to the accumulation of high amounts of soluble HimA protein which could be purified relatively easy. Overexpression of *himD* resulted in the formation of HimD aggregates in the cell. Using urea these aggregates could be regenerated to a soluble form, which could subsequently be purified. The purified HimA and HimD proteins could be reconstituted to an active IHF dimer. Using gel retardation we could show that at high concentrations of HimD, this protein binds specifically to the Mu *ihf* site. It also induces the same bending in the DNA as IHF does, indicating that it is binding as a homodimer. HimA did not form a specific complex with this site. From the properties of HimA-HimD fusion proteins we could show that an IHF molecule which has the putative DNA binding domains of HimA only can bind specifically to the DNA. From these results we postulate that the inability of HimA to bind the *ihf* site is due to the inability to form proper homodimers. Using IHF-mediated activation of Mu transcription as a test-system, we isolated a set of IHF mutants. Some of the mutants have been purified and studied for DNA binding and transcription activation *in vitro*. These mutants give indications about the orientation of the IHF dimer with respect to the binding sequence. Interestingly one of the mutants is unable to bind to the DNA by itself, but can bind when also RNA polymerase is present. We present evidence that interactions between IHF and RNA polymerase are involved in this phenomenon.

F 139 DONOR DNA LENGTH EFFECTS ON IS50 TRANSPOSITION, Igor Yu Goryshin[1,2] And William S. Reznikoff[2], [1] St. Petersburg Nuclear Physics Institute, St. Petersburg, Russia. [2] Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

We have been using the prokaryotic transposable element IS50 to study the importance of the timing of the transposase oligomerization step. In this communication we present the first *in vivo* evidence indicating that transposase oligomerization must occur prior DNA cleavage in order for transposition to occur.

The test system that we have used is conceptually quite simple. If the ability of the 2 end sequences to come together through space is constrained, oligomerization of the end bound transposase molecules can be prevented. This constraint can be imposed by reducing the length of donor DNA between transposable element ends. An experiment shows that for very short DNA molecules (9 - 63bp) the intervening DNA sufficiently inflexible to prevent the end bound transposase molecules from oligomeriziang. At slightly longer DNA spacing, we would predict that oligomerization (and transposition) would exhibit a periodicity reflecting the structure of the DNA helix. This is because the desired transposase oligomer probably must assume a precise structure but that at short distances the angular flexibility of DNA is sufficiently limited to prevent the transposase molecules bound to ends in incorrect orientations from forming this oligomeric structure. At still longer DNA spacings, transposition should occur at a high frequency. In the experiment, a strong phasing effect has been shown for the region of spacing DNA from 63 to 94bp with the step between points in general being 1bp.

The phase dependence of transposition suggests, in addition, that this system can be used to study the *in vivo* angular structure of defined DNA sequences.

F 140 EXCISION OF AN AMIKACIN RESISTANCE GENE CASSETTE AS A CIRCLE BY THE ACTION OF THE INTEGRON INTEGRASE PROTEIN. Annie Gravel and Paul H. Roy. Department of Biochemistry (FSG) and Infectiology Laboratory, CHUL Research Center, Laval University, Quebec City, Canada.

Integrons are mobile elements, efficiently spread by their insertion into broad-host-range plasmids and transposons, into which antibiotic resistance genes are inserted as cassettes by a site-specific recombinational mechanism. Integrons contain a gene coding for a lambda-like integrase, which is believed to interact with two target sites, GTTPuPuPuPy, located at the 5' end of each cassette, and a palindromic sequence (59-base element) at the 3' end of each cassette. We cloned the Tn*2424* *aacA*1a amikacin resistence cassette, with flanking sequence from adjacent cassettes, into a clone in which expression of the integrase is inducible from a lambda P_L promoter. When integrase production was induced, we observed, by inverse PCR, the excision of the *aacA*1a cassette as a circle from this clone. We also observed the diminution of the size of the original clone by a length equal to that of the cassette. We cloned, by PCR, the pVS1 integrase gene into the pET-3d vector and induced the expression of the protein, in *Escherichia coli* BL21(DE3)-pLysS, with IPTG. We have observed that the integrase represents as much as 50% of total protein but all of it is in inclusions bodies. We have purified the protein from inclusion bodies, by a sarcosyl method, and we have done mobility shift experiments to demonstrate that integrase binds to its recombinational target sites.

F 141 VDJP: A PROTEIN CAPABLE OF IMMUNOGLOBULIN GENE RECOMBINATIONAL SIGNAL SEQUENCE DIRECTED DNA JOINING

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To study V(D)J recombination at the molecular level, we have focused on a protein that interacts with the nonamer element, VDJP. We have obtained cDNA clones encoding a protein that specifically binds the nonamer element, VDJP. VDJP has a region of homology with bacterial DNA ligases, located at the C-terminus of the molecule. Within this region of 80 amino acids, 53 of the positions are identical with one or more of the three bacterial DNA ligases for which sequence data is available.

We have assayed VDJP for DNA ligase activity, but did not detect any . To determine if site directed DNA joining activity was present we devised an assay for V(D)J RSS dependent DNA joining. We have shown that DNA joining takes place between DNA fragments that both contain the RSS element, but not when one or both fragments lack the RSS sequence. Furthermore, both the heptamer and nonamer sequences are required for efficient joining to take place. Joining has been demonstrated to take place between DNAs containing RSS elements from different Ig gene segments and different gene segment families. These gene segments share sequence only at the heptamer and nonamer sequence elements, leading to the conclusion that homology is not required for DNA joining and that joining is dependent on the RSS and not other DNA elements. This joining activity in addition to being site directed, also joins DNA fragments in an orientation specific fashion. Because of this activity, we have named the protein V(D)J DNA Joining Protein (VDJP).

We are currently studying the mechanism of the joining reaction. We have sequenced more than 50 DNA junctions derived from the joining reaction and found they have features associated with coding junctions, including variable loss of nucleotides at the junction, the use of short regions of homology to direct joining, and the loss of an internal nucleotide near the heptamer region. We have examined some of the biochemical requirements of the reaction, and have found that there is no requirement for a high energy cofactor such as ATP or NAD and preincubation with NMN or PPi does not inhibit binding. Recently, we have found that VDJP may be related or identical to the PO-GA protein and Replication Factor C identified from human cells as well as a branched DNA structure binding protein identified from Drosophila. We are currently investigating the relationships between these proteins and V(D)J recombination.

F 142 THE TRANSPOSITIONAL ACTIVITY OF IS*200*, A NOVEL INSERTION SEQUENCE WHICH HARBORS NO TERMINAL REPEATS, Kenneth R. Haack and John R. Roth, Department of Biology, University of Utah, Salt Lake City, UT 84112

IS*200* is a 708 bp insertion sequence, lacking terminal repeats, found in *Salmonella typhimurium*. IS*200* transposes very rarely under all conditions tested; only two insertion events have previously been observed which resulted in a mutant phenotype. To facilitate assays of IS*200* transposition, we constructed IS*200* composite transposons containing a kanamycin resistance determinant flanked by IS*200* (in both direct and inverted orientations) in pUC plasmids. The transposition activity of these constructs has been tested by two independent assays. Transduction of these plasmids into *polA* cells, which do not allow Col E1 plasmids to replicate, gave Kan^R, Amp^S transductants. Several transposons had inserted at novel sites in the chromosome, including one insertion which resulted in a leucine auxotrophy. The composite transposon containing direct repeats of IS*200* also gave transductants that had inherited Kan^R by recombination at existing chromosomal IS*200* sequences. One of these recombinants was used in the second transposition assay which selects for transposition from a linear transduced fragment. A high frequency of transduction was achieved by the use of a stable Mud-P22 prophage that amplifies and packages, in 100-1000 fold excess, a specific region of the chromosome. Kan^R transductants were recovered in *recA* cells that have inherited circularized derivatives of these transducing fragments. These structures are consistent with a replicative-cointegrate mechanism for IS*200* transposition. Data suggests that the RecA protein, not a resolvase, is responsible for resolving these intermediate structures.

F 143 INTEGRON STRUCTURE AND MOBILITY, Ruth M. Hall, David O'Gorman, Gavin D. Recchia, Heidi J. Brown and H. W. Stokes, CSIRO Division of Biomolecular Engineering, P.O Box184, North Ryde 2113 and School of Biological Science, Macquarie University, Sydney 2109, Australia

Integrons are genetic elements which include a gene, *int*, encoding a site -specific recombinase and an adjacent site at which many different gene cassettes can be inserted. The genes found in the cassettes generally encode an antibiotic resistance determinant. The integrase which catalyses cassette insertion or deletion recognises a family of recombination sites, known as 59-base elements, located at the 3'-end of the genes and a unique site located adjacent to the *int* gene, designated the 5'-conserved segment site (5'-CS site), which does not have the recognisable features of 59-base elements. The region required for full 5'-CS site activity has been confined to between 40 and 69 bases. Both classes of recombination site have a core sequence GTTRRRY at the 3'-end, and the recombination crossover occurs between the G and the first T. Both classes of recombination site exhibit strong orientation specificity and the features of 59-base elements required for orientation specificity have been investigated.

Integrons of the class whose members generally include the *sull* gene in the conserved region 3'- to the insertion site are found in many different locations indicating that they are mobile genetic elements. Sequencing of the outer boundaries of the conserved sequences of several integrons found in a variety of locations in plasmids and transposons revealed that these integrons are indeed independently located and in some cases bounded by a 25 base inverted repeat and flanked by a 5 base direct duplication. However, both deletions and insertions have occurred in the 3'-conserved segment. Two distinct *sull* integron structures, which differ in the configuration of the 3'-conserved segment, have been identified and both appear to be mobile elements. The first structure contains between 6-7 kb 3' to *sull*, but deletions and/or insertions present in this region currently prevent conclusive identification of the complete sequence. The integron In2 found in Tn*21* is one member of this group, but In2 contains a deletion of 359 bases of the identified 3'-conserved segment sequences. The second structure contains only the first 0.95 kb of the 3'-conserved segment sequences beyond the *sull* gene followed by an IS*6100* element flanked by short segments (122 and 152 bases) of the outer end of the 3'-conserved segment in inverse orientation. The integron In4 found in Tn*1696* is a member of this group.

F 144 THE FUNCTION OF MUTAGENIZED Tn7 INTEGRASE IN SITE SPECIFIC RECOMBINATION OF ANTIBIOTIC RESISTANCE CASSETTES, Karin Hansson, Paul Roy, Ola Sköld and Lars Sundström, Department of Pharmaceutical Biosciences, Division of Microbiology, Uppsala University, P.O. Box 581 Biomedical Center, S-751 23 Uppsala Sweden.

Tn*7* appears to be a second type of integron able to integrate some of the same antibiotic resistance cassettes found in Tn*21*-like integrons. Comparison of the conserved sequences 5′ of the cassettes of Tn7 and Tn*21* indicates the likely extent of the core site which interacts with integrase. The minimal core site is being determined by analysis of deletions of the Tn*21* integron and seems to include at the most 8 nucleotides on the 5′side of the *aadA1* cassette and 13 nucleotides on the 3′ side of the cassette. The ability to integrate identical cassettes indicates an identical site specificity of the Tn*21* and Tn7 integrons. The limited variety of Tn7-like integrons is believed to be due to a mutation which has placed a stop codon in the center of the Tn7 integrase. We have mutagenized the Tn7 integrase pseudogene to produce a functional protein. Complementation of the Tn*21* integrase for cassette integration and excision is being tested. The Tn7 integrase may prove to be a useful alternative to the Tn*21* integrase for overexpression and purification for structural and DNA binding studies. The role of different host factors in the cassette recombination is being studied using a conjugation-mobilization assay.

F 145 DYNAMICS OF LINE-1 TRANSPOSITION IN THE MOUSE, Stephen C. Hardies, N. Carol Casavant, Lorrie P. Daggett, Brad A. Rikke, Department of Biochemistry, Univ. of Texas Health Science Center, San Antonio, TX 72824

The LINE-1 transposon has distributed 100,000 copies of itself within the mouse genome. The dynamics of the amplification process will be discussed in light of the following observations: 1) The age distribution is an exponential curve increasing towards the present, extrapolating to a current rate of expansion of 25,000 copies/Myr. This curve has been extended to within 0.2 Myr of the present. We believe that this curve applies equally well to fixed and unfixed copies. 2) New subfamilies expand to >2000 copies/haploid genome within 0.2 Myr. We believe that this level of amplification precedes global fixation (see related poster by Casavant and Hardies). 3) Accelerated divergence within successful lineages indicates a need for repeated movement to maintain function. We believe that LINE-1 is a selfish transposon. 4) The presence of some Mus spretus LINE-1 elements in the hybrid M.m. domesticus/M.m. musculus inbred strain C57BL indicates a breach of the reproductive barrier with Mus spretus within the last 0.2 Myr. [supported by NIH HG00190].

F 146 The Characterization of a Mammalian Structure-Specific Endonuclease, John J. Harrington and Michael Lieber, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

The use of homology in the resolution of V(D)J coding ends and other DNA double-strand breaks has been proposed to proceed through DNA flap structure intermediates. To identify DNA flap cleaving activities in mammalian nuclear extracts, we created a synthetic DNA substrate. Using this assay, we have purified the first mammalian DNA structure-specific nuclease. This enzyme, Flap EndoNuclease-1 or FEN-1, cleaves DNA flap strands which terminate with 5' single-stranded ends. As expected for an enzyme which functions in homology-dependent double-strand break repair (DSBR), FEN-1 cleavage is flap strand-specific and independent of flap strand length. Furthermore, efficient flap cleavage requires the presence of the entire flap structure. Substrates missing one strand are not cleaved by FEN-1. Other branch structures, including Holliday junctions, also are not cleaved by FEN-1. In addition to endonuclease activity, FEN-1 has a 5'-3' exonuclease activity which is specific for double-stranded DNA. The endo- and exonuclease activities of FEN-1 are discussed in the context of V(D)J coding end resolution and DSBR.

F 147 *Abstract Withdrawn*

F 148 DNA BRANCHING AND ASSEMBLY OF THE HIN INVERTASOME.

Michael J. Haykinson and Reid C. Johnson.
Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024

The formation of the invertasome complex is a prerequisite for the Hin-mediated DNA inversion reaction. This complex consists of two Hin-bound recombination sites (*hix*) and the recombinational enhancer, bound by Fis protein. Previous experiments have also shown that Hin can associate the two *hix* sites into a 'paired-*hix*' complex without the activity of Fis or the enhancer. In order to determine the sequence of events leading to invertasome formation *in vitro*, a pair of *lac*-operator sequences have been introduced at different positions in the DNA substrate. *Lac* repressor bound to both operator sites is able to form a tetramer which in turn results in efficient tethering of *lac*-operator sites within the DNA molecule. The results indicate that the paired-*hix* complex is very unstable relative to the time required for invertasome assembly suggesting that the enhancer may not be incorporated into a paired-*hix* complex as previously proposed. Rather, the invertasome may be assembled by nearly simultaneous collision of three sites (two *hix* sites and the enhancer) that are transiently co-localized at the base of a supercoiled DNA branch. Experiments will be presented on DNA branching and its effect on the DNA inversion reaction.

F 149 A NOVEL ASSAY FOR HIV-1 INTEGRASE AND CHARACTERIZATION OF THE DNA STRAND TRANSFER REACTION, Daria J. Hazuda, Jeffrey C. Hastings, Abigail L. Wolfe and Emilio A. Emini, Department of Antiviral Research, Merck Research Laboratories, West Point, PA 19486

The replication of retroviruses is contingent upon integration of a DNA copy of the viral genome into the genome of the host cell. Integration encompasses a series of defined cutting and DNA strand transfer reactions that are catalyzed by the virally encoded enzyme integrase. The absolute requirement for integrase activity in the propagation of HIV-1 in cell culture defines integrase as an attractive target for antiviral chemotherapeutic intervention. Toward this aim, we developed a novel, non-radioisotopic microtiter assay for integration. The microtiter assay uses immobilized synthetic oligonucleotides to represent the specific viral LTR substrates and heterologous biotinylated oligonucleotides to represent the integration targets. The resulting integrase-mediated covalent linkage between the biotinylated target and the immobilized LTR donor DNA is detected using an avidin-linked colorimetric assay. The microtiter integration assay has been useful for characterizing the HIV-1 integrase mediated strand transfer reaction and defining requirements for target site selection. In addition, the general configuration of this method should prove applicable in the study of a number of DNA processing enzymes, including transposases.

F 150 MAPPING INTEGRASE-DNA INTERACTIONS BY SPECIFIC CROSSLINKING Timothy S. Heuer and Patrick O. Brown, Departments of Pediatrics and Biochemistry, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305.

Retroviral integration occurs by a concerted cleavage and ligation mechanism that is mediated by retroviral integrase. In the first step of integration, integrase processes retroviral DNA by removing the two terminal nucleotides from the 3' end of each viral DNA strand. Integrase then mediates the joining of processed viral DNA 3' ends to newly created 5' ends in the target DNA. This latter step is referred to as strand transfer. Strand transfer is reversible, and the reverse reaction has been termed disintegration. Integrase mediates the cleavage and ligation reactions of integration and disintegration through specific protein-DNA interactions that remain to be elucidated. The identification of functional domains or residues of integrase will enhance our understanding of the integration process.

To identify specific integrase-DNA interactions we have conjugated the photoreactive azidophenacyl group to a disintegration substrate. The position of the azidophenacyl group on a DNA molecule is easily varied by virtue of its reactivity with sulfur, which can be introduced into DNA as a phosphorothioate. Disintegration substrates mimic the strand transfer intermediate, and contain both viral and target DNA elements. The disintegration substrate is attractive for integrase crosslinking studies because the photoreactive group can be positioned to identify either viral or target DNA interactions. The azidophenacyl-disintegration substrate is crosslinked to integrase by UV irradiation. Integrase retains disintegration activity following crosslinking. Crosslinking of the disintegration substrate to integrase requires an active enzyme, and displays salt sensitivity that parallels disintegration and re-integration activity. We have shown that the disintegration substrate crosslinks to integrase in a functionally relevant orientation because the crosslinked substrate can be processed by integrase to yield the viral and target DNA disintegration products. To identify the site of interaction between integrase and the disintegration substrate we are currently mapping the site(s) of crosslinking by proteolytic peptide mapping.

F 151 SOLUBILITY AND ENZYMOLOGY OF N- AND C-TERMINAL TRUNCATED VERSIONS OF HIV1 INTEGRASE. Alison B. Hickman, Alan Engelman, and Robert Craigie, LMB, NIDDK, NIH, Bethesda, MD 20892.

Efforts to crystallize HIV integrase have been hampered by the low solubility of the isolated protein. Our approach to overcome this problem has been to purify N- and C-truncated versions of HIV1 integrase as well as fusion proteins of integrase to soluble proteins, and to investigate their solubility properties under various buffer conditions.

The availability of soluble versions of HIV1 integrase in large quantities has also allowed us to compare aspects of their enzymatic properties to each other and also to those of the wild-type protein, with particular focus on their metal-binding requirements.

Truncated proteins that will be discussed include IN^{50-212}, IN^{1-173}, $IN^{106-288}$, $IN^{213-288}$, and fusion proteins.

F 152 "MUPRINTS" DEMONSTRATE PHYSIOLOGICAL CONTROL OVER THE RANDOMNESS OF IN VIVO TRANSPOSITION. N. Patrick Higgins and Xiu Hu Wang. Department of Biochemistry. University of Alabama at Birmingham. Birmingham AL 35294

A method called Muprinting has been developed that uses PCR to generate a detailed picture of the Mu transposition sites in any chosen domain of the bacterial chromosome. Muprinting experiments in *Escherichia coli* show that the frequency of phage integration changes dramatically near two repressor binding sites in the *lac* operon. When the *lac* operon was repressed, hotspots for Mu transposition were found near the O_1 and O_2 operators that are proposed to make a repression loop. When cells were grown in lactose, Mu transposition near these operators was greatly diminished. Striking changes in transposition frequencies were limited to the control region and were not found throughout most of the 2500 bp segment of the *lac*Z gene beyond the O_2 operator. Muprints of the *bgl* operon showed a similar pattern; hotspots for Mu transposition were detected in sequences upstream of the *bgl*C promoter when the operon was silenced and these sites disappeared when the operon was activated. By targeting transposition to the regulatory regions around non-expressed genes, Mu may demonstrate a self-restraint mechanism proposed previously (1) that allows the virus to move through its host genome without disrupting functions contributing to a healthy cell physiology.

1. Casadesus J., and Roth J.R. 1989. Transcriptional occlusion of transposon targets. Mol. Gen. Genet. 216:204-209.

F 153 ANALYSIS OF RETROTRANSPOSITION IN A HETEROLOGOUS SYSTEM, Eleanor F. Hoff, Henry L. Levin* and Jef D. Boeke, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, * National Institutes of Child Health and Human Development, Bethesda, MD 20892

Tf1-107 is a functional retrotransposon of 4.9 kb found in multiple strains of the fission yeast *Schizosaccharomyces pombe*. It has long terminal repeats, and its encoded proteins show strong homology to retroviral protease (PR), reverse transcriptase (RT), and integrase (IN); however, the element is unusual in that it contains a single open reading frame (ORF) of 1340 amino acids. When expressed in multi-copy in *Saccharomyces cerevisiae* as a *neo*-marked element under the inducible *GAL1* promoter, it is transcribed; 3'-end formation appears to occur at the proper site, and the transcript is stable. In addition, the Tf1 proteins appear to be made and properly processed. In this system, however, Tf1-107 does not show levels of putative transpositon events comparable to the *S. cerevisiae* Ty1 retrotransposon, nor to Tf1 in its natural host. Isolation of the primer of reverse transcription of Tf1-107, well as screening of *S. pombe* genomic and cDNA libraries through cotransfection with the pGAL-Tf1 plasmid, may elucidate the *S. pombe* host factors essential for Tf1-107 transposition in a heterologous system.

F 154 IDENTIFICATION OF A NEW HUMAN ACTIVE TRANSPOSABLE ELEMENT. Susan E.Holmes, Beth A.Dombroski, Claudia M.Krebs, Corinne D.Boehm, and Haig H.Kazazian, Jr. Center For Medical Genetics, Johns Hopkins Hospital, Baltimore, MD 21205

In a patient with muscular dystrophy we found a 2 kb insertion containing a rearranged L1 element plus unique flanking sequence in exon 48 of the dystrophin gene and we used oligonucleotides from the flanking sequence to clone the precursor of this element, the second known active human L1. We screened a human genomic placental library, sequenced the clones identified on a DNA sequencer, used PCR to characterize several polymorphic sites within the precursor element, and mapped the element using somatic cell hybrid panels. The locus LRE-2 (L1 Retrotransposable Element-2) maps to chromosome 1q. Two alleles of the precursor element were identified among the clones, and subsequent analysis of various individuals has shown the element to be polymorphic in at least 6 locations, including the polyA tail. In composite, an allele matching the insertion was found, which is presumably present in the individual whose element gave rise to the insertion. LRE-2 is definitely the precursor, as the unique sequence was transposed next to it and serves as an identifying 'tag'. The precursor has a perfect 13-15 base pair target site duplication, and an unusual 21 base pair truncation of the 5' end,which presumably occurred during its own transposition. No individuals so far examined by PCR and blotting show an allele containing L1 nucleotides 1 to 21. LRE-2 has 2 ORFs, so it appears likely that a slightly truncated element can still be functional. It belongs to a different subfamily from LRE-1, the first active element previously identified, differing in sequence by about 0.7%. The element is polymorphic as to presence in human genomes, suggesting that it is 1-2 million years old. Blotting revealed empty sites on about 33% of human chromosome 1s, with a slightly higher presence of the L1 element in the patient's ethnic group. 20 chimps and gorillas analyzed were all lacking the element. Identification of LRE-2 provides further evidence that previously transposed L1 elements can themselves transpose, and indicates that read-through transcription can carry along flanking single-copy sequence in a retrotransposition event. It also suggests that priming of reverse transcription does not require an L1 sequence itself.

F 155 The Nucleotides of the Tn*5* OE Required For Specific Binding By Tn*5* Transposase, Ross A. Jilk and W.S. Reznikoff, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706

The outside end (OE) sequences of the Tn*5* transposon contain information required for specific transposase (Tnp) and DnaA binding, and also base pairs which are necessary for proper excision and insertion of the transposon. We have determined which of the 19 base pairs of the OE are involved in the binding interaction with the transposase protein. A missing nucleoside experiment was used to show that the presence of the base pairs at positions 6-8 and 13-16 are required for Tnp binding. These positions overlap with the sites previously shown to be involved in DnaA binding (8-16). In addition, various single base substitutions scattered throughout the OE were analyzed, by gel retardation techniques, for their relative affinities with the Tnp. These experiments also suggest the importance of base pairs 5-8 and base pair 13 in Tnp binding and perhaps hint at minor groove binding. Base substitutions which apparently allow Tnp binding but are otherwise defective in transposition give evidence towards their importance in either DnaA binding and function or in a subsequent transposition process.

F 156 DEFINING DNA BINDING, CATALYTIC, and DOMAINS FOR PROTEIN-PROTEIN INTERACTION IN THE RETROVIRAL INTEGRASE, M-MULV Colleen B. Jonsson*, George A. Donzella, Elena Glaucan, and Monica J. Roth, Dept. Biochemistry, UMDNJ-R. W. Johnson Medical School, Piscataway, NJ 08854; *Dept. of Chemistry-Biochemistry, New Mexico State University, Las Cruces, NM 88001

Integration and disintegration activities associated with the Moloney murine leukemia virus integrase protein have been recently characterized by our laboratory. Although the activities of the retroviral integrases have distinct reaction similarities, we have observed differences in the substrate requirements of M-MuLV IN compared with HIV-1 IN. Currently, studies are focused on defining regions of M-MuLV IN required for catalysis and DNA binding by mutational and southwestern analysis. An N-terminal deletion of the zinc finger region of IN, Δ105, has near wild type levels of disintegration activity. Interestingly, this mutant is able to catalyze integration reactions at a low level, in contrast with HIV IN, where this region is required for integration activity. Complementation analysis with N and C-terminal deleted proteins suggest that a minimum of one amino and one carboxyl terminus is required for one LTR reaction.

In addition to defining the catalytic domains of IN, we have been using hairpin substrates (crossbones, P. Brown, Stanford) to look at protomer-protomer interaction. Dimerization of the crossbone substrate by M-MuLV IN protomers generates an integration-intermediate structure and promotes specific disintegration reaction products. Structurally modified crossbone substrates designed to alter the presumed spacing in the active site were used to probe reaction flexibility. Finally, far-western analysis has shown the ability of the N-terminus to interact with the entire IN. Studies are now aimed at defining the domain(s) involved in protein-protein interactions.

F 157 PROTEIN-PROTEIN INTERACTIONS OF HIV-1 IN: *ini-1* (IN INTERACTOR-1), A NOVEL HUMAN GENE WITH SEQUENCE SIMILARITY TO YEAST TRANSCRIPTION FACTOR SNF5, BINDS TO HIV-1 INTEGRASE. Ganjam V. Kalpana and Stephen P. Goff, Columbia University College of P & S, New York.

The retroviral integrase protein (IN) catalyzes concerted integration of linear viral DNA into host DNA. To study multimerization of IN, we made use of the yeast two hybrid system. Co-expression of a GAL4 DNA binding domain-IN (GAL4DB-IN) fusion protein and a GAL4 activation domain-IN (GAL4AC-IN) fusion protein activated a GAL4-responsive *lacZ* reporter gene. Using this system we have demonstrated that the central region of IN is necessary for multimerization.

The yeast system was also used to fish for a host protein interacting with HIV-1 IN. GAL4DB-IN was used as a bait to screen a human HL60 library of cDNAs fused to GAL4AC. Examination of 600,000 transformants of yeast resulted in 3 independent but identical cDNA clones encoding a potential IN interactor (*ini-1*). We have also demonstrated that the GST-Ini fusion protein expressed in *E. coli* specifically binds to recombinant IN. Examination of a panel of IN mutants for their ability to bind to Ini-1 indicated that central domain of IN is involved in binding to Ini-1. Sequence comparison of *ini-1* revealed a region of similarity to yeast transcription factor SNF5. In yeast, genetic evidence indicates that SNF5 activates transcription by complexing with proteins one of which, SWI2/SNF2, has similarity to helicases (Laurent *et. al. PNAS* **88**:2687). Both SNF5 and SWI2/SNF2 are thought to alter the chromatin structure by affecting the arrangement of histones in the nucleosome (Hirschhorn *et. al. Genes & Dev.* **6**:2288). Like *SNF5*, *ini-1* activated GAL4 dependent reporter gene in yeast when tethered to GAL4DB. Based on these data we suggest that *ini-1* might be involved in targeting the incoming viral DNA to an open chromatin region for integration. This finding might explain the fact that Mo-MLV preferentially integrates into DNase-1 hypersensitive regions (Vijaya *et. al. J. Virol.* **60**:683).

F 158 MUTATIONS IN THE tRNA $_i^{Met}$ PRIMER OF Ty1 WHICH ABOLISH TRANSPOSITION, Jill B. Keeney, Karen Chapman, Anders Byström*, and Jef Boeke, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205. *Department of Microbiology, Umeå University, S-90187 Umeå, Sweden.

The Ty1 retrotransposon of *Saccharomyces cerevisiae* uses tRNA$_i^{Met}$ as its minus strong-stop primer, but little is known about what sequences outside the primer binding site (PBS) complementarity region of the tRNA are important for this priming function. We have undertaken a genetic study of this interaction. A system has been devised by which any tRNA mutation can be assessed for its ability to support transposition, regardless of whether it is a viable mutation. A tester yeast strain has been constructed in which all four chromosomal *IMT* genes, encoding tRNA$_i^{Met}$, are disrupted. A tRNA mutant, *imt4-9*, which can support growth of the tester strain, but is unable to support transposition due to non-complementarity to the PBS of Ty1 (Chapman et al., 1992, *PNAS* **89**:3236-3240.), is being used to study a battery of tRNA$_i^{Met}$ mutations for their ability to support transposition. Four double mutations with transposition levels about 1% of wild-type were identified: G1/C72, A3/T70, C54/G60, and G54/C60. G1/C72 and T3/A70 are located within the PBS complementarity region in the tRNA amino acid stem and G54/C60 and C54/G60 are mutations in the TΨC loop. None of these mutations affect tRNA stability. Mutations within the anticodon loop, the anticodon stem, the D-loop and the TΨC stem all demonstrated near wild-type levels of transposition. These results suggest that the amino acid stem and the TΨC loop are important for supporting transposition. Compensatory changes of the G1/C72 and T3/A70 mutations were introduced into the Ty1 sequence. Surprisingly, these mutations were transposition competent, indicating that a one basepair mismatch between the PBS and the tRNA primer reduces transposition 100-fold. Work is in progress to differentiate whether the primer-template interaction is dramatically reduced by the mismatches or whether the complex forms, but reverse transcriptase cannot recognize the resultant structure.

F 159 DNA-PROTEIN COOPERATIVITY IN THE ASSEMBLY AND STABILZATION OF MU STRAND TRANSFER COMPLEX: RELEVANCE OF DNA PHASING AND *ATT* SITE CLEAVAGE. Keetae Kim, Soon Young Namgoong, Makkuni Jayaram and Rasika M. Harshey, Department of Microbiology, University of Texas at Austin, Austin, TX 78712.

We have extended the studies on the transposition pathway using DMSO-assay conditions (Mizuuchi and Mizuuchi, Cell 58:399, 1989). This assay has the advantage of revealing many subtle aspects of the reaction that the standard assay fails to demonstrate because of its stringent requirements for DNA superhelicity and an enhancer element. We show that *att* DNA and Mu A protein mutually promote the assembly of 'higher order' complexes held together by non-covalent protein-DNA and protein-protein interactions. A large subset of these complexes is competent in mediating strand transfer. The complexes are built almost exclusively from *att* R-containing DNA fragments. While the R3 subsite is dispensable, proper phasing and spacing between R1 and R2 subsites are central to the protein-DNA oligomerization steps as well as to the strand transfer reaction. A single base pair change in the terminal nucleotide that renders *att*-R non-cleavable prevents the assembly of stable higher-order complexes, showing that strand cleavage and stabilization of higher-order complexes are tightly coupled events (Surette et al. JBC 266:3118, 1991). Conversely, pre-cleavage at the *att* L site allows it to function in the assembly process, a reaction enhanced by the HU protein. Tight coupling between DNA cleavage and protein assembly have also been observed with the Flp recombinase and the integrase of Moloney Murine Leukemia virus, suggesting that this aspect of the recombination reaction may have more general implications than had been suspected.

F 160 MUTATIONAL ANALYSIS OF RAG-1, Susan A. Kirch and Marjorie A. Oettinger, Department of Molecular Biology, Massachussetts General Hospital, Boston, MA 02114

V(D)J recombination in vertebrates creates much of the diversity in the binding specificities of immunoglobulins and T-cell receptors. Together, RAG-1 and RAG-2 are necessary and sufficient to stimulate V(D)J recombination in fibroblastoid cells.

In order to learn more about the role of RAG-1 in V(D)J recombination, we have carried out a mutational analysis of this gene. As has also been reported by others, we have found that surprisingly large deletions within RAG-1 retain function. In addition we have identified a number of single amino acid substitutions and insertions that substantially alter the ability of RAG-1 to induce the rearrangement of different substrates. A detailed characterization of these mutants will be presented.

F 161 IN VITRO INTEGRATION OF TY3: DEFINING THE TARGET, Jacqueline Kirchner, Tom M. Menees, Charles M. Connolly, Suzanne B. Sandmeyer, Department of Microbiology and Molecular Genetics, University California, Irvine, Irvine, CA, 92717.

Ty3, a retrotransposon in *Saccharomyces cerevisiae*, integrates in a position-specific manner one to three nucleotides upstream of the transcription initiation site of genes transcribed by RNA polymerase III. We developed an in vitro integration assay using Ty3 viruslike particles as the source of integration machinery and Ty3 DNA together with a target plasmid which contains a modified version of a tRNA gene. Integration was monitored by PCR amplification of the Ty3/tDNA junction. By this assay, integration was observed into the tRNA gene target when the plasmid was isolated as chromatin. In a wild-type tDNA, TFIIIC binds to the internal promotor, allowing TFIIIB to bind upstream of the initiation site, followed by binding of RNA polymerase III. A C56G mutation was made in the internal promotor of the tDNA. This mutation causes a 300-fold decrease in binding of TFIIIC. When the C56G mutant was isolated as chromatin and used as a target it supported integration at a significantly lower level. A low level of integration was also observed when naked target DNA was used instead of chromatin. These events may represent non-specific integration occurring at several positions.

To further define the cellular targeting components, we developed a second in vitro assay using the same target tDNA plus RNA polymerase III transcription extract. The extract was shown to transcribe the wild-type original target tDNA, but not the C56G mutant. In the presence of transcription extract, position-specific integration was greatly increased over integration occurring into the naked DNA target. The level of integration into the target C56G tDNA was not enhanced by the addition of extract and was comparable to the level of integration into the wild-type tDNA target in the absence of extract. We are currently fractionating the RNA polymerase III extract into TFIIIB-, TFIIIC-, and polymerase III-containing fractions. These will be used separately and in combination to identify the target component(s) required for integration.

F 162 EXPRESSION OF L1 IN A MOUSE EMBRYONAL CARCINOMA CELL LINE, F9: THE SEARCH FOR AN ACTIVE ELEMENT, Vladimir Kolosha and Sandra Martin, Department of Cellular and Structural Biology, School of Medicine, University of Colorado Health Sciences Center, Denver, CO 80262

LINES (L1) are highly-repetitive sequences, with a copy number of about 10^5, present in the mammalian genome as truncated, rearranged and full-length elements. Only a small subset of the full-length L1, the so-called active elements, are capable of undergoing retrotransposition. The retrotransposition process is thought to require element-encoded proteins (ORF1 and ORF2).

In order to identify the active elements, we developed a screening procedure to isolate copies that encode ORF1 proteins that are expressed *in vivo*. Three proteins, with molecular weights ranging from 41.3 to 45.0 kD, were detected in F9 cell extracts with a polyclonal antibody against ORF1 protein expressed in bacteria. When F9 cell extracts are fractionated on a sucrose gradient, similar proteins are enriched in the fraction containing the full-length (7.5 kb) L1 RNA, associated as the ribonucleoprotein particles (RNPs). We hypothesized earlier that these RNPs are intermediates involved in retrotransposition of L1.

Here we provide evidence that poly(A)+ RNA isolated from the sucrose gradient fraction containing L1 RNPs yields, after *in vitro* translation followed by immunoprecipitation, two proteins with molecular weights of 41.3 and 43.0 kD. To isolate elements encoding these two forms of ORF1, RT-PCR was performed, followed by cloning, *in vitro* transcription-translation, immunoprecipitation, and SDS-PAGE electrophoresis. Most of the 30 tested clones were found to contain plasmids encoding the 41.3 kD protein. We are currently sequencing cloned fragments to identify whether they derived from one or several elements. Alternative approaches are under way to identify sequences encoding the 43kD protein.

F 163 HORIZONTAL TRANSFER OF *mariner* TRANSPOSABLE ELEMENTS ACROSS ORDERS OF INSECTS, David J. Lampe and Hugh M. Robertson, Dept. of Entomology, University of Illinois, Urbana, IL 61801.

We have discovered representatives of five major subfamilies of *mariner* elements widely distributed in insects, and closely related arthropods such as a centipede and a mite. Sequences of PCR fragments that comprise the central half of the transposase gene from at least six clones from each of 40 species delimit these subfamilies on the basis of both sequence differentiation (25-35% amino acid identity) and length differences (1-5 amino acids) in their transposases. One large subfamily is characterized by the *mariner* element from *Drosophila mauritiana*, while members of a second smaller subfamily are similar to the element from the cecropia moth, *Hyalophora cecropia* (the PCR primers were designed from comparison of the published sequences of these two elements).

A third small subfamily is named for elements from the Med fly, *Ceratitis capitata*. A fourth large subfamily consists of elements similar to that of the honey bee, *Apis mellifera*. Elements unusually similar to the honey bee element (eg. 90-95% DNA identity) have been found in insects from four other orders (Coleoptera, Diptera, Dermaptera, and Neuroptera), indicating recent horizontal transfers between these ancient lineages. A fifth subfamily is named for the elements comprising 1% of the genome of the horn fly, *Haematobia irritans*, and a second set of recent horizontal transfers involves this particular element. Multiple full length copies have been cloned and sequenced from genomic libraries of five flies (horn fly, Med fly, *Anopheles gambiae*, *Rivellia quadrifasciata*, and *D. ananassae*) and two neuropterans (a green lacewing, *Chrysoperla plorabunda* and a mantispid, *Mantispa pulchella*). These have been used to generate a consensus sequence for a *mariner* element that probably represents the horizontally transferred element, and may be capable of transformation of many insect germlines. We are also seeking active versions of the honey bee horizontal transfer element that might provide a second independent system.

F 164 CHARACTERIZATION OF *SIRE-1*, A LONG INTERSPERSED REPETITIVE DNA ELEMENT FROM SOYBEAN WITH WEAK DNA SEQUENCE SIMILARITY TO RETROTRANSPOSONS, Howard M. Laten, Yi-An Bi and Arpita Majumdar, Department of Biology, Loyola University Chicago, Chicago, IL 60626

SIRE-1 (soybean interspersed repetitive element) is a family of dispersed repeated elements found in *Glycine max* and its wild progenitor, *Glycine soja*. The repeat is at least 10.6 Kb in length, and copy numbers range from 200 per haploid genome in *G. max* cv Mandarin to 1600 in *G. max* cv Richland. A 776-bp PCR-amplified fragment from this family has been sequenced[1]. Two overlapping 300-bp stretches of this sequence exhibit suggestive DNA sequence similarity to adjacent regions of the RNA binding domain of Tal from *A. thaliana*. A soybean cDNA library was probed using a cloned copy of the PCR-amplified fragment and a 3.5-Kb cDNA was recovered. Its nucleotide sequence and derived amino acid sequence will be presented. Experiments to recover family members from a genomic library are under way.

[1]H.M. Laten and R.O. Morris. 1993. *Gene*, in press.

F 165 Role of Spacer Homology in Flp Site-specific Recombination: in Synapsis and Cleavage or Post-Cleavage steps? Joonsoo Lee and Makkuni Jayaram, Department of Microbiology, U. T Austin, Austin, TX 78712.

Efficient recombination between two recombination "full-sites" by the Flp site-specific recombinase requires that the strand exchange regions (spacers) of the substrates be completely homologous. Which of the steps of recombination (synapsis, strand cleavage, strand exchange and branch migration) require homology? Although there is suggestive evidence that synapsis occurs independent of homology, results with recombination "half-sites" (especially those from the Cox laboratory) necessitate a reinvestigation of this issue. We have constructed a number of half-site and full-site substrates with altered spacer sequences and carried out recombination reactions within a half-site, between two half-sites and between half-sites and full-sites. The results so far are consistent with the following conclusions: 1. Homology at the first two or three spacer nucleotides is critically sensed during recombination. 2. Synapsis and strand cleavage steps are not exquisitely sensitive to spacer homology between partners. 3. The homology requiring step is a post-cleavage step. It is possible that the strand joining reaction requires pairing of the exchanged strand with its new partner at the first 2-3 nt positions of the spacer. The pairing may provide the proper relative orientation between the 5'-hydroxyl group (the active nucleophilic species) and its target (the 3'-phosphotyrosyl linkage between Flp and the cleaved strand). This would be analogous to the patch/join model proposed by the Nash laboratory for the λ Int reaction. We also noticed that, within a half-site, strand cleavage by the active site tyrosine is impeded if the first 2-3 spacer positions are not mutually complementary. In a simplistic sense, strand pairing may be utilized to to align the phosphodiester at the cleavage point and the active site nucleophile provided by Tyr-343 of Flp. These results demand a reconsideration of the generally accepted role for DNA homology in facilitating branch migration across the entire spacer. If 2-3 nt pairing between spacer strands from partner substrates is necessary for the joining reaction at either end of the 8 bp spacer (top and bottom strands), true branch migration within the Holliday intermediate must be limited to the central 2-4 bp region.

F 166 MOLECULAR BASIS OF A BARRIER TO AUTO-INTEGRATION OF MOLONEY MURINE LEUKEMIA VIRUS DNA, Myung Soo Lee, Kiyoshi Mizuuchi and Robert Craigie, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

As an essential step in the retrovirus life cycle, retroviral DNA integrates into the host genome. It is important for the retrovirus to ensure that the viral DNA does not integrate into itself (termed auto-integration), a process that would result in suicide of the virus in the infected cell. What is the mechanism that prevents auto-integration of the retroviral DNA?

Retroviral DNA integration is mediated by a nucleoprotein complex [termed the integration-competent nucleoprotein complex (INC)]. The INC, isolated from cells after infection with Moloney murine leukemia virus, efficiently integrates its endogenous copy of viral DNA into an exogenously introduced target DNA *in vitro*. Utilizing preparations of isolated INCs, we have developed a simple assay to detect auto-integration of the retroviral DNA into itself and discovered that the degree of the auto-integration can be modulated by the ionic strength of the reaction mixture. In the presence of KCl at 500 mM the auto-integration reaction is highly preferred to the intermolecular reaction, whereas the products are almost exclusively intermolecular when reactions are performed at lower ionic strength. Auto-integration events also predominate when the INC is preincubated with high salt and the reaction is subsequently carried out at low ionic strength. This suggests that the high ionic strength causes a change in the organization of the INC that disrupts a barrier to auto-integration.

Auto-integration complexes were purified by velocity sedimentation in sucrose gradients after high salt treatment. These complexes can restore their barrier to auto-integration upon incubation with a fraction from the sucrose gradient that contains free proteins. We conclude that a factor(s) that prevents auto-integration is stripped from the INC by high salt treatment and separated by velocity sedimentation. We are in the process of determining the identity of this factor that can protect the viral DNA from autointegration and the nature of its participation in the complex.

F 167 MUTATIONS IN THE Tf1 RETROTRANSPOSON OF *S. POMBE* THAT REDUCE TRANSPOSITION ACTIVITY, Henry Levin and Erin Sweeney, Lab of Molecular Genetics, NIH, Bethesda, MD 20892

The *Schizosaccharomyces pombe* retrotransposon Tf1, has one 1,340 amino acid open reading frame with strong homology to the protease (PR), reverse transcriptase (RT), and integrase (IN) proteins of retroviruses and retrotransposons. The results of *in vivo* experiments designed to detect the transposition of Tf1 show that Tf1 is indeed active and can insert itself into the host genome via a true retrotransposition process. Transposition frequencies as high as 20% have been observed in cells containing a high-copy plasmid with the *nmt1* promoter that served to overproduce the neo-marked Tf1 transcript. Cells suffering transposition events became resistant to the drug G418 because the *neo* gene within the transposon was carried with the element into new positions within the host genome.

We have isolated 135 mutant Tf1 elements that exhibit reduced transposition frequencies. About half of these are unable to produce reverse transcripts suitable for homologous recombination as determined by the intron assay described below. An artificial *S. pombe* intron was introduced into the *neo*-marker of Tf1 to allow for the direct and efficient screening of transposition events. The orientation of the intron was such that it could only result in $G418^R$ if the *neo* gene was reverse transcribed from a Tf1 transcript that had the intron spliced out. Although cells became resistant to G418 at high frequencies, the cause was not transposition events as predicted, but was homologous recombination between the reverse transcripts with the intron removed and plasmid or genomic copies of Tf elements. A frameshift mutation in Tf1 IN had no effect on the frequencies of $G418^R$ while a similar mutation just upstream of RT drasticaly lowered the frequency. Further characterization of these mutations including immunoblots will be presented.

F 168 NON-LTR RETROTRANSPOSONS IN PLACE OF TELOMERIC REPEATS AT A DROSOPHILA TELOMERE

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Loss of DNA from the termini of a linear chromosome is predicted to occur at each round of replication. Telomeric DNA must therefore be elongated by a specialized mechanism to counterbalance this loss. In most eukaryotes, telomerase is thought to elongate the telomeric DNA by repeatedly polymerizing a short sequence onto the end of the chromosome, creating an array of short tandem repeats. We have isolated the DNA of a Drosophila telomere and found that it differs from those isolated from other eukaryotes by its lack of short tandem repeats at the terminus. The terminal 14.5 kb is instead composed of four long tandem elements derived from two families of non-LTR retrotransposons called HeT-A and TART. Both families of elements have been shown to transpose onto broken chromosome ends. We hypothesize that they also transpose to the natural ends of Drosophila chromosomes as part of the process by which telomeric DNA is maintained. TART elements have been localized to the telomeric regions and the Y chromosome; HeT-A elements are found at these locations and in the pericentric heterochromatin of the autosomes. These elements are particularly interesting both because they carry out an essential cellular function and because their transposition appears to be highly site-specific but not sequence-specific. A standard mechanism proposed for the integration of non-LTR retrotransposons at internal sites can be adapted for transposition to the terminus. This mechanism for addition of a retrotransposon to a telomere is analogous in its basic features to the addition of short telomeric repeats by telomerase.

Supported in part by a grant from the NIH (GM38259).

F 169 CELL CYCLE CONTROL OF V(D)J RECOMBINATION ACTIVATOR PROTEINS RAG-1 AND RAG-2.

Weei-Chin Lin and Stephen Desiderio, Department of Molecular Biology and Genetics and Howard Hughes Medical Institute, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

The antigen receptor genes of B and T cells are assembled by a series of site-specific DNA rearrangements; the products of the recombination activator genes RAG-1 and RAG-2 are essential for this process. We have observed an association between CDK phosphorylation and Ädegradation of RAG-2 protein (Science 260:953-959), which suggested that expression of RAG-2 may be regulated in the cell cycle. To test this, we have analyzed expression of RAG proteins in lymphoid cells or transfected fibroblasts as a function of cell cycle stage. In immature B cells or thymocytes, expression of RAG-2 protein is restricted to the G0/G1 phases of the cell cycle, despite the presence of RAG-2 RNA at all cell cycle phases. In contrast, levels of RAG-1 protein are similar across the cell cycle. Expression of RAG-2 protein in transfected fibroblasts showed similar cell cycle dependence; as observed in lymphoid cells, RAG-1 protein did not vary appreciably during cell cycle. These observations imply that V(D)J recombination is restricted to G0/G1 through post-transcriptional control of RAG-2 expression, possibly by a CDK-targeted degradation mechanism.

F 170 EVIDENCE FOR THE TRANSPOSITION OF L1 ELEMENTS IN INTERSPECIES HYBRID CELL LINES.

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Five to 10% of the human genome consists of L1 retrotransposon sequences. While there are some documented examples of L1 transposition, detecting new insertions is extremely difficult due to the high copy number of these sequences. To identify *de novo* transposition events, we have used the sequence divergence between human and hamster repetitive elements to detect human L1 elements that have transposed into hamster chromosomes in interspecies mammalian cell hybrids. Genomic libraries in λ were prepared from two human/hamster hybrid cell lines. These libraries have been screened for clones containing both human and hamster DNA using a probe specific for the 3′ end of the human L1 element and hamster inter-*B2* probe generated by PCR. Four clones containing human L1 elements plus hamster repetitive DNA were recovered from screening 117,000 clones from the human/hamster genomic libraries. Restriction mapping and Southern hybridization of these four λ clones showed human L1 sequences flanked by hamster DNA. Sequence obtained from one of these reveals a human L1 element comprising 1.3kb of the 3′ end of a full length element. This was a genuine human L1 element with ~95% homology to the human L1 consensus. This truncated L1 element is flanked by a hamster *B2* repeat on the 5′ side. Insertion into the *B2* repeat appears to have been accompanied by a deletion in the hamster chromosome. PCR amplification showed that this sequence was not a cloning artifact as it was present in the genomic DNA of the cell hybrid. These data indicate that human mobile elements transpose between the chromosome sets in mammalian interspecies cell hybrids. This is the first experimental system in which it is possible to readily demonstrate the active transposition of endogenous human mobile elements.

F 171 CONJUGATIVE TRANSPOSITION:CHARACTERIZATION OF Tn916 INTEGRASE, Fang Lu and Gordon Churchward, Department of Microbiology and Immunology, Emory University School of Medicine, Atlanta, GA30322

Conjugative transposon Tn916 contains two genes, *xis* and *int*, which are implicated in conjugative transposition. However *int* is the only transposon factor essential for integration and excision in an *E.coli* model system. We have cloned the *int* gene into a maltose binding protein(MBP) fusion protein vector. The fusion protein MBP-INT_{3-405} contains almost the whole integrase fused to MBP. We were able to detect excision activity of MBP-INT_{3-405} in vivo, which suggests that the fused integrase retains biological function. Using DNase I footprinting analysis, we found that MBP-INT_{3-405} binds at least four sites on an integrated form of transposon Tn916. Sites C_L and C_R extend about 40bp from the transposon end into the adjacent bacterial DNA. Sites R_L and R_R are located inside the transposon DNA, 50-120bp away from the C sites. We also used the MBP fusion system to express a shortened form of the integrase protein lacking the first 82 amino acids of *int*. The resulting protein(MBP-INT_{82-405}) has completely lost its binding activity to sites R, but retained binding activity to sites C. The different sequence patterns of sites R and sites C suggested that Tn916 integrase contains two domains which bind independently to sites R and sites C. Competition binding experiments show that two DNA fragments containing either site R or site C do not cross-compete with each other for binding MBP-INT_{3-405}. We also observed binding of both fusion integrases to a preferred target site for Tn916 transposition.

F 172 THE TOBACCO RETROTRANSPOSON Tnt1 IS FUNCTIONAL IN THE HETEROLOGOUS HOST *Arabidopsis thaliana*, Hélène Lucas, Jean-Benoit Morel, Michel Caboche, Marie-Angèle Grandbastien, INRA, Laboratoire de Biologie Cellulaire, 78026 Versailles Cedex, France.

We have studied the behaviour of the tobacco retrotransposon Tnt1 in the heterologous host *Arabidopsis thaliana* to determine whether it could be used as a gene-tagging tool in this species.
The use of an LTR-GUS translational fusion construct in transgenic plants allowed us to define the expression pattern of Tnt1 in *Arabidopsis thaliana*. Tnt1 promoter is activated in the roots, the cotyledons and some floral organs of transgenic plants harboring the LTR-GUS construct. It is strongly activated in protoplasts and when the plants are in contact with pathogens or pathogenic elicitors.
The 35S promoter of the Cauliflower Mosaic Virus is expressed almost constitutively in transgenic plants of *Arabidopsis thaliana*. That is the reason why we looked for reverse transcribed copies of the retrotransposon in plants containing a modified Tnt1, in which the U3 region of the LTR was replaced by the 35S promoter of the CaMV. We were able to discriminate the construct introduced in the plants from the amplified copies by using specific primers in a PCR experiment. Our results indicate that Tnt1 is indeed replicated in *Arabidopsis thaliana*. This is the first example of the functionality of a plant retrotransposon in a heterologous host.

F 173 CONTROL OF FLP RECOMBINASE GENE EXPRESSION IN MAIZE CELLS BY SOYBEAN Gmhsp 17.5-E GENE PROMOTER, Leszek A. Lyznik, Lynne Hirayama and Thomas K. Hodges, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

We have demonstrated that a yeast FLP/FRT site-specific recombination system functions in maize and rice protoplasts. FLP recombinase activity was monitored by reactivation of ß-glucuronidase (GUS) expression from vectors containing the gusA gene inactivated by insertion of two FRTs and a 1.31 kb DNA fragment. The stimulation of GUS activity in protoplasts co-transformed with these vectors and plant FLP expression vectors (containing the FLP gene controlled by maize ubiquitin or adh-1 gene promoter) was dependent on both the expression of the FLP recombinase and the presence and structure of the FRT sites. The FLP enzyme could mediate inter- and intramolecular recombination reactions. To test activity of the FLP recombinase gene in maize cells, a chimeric FLP gene was constructed in which expression was controlled by the soybean Gmhsp 17.5-E gene promoter. This gene was co-transformed into maize protoplasts together with a test plasmid containing the neo gene flanked by two FRT sites. Site-specific recombination mediated by the FLP protein should lead to the excision of the neo gene and activation of GUS expression from otherwise promoterless gusA gene positioned behind the neo gene in the original vector DNA. Protoplasts were co-transformed with these vectors and incubated at 42°C for 2 hrs. These protoplasts showed high GUS activity equivalent to 50% of GUS activity observed in protoplasts co-transformed with the constitutively expressed FLP gene under control of the maize ubiquitin gene promoter. In addition, as a result of heat shock treatment of stably transformed and kanamycin resistant maize cells, the activation of gusA gene expression and the decrease, or even elimination, of NPTII activity in some of transgenic maize lines was observed. Experiments are ongoing to provide molecular evidence of a DNA excision process catalyzed by the FLP protein in mazie transgenic cells. If successful, these experiments will indicate that the FLP protein can recognize and subsequently recombine the target sites integrated into plant genomic DNA.

Presenters M-Z

F 200 EVIDENCE FOR RETROTRANSPOSITION OF THE RTVL-H FAMILY OF PRIMATE ENDOGENOUS RETROVIRUS-LIKE SEQUENCES, Dixie L. Mager and Nancy L. Goodchild, Terry Fox Laboratory, B.C. Cancer Agency & Dept. of Medical Genetics, University of British Columbia, Vancouver, B.C., Canada V5Z1L3

RTVL-H elements are found in ~1000 dispersed copies in the genomes of humans and other primates. The most abundant subclass of these elements is 5.7 kb and is transcribed in certain cell types primarily as a 5.6 kb unit length RNA and a 3.7 kb spliced derivative. The proviral-like structure of these elements, including the presence of LTRs and flanking 5 bp direct repeats, suggests that their numbers have increased in the genome through retrotransposition. However, this has not been formally demonstrated for RTVL-H. As one way of determining if these sequences have amplified via a retro-virus-like process, we screened primate DNAs for RTVL-H elements that were transcribed, spliced, reversed transcribed and integrated back into the genome at some point during evolution. To do this, we performed genomic PCR using primers 5' to the splice donor site and 3' to the splice acceptor site. Several PCR fragments in the expected size range to represent spliced elements were found and 11 were analyzed further. Five of these (one human, one orangutan and three gibbon) had the expected sequence for a spliced element, whereas the others appeared to have been generated from elements with similar sized deletions. Presence of the human spliced element is polymorphic, suggesting that its integration was a relatively recent event. This element and the 3 gibbon clones are most probably RTVL-H "processed pseudogenes" as opposed to retrotransposed elements since no evidence for associated intact 5' LTRs could be obtained using PCR. However, PCR and sequencing demonstrated that a 5' LTR is associated with the orangutan element. The finding of a spliced integrated RTVL-H element with intact LTRs strongly suggests that it arose through a retrovirus-like transposition event.

F 201 DEVELOPMENTAL ANALYSIS OF LINE-1 EXPRESSION IN THE MOUSE, Sandra L. Martin, Stephanie A. Trelogan and Dan Branciforte, Department of Cellular and Structural Biology and Molecular Biology Program, University of Colorado School of Medicine, Denver, CO 80262

LINE-1, or L1, is a ubiquitous repetitive DNA family in mammalian genomes that achieved its high copy number through a duplicative transposition mechanism. Transposition appears to occur in both somatic cells and cells destined to form the next generation. The latter is of particular interest because it is responsible for both new germ-line mutations and for the evolutionary maintenance and propagation of L1. Expression of full-length, sense-strand L1 RNA, as well as L1-encoded proteins (ORF 1 and ORF 2), are prerequisites for transposition. For this reason, we screened a variety of mouse tissues at different developmental times for the presence L1 transcripts and ORF 1 protein. Full-length, sense-strand L1 RNA was detected on Northern blots using strand-specific probes from the 5' and 3' ends of the complete, 7+kb structure of L1. ORF 1 protein was detected on Western blots and by immunocytochemistry using an affinity-purified ORF 1 antibody. Expression of discrete L1 transcripts and/or L1-encoded ORF 1 is detected only rarely. Thus, we conclude that L1 expression, and hence transposition, is restricted in most cell types at most times during development. However, expression of L1 RNA and protein can be detected in a few cell types; these belong to both somatic and germ-cell lineages. Included among the former are interstitial cells of the testis and ovary. Among cells that are destined for the next generation, spermatids and spermatocytes of the testis both express L1 ORF 1 protein. However, the truncated form of L1 transcript that is present in spermatids is not likely to be involved in productive transposition of L1. Spermatocytes, in contrast, do express a full-length, sense-strand L1 RNA. This makes the spermatocyte a strong candidate to be a cell type that supports transposition of L1 and its propagation to subsequent generations. The relationship between expression and transposition of L1 and the consequence of somatic cell vs. germ cell transposition will be discussed.

F 202 NOVEL INTRA- & INTERMOLECULAR STRAND TRANSFER & DISINTEGRATION ACTIVITIES OF WILD-TYPE AND MUTANT HIV-1 INTEGRASE,
Abhijit Mazumder, Mark Fesen and Yves Pommier, Laboratory of Molecular Pharmacology, DTP, DCT, National Cancer Institute, Bethesda, MD 20892

The integrase protein of human immunodeficiency virus type one (HIV-1 IN) integrates the double-stranded DNA copies of the viral RNA genome into host chromosomes. When presented with two gapped duplex oligonucleotide substrates (which contain a 5 base single-stranded region flanked by either the U5 or U3 end of the HIV LTR and nonspecific DNA sequences), HIV-1 IN annealed their single-stranded regions, generating a X structure. This novel structure resembles the *in vivo* end product of integration. The HIV-1 IN then catalyzed a reverse or "disintegration" reaction of one or both ends, generating a "Y" structure or a linear 30 base pair double-stranded oligonucleotide, respectively. Unexpectedly, HIV-1 IN could also catalyze a strand transfer on each individual gapped duplex by cleaving the viral DNA end from the molecule and joining the resulting 5'-phosphate end to the 3'-hydroxyl end of the nonspecific DNA sequence, generating a hairpin structure. Deletion mutants of the HIV-1 IN which lacked the zinc-finger and/or the carboxy-terminal DNA-binding domains still annealed and disintegrated the X substrates. However, these mutants were unable to generate significant amounts of the hairpin structure from each gapped duplex. This intramolecular strand transfer activity could be partially restored by mutants which could complement each other *in trans*, consistent with HIV-1 IN acting as an oligomer. In addition to providing novel substrates for biochemical assays, these heretofore undemonstrated activities should allow a more detailed understanding of the integrase enzymology and aid in the development of pharmacologically active compounds.

F 203 DOUBLE STRAND BREAKS AT THE TCRß LOCUS SUPPORT A DOUBLE STRAND CLEAVAGE MODEL FOR V(D)J RECOMBINATION
J. Fraser McBlane[1], David B. Roth[2], Joseph P. Menetski[1], Pamela B. Nakajima[3], Melvin J.Bosma[3], and Martin Gellert[1]. 1) Laboratory of Molecular Biology, NIDDK, NIH, Bethesda, Md 20892; 2) Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030; (3)Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania, PA 19111.

Functional immunoglobulin and T cell receptor genes are assembled from pools of germline gene segments during lymphocyte development by a site-specific recombination mechanism: V(D)J recombination. Short signal sequences are located immediately adjacent to each coding segment and target the recombinase activity, although details of the recombination mechanism remain unclear. Previously, double strand breaks (DSB) at signal sequences were observed within the mouse TCR delta locus in thymocyte DNA (Roth et al, 1992, Cell, **69**, 41-53), consistent with a double strand cleavage model for V(D)J recombination. In SCID mice, which are impaired in the ability to join coding segments, broken molecules terminating in coding ends were found to have a hairpin structure (Roth et al, 1992, Cell, **70**, 983-991).

If double strand breaks are true intermediates in the V(D)J recombination pathway, rather than unique structures at the TCRδ locus, it should be possible to locate similar broken molecules at signal sequences within other rearranging loci. Here we report the finding of double strand breaks at signal sequences within the mouse TCRß locus. By hybridization analyses of thymocyte DNA, double strand breaks were observed adjacent to Dß1 and Dß2 gene segments. Broken molecules terminating within the Jß1 or Jß2 clusters could not be detected, probably due to the large number of Jß segments. No double strand breaks were observed in non-rearranging cell types. Also similar to the TCRδ locus, a significant fraction of Dß1 coding ends in SCID mouse thymocyte DNA are in the form of hairpins.Using ligation mediated PCR, it was found that blunt-ended linkers could ligate directly to signal ends generated by cleavage at Dß1. No nucleotides had been gained or lost from the blunt signal end.

These results support the findings at the TCRδ locus and strengthen the view that double strand breaks are intermediates in the V(D)J recombination mechanism.

F 204 XER-MEDIATED SITE-SPECIFIC RECOMBINATION EFFICIENCY IS UNAFFECTED BY SEQUENCE ALTERATIONS TO THE CER OVERLAP. R.McCulloch, L.Arciszewska, G.Blakely, K.Grant, N.Leslie, G.May, A.Merican, J.Roberts, D.Sherratt. Dept. of Genetics, University of Glasgow, Church Street, Glasgow, Scotland.

The Xer site-specific recombination system of *Escherichia coli* is required for the stable inheritance of multicopy plasmids related to ColE1 and for the normal segregation of the bacterial chromosome during cell division. Recombination of the plasmid-borne recombination sites, for example *cer* of ColE1, requires four host proteins: ArgR, PepA, XerC and XerD. In contrast, recombination of the chromosomal locus, named *dif*, needs only XerC and XerD. The different protein requirements of the two sites are reflected in differences in their sizes (*cer* is approximately 220bp in size, while *dif* is only 33bp), and differences in the types of recombination they can undergo (*cer* sites only recombine intramolecularly whilst *dif* will recombine both intra and intermolecularly). Genetic evidence has implicated ArgR and PepA as accessory factors that ensure the intramolecular nature of Xer-mediated recombination at *cer*. Both XerC and XerD have sequence homologies with the lambda integrase class of recombinases and both bind *cer* and *dif* sites *in vitro*. Consequently we believe that the Xer recombination reaction utilises two recombinases during strand exchange.

Comparison of *cer* and *dif* to other recombination sites in the lambda integrase family suggests a similar organisation; each appears to consist of two recombinase binding sequences flanking a spacer of variable size (8bp in *cer* and 6bp in *dif*) and sequence. We believe that this spacer corresponds to the "overlap" sequence that has been described in other systems. By analogy, therefore, we hypothesise that the points of strand exchange will flank the spacer and Holliday junction recombination intermediates will branch migrate across it. To attempt to address this, we have performed site-directed mutagenesis and created *cer* site variants with sequence alterations in their spacers. Despite all having at least three bp alterations, the variants all recombine proficiently with themselves *in vitro* and *in vivo*. Similar results have been reported in other systems and we conclude that the sequence of this region is not critical to its function. Surprisingly, we find that the variant sites will also recombine efficiently with wild type *cer*. This therefore suggests that either our predictions regarding the positions of strand exchange in *cer*-like sites are incorrect, or the Xer system does not require sequence homology in the overlaps of recombining sites. Furthermore, it is possible that the extent of Holliday junction branch migration in this system may have to be reassessed.

F 205 STRATEGIES OF IS*5* AND IS*150* TO RESPOND TO HOST PHYSIOLOGY. Carl-Georg Meinhof, Karl Vögele, Caroline Welz, Klaus Fuchs, and Bodo Rak, Institut für Biologie III, Universität, Schänzlestr. 1, D-79104 Freiburg, Germany.

IS elements should be able to sense the physiological state of their host and adapt their transpositional activity accordingly. We have discovered two such strategies used by the *E.coli* elements IS*5* and IS*150*.

IS*150* expresses three proteins from two partially overlapping reading frames (*ins150A* and *ins150B*): Ins150A, which is an inhibitor of transposition, Ins150AB, the transposase, which is jointly encoded by both reading frames, and Ins150B, the concentration of which determines the rate of cointegrate formation, but not that of simple transposition. Both, Ins150B and Ins150AB require ribosomal -1 frameshifting within the *insA/B* overlap for their expression[1]. Translation of *ins150B* is stimulated by stress conditions (starvation, ethanol, shift-down). This response is mediated by a new translational initiation mechanism which utilizes signal sequence located downstream of the start codon of *ins150B*. The modulation of InsB expression allows IS*150* to couple its activity to form cointegrates to the host physiology.

IS*5* expresses its transposase at a high level. The C-terminal half of the transposase is rapidly and unspecifically degraded, whereas the N-terminal half is stable. Through this processing reaction IS*5* produces two proteins from one gene: the rapidly degraded transposase Ins5A and the stable N-terminal fragment, Ins5A'. Ins5A' acts as an inhibitor of IS*5* transposition. The half-life of the transposase and thus the amount of transposase relative to that of the inhibitor is dependent on the host physiology. We have found that the IHF and Hfl proteins are involved in modulation of the processing reaction.

In addition to the transposase both IS elements thus synthesize extra proteins encoded by part of the transposase gene. These extra proteins control the activity of the elements. In response to changes in host physiology both elements modulate the concentration of these extra proteins relative to the transposase. This response is achieved by two completely different mechanisms.

[1]K. Vögele et al., 1991, Nucleic Acids Res. 19: 4377-4385.

F 206 ASSEMBLY AND ORGANIZATION OF THE MU TRANSPOSASE TETRAMER

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MuA transposase (MuA) is a large multi-functional protein. It binds specifically to the two ends of Mu DNA to pair them together, cleaves the end sequences, and then promotes strand transfer. The donor DNA cleavage and strand transfer reactions occur within stable complexes between MuA and the DNA: the complex at each reaction stage is called the stable synaptic complex (SSC), the cleaved donor complex (CDC), or the strand transfer complex (STC). In these complexes a tetramer of MuA stably pairs the two ends of the Mu DNA by binding tightly to R1, R2 and L1 sites. Tetramer formation is intrinsic to the MuA protein and is essential for its catalytic activities in the transposition reaction.

In order to analyze the functional organization of the MuA tetramer and its assembly process, we have used several truncated MuA proteins that were labeled with S-35 methionine. A truncated protein (A615) that lacks the C-terminal domain (aa616-663) is unable to carry out the intermolecular strand transfer reaction, which requires activation of the MuA tetramer by MuB (the second phage encoded transposition activator protein) bound to target DNA. However, A615 retains the donor cleavage and intramolecular strand transfer activities. Further, when mixed with wild type MuA, A615 protein inhibits the formation of the intermolecular STC by displacing some monomers within the tetramer. Another truncated protein that lacks the N-terminal domain (aa1-76) which binds to the IAS (enhancer-like element) can not efficiently carry out transposition due to inefficient SSC formation. However, MuB protein and the target DNA can help the formation of the SSC by this protein. Based on the analysis of the activity and composition of mixed tetramers, involving these proteins and wild type MuA, we will discuss how the interactions with the accessory factors, IAS and MuB, are used in the tetramer assembly process and activation of the tetramer for strand transfer.

F 207 INTRON MOBILITY IN BACTERIOPHAGE T4 REQUIRES ENDONUCLEASE PLUS RECOMBINATION AND REPLICATION FUNCTIONS, John E. Mueller, Jonathan Clyman, Mary Bryk† and Marlene Belfort, Molecular Genetics Program, Wadsworth Laboratories, New York State Department of Health, Albany, NY 12201, †Department of Microbiology, Immunology and Molecular Genetics, Albany Medical College, Albany, NY 12208

Intron mobility or homing, a process whereby group I introns are transferred in a site-specific fashion from intron-containing to intronless alleles, is believed to occur via a double-strand-break repair mechanism. The enzymes responsible for the initial cleavage event at the "homing site" are intron-encoded endonucleases. In the case of the *td* intron from bacteriophage T4, the endonuclease I-*Tev*I is responsible for the break in its homing site, an intronless *td* allele. Gel mobility shift assays reveal that I-*Tev*I binding induces a bend within the *td* homing site to facilitate cleavage. Genetic and physical analyses of I-*Tev*I-DNA complexes indicate that the endonuclease interacts with its target across the relatively non-discriminative minor groove in a sequence-tolerant fashion. Such promiscuity in target selection may impact the ability of endonucleases to promote mobility.

In contrast to typical transposases that are involved in DNA exchanges, I-*Tev*I is not required for intron mobility beyond the cleavage event. However, *in vivo* studies have identified T4 activities essential to subsequent repair of the cleaved *td* homing site. These include the *uvs*X recombinase, subunits of a putative 5'-3' exonuclease (gp46 and gp47), T4 single-strand binding protein (gp32) and T4 DNA polymerase (gp43) with associated accessories (gp44, gp45 and gp62). Thus, these genetic analyses reveal that *td* intron mobility requires both recombination and replication functions and suggest that intron mobility is closely linked to recombination-dependent replication in bacteriophage T4. To provide further insight into the recombination mechanism central to intron homing, an *in vitro* homing system is being developed.

F 208 RNA-MEDIATED TRANSPOSITION OF GROUP II INTRON *aI1* IN YEAST: SITE-SPECIFIC INVASION OF MITOCHONDRIAL GENES AT NEW LOCATIONS, Mueller M.W. Allmaier M. Eskes R. Schmidt W.M. and Schweyen R.J., Vienna Biocenter, Institute of Microbiology and Genetics, University of Vienna, A-1030 Vienna Austria

Intron mobility at the RNA-level *via* splicing reversal at allelic (homing) and non-allelic locations (transposition) has been reported to occur *in vitro*. In the living cell, however, only intron homing *via* unidirectional gene conversion has been described. Supposing that intron insertions at non-allelic sites might occur *in vivo*, we speculated that group II splice-site associated macro-deletions in fungal mitochondria DNA (mtDNA) might result from group II intron transposition to new locations followed by recombination. We have applied PCR-techniques to detect this critical, infrequent intermediate in mtDNA populations. Here we report on group II intron *aI1* transposition to non-allelic, splicing compatible locations within the *cox1* gene of yeast mtDNA. The identified integration sites are preceded by motifs reminiscent of the upstream exon *A1*. Sequences flanking intron *aI1* are not co-converted to the insertion sites and *cis*- and *trans*-acting mutations within *aI1* reduce intron mobility below detection levels. These findings suggest the involvement of an RNA intermediate in group II intron transposition. We present an RNA-based model for group II splice site associated deletions observed in fungal mitochondria which invokes intron transposition to IBS-like locations by splicing reversal followed by cDNA synthesis DNA recombination.

F 209 FACTORS ENCODED BY THE BACTERIAL INSERTION SEQUENCE IS*2* SERVE SYNONYMOUS ROLES IN TRANSPOSITION AND PRECISE EXCISION, Richard E. Musso, Tara Hodam and Shu-Yi Wang, Department of Botany and Microbiology, Auburn University, AL 36849.

IS*2* is a 1.3 kb transposable element present in multiple copies in the chromosome of common *Escherichia coli* K-12 strains but not in *E. coli* C. Based on primer extension and *lacZ* operon fusion analyses, IS*2* transcription originates from a single promoter region near one end of IS*2*. Using *lacZ* gene fusions to the various ORFs of IS*2* as well as an *in vivo* T7 expression system, we have demonstrated two translation products: a 14-kDa protein, encoded proximally to the IS*2* promoter region, and a 46-kDa protein, apparently expressed by translational frameshifting from the latter ORF to a partially overlapping, downstream ORF. By constructing derivative strains of *E. coli* C with IS*2* insertions in the *galETK* operon, we have shown that Gal+ revertants occur by IS*2* excision. Both the rate and the precision of excision depend on the 5 bp direct repeats and other sequences flanking IS*2* as well as on the relative levels of IS*2*-encoded factors. Our studies indicated that excision depends on the IS*2*-encoded 46-kDa factor but is inhibited by the 14-kDa factor. Parallel studies on IS*2* transposition also indicated that the 46-kDa protein is required and the 14-kDa protein is inhibitory. The possibility that the 14-kDa protein acts as a transcriptional repressor to inhibit transposition and excision has been contra-indicated by primer extension and operon fusion studies. We suggest that inhibition may be achieved by competition for a common DNA binding site or by association of the 14-kDa and 46-kDa proteins in an inactive complex.

F 210 FINE-RESOLUTION MAPPING OF SPONTANEOUS AND DOUBLE-STRAND BREAK-INDUCED GENE CONVERSION TRACTS, Jac A. Nickoloff, Douglas B. Sweetser, Heather Hough, Jennifer Whelden, Laura Gunn and Melissa Arbuckle, Department of Cancer Biology, Harvard University School of Public Health, Boston, MA 02115

Spontaneous and double-strand break (DSB) induced gene conversion was examined in an allele of the *S. cerevisiae ura3* gene containing nine silent mutations consisting of restriction fragment length polymorphisms (RFLPs). RFLP mutations at approximately 100 bp intervals flanked HO nuclease recognition sequences in recipient alleles carried on *ARS1/CEN4* plasmids. Donor alleles were present at the normal chromosome V locus. DSBs were delivered to recipient alleles *in vivo* by HO nuclease regulated by the *GAL1* promoter. Crossovers were not detected since the resultant dicentric chromosomes were lethal. Plasmids with recombinant *ura3* alleles were transferred to *E. coli* for analysis of gene conversion tracts. The analysis of 439 recombinant products from 5 crosses revealed several new features of gene conversions. DSB-induced gene conversion tracts are asymmetric: RFLPs 5' to a 5' HO site were more likely to be converted than equidistant RFLPs 3' to a 5' HO site, and vice versa for RFLPs flanking a 3' HO site. These asymmetries were independent of the orientation of HO sites. In contrast, spontaneous tracts are symmetric. The majority of DSB-induced conversion tracts extend unidirectionally from HO sites, but most spontaneous tracts are bidirectional. DSB-induced tracts averaged 200 bp in length, while spontaneous tracts in an identical cross were 40% longer. Conversion tracts greater than 350 bp were found in only about 14% of DSB-induced products, but in 45% of spontaneous products. Most tracts were continuous, consistent with gap repair events, but 3% of spontaneous and DSB-induced tracts were discontinuous, suggesting that heteroduplex DNA (hDNA) is involved in at least this fraction of events. Four additional crosses were performed in which gap repair initiated from HO sites could not produce functional *URA3* genes. In these crosses, all DSB-induced products exhibited discontinuous tracts. Discontinuous tracts were also frequent among spontaneous products in crosses with mutations in donor and recipient alleles separated by only 31 bp. These results indicate hDNA is usually involved when very close markers are not coconverted, and that mismatch repair tracts may be as short as 30 bp. This research was supported by Public Health Service award CA 55302 from the National Institutes of Health.

F 211 *HOBO* AND *HOBO*-RELATED ELEMENTS IN INSECTS, David A. O'Brochta, William D. Warren, Kenneth J. Saville and Peter W. Atkinson*, Center for Agricultural Biotechnology, Maryland Biotechnology Institute, University of Maryland, College Park, MD 20742, * Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Canberra, ACT 2601, Australia

The *hobo* element is a short, inverted-repeat type transposable element originally isolated from *Drosophila melanogaster* and subsequently found to have similarities to the *Ac* element from maize and the *Tam3* element from snapdragons. *Ac* and *Tam3* have the interesting property of being able to function in heterologous plant species and are potentially useful as gene tagging agents. We have identified similar elements in a number of insects distantly related to *D. melanogaster*. Like *Ac* and *Tam3*, *hobo* and *hobo*-related elements may be useful as gene vectors and gene tagging agents in insects other than *D. melanogaster*. We refer to this group of elements as the *hAT* (*hobo*, *Ac*, *Tam3*) family of transposable elements.

The *Hermes* element, a member of the *hAT* family of transposable elements, from the housefly, *Musca domestica*, is approximately 66% identical to *hobo* at the nucleic acid level and has 12bp terminal inverted repeats that differ from those of *hobo* by on 2 nucleotides. The putative *Hermes* transposase region is approximately 70% similar to *hobo* transposase at the amino acid level. The *Hermes* system appears functionally related to the *hobo* system and to be capable of driving excision but not transposition of *hobo*. *Hobo* can transpose in *M. domestica* if *hobo* transposase is expressed *in trans* and transposition of *hobo* in this species is indistinguishable from that observed in *D. melanogaster*. Only sequences delimited by *hobo* terminal sequences transpose, and insertion results in an 8bp duplication of the target site. *Hobo* transposition also shows a high degree of insertion site preference and some of the factors influencing target site selection will be discussed.

F 212 REGULATION OF GYPSY EXPRESSION AND TRANSPOSITION BY THE DROSOPHILA FLAMENCO GENE, Alain Pélisson(1,2), Nicole Prudhomme(1), Patricia Smith(2), Alain Bucheton(1) and Victor G.Corces(2); (1):Centre de Génétique Moléculaire, C.N.R.S., 91190 Gif Cédex ,FRANCE, (2):Dept of Biology, Johns Hopkins Univ., Baltimore, MA 21218, USA.

Gypsy is one of the Drosophila transposable elements that are the most similar to endogenous retroviruses. It is 7.5 kb long and has two LTRs which are 482 nucleotides long. It contains three large open reading frames. ORF1 presumably encodes a polypeptide related to GAG proteins of retroviruses, and ORF2 encodes a potential polypeptide showing similarities with viral reverse transcriptases, proteases and endonucleases. Moreover, the putative product of ORF3 discloses transmembrane features of viral envelope polypeptides.

Most laboratory stocks of Drosophila melanogaster contain a few euchromatic strain-specific insertions of this element (0 to 5) and many defective copies located in pericentromeric heterochromatin. However, some strains contain many more euchromatic copies (20 to 50), suggesting that deregulation can sometimes occur.

We have identified a gene, flamenco (flam), involved in the control of gypsy activity. Crosses between females homozygous for a mutant allele of flam and males carrying the female sterile dominant mutation ovoD1 produce up to 10-15 % daughters with one ovary. They result from mutation of the dominant towards a nul allele, due to insertion of gypsy into the ovo gene occurring early in development. This indicates that ovo is a hotspot of insertion of gypsy.

Gypsy transposition is correlated with increased accumulation of gypsy transcripts in flam females. More specifically, a new 2 kb transcript is only found in homozygous flam females. This is a spliced message similar to the one used by retroviruses to express the envelope product of their ORF3. Hence the possibility that, just like retroviruses, an extracellular particule is required for gypsy to achieve its whole retrotransposition cycle in flies.
Analysis of the regulatory mechanisms involved by the flam gene will be presented.

F 213 INHIBITION OF HIV-1 INTEGRASE BY FLAVONES AND RELATED COMPOUNDS, Yves Pommier, Mark R. Fesen, Jessie Yung and Kurt W. Kohn, Laboratory of Molecular Pharmacology, DTP, DCT, National Cancer Institute, Bethesda, MD 20892.

The inhibition of HIV-1 integrase by flavones and related compounds was investigated biochemically and by means of structure-activity relationships. Purified enzyme and synthetic oligonucleotides were used to assay for 3 reactions:
(1) processing of 3' termini by cleavage of the terminal dinucleotide;
(2) strand transfer which models the integration step; and
(3) 'disintegration' which models the reversal of the strand transfer reaction.
Inhibitions of all 3 reactions by flavones generally occured in parallel, but caffeic acid phenethyl ester (CAPE) appeared to inhibit reaction 2 selectively. CAPE however inhibited reactions 1 and 3 effectively when preincubated with the enzyme, suggesting that this compound differs from the flavones primarily in requiring more time to block the enzyme. The core integrase fragment consisting of amino acids 50-212 retains the ability to catalyze reaction 3, and flavones and CAPE retained the ability to inhibit a putative Zn-finger regions which is deleted in this fragment hence is probably not the target of inhibition.
Inhibition by flavones usually required the presence of at least one ortho pair of phenolic hydroxyl groups and at least one or two additional hydroxyl groups. Potency was enhanced by the presence of additional hydroxyl groups, especially when present in ortho pairs or in adjacent groups of three. Inhibitory activity was reduced or eliminated by methoxy or glycosidic substitutions or by saturation of the 2,3 double bond. These structure-activity findings for flavones were generally concordant with those previously reported for reverse transcriptase and topoisomerase II. These findings are discussed in the context of a review of the effects of flavones on various enzymes and the possible mechanisms of inhibition.

F 214 Tx; TRANSPOSABLE ELEMENTS OF *XENOPUS LAEVIS*

Geneviève Pont-Kingdon, Shawn Christensen, Enxi Chi, Norma Wills and Dana Carroll, Department of Biochemistry, University of Utah School of medicine, Salt Lake City, UT 84103.

Tx1L and Tx2L are two distinct families of non-LTR retrotranposons from *Xenopus Laevis*. Like the ribosomal DNA insertions of insects and several elements found in the spliced-leader genes of trypanosoma, TxL elements appear to be site-specific. They are only found inserted into the tandemly repeated sequences (PTR-1) of another type of transposable element, Tx1D and Tx2D. TxL elements encode two ORFs that overlap by 1 nucleotide. The putative protein encoded by ORF1 (775 aa) contains a "zinc finger" motif comparable to the CCHC box found in retroviral gag proteins. The second ORF (1304 aa) encodes a putative protein that shows strong homology to the reverse transcriptase domain of the pol gene of retroviruses.

Site-specific transposition of Tx1L is being tested by injection of donor and target plasmid into Xenopus oocytes. These germ line cells can accommodate nanogram quantities of injected material and they contain active metabolic machinery that allows transcription and translation of appropriately designed substrates. A donor substrate has been engineered which will allows, upon injection, the transcription of a full length element. This RNA is believed to represent the RNA intermediate of retrotransposition. A target plasmid containing a complete PTR-1 sequence provides the target site for Tx1L insertion. Both ORFs have been epitope tagged and placed in expression vectors. Injection into oocytes of RNA made *in vitro* from one of the ORF2 construct is followed by synthesis of proteins detectable with an anti-epitope antibody. Experiments are underway to determine if the ORF2 proteins have activity such as reverse-transcriptase, RNAse H and endonuclease. Using translation in reticulocytes, we are examining the potential from frame shifting of the sequence at the junction of the 2 ORFs. Preliminary data indicate that no frameshift occurs and that expression of ORF2 might require re-initiation of translation.

F 215 TRANSPOSASE OLIGOMERIZATION AND ITS ROLE IN REGULATING Tn5 TRANSPOSITION,

W. S. Reznikoff, M. D. Weinreich, A. Gasch, N. de la Cruz, L. Mahnke and T. Wiegand, Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

Transposase oligomerization is thought to be a critical step in transposition; transposase monomers bound to the ends of Tn5 dimerize to form a transposing complex. However, premature dimerization (prior to end sequence binding) is believed to be an important regulatory event that inhibits transposition and is the basis for the *cis* active nature of transposase. The role of transposase oligomerization in Tn5 transposition regulation is suggested by the following: 1- Transposase acts in *trans* as an inhibitor of transposition. 2- The inhibitor (an N-terminal deletion of the transposase) forms mixed oligomers with transposase. A domain involved in transposase oligomerization is thought to be located near residue 372 since mutation LP372 results in a *trans* active transposase with reduced inhibitory activity and reduced sensitivity to inhibition. In addition C-terminal deletions into this sequence encode proteins that form monomer (instead of the wild type oligomer) transposase complexes with DNA.

F 216 IS231 FROM BACILLUS THURINGIENSIS: STRUCTURAL AND FUNCTIONAL ORGANIZATION.

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IS231 defines a family of eight insertion sequences (IS231A to F, V and W) originating from the Gram-positive entomopathogen Bacillus thuringiensis. These elements display similar transposases with an overall 40% identity. Comparison with all the prokaryotic transposable elements sequenced so far reveals that the IS231 transposases share two conserved regions with those of 35 other insertion sequences of wide origins. These sequences, defining the IS4 family, have a common bipartite organization of their ends and are divided into two similarity groups. Interestingly, the transposase domains conserved within this family display similarities with the integrase domain shared by the transposases of the IS3 family and integrases of retroelements.

In its original host, IS231A is found inserted into the terminal inverted repeats of the transposon Tn4430. Demonstration of its transposition in Escherichia coli has confirmed that the Tn4430 ends behave as insertion hot spots for IS231A. Sequence analysis of numerous target sites reveals that IS231A inserts preferentially at sites matching the 5'-GGG(N)5CCC-3' consensus. However, this consensus is not the only determinant of IS231A insertion specificity. Although both Tn4430 ends have identical sequences, one is strongly preferred to the other. This preference is determined by the flanking regions. DNA conformation analysis by polyacrylamide gel electrophoresis show that the preferred target site of IS231A is organized as a symmetrical "S"-like structure where the central consensus is flanked by DNA segments curved in opposite directions.

F 217 FORMATION OF ACTIVE COMPLEXES BY MUA PROTEIN WITH SHORT LINEAR DNAs

Phoebe Rice, Harri Savilahti, and Kiyoshi Mizuuchi, NIDDK / NIH, Bldg 5/Rm 237, Bethesda, MD 20892.

Transposition of phage Mu is catalyzed by the MuA protein. This protein facilitates the sequence-specific formation of nicks at the ends of the Mu genome in the donor DNA, and the subsequent strand transfer reaction in which the Mu ends become covalently joined to the target DNA.

The catalytic form of the MuA protein is a tetramer bound to both ends of the Mu DNA. Under standard reaction conditions, formation of the active complex requires supercoiling of the donor DNA, divalent cations, host proteins HU and IHF, and the presence of an internal activating sequence (the IAS). After the nicking reaction has taken place, the complex becomes more stable and supercoiling is no longer required to maintain it.

We have investigated the requirements for complex formation using short DNA oligonucleotides carrying the R1 and R2 MuA binding sites from the right end of the Mu genome. We have determined the minimum length necessary for the DNA. If the DNA used is pre-nicked as in the cleaved donor complex (the CDC), or branched as in the strand transfer complex (the STC), competitor DNA-resistant complexes form readily in the absence of DMSO. We have shown that the complexes with nicked DNA are competent for strand transfer, while the complexes formed with the branched oligos catalyze removal of the branch segment corresponding to the target DNA part. While this reaction mimics reversal of the strand transfer reaction, product analysis indicates that each half of the "target" is released as an intramolecular hairpin rather than as two halves joined together to restore the original continuous target sequence.

We have also begun structural studies of the MuA protein. Crystals that diffract to very low resolution have been obtained with a fragment of the protein, and crystallization experiments with protein-DNA complex are underway.

F 218 EFFICIENT TEMPLATE SWITCHING OF A THERMOSTABLE DNA POLYMERASE DIRECTED BY DOWNSTREAM ANNEALED OLIGONUCLEOTIDES, Sam J. Rose, Rajesh Patel, Yan Chen, Nurith Kurn and Ted Ullman, Nucleic Acids Group, SYVA Co., Palo Alto, CA 94303

Template switching of DNA polymerases upon primed DNA has been known since 1964 (Schildkraut, C. L. et al., (1964) J. Mol. Bio. 9, p. 24) but the factors that might prompt such switching are unknown. We have discovered a method of designing oligonucleotide sequences which hybridize downstream of primed templates and induce efficient switching of Pfu Polymerase from its original DNA template to a second DNA template. The sequence elements and reaction conditions required for template switching have been explored. The method is broadly applicable to a variety of templates including short synthetic sequences, plasmids, and bacterial or eucaryotic genomic DNAs.

F 219 EXPRESSION OF A YEAST INTRON-ENCODED ENDONUCLEASE IN MAMMALIAN CELLS ENHANCES HOMOLOGOUS RECOMBINATION IN VIVO. P. Rouet, F. Smih-Rouet and M. Jasin, Sloan-Kettering Institute, 1275 York Ave., New York, NY 10021. FAX (212) 717-3317

Double strand breaks (DSB) in DNA introduced into mammalian cells and in chromosomal DNA in mitotic and meiotic yeast are known to enhance homologous recombination. In order to study the effects on recombination of an *in vivo* DSB in mammalian chromosomal DNA, we have expressed a site-specific endonuclease. We used the universal code equivalent for the yeast mitochondrial intron encoded enzyme I-*Sce* I, which was provided by B. Dujon (Pasteur Institut, France). We have built a mammalian expression vector, pCMV-I-*Sce* I which harbors a cytomegalovirus promoter and an SV40 origin of replication. The I-*Sce* I ORF was modified by adding sequences encoding a nuclear localization signal and a hemagglutinin epitope tag.

Our initial assay for *in vivo* cutting and enhanced recombination uses an extrachromosomal recombination assay, since this form of recombination is very efficient in mammalian cells. The assay utilizes RSVCAT plasmid substrates consisting of overlapping chloramphenicol acetyltrasferase (CAT) gene fragments transiently transfected into cells (Jasin and Liang, *Nucleic Acids Res. 19*, 7171-7175). When the plasmid substrates are cut *in vitro*, approximately 10% of the introduced DNA is recombined, as assayed by CAT activity and Southern analysis. Recombination is much less efficient if the DNA is uncut. Therefore, extrachromosomal recombination is sensitive to the presence of DSBs. The RSVCAT plasmids were modified by the insertion of a synthetic I-*Sce* I site at the end of the homology repeats. Co-transfections were carried out in COS1 cells with pCMV-I-*Sce* I and the CAT DNA substrates, along with a control *pgk-lacZ* plasmid to monitor the transfection efficiency. We observed a strong increase of CAT activity in co-transfections of pCMV-I-*Sce* I with CAT substrates containing the I-*Sce* I site versus plasmids not containing the site. Southern analysis clearly showed cutting *in vivo* of the DNA substrate, as well as the appearance of the recombined products due to intermolecular and intramolecular recombination events. These extrachromosomal recombination experiments have shown that it is possible to generate a DSB *in vivo* in mammalian cells and that the breaks can enhance recombination.

We are now developing chromosomal substrates for I-*Sce* I in order to determine if gene targeting and other events involving the chromosome can be enhanced by expression of the endonuclease.

F 220 *IN VITRO* ACTIVITIES OF Tn*552* TRANSPOSASE AND RESOLVASE, Sally-J. Rowland and David Sherratt, Institute of Genetics, Church St., Glasgow, Scotland, G11 5JS.

Tn*552* is a 6.5 kb transposon from *Staphylococcus aureus* that encodes three putative transposition proteins: a site-specific recombinase, a transposase and a potential nucleotide-binding protein. These have been overexpressed and purified. The *in vitro* activities of the two recombination proteins are reported here.

The putative transposase (p480 or "**pA**") is related to the integrase function of retro-elements and to the transposases of IS3 family elements and Tn7; these elements also have in common the 3' terminal dinucleotide CA. pA has been purified both as encoded by *orf480* and with an N-terminal "histidine tag". Gel retardation and DNAse I protection analyses have demonstrated that pA binds specifically to, and induces a bend in, two directly-repeated 23 bp motifs at each end of Tn*552*. pA also exhibits Mn^{2+}-dependent strand-transfer activity *in vitro* with DNA fragments that have pre-cleaved 3' ends generated by restriction endonuclease digestion. The exposed 3' ends are specifically transferred to target sequence.

The site-specific recombinase, **BinL**, acts at a recombination site, *resL*, located upstream of its gene in Tn*552*. BinL is a member of the Tn3 family of resolvases and DNA invertases, as are BinR and Bin3, the genes for which flank a hotspot for Tn*552* insertion in the *S.aureus* plasmid pI9789. Although initially characterized as DNA invertases *in vivo*, both BinL and BinR exhibit strict resolvase activity *in vitro*, recombining substrates that contain two complete *res* sites in direct repeat to generate simple catenenes; no activity was detected with any other substrate.

F 221 CIN MEDIATED INVERSION AT SECONDARY SITES ON THE Escherichia coli CHROMOSOME, Frank Rozsa, Rosemarie Hiestand-Nauer and Werner Arber, Biozentrum, Dept. of Microbiology, University of Basel, Switzerland CH-4056

The C segment on the bacteriophage P1 genome can be inverted by the adjacent *cin* gene product, resulting in a rearrangement of tail fiber structural genes which control the host range of the phage. The two normal crossover sites (*cix*) for inversion consist of two imperfect 12 bp inverted repeats, separated by a dinucleotide core. Studies using inversion tester plasmids containing only one crossover site have shown that inversion can occur rarely at secondary or "quasi" *cix* sites (*cixQ*) that have a lower homology to the normal *cix* sites.

Cin-mediated inversion at chromosomal quasi-sites on *E. coli* N100 (*recA galK str*) was tested by integrating an ori-less inversion tester plasmid into the attB site using lambda integrase. In the presence of Cin, a color change indicated that a promoterless *gal*K gene was being expressed after a putative inversion. Rearrangements were found at two independent *cixQ* sites by genomic southern blot analysis.

The first *cixQ* site (*cixQlac*) corresponds to an inversion of 2.4 kb from the primary site and is located on the tester plasmid itself, even though the inversion occurred after the plasmid was integrated into the chromosome. The segment containing the *cixQlac* site was cloned back from the chromosome and sequenced. The chromosomal crossover site was identical to that found on the inversion tester plasmid. This *cixQlac* site was the preferred location for inversion using plasmid-based studies, despite the presence of other *cixQ* sites with a higher homology to the normal *cix* sites. The reason for the relative efficiency (versus other *cixQ* sites) remains unclear.

The other *cixQ* site (*cixQompA*) was mapped to the *omp*A gene, representing an inversion of over 215 kb. Sequencing *cixQompA* confirmed the crossover in *omp*A. The inversion divides the *omp*A gene into two segments, separated by 215 kb. The *cixQompA* site has a much better match to the consensus *cix* sequence than all previously described secondary sites. Since the *omp*A and *gal*K promoters are quite similar, detection of *gal*K expression is excellent. Inversion at this exact site has occurred on twice in independent studies and suggests that distance is less relevant than homology for this type of site-specific recombination. These chromosomal studies may more accurately reflect on a mechanism of inversion which have until now been restricted to plasmid based studies.

F 222 EXPRESSION AND V(D)J RECOMBINATION ACTIVITY OF MUTATED RAG PROTEINS

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The products of the RAG-1 and RAG-2 genes are essential for the recombination of the DNA encoding the antigen receptors of the developing immune system. Little is known of the specific role these genes play. We have explored the sequences encoding the mouse RAG genes by introducing deletions and local sequence changes. Activity of the resulting constructs was measured in fibroblasts using the plasmid recombination assay. We find that a RAG-1 gene with 40% of the coding region deleted still retains its recombination function. In addition, of a series of ten small deletions within the strongly conserved remaining 60% of the coding region, only one was active. The RAG-1 protein generated from this expression system is transported to the nucleus and is degraded with a 15 minute half-life. The same is true of the most extensive deletions studied. The functionality of the deleted proteins is discussed with relation to an alignment of RAG-1 sequences from five animal species.

A large portion of the RAG-2 gene can also be removed while retaining function. We find that high activity is obtained from a construct that deletes 25% of the coding region, and low activity can be detected from a construct that deletes 35% of the coding region.

F 223 THE FATE OF DELETED DNA PRODUCED DURING PROGRAMMED GENOMIC DELETION EVENTS IN *TETRAHYMENA THERMOPHILA*.

Serge V. Saveliev, Michael M. Cox, Department of Biochemistry, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706

Thousands of DNA deletion events occur during macronuclear development in the ciliate *Tetrahymena thermophila*. Using a sensitive PCR method, we analyzed the structure of deleted sequences released during this process. In two deleted genomic regions, designated M and R, the eliminated sequences form circles. The circular forms occur at very low level in conjugating cells, but are surprisingly stable. These circles first appear about 10 hours after cell mating, and they can be detected for many subsequent hours. Sequencing analysis showed that many of eliminated sequences have reflected a precise deletion in the M and R regions. However, the remaining circles were either smaller or larger. Larger circles contained varying lengths of sequences (up to nine hundred bp) derived from chromosomal DNA surrounding the eliminated region. Some of the large circles had internal deletions, corresponding to precise chromosomal deletions. The junction of almost all the circles occured within short stretches of homology (ranging up to 15 bp). The inclusion of flanking DNA in the released recombination product is not reflected in the chromosomal joints present in mature macronuclear DNA. The macronuclear junctions were precise and structured as reported previously (Yao, 1989), although we detected a new junction in the M region. A model based on these results is presented that includes replication of chromosomal sequences flanking the eliminated region and generation of long 3' single-stranded ends during the deletion event.

F 224 ANALYSIS OF BARLEY RETROTRANSPOSON BARE-1 EXPRESSION,

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The copia-like retrotransposons, or at least sequences similar to reverse transcriptase, appear to be ubiquitous in plants. We have isolated and sequenced a complete retroelement from barley (Hordeum vulgare L.), named BARE-1, with all the features of a copia-like retrotransposon. The long terminal repeats (LTRs) of BARE-1 are 1829 bp, contain 6 bp inverted repeats at their ends and flanked by 4 bp direct repeats in the host DNA. Between the long terminal repeats is an internal domain with a derived amino acid sequence of 1301 residues, with well conserved gag, aspartic proteinase, endonuclease, and reverse transcriptase/RNase H domains. We have detected transcripts for BARE-1 in several tissues as well as in protoplasts. Retroelement LTRs should be functional as promoters and in transcription termination. In order to analyze BARE-1 expression, we have made reporter-gene constructs containing uidA (β-glucuronidase) or luc (luciferase) fused to the intact LTR and to a series of LTR deletions. These were transferred by electroporation into barley leaf protoplasts, which were then incubated for 12-48 h and assayed for the reporter enzyme. The BARE-1 LTR is able to drive reporter-gene expression in barley protoplasts, and the deletion analyses have defined regions critical regions for promoter function. One domain was shown to partially restore function of a deleted CaMV 35S promoter in protoplast assays. Partial cDNA clones for BARE-1 have now been isolated; this confirms that this retroelement is indeed transcribed in the intact plant. We are currently analyzing both the element's expression and its evolution.

F 225 DISSECTING LAMBDA INTEGRASE-MEDIATED RECOMBINATION BETWEEN TWO *attL* SITES,

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Lambda integration (*attP* x *attB*) and excision (*attL* x *attR*) are mediated between targets known as attachment (*att*) sites by a recombinase, Int, in conjunction with one or several accessory proteins. Here, we present studies of a simpler reaction of the lambda system, one that is carried out between two *attL* sites by Int alone.

High affinity binding sites for Int flank the strand exchange, or core, region of *attP*, *attL*, and *attR*. These "arm" sites are thought to deliver Int to the core, which has a low affinity for Int. In *attL*, Int is delivered to the core in two distinct ways. In the first, Int makes *intra*molecular bridges between its binding sites on a single *attL* molecule, contingent on the presence of a bending protein (IHF, HU, and HMG proteins all serve this role). Landy and colleagues have shown that this delivery mode is used in L x R recombination. In the second delivery mode, Int makes *inter*molecular core contacts in the context of a bimolecular complex, in which two molecules of *attL* are aligned by several protomers of Int. We have shown that this delivery mode is used in recombination between two *attL* sites *in vitro*, a reaction mediated by Int alone and inhibited by IHF. Unaided L x L recombination is inefficient both *in vitro* and *in vivo*. Although IHF can stimulate *in vivo* recombination 5-10 fold, the stimulation had not been reproduced *in vitro*. We now report that IHF can stimulate the *in vitro* recombination between two *attL*s only if they are defective in the core-proximal Int arm binding site. The efficiency of this *in vitro* IHF-stimulated L x L reaction is comparable to the *in vivo* IHF-stimulated reaction, and the *attL* variants that are active *in vitro* are also active *in vivo* for L x L recombination, but not for efficient L x R recombination. We are characterizing the IHF-stimulated L x L reaction in parallel with the Int-only reaction, and comparing *in vivo* and *in vitro* conditions in order to better understand the various active conformations of the *attL* site.

F 226 PURIFICATION AND PROPERTIES OF THE NATIVE IS1 TRANSPOSASE, Marie-Claude SERRE, Catherine TURLAN and Michael CHANDLER, Laboratory of Molecular Genetics and Microbiology (UPR9007 du C.N.R.S.),118 Rte. de Narbonne, 31062 Toulouse CEDEX, FRANCE.

IS1, 768bp, is one of the smallest known bacterial insertion sequences. It is highly compact and encodes two consecutive open reading frames (*ins*A and B) whose integrity is essential for transposition. The product of the upstream frame, InsA, binds specifically to the ends of the element and has been shown to act as a repressor of gene expression and as an inhibitor of transposition. The transposase, InsAB', is a fusion protein produced by a programmed -1 translational frameshift between the upstream and downstream (*ins*B) frames. Constitutive production of this transframe protein in the absence of InsA leads to levels of transposition of 1% in vivo.

We report a simple procedure for purification of the transposase in native form and an analysis of its biochemical properties. The endonucleolytic properties of InsAB' will be presented and discussed.

Inspection of the primary amino acid sequence of the carboxy-terminal part of InsAB' reveals the presence of a signature resembling that of the phage λ integrase family of proteins. Mutants at each of the three phylogenetically conserved residues (His, Arg and Tyr) have been constructed and their behavior investigated *in vivo*.

F 227 TERMINAL TRANSPOSITION OF TART, A TELOMERE-ASSOCIATED RETROTRANSPOSON IN DROSOPHILA MELANOGASTER, Fang-miin Sheen, Robin Ganesan, Leigh A. Tolar, Robert W. Levis, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104

We report a new family of non-LTR retrotransposons called TART (Telomere-Associated Retrotransposon) in *Drosophila melanogaster* with an unusual chromosomal distribution. A probe for TART hybridized exclusively to a subset of the tips of polytene chromosomes, and that subset differed among strains. This suggests that TART elements are transposable and are preferentially targeted for telomeric regions. Southern blot analysis indicates that there are also many cross-hybridizing sequences on the *Y* chromosome. A 5' truncated copy of TART was originally discovered at the terminus of chromosome *3R* in a walk from the subterminal insertion of a *P* element. Complete copies of TART have been cloned from the DNA of Oregon R females, where most if not all of them are believed to be telomeric. There are two major subfamilies of TART elements, TART-A and -B. Each subfamily has two ORFs, as do most other non-LTR retrotransposons. The ORFs of TART-A are >90% identical to the corresponding ORFs of TART-B. ORF 1 (970-1043 aa) is 2-3 times longer than that in most other non-LTR retrotransposons, but contains 3 cys-his motifs characteristic of ORF 1 of non-LTR retrotransposons and of the *gag* polypeptides of retroviruses. ORF 2 (1080-1084 aa) is similar to those of other non-LTR retrotransposons and has motifs conserved in the reverse transcriptases of retroviruses and in the capsid-like domain in non-LTR retrotransposons. Each family has a long (3.2-5.4 kb) 3' untranslated extension, ended with an oligo-A tract. In contrast to the ORFs, most of the 3' untranslated regions of TART-A and -B are dissimilar. Two regions are closely related: the 0.5 kb adjacent to ORF 2 and 97 bp adjacent to the 3' oligo-A tract. The sequence of TART is very different from that of another telomere-associated element, HeT-A, which also ends with an oligo-A tract. The only sequence similarity we recognize is between a 482 aa region of ORF 1 of TART and a 544 aa region of the single ORF associated with some HeT-A elements. Terminal additions of DNA can occur onto the ends of broken chromosomes. We have found two such cases in which a TART-B element is joined to the broken end. This is evidence that, as has been shown for HeT-A, TART elements can transpose to the termini of broken chromosomes. Studies on an intact telomere indicated that TART elements can also transpose to native telomere ends (see poster by Levis *et al.*). We postulate that terminal transpositions of TART, HeT-A, and possibly other telomere-associated retrotransposons play a role in maintaining telomeres.

F 228 SITE SPECIFICITY AND INSERTION OF A MOBILIZABLE *BACTEROIDES* INSERTION ELEMENT, NBU1, IN *BACTEROIDES* AND *E.coli* HOSTS, Nadja B. Shoemaker, Gui R. Wang, and Abigail A. Salyers, Department of Microbiology, University of Illinois, Urbana, IL 61801

Conjugal tetracycline resistance transposons (TET elements) found in the human colonic *Bacteroides* species are capable of tetracycline inducible self-transfer, mobilization of co-resident plasmids and excision and mobilization of at least two classes of mobilizable insertion elements. One class of the insertion elements called nonreplicating *Bacteroides* units, NBUs, are grouped because they share a small (<2kbp) region of homology. NBU1 has been trapped on a shuttle vector and shown to be mobilized by the TET elements and by IncP plasmids. The region of NBU homology is not part of the mobilization or the insertion regions of NBU1 but may be the region recognized by regulatory functions on the TET elements necessary for the excision of the NBU1 from the chromosome to form the circular mobilization intermediate. The attP of the NBU1 and the attB of the primary insertion region in *B. thetaiotaomicron* have been sequenced. The NBU1 insertion region does not resemble that of the TET element, XBU4422. NBU1 inserts within a 14bp sequence contained on attP and attB near the 3' end of a gene that is conserved in all 10 of the human colonic *Bacteroides* species. NBU1 can also insert into secondary site(s) in both *Bacteroides* and *E. coli* hosts, resulting in 4bp and 2bp terminal repeats respectively. Sequence analysis of these sites indicate that both specificity and mechanism of insertion may be different from the primary site insertion. Since insertion (but not the excision) of the NBU1 into its target sites is independent of the TET elements, a system to study the insertion requirements for NBU1 into the two types of sites may be possible in *E. coli*.

F 229 MaLRS ARE PROBABLY ACTIVE LTR-TRANSPOSONS IN RODENTS, Arian F.A. Smit and Arthur D. Riggs, Department of Biology, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

MaLRs are a family of mammalian LTR-transposons represented by THE1 and MST elements in primates, ORR1 and MT elements in rodents, and the ancestral MLT1 elements in all mammals (1). The length of a full element is 2-2.5 kb, but copies are more often found as 350-500 bp solitary LTRs. The general structure of MaLRs resembles that of LTR-retrotransposons, but no homology can be found to reverse transcriptase in the internal sequence. Distribution of MaLRs started more than 100 million years ago (MYA), and appears to continue to this day in some mammals. The youngest elements in primates (THE1a) seem to have ceased to transpose about 25 MYA, but some copies of MT and ORR1 elements in mouse are of very recent origin, as inferred from their virtual identity to their consensus sequence. Multiple clones have been obtained of these most recently distributed copies, and their sequences will be reported. The ORR1 sequence has good homology to that of the primate THE1, which we found to contain a 1350 bp open reading frame encoding a protein that lacks significant homology to any of the proteins presently in the databases. However, the MT internal sequence has a completely different structure, and the homology of the MT-LTR to the ORR1-LTR may thus be the result of an ancient recombination event. Nevertheless, a similar integration site specificity and the apparent transposition of some ORR1-MT hybrid elements suggest that MT elements use the same mode of transposition as other MaLRs.

1) Smit, A.F.A. Identification of a new, abundant superfamily of mammalian LTR-transposons. Nucleic Acid Res. 21, 1863-1872.

F 230 DEVELOPMENT OF AN INTEGRASE DEFICIENT RETROVIRAL TRANSDUCTION SYSTEM; NEW STRATEGIES FOR LOCUS TARGETING.

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Retroviruses provide an attractive feature that might be useful for gene targeting. Target cells within the host range are infected with a high efficiency. Delivery of DNA into mammalian cells can be made more efficient than permitted by the widely used physical methods through the use of retroviral vectors.

By impairing the *cis*-sequences for integrase (IN) mediated integration in a retroviral vector Ellis and Bernstein [Mol. Cell. Biol.(1989) 9:1621-1627] demonstrated gene targeting by homologous recombination. The accessibility of the proviral DNA in the aberrant integration complex may however vary with specific *cis* or integrase mutations.

The establishment of an integrase deficient retroviral packaging cell line is in progress. The purpose is to abrogate the normal integrative pathway without interference with the synthesis of the viral DNA. In future experiments an integrase deficient packaging cell line might be useful for high efficiency and low copy delivery of substrate vector for gene targeting by homologous recombination in mammalian cells.

In addition, a new two-gene system employing site-specific recombination in rearrangement of an integrated proviral vector is under development.

F 231 THE V(D)J RECOMBINATION ACTIVATING GENES RAG-1 AND RAG-2.

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Variable regions of diverse antigen-binding specificity are generated when different V, D and J coding elements are joined by somatic recombination. Two genes, RAG-1 and RAG-2, were identified in our laboratory, the products of which can promote V(D)J recombination on a recombination substrate. In RAG-1 or RAG-2 deficient mice lymphoid development is arrested in a very early stage, indicating the indispensable role of these two gene products in allowing antigen-receptor assembly.

The physical distribution of both proteins was studied by Western, immunoprecipitation and immunofluorescence analyses. These studies revealed that RAG-1 and RAG-2 are present in multiple phosphorylated forms and at very low levels in V(D)J recombination competent primary cells. The two proteins can be co-precipitated in lysates derived from primary thymocytes as well as overexpressing fibroblasts, suggesting a direct interaction. Endogenous RAG-1 localizes in the nucleus, the nucleolus as well as the nuclear periphery whereas RAG-2 is found mainly in the periphery of the nucleus. However, when overexpressed in fibroblasts, RAG-1 is seen mainly in the nucleolus while RAG-2 exhibits an even nuclear distribution pattern.

To address the ability of RAG-1 to localize in the nucleolus we performed deletion analysis of certain motifs that show homology to RNA binding motifs. These studies showed that RAG-1 contains three domains of basic amino acids that could have affinity for RNA or ssDNA.

Finally, we employed internal deletions and point mutations in order to analyze the structural characteristics of the two proteins. Deletions in the Zn-finger domain of RAG-1, and mutations at the C-terminus and the acidic region of RAG-2 result in decreased efficiency of V(D)J recombination. By contrast, deletions at the C-terminus of RAG-1 (homology to yeast topo I), and a nucleotide binding site at the N- terminus of RAG-2 proved to be dentrimental for the function of the two proteins.

F 232 ACTIVATION OF Tn7 TRANSPOSITION.

Anne Stellwagen and Nancy L. Craig, Dept. of Molecular Biology and Genetics and the Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The bacterial transposon Tn7 encodes five genes, tnsABCDE, whose protein products mediate its transposition. TnsA+B+C contain the strand breakage and joining activities which execute transposition; however, the TnsA+B+C machinery by itself is inactive. Either TnsD or TnsE is required to activate the core machinery and to direct transposition to different types of target sequences. We have now activated the core machinery genetically, generating mutations in tnsC which activate TnsA+B+C in the absence of TnsD or TnsE. These mutations fall into two classes. One class of mutants activates TnsA+B+C such that regulatory signals which influence wild type Tn7 transposition retain an influence over the mutant transposition reaction. For example, transposition remains sensitive to signals from the target DNA, i.e. transposition immunity. A second class of tnsC mutants activates transposition such that the reaction is no longer responsive to these regulatory signals.

TnsC is an ATP-dependent DNA binding protein. ATP is required for Tn7 transposition in vitro; however DNA breakage and joining can occur in the absence of ATP. Thus, ATP plays a regulatory role, likely in the choice of target sites. Intriguingly, one of the tnsC mutations lies within a consensus sequence motif for ATP hydrolysis. This mutant protein has been purified, and its altered role in Tn7 transposition is being pursued both biochemically and genetically.

F 233 MULTIPLE REPEATS CLOSE TO THE ENDS OF THE INTEGRON-TYPE TRANSPOSON Tn*5090*: RELATIONS TO Tn7 AND MU, Lars Sundström, Peter Rådström, Ola Sköld, Göte Swedberg and Karin Hansson. Department of Pharmaceutical Biosciences, Division of Microbiology, Uppsala University, P.O. Box 581 Biomedical Center, S-751 23 Uppsala Sweden.

A type of widespread recombination elements, integrons, are known for their ability to take up antibiotic resistance cassettes by integrase-mediated recombination. The nucleotide sequence of the integron of R751, here called Tn*5090*, shows a backbone in common with the integrons on mercury transposons Tn*21*/Tn*5086*. The conserved backbone was found to be flanked by 25 bp inverted repeats and to carry two putative transposition genes, *tniA* and *tniB*. The deduced protein structures of the probable transposase encoded by *tniA* and the ATP-binding protein encoded by *tniB* indicated that Tn*5090* and Tn7 are remotely related mobile elements. The products of *tniA* (559 amino acids) is 25% identical to TnsB from Tn7. Both proteins contain the D,D(35)E motif of a recently defined protein family which includes the retroviral IN proteins and some bacterial transposases such as those of Tn*552* and IS3. The ATP-binding protein probably coded for by *tniB* is weakly related to both TnsC of Tn7 and protein B of phage Mu. Tn*5090* and Tn7, but also phage Mu, carry 5'-TG-CA-3' ends which dinucleotides are found at the ends of most elements producing a protein of the D,D(35)E type. Both Mu and Tn7 carry multiple repeats of approximately 20 bp near the ends. In both instances these repeats have been shown to bind transposase and to be necessary for the assembly process of the active form of Mu transposase (MuA) tetramer. We observed here that Tn*5090* carries similar multiple repeats of 19 bp; four at the left end and three at the right end. Those repeats proximal to the ends include part of the 25 bp terminal IR's. The promoter for the *tniA* gene overlaps with one repeat, an arrangement putatively involved in autoregulation of transposase expression. The presence of multiple repeats at the ends indicates a relation between Mu, Tn7 and Tn*5090*. The occurrence of a gene (*tniC*) for a protein of the invertase /resolvase family in Tn*5090* infers that the transposition mechanism might be replicative and requires efficient resolution of cointegrates. This would differ from Tn7 which transposes by simple insertion.

F 234 A TEST FOR P ELEMENT-INDUCED SISTER-STRAND RECOMBINATION IN *DROSOPHILA MELANOGASTER* USING A RING-X CHROMOSOME. John A.Sved, Merryl K. Robson and Yasmine H.M. Svoboda, School of Biological Sciences, University of Sydney, NSW 2006, Australia.

P elements are known to cause recombination in *D. melanogaster*. A single element in heterozygous condition leads to about 0.5 - 1% recombination, while homozygous elements can lead to around 20% recombination. The occurrence of clusters of recombinant products suggests that this recombination is frequently mitotic (pre-meiotic) rather than meiotic.

Models in which recombination is caused by *P* elements, particularly in the case of homologous elements, suggest that high levels of sister-strand recombination should also occur. This prediction is also a consequence of the model of Engels et al, 1991, *Cell* 62:515-525, in which 80-90% of repair of double strand gaps produced by *P* element excision comes from the sister rather than a homologous chromatid.

We have recombined a *P[CaSpeR]* element onto a ring-*X* chromosome. The presence of this element has been confirmed genetically and by PCR. Crosses have been made to produce males containing this element together with the transposase-producing element *P[Δ2-3](99B)*. Sister-strand recombination in the ring-*X* chromosome should then lead to double-sized rings containing two centromeres, which is expected to lead to loss of the ring chromosome. The prediction is thus for a biased sex ratio from males containing such chromosomes.

Preliminary results from a number of males grown at different temperatures have indicated that there is not a strong effect on the sex ratio. Possible interpretations of this result in terms of models of *P* element-induced recombination and the timing of the cell cycle will be discussed.

F 235 CAN P ELEMENT ACTIVITY BE PRODUCED BY COMBINING END-DEFICIENT ELEMENTS IN *DROSOPHILA MELANOGASTER*?, Yasmine H.M. Svoboda, Merryl K. Robson, and John A. Sved, School of Biological Sciences, University of Sydney, NSW 2006, Australia

It is known that incomplete P elements at exactly homologous sites on a chromosome can produce up to 20% male recombination between outside markers. We have used this system to test the effects of deficient P element ends on recombination. We used the procedure outlined by Johnson-Schlitz and Engels (*Mol. Cell Biol.*, in press) of screening for derivatives following mobilisation of a particular P element insertion. The P[CaSpeR] element insertion at 50C, labelled Ca31.4, was used. We screened for chromosomes which lacked the CaSpeR eye colour phenotype indicating that at least some of the element had been deleted. These chromosomes were then made homozygous and characterised by PCR. Use of the CyO balancer chromosome in these crosses ensured that only 3 of the 64 chromosome lines showed precise excision of the element. Amongst the remaining chromosomes, a number were found which lacked either the 5'- or 3'-ends, while others had internal deletions with both ends remaining intact.

We have characterised some of the end-deleted elements. Preliminary results indicate that the homozygous combination of end-deleted elements leads to no recombination. However, the heterozygous combination of 5'- and 3'-deleted end elements has been capable of restoring approximately the same level of recombination given by the original Ca31.4 element. Furthermore, PCR results show that the element ends are present but displaced in about 10% of progeny chromosomes from this heterozygous combination. This leads to a model in which the combination of ends from different P elements can function normally in excision and insertion.

We have also studied the effects of combining end-deleted elements with the complete Ca31.4 element. High levels of recombination resulted, but PCR results in this case were consistent with the model of excision of the Ca31.4 element followed by repair from the region of the end-deleted element. We discuss the difference between the two sets of results in the light of work by Mullins, Rio and Rubin (Genes & Devel. 3:729-738).

F 236 EXPRESSION OF HUMAN LINE-1 ELEMENTS: LESSONS FROM TESTICULAR GERM CELL TUMORS AND THE PALLISTER-KILLIAN SYNDROME. Gary D. Swergold, Food & Drug Administration, CBER, Division of Cell and Gene Therapy, Building 37, Room 4A-01, Bethesda, MD 20892

Expression of the human LINE-1 (L1Hs) transposable element is restricted to a narrow range of cell types. Specific expression of either endogenous elements or transfected recombinant elements has been reported primarily in tumors and cell lines of germ cell origin, including the NTera2D1, 2102EP, and JEG3 cell lines. These tumors and cell lines often contain one or more copies of isochromosome 12p, or translocations of 12p. Another human condition, the Pallister-Killian syndrome, is also characterized by the mosaic presence of an isochromosome 12p in patient's cells. M28, a previously described somatic hybrid cell line, contains a human isochromosome 12p derived from fibroblasts of a patient with the Pallister-Killian syndrome in a mouse LMTK- background. I asked whether the M28 cell line would exhibit enhanced expression of endogenous or transfected L1Hs elements.

Expression of transfected recombinant L1Hs elements was 10-20 fold higher in M28 than in LMTK- cells. Expression of L1Hs elements was not increased in the GM10868A somatic cell hybrid line which contains a complete human chromosome 12 in a Chinese Hamster Ovary background. Somatic cell hybrid lines containing various human chromosomes in an LMTK- background also exhibited no enhanced L1Hs expression. p40, the protein encoded by the L1Hs first open reading frame, was detected in NTera2D1 but not in non-transfected M28 cells. Preliminary promoter deletion experiments indicate that similar, but non-identical regions of the L1Hs 5' UTR, contribute to high level expression in the NTera2D1 and the M28 cell lines. These data suggest that the enhanced expression of human LINE-1 elements in tumors of germ cell origin is due in part to the presence of the isochromosome 12p.

F 237 RETROVIRUSES IN INVERTEBRATES: THE *GYPSY* RETROTRANSPOSON IS AN INFECTIOUS RETROVIRUS OF *DROSOPHILA MELANOGASTER*, Christophe Terzian, Alexander Kim, Pedro Santamaria, Alain Pélisson, Nicole Prud'homme and Alain Bucheton, Centre de Génétique Moléculaire CNRS 91198 Gif-sur-Yvette cedex FRANCE.

gypsy is 7.5 kb long and has two LTRs which are 482 nucleotides long. It contains three open reading frames; ORF1 presumably encodes a polypeptide related to gag proteins of retroviruses, and ORF2 encodes a potential polypeptide showing similarities with viral reverse transcriptases, proteases and endonucleases. The putative product of ORF3 has some characteristics of envelope polypeptides.

Most laboratory stocks of *Drosophila melanogaster* contain apparently only a few euchromatic copies of this element (up to 5) and many defective copies located in pericentromeric heterochromatin. However, some strains contain many more euchromatic copies (20 to 50), suggesting that deregulation of gypsy occurs frequently.

MS is a stock exhibiting high levels of transposition of *gypsy*. It contains many euchromatic copies of this element. SS is another strain devoid of active *gypsy*. We have performed three experiments to demonstrate infective properties of *gypsy*. In experiments 1 and 2, we have injected cytoplasm from MS into SS embryos, before (exp. 1) and after (exp. 2) cellularization. In experiment 3, SS individuals were raised from first instar larvae to adults on a medium made of standard *Drosophila* food mixed with homogeneized MS pupae. Transfer of *gypsy* in these conditions was checked by the ability of females resulting from all experiments to induce null alleles of *ovo* from the *ovo D1* mutation. We have recovered such events and have shown by Southern blot and in situ hybridization experiments that they are due to *gypsy* insertions at the *ovo* locus. These results indicate that *gypsy* is a retrovirus that can be transmitted by infection. The infectious properties of *gypsy* are currently examined within and between species of *Drosophila*.

F 238 COMPARISON OF PRIMATE CELLULAR SEQUENCES HOMOLOGOUS TO HERV-K REVERSE TRANSCRIPTASE,
Ralf R. Tönjes and Reinhard Kurth, Paul-Ehrlich-Institut, Department of Biotechnology, Paul-Ehrlich-Str. 51-59, D-63225 Langen.

The human genome contains numerous endogenous retrovirus sequences. Most of them seem to be defective due to deletions, multiple termination codons, or the lack of a 5'LTR. Replication-competent human endogenous retroviral (HERV) genomes have not yet been isolated. HERV-K represents a family of endogenous retroviruses with limited sequence homology to the B-type mouse mammary tumor virus (MMTV) showing long open reading frames for *gag*, *pol* and *env* genes.

Southern blot analyses employing HERV-K specific probes revealed similar restriction patterns in the genomes of different old world monkeys compared with human DNA. In DNA of new world monkeys HERV-K sequences were not detectable under identical conditions. We have isolated a subset of HERV-K sequences encoding reverse transcriptase (RT) from different species. Individual clones isolated by genomic cloning and PCR techniques were sequenced. Comparison of homologous RT sequences suggests extensive conservation of HERV-K *pol* genes during evolution of primates.

F 239FUNCTIONAL GEOMETRY OF BACTERIOPHAGE MU REPRESSOR
Ariane TOUSSAINT(1), Jamal LAACHOUCH(1), Amina Mhammedi-Alaoui (1), Marie-José GAMA (1), Lucie DESMET(1) and N.Patrik HIGGINS(2).
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(2) Department of Biochemistry, University of Alabama at Birmingham, Birmingham, AL 35294

Mu repressor binds to a tripartite 184 bp operator which consists of three boxes O1, O2 and O3, which contain 3, 4 and 2 consensus (CTTTTNNNWWW) repressor binding sites respectively. O2 straddles the early phage promoter pE and O3 the repressor promoter pc. Four types of repressor mutants have been isolated and characterized which allow to devide the protein in three functional domains. The operator binding moiety resides in the 70 N-terminal amino-acid segment of the protein which shares homology with the phage transposase. Preliminary results indicate that a segment around residue 129 is involved in protomer/protomer interaction. Several types of mutations in the 3'-end of the repressor gene *c* were characterized. The unexpected properties of repressors with these mutations suggest that the carboxy-terminal part of repressor is important for the overall conformation of the protein.

We devised a selection procedure to isolate operator mutations leading to constitutive expression of the phage lytic functions. Two out of three such mutations so far sequenced reside in O2 and are G->T transversion in one of the central N nucleotides. Such mutations should be very usefull to compare protease and repressor interactions with the operator/IAS region.

F 240 INSERTION OF FILAMENTOUS PHAGE ϕLf BY SITE-SPECIFIC INTEGRATION AND HOMOLOGOUS RECOMBINATION INTO THE *XANTHOMONAS CAMPESTRIS* CHROMOSOME, Yi-Hsiung Tseng, Nien-Tsung Lin, Su-Jean Lee, Ruey-Yi Chang and Fu-Shyan Wen, Institute of Molecular Biology, National Chung Hsing University, Taichung 402, Taiwan, R.O.C.

ϕLf is a filamentous phage of *Xanthomonas campestris* which contains a single-stranded circular genome of DNA molecule (6.0 kb). The RF DNA of ϕLf was able to integrate into the host chromosome. Two types of integration, site-specific integration and homologous recombination, were detected. The host and the phage DNA regions mediating the integrations were sequenced. The *att*P for site-specific integration possessed a AT rich region homologous to the *dif* site of *E. coli* chromosome and the *att*P for integration of the filamentous phage Cf16-v1 into *X. citri* chromosome. A putative IHF binding site was found near the *att*P, although the role it plays remains unknown. The region mediating homologous recombination was located near the *att*P in the RF DNA. In the host chromosome, a 4.4 kb ϕLf-homologous region was found which contains the *att*B and the site for homologous recombination. Deletion of this 4.4 kb region caused filamentation of the cell, similar to the consequence of *dif* deletion in *E. coli* which loses normal partitioning of the chromosome. Occurence of the ϕLf-homologous region in the *X. campestris* chromosome, which can be considered as a defective phage, might have significant meaning in evolution of the filamentous phage.

F 241 SEQUENCES CONTROLLING EXPRESSION OF THE *I* FACTOR, A LINE-LIKE ELEMENT IN *DROSOPHILA MELANOGASTER*. Apinunt Udomkit, Susan Forbes, Carol McLean, Irina Arkhipova and David J. Finnegan, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, Scotland.

I factors of *Drosophila melanogaster* are transposable elements related to mammalian LINEs (1). They transpose at high frequency in the germ line of the female progeny of crosses between inducer males and reactive females where full-length RNA is detected. Intact *I* factors are 5.4kb long and have two open reading frames, ORF1 and ORF2, which enable polypeptides similar to those of the *gag* and *pol* genes of retroviruses. (2) Transposition of *I* factors involves reverse transcription of an RNA intermediate probably using the reverse transcriptase encoded by ORF2 (3).

The first 186 nucleotides of the *I* factor contains a promoter that is most active in the ovaries of reactive females (4). This high level of activity in ovaries is controlled by two regions downstream of nucleotide 40. One lies between nucleotides 40 and 100 and the other between nucleotides 100 and 186.. The latter regions conatains a sequence, position 138-157, that is recognised by a sequence specific binding activity found in all *Drosophila* cells tested. This sequence is required for high level expression in ovaries.

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F 242 GENETIC AND STRUCTURAL STUDIES ON THE Mu Gin PROTEIN, Picter van de Putte, Liesbeth Spaeny-Dekking, and Nora Goosen, Department of Molecular Genetics, Leiden Institute of Chemistry, Leiden University, 2300 RA Leiden, The Netherlands

Site-specific recombination involves the formation of ordered nucleoprotein complexes. For the recombination process that leads to the inversion of the G-region in Mu, a synaptic complex is formed between two Gin dimers bound to the inverted repeats that flank the G-region, and Fis molecules bound to the enhancer. Until now it is not clear what kind of interactions between the different molecules of Gin take place during the subsequent steps of the recombination reaction, and whether Fis is only needed for the formation of the complex or whether it has some additional role in the strand exchange.

To get more information about the protein interactions between the different molecules of Gin we did *in vitro* cross-link experiments on the purified protein in solution. We found that in the Gin molecule the N-terminal region of 61 AA is in close contact with a second Gin molecule upon dimer formation. Surprisingly we found that under oxidizing conditions, a dimer of Gin molecules is also formed without the presence of a chemical crosslinker. We can show that this dimerisation under oxidizing conditions is caused by disulfide bond formation between one or two of the cysteines which are present in the wildtype protein at the positions 24 and 27.

We have also tried to isolate mutants in Gin which are disturbed in the putative interaction with Fis. For this we have isolated 25 independent Gin mutants that still normally bind to the recombination site but are affected in one or more of the subsequent steps in the reaction. In these mutants we introduced a mutation which in the wildtype Gin protein causes a Fis-independent phenotype (A. Klippel et al., EMBO J. **7** (1988) 3983-3989). This was done because we expected that mutants that were only disturbed in the interaction with Fis will function again when they are Fis-independent. Among the Gin mutants we have isolated, we found two mutants with the expected phenotype. The location of these mutations in the protein structure and their properties will be discussed.

F 243 MECHANISM OF Tc3 TRANSPOSITION, Henri G.A.M. van Luenen and Ronald H.A. Plasterk, The Netherlands Cancer Institute, Division of Molecular Biology, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

The Tc1 and Tc3 transposable elements of the nematode *C. elegans* are members of a class of transposons found in diverse phyla such as nematodes, arthropods and vertebrates. The Tc3 element is 2335 bp long and is flanked by inverted repeats of 462 bp. The element contains one major open reading frame (*Tc3A*). This gene encodes the Tc3 specific transposase. The Tc3A protein binds specifically to the Tc3 inverted repeat and forced expression of Tc3A results in transposition of Tc3 elements (Van Luenen *et al.* (1993), EMBO J. **12**:2513-2520). Transposition of Tc3 is accompanied by the appearance of extrachromosomal Tc3 elements. These are the result of double strand DNA breaks at the ends of the inverted repeat. We have isolated extrachromosomal Tc3 elements and determined the exact cleavage sites which led to excision. The 3' end of the excised transposon coincides with the last nucleotide of the Tc3 sequence and contains a 3'OH-group. The last 2 nucleotides of Tc3 are missing at the 5' end. In other words: the excised Tc3 element has a two nucleotide 3' extension. This has several implications for the mechanism of Tc3 transposition: 1) The transposon has to use the 3' end for integration and not the 5' end, otherwise transposon sequences will be lost. 3'OH-groups carry out the nucleophilic attack during the strand transfer reaction in all other transposition systems analyzed to date. 2) Tc3 elements integrate into the sequence TA and after insertion the element is flanked by two copies of the TA dinucleotide. These TA dinucleotides are the result of target site duplication since they are not part of the excised transposon. 3) With the determination of the cleavage sites we can explain the vast majority of somatic footprints found in the DNA after Tc3 excision. After excision the host is left with a double strand break containing at each end a 2 nucleotide 3' extension of transposon sequences and a TA dinucleotide. Several footprints can be expected depending on the cellular repair process; these have all been found.

F 244 MUTAGENESIS STUDY OF THE XerC SITE-SPECIFIC RECOMBINASE, Ian Viney and David Summers, Department of Genetics, University of Cambridge, Downing St., Cambridge, CB2 3EH. U.K.

Multimerisation of high copy number plasmids in *E.coli* decreases their segregational stability[1]. Stable inheritance of the natural plasmid ColE1 requires site-specific resolution of multimers to monomers. Resolution is mediated by four chromosomally-encoded proteins (ArgR, PepA, XerC and XerD) and a plasmid-borne site *cer* [2]. XerC and XerD are members of the lambda integrase family of site-specific recombinases which in addition to their role in plasmid multimer resolution have been shown to act on a *cer* -like site (*dif*) at the replication terminus region; a process required for normal partition of the *E.coli* chromosome[3].

cer recombination is topologically constrained, occurring only between directly repeated sites in the same molecule. However a hybrid site generated by recombination between *cer* and the *parB* site of CloDF13 (type II hybrid[4]), and also the *dif* site are unconstrained. These sites undergo both intra- and intermolecular recombination independent of the accessory proteins ArgR and PepA. To investigate the role of XerC in topological constraint of *cer* -mediated recombination and its interaction with other proteins involved in the process, we have undertaken an extensive random chemical mutagenesis of the *xerC* gene. We describe the isolation and characterisation of mutant XerC proteins with altered *in vivo* recombination activity.

1 Summers *et al.*, (1993) *Mol. Micro.* **8**(6) 1031-1038
2 Summers and Sherratt (1984) *Cell* **36** 1097-1103
3 Blakely *et al.*, (1991) *New Biologist* **3**(8) 789-798
4 Summers (1989) *EMBO J.* **8**(1) 309-315

F 245 ANALYSIS OF FUNCTIONAL DOMAINS OF HIV INTEGRASE, Cornelis Vink, Fusinita M.I. van den Ent, Ramon A. Puras Lutzke and Ronald H.A. Plasterk, Division of Molecular Biology, The Netherlands Cancer Instite, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

The retroviral integrase (IN) protein is the only protein known to be required for specific cleavage of the viral DNA ends, and integration of these ends into target DNA. Previously, three distinct regions in HIV IN were identified: (i) the N terminus, which might be involved in correct positioning of the viral DNA ends, (ii) the central region, which is required for all known catalytic activities of IN, and probably contains the complete (and single) active site of the protein, and (iii) the C terminus, which contains a DNA-binding domain. We found, by analysis of both point and deletion mutants of HIV IN, that mutants that are mutated in different regions, and therefore are not specifically active, exhibit full activity when they are tested as mixtures. This indicates that IN is active as an oligomer, in which the different subunits can have different functions.

We fine-mapped the DNA-binding domain in the C terminus of IN to a polypeptide stretch of approximately 40 amino acids. We are attempting to purify this polypeptide fragment to use it for structure determination.

The contacts between IN and the viral DNA termini were studied by missing-base analysis. Several nucleotides were found to be important for specific recognition by IN. Similar results were obtained by studying heteroduplex DNA substrates.

The IN protein of another lentivirus, feline immunodeficiency virus (FIV), was investigated. FIV IN is active in cleavage, integration and disintegration. FIV IN differs from HIV IN in site-specific cleavage activity: cleavage by the FIV protein generates relatively more of a specific cyclic dinucleotide product, whereas cleavage by HIV IN generates higher relative amounts of the "normal" dinucleotide and glycerol-dinucleotide products. A similar phenotype was observed for a certain class of active-site mutants of HIV IN.

F 246 THE TY5 RETROTRANSPOSONS OF *SACCHAROMYCES CEREVISIAE* AND *SACCHAROMYCES PARADOXUS*,
Daniel F. Voytas and Sige Zou, Department of Zoology and Genetics, Iowa State University, Ames, IA 50011
The Ty5 retrotransposon family of *S. cerevisiae* was revealed through analysis of the chromosome *III* (chr *III*) nucleotide sequence. A Ty5 insertion (Ty5-1) was found near the left telomere that encodes open reading frames with similarity to protease, reverse transcriptase and RNase H of Ty1/*copia* group retrotransposons. A novel feature of Ty5-1 is its minus strand primer binding site (-PBS). In contrast to most retrotransposons and retroviruses in which the -PBS is complementary to the 3' acceptor stem of a host tRNA, the Ty5-1 -PBS is complementary to the anticodon stem/loop of the *S. cerevisiae* initiator methionine tRNA (IMT). The Ty5-1 -PBS begins at the same nucleotide as the -PBS of the *Drosophila melanogaster copia* elements and related retrotransposons from *Volvox* and *Physarum*, suggesting that priming by half-IMTs is widespread. Structural organization of Ty5-1 indicated that it is no longer functional and has accumulated mutations and deletions since its insertion on chr *III*. To identify transpositionally active Ty5 elements, Ty5 copy number was assessed among diverse *S. cerevisiae* strains and found to vary from zero to two. In those strains that carry two insertions, one is the telomeric Ty5-1 element on chr *III* and the other resides near the telomere of chr *XI*. This element, however, did not arise by transposition, but rather by duplication of telomeric sequences that include Ty5-1. We have extended our survey to other species in the genus *Saccharomyces*, and have discovered Ty5 elements in the closely related species *S. paradoxus*. In contrast to *S. cerevisiae*, Ty5 elements vary in copy number among *S. paradoxus* strains, and structural characterization of two insertions suggests they are capable of additional transposition. We report here the features of the *S. paradoxus* Ty5 elements and discuss on-going experiments directed toward understanding mechanisms of half-IMT priming.

F 247 A PROTEIN DISSOCIATION STEP LIMITS TURNOVER IN FLP RECOMBINASE-MEDIATED SITE-SPECIFIC RECOMBINATION, Leslie L. Waite, Michael M. Cox, Department of Biochemistry, University of Wisconsin-Madison, 420 Henry Mall, Madison, WI 53706
We have compared the initial rates of product formation in FLP recombinase-mediated recombination reactions to rates of cross product formation when two ongoing reactions are mixed. We find that cross-product formation is subject to a lag of several minutes. The length of the lag increases with increasing FLP protein concentration. In experiments in which FLP protein concentration is limiting, burst type kinetics are observed. The results provide evidence that at least one FLP protein monomer is released from reaction complexes in a discrete step that occurs after products are formed, but before products are released in a form that is free to react with other FRT-containing DNA molecules. Further, this protein dissociation step is rate limiting under conditions often used to monitor this reaction *in vitro*.

F 248 INTERACTION BETWEEN NEIGHBORING *Ds* ELEMENTS IN MAIZE AND BETWEEN *Ds* AND ITS HOST,
Clifford F. Weil, Marlyse P. Ndi, Christine A. Vogel and Lucille A. Scott, Dept. of Biological Sciences, Univ. of Idaho, Moscow, ID 83843
Unlike most *Ds* alleles of maize, the *waxy (Wx)-m5* allele displays an unstable somatic phenotype in which background gene expression appears wild-type and somatic sectors of mutant tissue are formed. These sectors appear to be the result of short-range transposition by *Ds* from a site at position -470 into the *Wx* coding region.

Germinal derivatives in which *Ds* has transposed to sites throughout the *Wx* coding region can be isolated readily. Approximately 10% of these derivatives contain two copies of *Ds*, one at the -470 site and a second copy within the gene. At least two such alleles result in chromosome breakage at the *Wx* locus despite the fact that the *Ds* elements are physically separated and do not form a "double *Ds*", a previously identified chromosome-breaking *Ds* structure. Molecular analysis suggests these alleles undergo aberrant transposition events involving both elements simultaneously, resulting in sister chromatid fusion and dicentric chromosome formation. These results will be discussed in the context of interactions between transposase molecules and a mechanism by which *Ds* elements acquire competence to serve as transposition substrates.

Unstable *Ac*/*Ds* alleles that display frequent somatic reversion generally yield germinal revertants at high frequency (10^{-2} to 10^{-3}). Three *Ds* insertions near the 3' end of the *Wx* gene, *wx-m5:8311B*, *wx-m5:CS8* and *wx-m5:CS23* fit this pattern. In contrast, a fourth insertion in this region, *wx-m5:CS7*, shows frequent somatic but little or no germinal reversion ($\leq 10^{-5}$). Genetic data suggest this phenomenon does not depend on the source of *Ac* transposase. The *CS7* allele may thus provide insight into *cis*-acting *Ds* sequences or *trans*-acting host factors involved in regulating element activity during plant development.

F 249 WITHIN-SPECIES VARIATION: EVIDENCE FOR RECENT *MYS* TRANSPOSITION, Holly A. Wichman, Rhonda N. Lee, Jacqueline C. Jaskula, and Ryan S. Sawby, Department of Biological Sciences, University of Idaho, Moscow, ID 83843.
Mys, a retrovirus-like transposable element, is found throughout the genus *Peromyscus* and is present in at least 4,700 copies per haploid genome in the white-footed mouse, *P. leucopus*. Several *mys* subfamilies identified on the basis of restriction site variation occur in more than one species; the distribution of these subfamilies is consistent with the accepted species phylogeny. This suggests that *mys* was present in the ancestor of *Peromyscus* and has been active through much of the evolution of this genus. Two approaches have been used to examine the extent to which *mys* has been active since the speciation of *P. leucopus*. Both approaches are based on the assumption that elements which are fixed within a species are likely to be older than elements which are variable in the same species. First, we used quantitative Southern blot analysis of mice from across the species range to determine if some subfamilies of *mys* were more variable than others within *P. leucopus*. We found that a subfamily defined by a 1.3 kb *Eco*RI band in genomic Southern blot analysis was more constant in copy number than a subfamily defined by a 1.8 kb *Eco*RI band. Additionally, the 1.3 kb *Eco*RI subfamily had a more narrow phylogenetic distribution than the 1.8 kb *Eco*RI subfamily. This observation is consistent with the original assumption. Secondly, we used PCR to examine individual *mys* insertions in *P. leucopus*. We found that two inserts were limited in distribution, while a third could be found in most mice from the northern part of the species range. In these experiments, the age of individual *mys* inserts could be estimated by differences in the LTRs, because these differences represent mutations which occurred following insertion. As predicted, the widely distributed *mys* insert was older than the two narrowly distributed inserts. Taken together, these data suggest that *mys* was active subsequent to the speciation of *P. leucopus*, and has remained active in the very recent history of this species.
This work was funded by NIH grant GM38727.

F 250 REGULATION OF TRANSPOSABLE DNA ELEMENTS IN HETEROCHROMATIN: THE P ELEMENT OF *DROSOPHILA MELANOGASTER* AS A MODEL SYSTEM, R. C. Woodruff and Bethany Haller, Dept. of Biological Sciences, Bowling Green State University, Bowling Green, OH, 43403

The regulation of DNA elements, based on the synthesis of encoded gene products such as transposase and repressor, has been observed to be modified by genomic position. In addition, the majority of DNA elements localized in heterochromatin in Drosophila are inactive. These observations suggest that transpositions into heterochromatin, and possibly into highly repetitive DNA, may lead to temporary or permanent inactivation. Such inactivations may have evolutionary importance, because the elements may subsequently be activated by rearrangements that relocate elements to euchromatin.

We are currently studying the regulation of P elements of *D. melanogaster* in heterochromatin by measuring the frequency of movement of P[w^+] elements into the Y chromosome, which is almost entirely heterochromatin. Two questions are being asked. First, do P[w^+] transpositions into the Y chromosome inactivate the w^+ gene insert, and second, can P[w^+] elements located in the Y chromosome respond to P transposase.

From experiments with P[ry^+w^+] and P[lacZw^+] elements, we have recovered over 200 transpositions to the autosomes and none to the Y chromosome. These results suggest that transpositions into heterochromatin usually inactivate the w^+ gene in these inserts. In addition, we have attempted to remobilize presumed P[w^+] inserts in the Y chromosome that do not shown expression of the w^+ gene. Preliminary results show that P[w^+] inserts in the heterochromatin are usually not responsive to the P[$ry^+\Delta 2$-3](99B) transposase.

F 251 THE MOUSE DNA BINDING PROTEIN Rc FOR THE SIGNAL SEQUENCE OF V(D)J RECOMBINATION ALSO BINDS THE REGULATORY MOTIF FOR IG κ CHAIN TRANSCRIPTION. Lai-Chu Wu, Yiling Liu and Chi-Ho Mak. Department of Medical Biochemistry and Department of Internal Medicine, The Ohio State University, Columbus, OH 43210

The somatic V(D)J recombination for the assembly of the Ig and TCR genes is mediated by the recombination signal sequences (Rss) and the V(D)J recombinase. The Rss contains a conserved heptamer and a conserved nonamer. A cDNA clone for a putative zinc finger protein Rc was isolated from a λgt11 expression library, made from mouse thymocyte poly(A)$^+$ RNA, by the ability of its protein product to bind to the Rss. The deduced amino acid sequence for Rc reveals a pair of Cys_2-His_2 zinc fingers followed by an acidic domain. In addition, there are five copies of the Ser/Thr-Pro-X-Arg/Lys motif which are small DNA binding units. Rc transcripts of different sizes have been detected among different tissues or cells, and some of them appear to be restricted to the V(D)J recombination competent tissues or cells. Bacterial fusion proteins for Rc were produced to investigate its Rss DNA binding properties. These fusion proteins bind preferentially to the Rss heptamer. Moreover, the Rc-Rss DNA-protein complexes tend to multimerize in an order fashion. The data shows that Rc binds DNA as dimers, tetramers and multiples of tetramers. We speculate that Rc could act to create higher order nucleoprotein structures by bringing together distant binding sites which is an apparent requirement for V(D)J recombination. The zinc-finger and acidic domain structures of Rc are also found in several enhancer binding proteins, such as those for the κB motif of the Ig κ light chain enhancer and related sequences. Subsequently, the Rc fusion protein has been shown to bind the κB motif. The dual binding affinities of Rc for the Rss heptamer and the κB motif suggest a possible link between Ig transcription and somatic recombination.

F 252 DNA-BINDING DOMAINS OF PHAGE MU AND D108 TRANSPOSASE PROTEINS. Jin Ying Yang, Keetae Kim and Rasika M. Harshey, Department of Microbiology, University of Texas at Austin, Austin, TX 78712.

Transposases of the two homologous phages Mu and D108 encode DNA binding to two separate sites - *att* ends and enhancer - in their respective genomes. The differences in these two proteins lie mainly in 60 amino acids specifying the enhancer-binding region. Protein hybrids at every 10 amino acid intervals within this region are helping us understand how enhancer-binding specificity is achieved. Deletion analysis of the *att*-binding region of the Mu transposase has revealed details of how *att*-binding specificity is achieved. Details of the results from these studies will be presented.

F 253 Tn5 Transposase and Chromosome Partition, H. Yigit, M. Weinreich and W. S. Reznikoff, Department of Biochemistry, University of Wisconsin, Madison, WI 53706 USA.

Overproduction of the Tn5 transposase kills host cells. This host cell killing is due to a block in cell septation probably resulting from an inhibition of chromosome partition. Host cell killing is not a consequence of transposition; killing occurs in the absence of a transposing element and results from overproduction of transposition defective transposase mutants. Deletion of the first three transposase codons blocks host killing. Host mutants resistant to transposase overproduction killing (but permissive for transposition) have been isolated and mapped to 28′ *(top A)*, 76′ *(fts S, Y, E and X)*, 85.5′ *(near oriC)* and 99.5′ *(near dnaK, J and dnaT, C)*. A more detailed analysis of the 76′ locus will be presented. It is possible that normal levels of transposase delay chromosome partition sufficiently to facilitate the inheritance of transposition products. Alternatively this host cell killing may reflect an interaction of transposase with host functions involved in transposition. Other transposase proteins appear to manifest the same property.

F 254 THE DICHOTOMOUS SIZE VARIATION OF THE HUMAN COMPLEMENT COMPONENT C4 GENES IS CAUSED BY AN ENDOGENOUS RETROVIRUS, C. Yung Yu, [1,2] Andrew Dangel,[1] Anna R. Mendoza,[1] Bradley J. Baker,[1,2] and Lai-Chu Wu.[2] [1]Children's Hospital Research Foundation, Columbus and [2]The Ohio State University Biochemistry Program, Columbus, Ohio 43205.

The highly polymorphic human complement component C4A and C4B are encoded by tandem loci located at the class III region of the major histocompatibility complex in the short arm of chromosome 6. The C4A and C4B proteins share 99% sequence identities but exhibit marked differences in chemical reactivities. Deletion of either C4 locus occurs in 10-20 % of the population. The size of the first C4 locus (Locus I) is invariably 21 kb (*long gene*), while the gene in the second locus (Locus II) can be either 21kb or 14.6 kb (*short gene*). Extensive variation in gene size and gene number make the C4 loci a highly polymorphic region of the human genome. Elucidation of the exon-intron structure of the long C4A gene revealed the presence of a large intron (Intron 9) of 6,784 bp in length. The corresponding intron in a short C4 gene is only 416 bp in length. Located in Intron 9 of the long C4 gene is a human endogenous retrovirus termed HERV-K(C4). The complete DNA sequence of this endogenous retrovirus has been determined and it is arranged in the opposite transcriptional orientation with respect to the C4 genes. HERV-K(C4) has many hallmarks of a retroviral genome. It is flanked by long terminal repeats (LTR) of 548 bp and 550 bp, respectively, which contain regulatory elements for gene expression such as a TATA box, an enhancer consensus and a polyadenylation signal. An 18 bp primer binding site that is complementary to the Lys-tRNA sequence is located downstream of the 5' LTR and a 22 bp polypurine track is located upstream of the 3' LTR. A comparison of HERV-K(C4) with databse sequences revealed that it is a novel endogenous retrovirus. Southern blot analysis of human genomic DNAs with an HERV-K(C4) specific probe suggests that there are 30-50 copies of this retroviral elements in the human genome. It also exhibits restriction fragment length polymorphisms in different individuals. The possible involvement of HERV-K(C4) in the high frequency of gene deletion of C4 and the neighboring genes steroid 21-hydroxylase CYP21A and CYP21B is being investigated.

F 255 THREE TYPES OF NOVEL MUTATIONS IN THE NH_2-TERMINUS OF TN5 TRANSPOSASE: STRUCTURE/FUNCTION OF TRANSPOSASE, Margaret Zhou and William S. REznikoff, Department of Biochemistry, University of Wisconsin at Madison, Madison, WI 53706

Transposition of bacterial transposon Tn5 requires two end sequences, namely, two OE sequences when the whole Tn5 transposes; or an OE and an IE when only its insertion sequence, IS50, transposes. Transposition also requires a transposase encoded by Tn5. The Tnp N-terminal sequences are believed to be critical for one or more transposase activities. Therefore, we have initiated a mutational analysis of this region. Three mutations in the NH2-terminus of transposase which confer novel phenotypes have been identified. TA47 (and TP47) increases transposition ~5 fold, and may have done so by a combination of enhanced end binding affinity and decreased inhibitory activity. EK 54 (and EV54) causes 5~10 fold increased transposition in trans of a construct that contains two OE ends, but does not have any effect in cis on the transposition of a construct with an OE and an IE. This mutation may have different effect in cis vs. in trans; or it may have different effect on OE vs. IE mediated transposition. A third mutation, PL90, decreases transposition as well as transposase binding to OE, and the transposase-OE complex migrates slightly faster than the wild type complex. This mutant transposase may be more sensitive to proteolytic cleavage, resulting in a smaller protein bound to DNA, or it may bind DNA in a different conformation (eg, different bending angle). The later possibility is more interesting. Investigations are under way to reveal the structure-function of the NH_2-terminal portion of transposase by studying these mutants.

Late Abstracts

EVIDENCE FOR TISSUE SPECIFIC ACTIVATION OF THE RETROPOSON L1 IN MICE, Wolf M. Bertling[1], Thomas Aigner[2], [1]Paul-Ehrlich-Institut, Unit Molecular Pathology, 63225 Langen, Germany, [2] Max-Planck Res. Units Rheumatology, Friedrich-Alexander-University, 91056 Erlangen, Germany

Observations of new integrations of L1 retroposons indicate that these elements are still actively moving within genomes. The main problem of studying the expression and activation of L1 by in situ hybridization is the enormous background of several thousand partially truncated DNA copies in the genome. We could, however, prove, that our technique is able to discriminate RNA from DNA signals. We used a sequence corresponding to the NH_2-terminal part of the first ORF in both orientations. We detected sense and antisense signals in testis in different stages of spermatocytes, and to a lesser extent in epidydimis. We found only signals for the sense orientation in brain and no signals in any of the other investigated organs. At this point we do not know if the antisense signal is due to a random transcription (which should then, however, be present in all tissues), due to a specific antisense promoter activation or if we can detect RNA/DNA heteroduplexes as a sign for reverse transcription.

ANALYSIS OF THE MODE OF DNA RECOGNITION AND MECHANISM OF RECOMBINATION BY THE FLP RECOMBINASE, Jehee Lee, Ilson Whang and Makkuni Jayaram, Department of Microbiology, U. T Austin, Austin, TX 78712

Binding of the Flp recombinase to its target sequence results in the activation of a single phosphodiester within a DNA chain for nucleophilic attack by the active site tyrosine. The specificity of phosphoryl transfer must be derived from the specificity of DNA contacts by Flp. A domain of Flp, approximately 200 amino acids long, that harbors target specificity has been identified. Swapping of peptide regions within this domain between Flp and a homologous yeast site-specific recombinase R has yielded further refinements of the DNA binding region. According to our model for the Flp reaction, activation of DNA phosphodiester and of the DNA-tyrosyl phosphodiester for the cleavage and joining steps, respectively, required for recombination of a single DNA strand is achieved by the same Flp monomer. Results from half-site-recombinase complexes immobilized in polyacrylamide gels and provided with tyramine as the active site tyrosine mimic are consistent with this idea. Recombinase-target complexes formed by a mixture of Flp and GST-Flp fusion proteins indicate that each binding element within the Flp target site is occupied by a protein monomer. Furthermore, half-site reactions using complementing pairs of Flp and GST-Flp step-arrest mutants support the trans-DNA cleavage model.

A V(D)J SITE-SPECIFIC RECOMBINATION MODEL INVOLVING NO COMPULSORY DOUBLE-STRANDED BREAK FORMATION AT THE CODING SEGMENTS, Sacha Kallenbach and François Rougeon, Département d'Immunologie, Institut Pasteur, Paris, France

Complete immunoglobulin and T-cell receptor genes are assembled by site-specific recombination of separately encoded gene segments. We present a novel recombination model which accounts for all the characteristics of V(D)J recombination that have been described. The sequence of events proposed implies no formation of double strand breaks at the coding ends, ensuring the continuity between the recombination partners during the reaction, and solves the problem of the ligation of extremities which have no complementarity. According to this recombination model the formation of covalently sealed coding ends does not contitute a compulsory step in the recombination process.