GENOME REARRANGEMENT

Ira Herskowitz and Mel Simon, Organizers

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Mutation I

1149 MECHANISM AND REGULATION OF Tn10 TRANSPOSITION. N.Kleckner, D.Morisato, D.Roberts, and J. Bender. Dept. of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA. 02138

PART I. Plasmids carrying a mini-Tn10 transposon and the IS10 transposase gene under ptac control give rise, upon transposase induction, to new DNA species. The major new species is a circle consisting of the mini-Tn10 element with its termini brought together. Each circle consists of one covalently closed strand and one strand containing an interruption at the Tn10 termini. In some molecules the latter strand has a second interruption near the first. The occurrence of this structure suggests that IS10 transposase can promote double strand breaks and single strand joints involving Tn10 termini. Such activities are consistent with many proposed transposition mechanisms. We think it unlikely that the circular structure is a true intermediate in Tn10 transposition in vivo (to be discussed).
PART II. By denaturation, renaturation and in vitro packaging we have generated lambda::Tn10 phages whose Tn10 elements contain single basepair heteroduplex mismatches. When these heterozygous elements transpose from lambda into the bacterial chromosome, information from both strands is recovered in the progeny. This observation argues against transposition models in which replication proceeds unidirectionally from one end to the other without generation of a cointegrate intermediate, and is easily explained if Tn10 insertion occurs by a non-replicative mechanism. Implications for other models are under investigation.
PART III. E.coli mutants have been isolated in which Tn10 transposition occurs at an elevated frequency. One class of mutants have acquired lesions in the dam gene, whose product methylates the A's in GATC sequences. dam mutations also elevate transposition of Tn903 and of IS elements inserting in gal. The effects of dam mutations on Tn10 transposition result from a direct effect of methylation on the interaction of RNA polymerase with the transposase promoter, pIN: (1) Expression of LacZ in pIN-LacZ fusions is increased in dam mutants. (2) pIN contains a methylated GATC site in its Pribnow Box, and a single base substitution that eliminates this methylation renders pIN activity and Tn10 transposition insensitive to the Dam phenotype of the host. (3) Abortive initiation by purified RNA polymerase in vitro is much more frequent on an unmethylated than a methylated template (B.C.Hoopes and W.McClure). In E.coli, DNA strands are unmethylated for a short time immediately after replication or synthesis. Dependence of transposase expression on absence of methylation may serve to limit transposase expression without losing promoter specificity. Alternatively, there may be evolutionary advantages to having a burst of transposase synthesis immediately after passage of a replication fork or upon entry of single stranded DNA into a new host during conjugation.

1150 TY TRANSPOSITION IN YEAST, Peter Philippsen, Hermann Eibel, Ursula Fleig, Jürg Gafner, David Pridmore, Felix Schirmaier, Agathe Stotz, Department of Microbiology, Biozentrum, Universität Basel, CH-4056 Basel, Switzerland.

TY insertions altering gene expression have been described for HIS4 (1), CYC7 (2), ADH2 (3), CAR1(4), LYS2 (5), PHO5 (6) and URA2 (7) in S. cerevisiae. We have been studying the frequency, target site specificity and secondary reactions of TY insertions at LYS2. This system offers an advantage, because mutants with either no or a much reduced LYS2 expression can be selected for (8). Such mutants occur spontaneously with a frequency of 5×10^{-6} in haploid lab strains and 5 TY insertions were so far found among 300 mutants. The TY transposition frequency in these strains, which harbor 30 to 35 TY elements is therefore 10^{-7}. Four of the five insertions were mapped in front of the LYS2 transcription start and one just after the transcription start (5). This preference for the gene start region was surprising. We are trying to repeat these experiments after exchanging the LYS2 promoter with promoters from other S. cerevisiae genes, in order to determine whether the observed regional specificity is due to AT-richness or to a more "open" chromatin structure around transcription start sites as compared to the structural gene. Complete excision of the TY elements was selected for, but was not observed in 10^9 lys2::TY mutant cells. We recovered one LYS2 revertant from a lys2::TY insertion mutant, which has an additional insertion of around 5 kb in the TY element. The origin of this secondary insertion is under investigation.

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1151 THE Ac/Ds INSERTION ELEMENT SYSTEM IN MAIZE, Peacock, W.J., Dennis, E.S., Gerlach, W.L., Howell, S., Sachs, M.M., and Schwartz, D., CSIRO Division of Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia.

We have sequenced a Ds controlling element from the Adh1-Fm335 allele in maize. The identity of the Ds segment was proven by analysis of progenitor, mutant and Ac-induced revertant alleles. The Ds segment is bounded by an 11 bp inverted repeat flanking 2 bp direct and 3 bp indirect repeats. The Ds element has a 373 bp AT-rich internal segment. In the Fm335 allele the Ds segment is inserted between the transcription and translation start points and the insertion has an 8 bp direct flanking repeat of Adh1 gene DNA. It severely affects mRNA production. Portion of the Ds segment appears to form a tenth intron in the Adh1 transcript.

Analysis of Ac-induced excisions of the Ds segment includes complete, partial and full revertants. Sequence data pertain to the mechanism of excision and to transcription of the gene segment. Most maize stocks have approximately 40 segments homologous to this Ds element. We have cloned and sequenced a number of them and characterized their flanking sequences. All have either 11-2-3 or 10-2-3 termini, the former being flanked by 8 bp direct repeats, the latter by 6 bp repeats. The length and sequence of the internal segments are similar to that of the Ds element at the Adh1 locus in the Fm335 allele.

The relation of this family of Ds elements to the Ds and Ac elements isolated from the Wx and Sh will be discussed. Engineering of the Ds element as a potential vector for gene transfer will also be outlined.

Mutation II

1152 **CHROMOSOME REARRANGEMENTS INDUCED BY P TRANSPOSABLE ELEMENTS IN DROSOPHILA**
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Massive genomic rearrangements are often characteristic of the activity of transposable genetic elements. This effect was first revealed from studies of maize transposons, and has been repeatedly observed in many other eukaryotes and prokaryotes. For eukaryotic elements such as those studied in maize, yeast and Drosophila, the details of rearrangement formation and how this process might be related to transposition remains unclear.

Members of the P family of transposable elements in Drosophila melanogaster (1,2) produce chromosome rearrangements at enormous frequencies. P factors have an elaborate mechanism of self-regulation resulting in two alternatve cellular states (cytotypes). In the P cytotype, transposition, rearrangement formation, and other observable aspects of element activity, collectively known as hybrid dysgenesis, are largely suppressed. The alternative state that supports these traits is called the M cytotype. When P factors and the M cytotype cohabit the germ cells of a fly, rearrangements can be observed in the next generation at the rate of up to 10% per chromosome arm, and approximately 85% of their breakpoints will be at the positions of pre-existing P elements (within a few hundred nucleotide pairs). The rest are scattered more or less evenly around the euchromatin. The kinds of rearranged chromosomes range from simple inversions to complex types with many breakpoints. Some are conservative, but others involve duplications and deletions. The distribution of complex types is close to that expected if each rearrangement comes about as a single multi-break event followed by random reunion of chromosome segments rather than through a sequence of simpler events. This distribution would not be expected if rearrangements came about from homologous recombination between P elements, or from formation of cointegrate structures. Closer examination of the breakpoints of rearrangements reveals that several kinds of molecular events are involved. In the majority of cases P elements that pre-existed at the points of breakage are still present in the rearranged chromosome, but loss of P elements is also very common. Gains of P elements have also been observed, but these events are relatively rare. Sometimes small duplications or deficiencies of genomic flanking DNA can be detected adjacent to breakpoints. When an inversion retains P elements at both its breakpoints, that inversion can revert to the normal sequence at high frequencies, restoring function of a gene at the breakpoint that had been previously disrupted by the rearrangement. Given the complexity and irregularity of rearrangement formation, the only direct relationship to element transposition remains the common control by cytotype.

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1153 P TRANSPOSABLE ELEMENTS IN DROSOPHILA, Gerald M. Rubin, Department of Biochemistry, University of California, Berkeley, Berkeley, CA 94720

The properties of P Transposable Elements will be discussed and related to those of other Drosophila transposable elements.

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1154 MOLECULAR ORGANIZATION AND EVOLUTION OF DROSOPHILA COPIA-LIKE MOBILE GENETIC ELEMENTS,17.6 AND 297,WHICH EXHIBIT A STRICTLY SITE-SPECIFIC INSERTION,Kaoru Saigo, Satoshi,Inouye,Katsuji Yoshioka,Shunji Yuki and Wataru Kugimiya,Department of Biochemistry,Kyushu University 60 School of Medicine,Fukuoka 812,Japan

In Drosophila melanogaster,about 5% of the genome DNA is formed of copia-like mobile genetic elements scattered at numerous sites along the chromosome. Recent analyses show that unlike the majority of copia-like elements,17.6,297 and H.M.S.Beagle are not bounded by 5'TG..CA3' but by 5'AGT..AYT3' (1,2,3). Interestingly,these three copia-like elements appear to have a strong tendency to insert into the TATA boxes. In fact,3 of the 4 insertion sites so far examined were the TATA boxes,2 for H3 histone genes and 1 for a cuticle gene (2,3). Because of the apparent biological importance of this type of insertion,we extensively analyzed the site-specificity of 17.6. Our data showed that (1) insertion of 17.6 takes place in a strictly site-specific fashion using as a target ATAT which corresponds to a considerable portion of the consensus TATA boxes and (2) in most cases,the target sequence,ATAT,is imbedded in 10-30bp purine pyrimidine alternating sequences,possible candidates for Z-DNA structure. We also noticed that the nucleotide sequences of the junctions between 17.6 (or 297) and host DNA are quite similar to those of the inverted heptamers for V-J joining in immunoglobulin gene. To get more information on the molecular mechanism by which the site-specific insertion of 17.6 (or 297) is carried out, the complete nucleotide sequences of 17.6 and 297 were determined. Our sequence data revealed that the molecular structure of 17.6 is as follows;5'LTR(512bp)-"pbs"(17)-AT rich Spacer(544)-Open Reading Frame I(1317)-Spacer(62)-O.R.F. II(3066)-Spacer(17)-O.R.F. III(1368)-Spacer(7)-Consensus(py)n(Pu)n(17)-LTR(512)3'. 297 was also found to have a similar molecular organization. Surprisely,from beginning to end all sequences in 297 were found to correspond base-to-base with those in 17.6,although the average homology between these two elements was about 70%. Since the amino acid sequences of the putative polypeptides corresponding to O.R.F. II and III were highly conservative,either of these coding regions may possibly for a putative enzyme responsible for the site-specific integration of 17.6 (or 297). During the course of sequence analysis, we also noticed that the overall nucleotide sequences of LTRs of avian leukosis-sarcoma viruses closely matched those of 17.6 and 297 LTRs(1). This finding together with our recent success of identification of retrovirus-like particles containing copia RNA(4) led us to the notion that copia-like mobile genetic elements might originate from infection of a progenitor Drosophila with a retrovirus from which the present-day AL-SV was derived.

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1155 INSERTIONAL MUTAGENESIS BY MURINE RETROVIRAL DNAs. Nancy A. Jenkins and Neal G. Copeland, Department of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, OH 45267

Retroviruses, like the transposable elements of Drosophila and yeast, have the capacity to be insertional mutagens. As a consequence of integration, retroviruses may interrupt (block) normal gene expression or activate the expression of cellular sequences flanking the proviral integration site. That spontaneous mutating insertions have occurred in the mouse germline by virus integration was first demonstrated by our studies of the recessive dilute (d) coat color mutation of DBA/2J mice.

The single endogenous ecotropic murine leukemia provirus of DBA/2J mice cosegregated with the d mutation of mouse chromosome 9. The d mutation is also relatively unstable and reverts at a frequency of 3×10^{-6}. In six independent dilute revertants (d^+) examined, reversion to wild type coat color was correlated with the loss of most of the ecotropic MuLV genome, indicating that the virus caused the mutation. Two revertant sites as well as the preintegration site were cloned and sequenced. Exactly one LTR remained in each revertant site, indicating that the virus was excised by homologous recombination involving the viral LTRs. In addition, sequence analysis of the proviral integration site indicated that the virus did not integrate in coding sequence. As the dilute mutation is recessive, the most likely interpretation of these results is that the provirus induced the dilute mutation by blocking normal gene expression via integration into either an intron or 5' or 3' non-coding sequence.

In addition to the original dilute (d) mutation, more than 300 spontaneous, X-ray, and chemically induced mutations at the dilute locus have been identified. Most of these mutations are either pre- or post-natally lethal. As we have identified a dilute mutation that was virally induced, it was possible to use the virus as a probe to clone sequences at this locus. These sequences have been used to analyze DNAs (in collaboration with L.B. Russell, Oak Ridge, TN) from mice carrying X-ray induced mutations (which are presumed to be deletions). Mutations that were genetically defined as large deletions lacked sequences homologous to the dilute probe. Mutations that were thought to be small deletions did not delete these sequences. Deletions thought to be intermediate in size in some cases deleted these sequences, and in others, did not. Furthermore, one deletion mutation appears to have its break point within the sequences recognized by this probe. Identification of multiple additional unique cell sequences from this locus will make it possible to further define the nature of the lethal regions and the product of the dilute gene. Finally, an unlinked recessive suppressor of dilute has been identified, but the mechanism of action of this locus is unknown.

Mice represent one of the most well studied and genetically characterized mammalian species. More than 800 mutant alleles have been identified, many of which are associated with neurological or developmental disorders. Mutagenesis of mice by retroviral DNAs promises to be an important model system for identifying and characterizing, at the molecular level, genes important in mammalian development.

Variation: Programmed Rearrangement I

1156 INVERTIBLE DNA IN BACTERIOPHAGE MU AND IN *E.COLI*, Pieter van de Putte and Ronald Plasterk, Laboratory of Molecular Genetics, State University of Leiden, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

Bacteriophage Mu contains in its genome an invertible region of 3000 bp; the orientation of this 'G-segment' determines the host range of the phage. The 'G-region' contains two sets of genes presumably coding for different tail fiber proteins. The expression of these sets of genes is controlled by the inversion process: the promoter of these genes is situated outside the G-region. The inversion process is catalysed by the phage-coded enzyme Gin. The *gin* gene is adjacent to the invertible region.

A plasmid was constructed containing the G-region, in which the *lac* operon was cloned, and a mutated *gin* gene. Inversion of the G-region on this plasmid is scored as a change in Lac phenotype. The construct is useful both for measuring quantitatively inversions *in vivo* and for serving as a substrate for the Gin protein *in vitro*. Using Gin amplified extracts inversions *in vitro* could be shown to occur. Probably no host factors are required for the reaction. The substrate on which Gin acts has to be supercoiled.

We found further that Gin^- mutants of Mu are complemented in certain *E.coli* K12 strains. The Gin complementing function, which we called Pin, was found on a clone in the *E.coli* colony bank of Carbon and Clarke. Pin acts on an adjacent invertible area of approximately 1800 bp, the P-region. The function was mapped on the *E.coli* chromosome and found to be located in the genetic element *e*14, which behaves as a defective prophage and which is excised from the chromosome after UV irradiation. Several features of the Gin system of bacteriophage Mu, the Pin system in *E.coli* and the Hin system in *S.typhimurium* will be compared.

1157 INITIATION AND RESOLUTION OF MATING TYPE SWITCHING IN YEAST, Jeffrey Strathern, Amar J.S. Klar, Carolyn McGill, Judith Abraham, Stuart T. Weisbrod, Margaret Kelly, Carmella Stephens, James B. Hicks, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Changes of cell type in *S. cerevisiae* are mediated by specific rearrangements of the DNA. The two alleles of the mating type locus MATa and MATα differ by substitutions of 642 and 747 base pairs respectively. Homothallic yeast are capable of switching between these two alleles by the transposition-substitution of these sequences from the unexpressed donor loci HML and HMR. These loci have been cloned and sequenced and hence have provided complete end-product descriptions of the switching reaction. These physical studies leave unanswered several questions about the mechanism by which this process occurs that we have attempted to answer. Do the donor HM and MAT loci physically interact? No diffusible copy of the mating-type cassettes has been observed and rare HM/MAT recombinants can be obtained. How is the process initiated? Homothallic cells have an endonuclease that recognizes a sequence in MAT and makes a double-stranded cut. This cut site is necessary for MAT to act as a recipient and occurs in strains lacking HML and HMR. HML and HMR have the site but it is not available to the endonuclease in MAR/SIR+ cells. How much of the DNA that is homologous between MAT and HML or HMR that flanks the substitution is moved? Does it represent a unique size? We have demonstrated that the length of DNA to the left of MAT that is moved is variable as assayed by the "healing" of linker-induced mutations. Finally, is there a specific mechanism that constrains the resolution of the recombination intermediate in this gene conversion-like process so that it does not lead to HM/MAT fusions (the equivalent of recombinations of outside markers resulting from resolution of isomerized Holliday junctions)? Our evidence suggests that the constraint against such events is not a trivial consequence of a general constraint against intra-chromosomal recombinations.

Variation: Programmed Rearrangement II

1158 GENE REARRANGEMENTS CONTROLLING THE SYNTHESIS OF SURFACE ANTIGENS IN TRYPANOSOMES, Piet Borst, André Bernards, Lex H.T. Van der Ploeg, Titia De Lange, Paul A.M. Michels, Alvin Y.C.Liu and Jan M. Kooter, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

We have found five types of DNA rearrangements involving the variant surface glycoproteins (VSGs) of trypanosomes:

A. A duplicative transposition of a chromosome-internal VSG gene to an expression site located at the end of a chromosome (1-3).
B. A (duplicative) telomere conversion transferring a VSG gene and surrounding sequences to another telomere, which may be the expression site telomere (4-6).
C. Addition of two extra copies to a tandem array of 70-bp repeats (2), associated with the non-duplicative activation of a telomeric VSG gene.
D. Telomere growth and contraction (7;8), probably mainly due to changes in the number of telomeric (CCCTAA) repeats and (CCCTAA)-derived sub-telomeric repeats present at trypanosome chromosome ends (4).
E. Extensive duplications/deletions/alterations of chromosome-internal genes, as indicated by strain comparisons, and dispersal of related genes throughout the genome (9).

VSG genes are incomplete in that they lack a mini-exon sequence present at the 5'-end of mature VSG mRNA but not in front of VSG basic copy genes. Processes A and B presumably transfer a VSG gene copy downstream of a promoter-mini-exon array in the expression site. The non-duplicative activation of telomeric VSG genes is not associated with a transfer to this site (4) and may involve a loss of base modification.

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Amplification and Diminution

1159 TRANSFORMATION AND AMPLIFICATION OF GENES FOR HUMAN DIFFERENTIATION ANTIGENS IN MOUSE FIBROBLASTS, Leonard A. Herzenberg and Paula Kavathas, Department of Genetics, Stanford University, Stanford CA 94305 USA

Following cotransfection of mouse L cells (TK^-) with the herpes simplex thymidine kinase (TK) gene and total human DNA from JM, a human T lymphoma line, we selected Leu-2^+ and other human lymphocyte membrane antigen transferents by FACS sorting[1]. We found the frequency of transferents for Leu-2 to be about 10^{-3} of the TK^+ cells selected in HAT medium. Though most cloned transferents had narrow ranges of antigen density per cell, one of the first four Leu-2^+ transferents found was strikingly more variable in the amount of Leu-2 antigen per cell. Further, this transferent, J10, had a mean Leu-2 staining per cell that was seven times greater than that of the other transferents[1].

We went through six cycles of selection of the brightest 0.1-0.3% of J10 cells stained with anti-Leu-2 and obtained a line which is 40 times brighter than the mean of J10. These cells have human DNA sequences which are amplified 10-50 fold and numerous double minute chromosome fragments, a common indicator of gene amplification in mouse cells. We feel that by these means, we have obtained the first example of spontaneous gene amplification for membrane antigens. These amplified cells should facilitate cloning of the Leu-2 gene and characterization of the Leu-2 molecule.

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1160 GENOMIC REORGANIZATION DURING MACRONUCLEAR DEVELOPMENT IN CILIATES, E. Blackburn, M. Budarf, P. Challoner, M. Cherry, E. Howard, D. Larson, T. Ryan and E. Spangler, Department of Molecular Biology, University of California, Berkeley, CA 94720

During the development of the somatic macronucleus in many ciliates, its genome is both rearranged and fragmented, with concomitant formation of new telomeres (1), and ribosomal RNA genes (rDNA) become amplified. To test the extent of genomic rearrangement, ten randomly chosen cloned DNA sequences were examined for their arrangements in the genomes of the macronucleus and the micronucleus (the germline nucleus) of a homozygous strain of Tetrahymena. Sequences found in all 10 clones underwent either rearrangement or elimination in macronuclear development, indicating such rearrangements are very common in the genome. Rearrangements were reproducible for 7 out of the 10 cloned DNAs; in the remaining 3 cases a unique sequence flanked by eliminated middle-repetitive sequences was found in a different sized restriction fragment in different macronuclei.

The formation of new macronuclear telomeres in macronuclear development was studied with particular reference to those in two forms of the amplified rDNA and three cloned non-rDNA telomeres. The sites to which the telomeric sequence (which consists of tandem repeats of the hexanucleotide CCCCAA) is added were found to be variable in sequence among macronuclear telomeres, although some common properties were found. Mutants defective or altered in their rDNA amplification properties (2) have been examined by molecular criteria, to identify DNA regions important for different aspects of amplification.

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Gene Activation I

1161 **GENE ACTIVATION BY YEAST TRANSPOSABLE ELEMENTS**, E.T. Young, A. Taguchi and J. Osterman, Department of Biochemistry, University of Washington, Seattle, WA 98195

The transposable element of yeast (Ty) causes gene activation and inactivation and genomic rearrangements. In common laboratory yeast strains there are approximately 35 Ty elements and 100 copies of solo delta sequences, the 340 bp direct repeat that borders the intact Ty element.

The ADH2 locus of Saccharomyces cerevisiae is a useful target gene for Ty insertion. Genetic selection can be used to identify regulatory mutations at this locus. ADH2 codes for the glucose-repressed alcohol dehydrogenase isozyme, ADHII. Selection for glucose-insensitive expression of ADHII allows the isolation of mutants most of which arise by Ty insertion into the promoter and regulatory regions of ADH2. In all nine cases studied the Ty element has inserted within 50 bp of the TATA box, and in each case the Ty element is oriented so that its own transcription is directed away from the ADH2 locus (1,2). The divergent transcription unit so created is regulated not by glucose or the normal activator of ADH2, ADR1, but by the same regulatory circuit that controls transcription of the Ty elements.

Ty elements, and the genes they activate by insertional mutagenesis, are regulated transcriptionally by the mating competence of the cell. In cells incompetent to mate, either as a consequence of diploidy or sterile mutations, Ty transcription is greatly reduced as is the transcription of genes activated by Ty insertion. Surprisingly, this block is itself catabolite controlled so that the inhibition of transcription is only significant in cells growing on a non-fermentable carbon source such as glycerol or ethanol. In cells growing on a fermentable substrate such as glucose, there is no inhibition of transcription (3). The Ty-activated ADH2 gene also escapes the "mating effect" when it is present on a centromere-containing plasmid in diploid cells.

The site of action of a putative regulatory protein for Ty activation has been sought by making deletions within a cloned Ty element. After creating the deletion, the mutated Ty element is replaced next to ADH2 and reintroduced into yeast cells by transformation. Deletion of a region about 500 bp from the internal border of the ADH2-proximal delta sequence causes loss of Ty activation of ADH2, and loss of transcription of the mutated Ty element. This result suggests that Ty transcription, and insertional activation requires positive activation mediated through a site which is within the region of Ty DNA that is transcribed since the Ty promoter is located within the delta sequence. A search for mutants unable to activate Ty-linked ADH2 genes has led to the isolation of mutants with a semi-dominant phenotype that effect both Ty-linked ADH2 and the wild-type locus.

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1162 CONTROL OF Ty REARRANGEMENTS IN YEAST, Gerald R. Fink, Jef Boeke, Karen Durbin, David Garfinkel and Fred Winston, Whitehead Institute/MIT, Cambridge, MA 02139

We have studied genes which affect Ty expression and transposition. The spt3* mutation, known to suppress Ty insertions and their solo delta derivatives at HIS4, can suppress at least one of the Ty insertions at LYS2 (Ty61) and also the Lys⁻ phenotype of a solo delta derivative of another Ty insertion (Ty128) at LYS2. These results demonstrate the spt3 mutations can suppress Ty and delta mutations at two different loci, suggesting that they are general for their effects on Ty and delta elements. The spt3 mutation has a profound effect on all Ty transcription in the cell. In SPT3⁺ cells a Northern of total RNA reveals a major RNA of 5.6 kb (presumably the aggregate product of some 35 Ty elements in the cell). In spt3⁻ strains this large transcript disappears and is replaced by a shorter transcript. This profound change is found in total Ty RNA and in the transcription pattern of a single Ty. Strains carrying the spt3 mutation show a number of anomalies; the most striking is a bilateral mating defect.

Transposition of Ty has been studied using the promoterless HIS3 gene on a centromere plasmid described by Scherer and Davis (1). Several modifications to the system have been made so that we can screen large numbers of transpositions. We now have large numbers of independent transpositions into the 5' non-coding region adjacent to the gene and are studying the effects of rad52, spt, and various environmental conditions on the transposition frequency.

1. Scherer, S., Mann, C. and Davis, R.S. (1982) Nature 298, 815-819.

* SPT3 = SPM3

Gene Activation II

1163 RETROVIRUSES AS INSERTIONAL MUTAGENS: H.E. Varmus[1], L.A. Donehower[1], D. Westaway[1], Y.K. Fung[1], A.A. van Ooyen[2], and R. Nusse[2], [1]Department of Microbiology and Immunology, University of California, San Francisco, CA; [2]Department of Virology, Antoni van Leeuwenhoekhuis, Amsterdam, The Netherlands.

The proviruses of retroviruses are structurally related to transposable elements of diverse origins and share with them the capacity to induce insertion mutations that inactivate or activate host genes (1). Integration of retroviral DNA appears to occur at random within host chromosomes, but fixed points in viral DNA are joined to host DNA and a short duplication of specific size is generated at the insertion site. A missense mutation we have introduced within the 3' region of the murine leukemia virus pol gene prevents integration of physically-normal, newly-synthesized viral DNA; thus a viral function is required for some step(s) in integration. Retroviruses without oncogenes may initiate tumorigenesis by insertion mutations that activate cellular genes through promoter or enhancer functions in the long terminal repeats (LTRs) of proviruses. (i) During the development of avian leukosis virus (ALV)-induced B cell lymphomas, ALV proviruses cause insertion mutations at the c-myc locus. The mutations are associated with efficient expression of c-myc, regardless of whether the proviruses are upstream from c-myc in either transcriptional orientation or downstream from the cellular gene (2). Analysis of cloned, insertionally-mutated c-myc alleles indicates that additional mutational events have occurred, both deletion mutations within proviral DNA and nucleotide substitutions in the c-myc coding domain (3). In one case, deletion of proviral DNA occurred by homologous recombination between LTRs. These secondary mutations may augment expression of c-myc, incapacitate expression of viral genes, or alter the product of c-myc. (ii) Tumorigenesis by the mouse mammary tumor virus (MMTV) is often accompanied by proviral insertions in a host locus called int-1 (4). This locus, on mouse chromosome 15, harbors a transcriptional unit that is generally silent, but is activated to produce small numbers of copies of a 2.6 kb polyadenylated RNA in tumors bearing insertion mutations. Gene activation most commonly occurs by an indirect mechanism (enhancement), with MMTV proviruses either upstream from the gene in the opposite transcriptional orientation or downstream in the same orientation (5).

(1) Varmus, Chapter 10 in Mobile Genetic Elements, Academic Press, 1983. (2) Payne et al. Nature 295:209, 1982. (3) Westaway et al. Proc. Natl. Acad. Sci. USA, in press, 1984. (4) Nusse and Varmus, Cell 31:99, 1982. (5) Nusse et al. Nature, in press, 1983.

Gene Acquisition

1164 THE MECHANISM OF TN3 TRANSPOSITION IMMUNITY. Heffron, F., Lee, C. H.*, Bhagwat, A.+, and Huang, C. Scripps Clinic and Research Foundation, Department of Molecular Biology, La Jolla, CA 92037. *Indiana University, Indianapolis, IN 46223. +Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Using deletion analysis we have determined that the terminal repeat of Tn3 is all that is required for transposition immunity (1). To verify this, we have used chemical synthesis of DNA to construct a 38 bp fragment identical to the terminal sequence of Tn3. We find that this sequence confers transposition immunity to most but not all plasmids we have tested. In addition a transposon containing two of these synthetic termini can transpose. Application of these micro-transposons to mutagenesis will be discussed. We find that direct repeats of the terminal sequence transpose whereas in the wild type transposon the sequence at the termini of Tn3 are perfect inverted repeats. Surprisingly, similar transposons made with restriction fragments from Tn3 will not transpose. We have determined the sequence of the junction fragments of several of these recombinants.

1. Lee, C. H., Bhagwat, A., and Heffron, F. Identification of a Tn3 sequence required for transposition immunity. Proc. Natl. Acad. Sci. 80: 6765-6769, 1983.

Genome Evolution

1165 THE Tn3 FAMILY OF BACTERIAL TRANSPOSONS AND ITS RELATIONSHIP TO THE DNA-INVERTASES. Nigel D.F. Grindley, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT 06510.

Many bacterial transposons have been identified that are related in structure and function to the ampicillin resistance transposon, Tn3. Transposons of the Tn3 family encode two proteins involved in the transposition process: the transposase that mediates cointegrate formation and the resolvase that breaks down the cointegrate into the final products of transposition. On the basis of gene organization and function complementarity, two subgroups of Tn3-like transposons can be distinguished. The major distinction is the orientation of the tnpR (resolvase) gene relative to the tnpA (transposase) gene. In Tn3 and γδ these genes are divergently transcribed from a region that contains the site of cointegrate resolution (res); expression of both tnpA and tnpR is regulated by resolvase. In the transposons Tn501, Tn21 and Tn1721 the orientation of tnpR is inverted so that both tnpA and tnpR are transcribed in the same direction; the res site lies just upstream of tnpR. The resolvase proteins of Tn3 and γδ complement one another as do those of Tn501, Tn21 and Tn1721. Although resolvase complementation is not detected between members of the different subgroups, all five proteins are clearly related. The resolvases are also related to a family of enzymes that are responsible for site-specific DNA inversion; these DNA-invertases are Hin, Gin, Cin and Pin. Hin controls flagellar phase variation in Salmonela, Gin and Cin mediate host range alternation in phages Mu and P1.

Comparison of their amino acid sequences shows that the resolvases and DNA-invertases contain two regions with distinctly different degrees of sequence conservation. The N-terminal portion (about 140 amino acids) is rather well conserved both within and between each of the three complementation subgroups. The C-terminal portion (between 45 and 60 amino acids) is much less well conversed. Studies of the γδ resolvase indicate that these two "homology domains" correspond to separate activity domains. DNase footprinting demonstrates that a 45 amino acid C-terminal fragment produced by proteolysis contains the DNA binding activity. The N-terminal fragment (140 amino acids) contains the sites of both protein-protein interaction (as shown by X-ray crystallography) and recombinational activity (as indicated by mutational studies.)

1166 MITOCHONDRIAL DNA SEQUENCES IN THE YEAST NUCLEAR GENOME, Ronald A. Butow, Cheryl Golden and Alan P. Hudson, The Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Previous work from this laboratory established the presence of a DNA sequence in the yeast nuclear genome with ∿85% homology to portions of three non-contiguous mitochondrial DNA (mtDNA) sequences: the varl and cytochrome b (cob) genes and a putative origin of mtDNA replication [F. Farrelly and R.A. Butow (1983) Nature 301, 296]. The arrangement of these sequences in the nuclear genome suggested that they might have arisen from a rearranged petite-like mtDNA that escaped the mitochondria and integrated into the nuclear genome. In some strains, this nuclear DNA fragment containing sequences homologous to mtDNA is found closely linked to a tandem pair of transposable (Ty) elements. Using 13 cloned mtDNA sequences from both exon and intron regions of the yeast mitochondrial genome as hybridization probes to Southern blots of nuclear DNA from three ρ° yeast strains, we have identified at least 9 additional nuclear DNA sequences in the nuclear genome with homology to mtDNA. These include portions of the coding regions of the genes for the 21S and 15S rRNAs, cytochrome oxidase III (oxi2), ATPase 9 (oli1) and cob, and to intron regions of the 21S ω^+, cytochrome oxidase I (oxi3) and cob genes. The hybridization signals show considerable restriction polymorphism among the strains and vary in intensity, probably reflecting differences in the extent and degrees of homology. Of the nine positively hybridizing probes, five (21S exon, upstream 15S, oxi2, oli1 and cob exon 6) react with nuclear DNA from all strains examined, while four (21S upstream ω^+, 21S downstream ω^+, downstream 15S, and oxi3 intron 4) react with nuclear DNA from just one or two of the strains. Preliminary restriction mapping suggests that these mtDNA sequences are not tightly linked, including the 21S exon and ω^+ intron sequences. We have obtained clones of some of these nuclear sequences by searching a λ Charon 4 library of partial EcoRI fragments of yeast nuclear DNA. One such clone (λ 6-1) contains ∿600 bp of DNA homologous to 21S exon sequences that is immediately flanked by two Ty elements; in addition, λ 6-1 contains sequences homologous to the oxi2 gene. Further screenings are in progress. Since some mtDNA intron sequences have been found in the nucleus, it seems unlikely that the transfer occurred through mature mtRNA intermediates. (Supported by Grants from the NIH and The Robert A. Welch Foundation)

Poster Session I

1167 A 140 BASE PAIR DNA SEGMENT FROM PLASMID R1 ACTS AS AN ORIGIN OF REPLICATION AND PROMOTES recA-INDEPENDENT SITE-SPECIFIC RECOMBINATION, Michel Clerget, Département de Biologie Moléculaire, Université de Genève, Genève, Switzerland.

A circular form of Tn2350, an IS1 flanked kanamycin resistance transposon carried by plasmid R1, was isolated and shown to be capable of autonomous replication (Clerget et al., J. Bacteriol. 151, 924, 1982). Essential replication functions of this plasmid (pTn2350) are contained within a 140 bp region that has been sequenced. This 140 bp segment acts as an origin of replication since it allows autonomous replication of a plasmid composed only of this sequence and the tetracycline resistance gene of pBR322. In addition this sequence promotes three kinds of recA-independent recombination events (fusion, deletion and inversion) that characterize other site-specific recombination systems. When cloned on pBR322, a single copy of this sequence permits multimer formation, in a recA strain. Two copies permit either deletion or inversion of the intervening region, depending on their respective orientation. DNA gyrase seems to be involved because the inversion rate in a plasmid carrying sequences in opposite orientation varies in different independently isolated spontaneous nalidixic acid resistant strains (gyrA mutants).

1168 TN916: A TRANSPOSON IN STREPTOCOCCUS FAECALIS WITH FERTILITY PROPERTIES, C. Gawron-Burke and D.B. Clewell, University of Michigan, Ann Arbor, Michigan, 48109

The chromosomal tetracycline (Tc) resistance determinant of Streptococcus faecalis DS16 is located on a 10 Mdal transposon, designated Tn916. Recent experiments have shown that Tn916 can be conjugatively transferred in the absence of plasmid DNA, and Southern blot hybridization experiments have demonstrated its insertion into different sites on the recipient chromosome. Certain Tc^r transconjugants of recipient strain JH2-2, such as CG110, transfer Tn916 at elevated frequencies: transposition of Tn916 to a subsequently introduced plasmid (pAD1) in strain CG110 is similarly increased. These studies imply that transfer and transposition share a common step; and recent data indicate that Tn916 transposition involves an excision-insertion process. It has been possible to clone fragments containing Tn916 in Escherichia coli on the pBR322-derived vector pGL101 and obtain expression of Tc-resistance; but an instability leads to rapid excision (and segregation) of the transposon under nonselective conditions. Interestingly, the DNA fragment into which Tn916 had been inserted is apparently regenerated on the vector. Plasmid DNA containing Tn916 can be used to generate plasmid-free Tc^r transformants of the oral bacteria, S. sanguis, which can be shown to harbor chromosomal Tn916 insertions at various sites. Thus, Tn916 may be a useful tool in the targeting of genes in gram positive bacteria for subsequent cloning in E. coli.

1169 THE CIS-ACTING TERMINAL SEQUENCES REQUIRED FOR BACTERIOPHAGES MU AND D108 DNA TRANSPOSITION AND MATURATION. Josée Harel, Jeffrey S. Kahn, George Szatmari, and Michael S. DuBow. McGill University, Montréal, Québec H3A 2B4 Canada.

Temperate bacteriophages Mu and D108 are heteroimmune, plaque-forming mobile genetic elements whose 37 kilobase double-stranded DNA genomes undergo approximately 100 cycles of DNA transposition during their one hour lytic cycles. In order to study the functional transposition and maturation domains that comprise the terminal cis-acting DNA sequences of these phages, we have subcloned large internally-deleted phage genomes (called Mini-Mu's or Mini-D108's) in the plasmid pSC101. These plasmids with fused phage termini are devoid of their transposition and packaging genes but are transpositionally proficient and capable of being assembled into virions in the presence of their respective helper phages in vivo. Plasmids pMD186 and pMD861 are isomers containing the Mu termini in close (or inverse) orientation (407 bp part) or in their normal orientation with the ends separated by 9.3 kb of pSC101 DNA, respectively. Though the outcomes of the vast majority of Mu and pMD861 Mini-Mu transposition events are replicon fusions (co-integrates), virtually all pMD186 transpositions result in simple linear insertions of the Mini-Mu plasmid. Moreover, we have further resected the ends of Mu (in both orientations) and plasmids (e.g. pJK36 containing 56 bp of attL and 116 bp of attR) do not detectably transpose or chromosomally associate upon provision of transposition functions from a helper phage. Ongoing experimentation with other subclones of Mini-Mu's, Mini-D108's and hybrid Mini-element plasmids will allow us to identify the functional terminal cis-acting DNA sequences and their spatial and orientation parameters required for DNA transposition and maturation.

1170 CHARACTERIZATION AND FUNCTIONAL DEFINITION OF THE ENZYMES WHICH REGULATE MU AND D108 DNA TRANSPOSITION. Michael S. DuBow, David Levin, Peter Tolias and Kelly Fox, McGill University, Montréal, Québec H3A 2B4 Canada.

Bacteriophages Mu and D108 are heteroimmune temperate coliphages whose 37 kilobase double-stranded DNA genomes behave as transposable genetic elements and whose regulatory and transposition functions do not complement one another in vivo. Lytic growth and lysogeny are controlled by two divergently transcribed genes, repressor (c), and ner, a λ cro-like function. We have cloned these genes and are isolating their gene products in order to characterize the mechanisms by which they regulate the lysis-lysogeny decision of a temperate transposable bacteriophage.

We have cloned the Mu repressor (pUD68), Mu ner, and the amino-terminal 20% of the Mu A gene (pUD88) under the control of the lac UV5 promoter in the expression vector pOP95-15. Furthermore, we have cloned the D108 repressor (pLV108) under λ CI857 control. These plasmid containing strains are all two to four orders-of-magnitude more immune and/or pseudoimmune than non-plasmid containing strains. Furthermore, chloramphenical-release assays demonstrate that these cloned genes greatly overproduce their respective polypeptides in vivo. We are currently using classical chromatographic techniques coupled with nitrocellulose filter-binding assays and sensitive protein-DNA blotting of polyacrylamide SDS-gels (versus radio-labelled Mu, D108, and λ (control) DNA's) to purify these (and other) regulatory enzymes. Our ultimate goal is to identify their mode of action, the DNA sequences to which they bind, and the phage and host-encoded enzymes with which they may associate and which may regulate their action and their specificity.

1171 NEW ORIGINS OF DNA REPLICATION IN Escherichia coli: INITIATION IN THE ABSENCE OF oriC. Tokio Kogoma[1], Bernard de Massy[2] and Olivier Fayet[2]. Department of Biology[1], University of New Mexico, Albuquerque, NM 87131 and Centre de Recherche de Biochemie et de Génétique Cellulaires du C.N.R.S.[2], 118 route de Narbonne, 31062 Toulouse Cedex, France.

Amplification of eukaryotic genes may result from abberant, repeated replication of certain DNA sequences and subsequent resolution into a tandem array by multiple step recombination. In Escherichia coli, initiation of a round of chromosome replication normally occurs exclusively at a unique site termed oriC. This tightly regulated event in the cell cycle involves an interaction between the oriC sequence and the dnaA protein. However, we have shown that sdrA (rnh) mutants of E. coli which lack ribonuclease H (RNasH) activity can initiate chromosome replication despite the absence of oriC from the chromosome. This suggests that the absence of RNase H allows initiation of DNA replication from certain, normally repressed site(s). The replication appears to require a recombinational activity. We will report evidance that in sdrA (rnh) mutants DNA replication is initated in at least four discrete regions of the chromosome in the absence of oriC. The locations of the new origins lend a support to a hypothesis that the E. coli genome may have evolved through two entire chromosome doublings. The possible involvement of this second mechanism of DNA initiation in gene amplification will be discussed.

1172 Transfer of the Bacterial Chromosome Mediated by Tn5 Containing the Origin of Transfer of RK2. Donald G. Guiney and Emmanuel Yakobson, U.C.S.D. Medical Center, San Diego, CA 92103.

The origin of transfer (oriT) of the broad host range plasmid RK2 has been cloned and sequenced. The fully functional oriT is located on a 760 bp HaeII fragment near one end of the tral region of the plasmid. This HaeII oriT fragment was cloned into a non-essential region of Tn5. The resulting Tn5 (oriT) transposon can transpose to other plasmids or onto the chromosome at normal frequencies. When present in the chromosome, the Tn5 (oriT) element provides an origin of transfer for chromosomal genes during conjugation mediated by a helper RK2 plasmid present in trans. This system has been shown to provide oriented, high frequency transfer of chromosomal genes in E. coli and Rhizobium melitoti. The broad host range properties of RK2 will allow this system of chromosome transfer to be applied to almost any gram-negative bacterium.

1173 EFFECTS OF THE BACTERIOPHAGE Mu c & ner GENE PRODUCTS ON EXPRESSION OF THE EARLY VIRAL GENES IN VITRO, H.M. Krause and N. Patrick Higgins, University of Alabama in Birmingham, Birmingham, Alabama 35294

Mu is a temperate bacteriophage which utilizes transposition to replicate its DNA during lytic growth. We are studying the transcriptional regulation of the Mu early region which carries the genes for viral integration and replication. The Mu early promoter P_E straddles a HindIII restriction site 1000 base pairs from the Mu left genetic end and initiates transcription rightward. Mu repressor (c) and a second protein (ner) are implicated in the transcriptional regulation of the Mu A gene product (the transposase) as well as the other early viral proteins involved in DNA regulation. Both proteins have been cloned in lambda expression vectors. The interaction of these proteins with DNA and their effects on transcription in vitro are being investigated to understand the regulation of Mu transposition and the switch between lysogenic and lytic pathways.

1174 ANALYSIS OF TARGET SITES FOR MU INTEGRATION, Hiroshi Nakai and Austin L. Taylor, Dept. of Microbiology/Immunology, University of Colorado Health Sciences Center, Denver, CO 80262.

The hypothesis that bacteriophage Mu preferentially integrates at host replication forks was tested. A two-step or sandwich hybridization assay was designed to analyze the relative kinetics of Mu integration into different parts of the host chromosome. An auxotrophic strain of E. coli K12 was starved for amino acids and infected with Mu upon release from starvation, and the relative amount of Mu integration into various parts of the chromosome was determined by the sandwich hybridization assay. The results indicated that Mu integrated into target sequences at various distances from oriC with similar kinetics. No evidence for preferential integration at sites of chromosomal DNA synthesis was observed. In an extension of these studies, auxotrophic Hfr strains were aligned for chromosomal DNA synthesis by amino acid starvation and nalidixic acid treatment and infected with Mu after allowing DNA synthesis to proceed for various times. The position of integrated copies of Mu was determined by DNA transfer kinetics of Mu sequences from the Hfr strains after treatment with chloramphenicol. This analysis again revealed that Mu does not preferentially integrate at regions of the chromosome being replicated. However, it revealed that the amino acid starvation and nalidixic acid treatment affected DNA transfer by each Hfr strain differently. These results indicate that host replication forks are not preferential target sites for Mu integration.

1175 PHASE VARIATION IN BORDETELLA PERTUSSIS, Sarah Goldman, Emanuel Hansky and Falk Fish, Tel Aviv University, Tel Aviv, Israel and the Weizmann Institute of Science, Rehovot, Israel

Pathogenic strains of Bordetella pertussis are known to undergo phase variation and to become non-pathogenic upon culturing in vitro. In order to study the variation process we have isolated phase variants from 2 different pathogenic strains of B. pertussis and characterized some of their virulence traits, namely: hemolysis, exotoxin, hemagglutinin and adenylate cyclase.

Only 4 phenotypes were identified within a sample of over 250 variant strains. Based on the analysis of the frequency and the interrelationship among the virulence traits, we concluded that phase variation in B. pertussis, as examplified by the loss of virulence traits, is a non-random, step-by-step sequence of events. Some events may, in addition, affect later events in a polar manner.

1176 DO CHANGES IN PILUS EXPRESSION FOR NEISSERIA GONORRHOEAE INVOLVE CHROMOSOMAL REARRANGEMENT? John Swanson, LMSF, Rocky Mountain Laboratories, Hamilton, MT 59840 and Michael Koomey, Stanford University, Stanford, CA 94305

Neisseria gonorrhoeae undergo relatively frequent changes in piliation ($pil^+ \rightleftharpoons pil^-$) as well as exhibiting transitions in the pilin subunit sizes they express. These changes in piliation and in pilus subunit size have been closely followed with Southern hybridization of parental and variant DNAs subjected to restriction endonuclease cleavage and probed with a recombinant plasmid (pVD203) containing a 1.2 kb fragment of N. gonorrhoeae strain MS11 DNA that encodes for pilin. Comparison of hybridization patterns coincident with single-step changes in piliation for several strains, including strain MS11 from which the cloned DNA was derived, reveal the following: (a) among assorted strains of N. gonorrhoeae, several different hybridization patterns can be observed; (b) five different hybridization patterns were observed for strain MS11; these did not correlate with particular phenotypes; (c) evidence of chromosomal rearrangement in the form of differences in Southern hybridization patterns were not consistently found for $pil^+ \rightleftharpoons pil^-$ transitions; (d) no changes in hybridization were observed coincident with changes in pilin subunit size among pil^+ organisms of the same strain; and (e) no changes in hybridization pattern were found during change in another phenotype (colony opacity) of N. gonorrhoeae which involves gain/loss of outer membrane protein II constituents. These findings raise doubt about the previously reported association between piliation change and chromosomal rearrangement in N. gonorrhoeae (Meyer, Mlawer & So. Cell 30:45, 1982).

1177 TWO-DIMENSIONAL S_1 NUCLEASE HETERODUPLEX MAPPING OF TRANSLUCENT VERSUS OPAQUE PHASE VARIANTS OF NEISSERIA GONORRHEAE, Fred Correia, Thomas Yee, and Masayori Inouye, SUNY at Stony Brook, Stony Brook, NY 11794

Colonies of Neisseria gonorrheae exhibit phase variation phenomena including switching between opaque (o^+) and translucent (o^-) phenotypes. Opacity is associated with the expression of a family of heat-modifiable outer membrane proteins. It is expected that switching between the o^+ and o^- phenotypes may be due to DNA rearrangements, like those associated with differential expression of pilus proteins in this organism. To identify those DNA segments which may be undergoing rearrangement in the o^+ to o^- switch, as well as other possible cryptic rearrangements, a two-dimensional S_1 nuclease heteroduplex mapping technique was developed in our lab (Yee and Inouye, submitted for publication). Genomic DNA restriction digests from two bacterial variants are mixed, denatured, renatured, then electrophoresed in a first dimension. In situ digestion by S_1 nuclease, followed by electrophoresis in a second dimension, resolves digested heteroduplexes as distinct spots or streaks beneath a main diagonal of unresolved homoduplex. We find that opaque and translucent DNA isolates of N. gonorrhoeae do indeed exhibit unique heteroduplex spots. When isolated and nick-translated, these spots hybridize differentially to genomic blots from opaque and translucent strains. We interpret these results as supporting evidence that genomic rearrangements may be responsible for the differential expression of the heat-modifiable outer membrane proteins of N. gonorrhoeae.

1178 AVIRULENCE IN GROUP A STREPTOCOCCI RESULTS FROM A DNA REARRANGEMENT IN SEQUENCES ADJACENT TO OR WITHIN THE M PROTEIN STRUCTURAL GENE, P. Cleary, S. Jones and J. Spanier, University of Minnesota, Minneapolis, MN 55455

Group A streptococci, best known for their propensity to cause pharyngitis and rheumatic fever have long been known to be genetically unstable. Cells from matt colonies are resistant to phagocytosis, a property dependent on the presence of the M protein on their surface (M+). These forms readily segregate cells that lack M protein (M-). These studies attempt to define the genetic switch that controls the synthesis of this important antigen. An E. coli clone derived from an M12 streptococcal genomic library was shown to synthesize M12 protein. A shortened segment of this streptococcal DNA was subcloned, and a PvuII-HaeIII map was developed. One fragment, PvuII B (3.1kb), is able to direct the synthesis of a cross reacting M12 protein, suggesting that one end terminates within the M12 structural gene, and that this fragment includes the promoter and 5' coding sequence. In order to detect alterations in the M structural gene or adjacent sequences in M- cells, the cloned PvuII B fragment was employed as labeled probe to compare DNA from M+ and M- isogenic strains. Chromosomal DNA's from paired strains were digested with HaeIII, an enzyme that cuts the cloned PvuII B sequence twice. When digestion products were electrophoresed, blotted onto nitrocellulose and hybridized to the PvuII B probe, three HaeIII fragments were revealed by autoradiography. The HaeIII fragment in DNA from M- cells that is thought to overlap the amino terminus of the M protein coding sequence was estimated to be 50 to 100 bp smaller than that in DNA from M+ cells. Moreover, PvuII digests of these DNA's also suggest that M- cells had undergone a rearrangement in or near the 5' end of the M12 structural gene. The molecular nature of this alteration is under investigation.

1179 CLONING OF THE PHASE VARIATION CONTROL REGION OF TYPE 1 FIMBRIAE IN E. COLI. Cynthia S. Freitag, John M. Abraham and Barry I. Eisenstein, University of Texas Health Science Center at San Antonio, Texas, 78284.

Expression of type 1 fimbriae in Escherichia coli is regulated by phase variation exhibiting an all-or-none phenotype. A strain was constructed which carries a fimD-lac operon fusion which undergoes $Lac^+ \rightleftarrows Lac^-$ phase variation. Specialized lambda transducing phage were isolated that contain the fimD-lac operon fusion. Integration of this phage into a Fim^+ strain results in the construction of a merodiploid strain with a double switching phenotype ($Fim^+ \rightleftarrows Fim^-$ and $Lac^+ \rightleftarrows Lac^-$) in which the two phase variations are independent and noncoordinated. These results demonstrate that the phase variation control region is carried on the specialized phage and that it is adjacent to the fusion. Light blue and dark blue plaques can be obtained from the specialized phage; both plaque types can alternate to the other phenotype indicating the phase variation may be occurring on the specialized phage. The entire fragment of bacterial DNA present on the phage was cloned into pBR322 and used as a hybridization probe in Southern blot analysis of a Fim^+ strain, detecting a restriction fragment that is not present in a Fim^- strain. The restriction fragment to which the probe is homologous is being cloned; the cloned DNA will be sequenced and analyzed genetically to determine the molecular mechanism of phase variation in E. coli.

1180 THE HIN PROTEIN AND PHASE VARIATION IN SALMONELLA, Michael F. Bruist and Melvin I. Simon, California Institute of Technology, Pasadena, CA 91125

The alternative expression of the Salmonella flagellin genes H1 and H2 is controlled by the orientation of a one kilobase pair invertible segment of DNA located at the 5' end of the H2 gene. The hin gene,which is encoded within the invertible region, is essential for inversion of the DNA. We have cloned the hin gene into E. coli and placed it under control of the P_L promotor of bacteriophage λ so that the Hin protein is copiously overproduced. A recombinant λ phage containing the H2 and the lac Z genes under control of the invertible region has been used to measure in vivo inversion activity catalyzed by Hin. Using this phage we have shown that the amount of inversion activity is proportional to the amount of Hin protein in the cell. An inactive form of the protein has been purified using its unusual solubility properties. The amino acid composition of this protien agrees with the DNA sequence. Antibodies have been made to the isolated protein and are being used in an assay for purification of the native protein.

1181 E. COLI INSERTION ELEMENTS ENHANCE EXPRESSION OF A GRAM-POSITIVE ERYTHROMYCIN RESISTANCE DETERMINANT, Francis Barany, Johns Hopkins Univ. Med. Sch., Baltimore, MD 21205

The gram-positive plasmid pFB9 (high copy number, constitutive erythromycin (Em^R) and chloramphenicol (Cm^R) resistance)can replicate (at low copy number) and express both resistance determinants in E. coli.[1] Spontaneous plasmid mutants arise in E. coli which demonstrate enhanced Em^R. These plasmid mutants (pBB plasmids) contain insertion elements IS1, IS2, or IS5 in front of the Em^R gene; 60 independent IS insertions which map throughout pFB9 have been isolated. Using a transformation frequency assay, it was demonstrated that Em^R enhancement is related to the distance of the IS element from the Em^R determinant, but independent of IS element orientation. This result (and additional constructions) make it appear unlikely that IS elements provide simple promotor activity or increase copy number. Enhancement of Em^R may be analogous to activation of the cryptic bgl operon in E. coli.[2] Insertion of foreign DNA into the IS element decreases Em^R expression. Recombinants in E. coli appear as small colonies on erythromycin containing plates. Plasmid pBB5 (pFB9::IS5) which contains unique Bgl II and Eco RI sites within IS5, and has a wide host range (related derivatives replicate in S. pneumoniae[1], S. aureus[3], B. subtilis[4], E. coli[1,5], and even the eucaryotic S. cerevisiae[6]) could become an attractive broad host range shuttle vector.

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1182 EVIDENCE OF AN INDUCIBLE PHASE-TYPE CONTROL SYSTEM IN ESCHERICHIA COLI K-12, Barry L. Wanner, Emory University, Atlanta, GA 30322

A mutanc search was conducted for a regulated gene whose induction involves a "change-in-state of the DNA. This was done in two stages. The first step involved fusing lacZ to inducible promoters by using the Mud1 (Amp, lacZ) transposon. Strains were identified that had twenty different phosphate-regulated promoter-lacZ fusions. These phosphate-starvation-inducible, or psi, promoters synthesize B-galactosidase when starved. They repress lacZ expression when phosphate is added back or when the strains are grown in high-phosphate media, in the absence of another inducing trigger (J. Mol. Biol. 166, 283(1983). Finally, the Mud1 fusions were converted to stable fusions to allow the isolation of secondary mutants showing altered psi-lacZ expression. In the second step, a search was made for mutants showing phase-type control of psi - lacZ. To do this, mutagenized cultures were grown in microtiter dish wells and then spotted onto high phosphate lactose indicator media. Mutants having a "speckled" appearance were further characterized. While some acted as simple unstable mutants, others showed bi-directional phase-type control of lacZ. According to a model for inducible phase-type control, the latter mutant class could be regulated by a site-specific DNA recombination in which a repressor binds DNA sequences which are substrates for a DNA rearrangement, thus preventing site-specific recombination. The mutants could be altered in a putative repressor protein, thus allowing constitutive switching. As predicted by this model, the switching frequencies vary and are dependent upon the growth media.

1183 A REPETITIVE PALINDROMIC EXTRAGENIC SEQUENCE IN E.coli, Maurice Hofnung and Eric Gilson, UPMTG, Institut Pasteur, 75015 Paris, France

We study a palindromic extragenic sequence which is found repeatedly in E.coli. Computer analysis of the available DNA sequence data as well as restriction and genetic analysis of some of the occurrences of this sequence allow to make hypotheses on its possible rôle in genome evolution and in gene expression.

1184 A VERTEBRATE SEQUENCE WHICH UNDERGOES SPECIFIC DELETIONS IN RESPONSE TO A PROKARYOTIC RECOMBINATION SYSTEM, Paul Keim, Andrew T. Thliveris, Edward A. Meenen and Karl G. Lark, University of Utah, Department of Biology, Salt Lake City, UT 84112

A sequence isolated from the Kangaroo Rat, D. ordii, has been found to undergo specific deletions in E. coli. These are mediated by the lambda Red or E. coli RecE functions (MGG (1982) 188:27-36). The specific nature of the deletions suggested a sequence dependent mechanism. Genomic DNA has been cloned into a Red- lambda vector and then subcloned into the plasmid pUC9. The plasmid can be grown on a RecE- host without deletion or in RecE+ host to obtain deletions. DNA can be prepared using the RecE- host. We have sequenced the deletion boundaries in several clones as well as deletions which were derived from these clones. The sequenced deletion boundaries are partially homologous and the size of the deletion product appears to be dependent upon this homology. In deletions, the actual switch occurs in or adjacent to a nonhomologous region. A 7 nucleotide sequence (or slight variations of it) is found at the homology-nonhomology junction. Such short sequences are found in other vertebrate genomic regions where specific rearrangements are known to occur including the immunoglobin and the histocompatibility genes.

1185 TRANSPOSITION OF Tn554 IN STAPHYLOCOCCUS AUREUS DOES NOT GENERATE A TARGET DUPLICATION, Ellen Murphy and Maria do Carmo de F. Bastos, The Public Health Research Institute, New York, N.Y. 10016

Tn554 is a transposable element in Staphylococcus aureus that encodes resistances to erythromycin and spectinomycin. It is both site- and orientation-specific, inserting into a single location in the S. aureus chromosome with a frequency approaching 100%; it also inserts very rarely into secondary sites. The chromosomal insertion site for Tn554 has been cloned, and the junctions of several independent Tn554 insertions were sequenced, revealing that Tn554 does not generate a duplication of the target DNA upon insertion, nor do its termini contain inverted or directly repeated sequences. These findings suggest that the mechanism of Tn554 insertion is significantly different from that of most transposons and may more closely resemble that of temperate bacteriophages such as λ. The left end of Tn554 contains an open reading frame, from bp 134 to 1216, which could specify a 361 amino acid polypeptide. This open frame is likely to encode a protein required for transposition, as *in vitro* mutatgenesis at a HindIII site located at bp 816 results in a transposition-defective phenotype. The probable promoter region for this transposase overlaps the terminal 89 bp of Tn554. This terminal region, when cloned to a plasmid containing no other transposon sequences, acts as an inhibitor of transposition: it reduces the transposition of an intact copy of Tn554 into its primary site by 2-3 orders of magnitude. The mechanism involved in this regulation is presently under investigation.

1186 INSERTION SEQUENCES PRESENT ON TWO IncN PLASMIDS, George M. Coupland, Anthony M.C. Brown and Neil S. Willetts, Dept. Molecular Biology, Edinburgh University, Edinburgh, Scotland.

Two IncN plasmids, R46 and N3, have been searched for transposable elements. Self annealing studies of R46, and the formation of co-integrates between R46 (or N3) and pME420 (a $Cm^R Ap^R Tc^S$ deletion of pBR325), revealed the presence of two insertion sequences on each IncN plasmid. The frequency of co-integrate formation was 10^{-5}-10^{-6}. R46::pME420 co-integrates could be resolved in Rec^+ backgrounds to yield pME420 carrying a copy of the insertion sequence. Restriction analysis of the products showed that the insertion sequence, IS46, contains 800 b.p., and single SalI and PstI targets. All four copies in R46 and N3 hybridised with one another and with IS15, but not with IS1, in Southern analyses. Restriction analysis of R46 showed that the two copies of IS46 are mutually inverted. An R46 derivative has been isolated in which the region between the two copies of IS46 has been inverted, presumably by reciprocal recombination. In N3, however, the two IS46 copies have the same orientation; and Tc^S derivatives have been isolated which have lost the intervening region, again by reciprocal recombination. We also propose that pKM101, an R46 derivative used in the Ames test for carcinogens, was formed from R46 by deletion mediated by IS46.

1187 CLONING OUTER MEMBRANE PROTEIN GENES OF GONOCOCCI WITH SYNTHETIC OLIGONUCLEOTIDES, Leonard W. Mayer, LMSF, RML, NIAID, NIH, Hamilton, MT 59840

Mixed, oligonucleotide probes are being used to identify genes coding outer membrane proteins of Neisseria gonorrhoeae. The P.II or opacity proteins of gonococci are a family of nonessential proteins on the bacterial surface. Amino acid sequence (provided by Milan Blake, Rockefeller University) of P.II protein from strain R10 is "reverse translated" to predict the possible m-RNA sequences. This mixture of oligonucleotides binds to 2-6 fragments of restriction endonuclease digested DNA in a Southern blot. The number of bands observed varies with the enzyme used for digestion of the DNA. The probe does not hybridize with DNA isolated from Escherichia coli, phage λ, or an unrelated gonococcal strain. Several other lines of evidence suggest that more than one gene coding for P.II proteins is present in the gonococcal genome. The expression of these genes appears to be independent of each other and not correlated with pilus gene expression. P.II protein expression may involve a mechanism similar to phase variation of Salmonella.

1188 THE TWO TN3 TERMINAL REPEAT SEQUENCES ARE THE ONLY DNA SEQUENCES REQUIRED FOR TN3 TRANSPOSITION, Chao-Hung Lee*, Jr-Shin Twu+, and Fred Heffron++, *Department of Pathology, +Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46223; ++Department of Molecular Biology, Scripps Clinic and Research Foundation, La Jolla 92037

Transposition of Tn3 is a two-step process. The first step is the formation of cointegrates. In the second step, the duplicated copies of the transposon resolve to yield a precise transposition. The two terminal inverted repeat (IR) sequences are essential for Tn3 transposition. However, it is unknown whether any internal sequences of Tn3 are required for the first step of transposition and whether the size of the transposon has any effect on its transposability.

To investigate these questions, we have chemically synthesized the 38 bp terminal sequence of Tn3 and made several constructions. The two 38 bp IR sequences were cloned in an inverted orientation and were separated either by a 8 bp BamHI linker or by a 1000 bp DNA fragment which encodes for B-lactamase. The transposition frequencies of these artificially constructed transposons were determined to be similar to that of the intact Tn3. These results demonstrate that no sequences other than the 38 bp IR sequences are required for the first step transposition of Tn3.

1189 INACTIVATION OF A MAJOR HOTSPOT FOR TRANSPOSON Tn5 INSERTION BY A SINGLE BASE PAIR CHANGE. J.K. Lodge, K. Weston-Hafer, J.B. Lowe and D.E. Berg, Washington University School of Medicine, St. Louis, MO.

The bacterial transposon Tn5 inserts into many sites in a genome. Our physical mapping of Tn5 insertions in plasmid pBR322 has revealed a set of 5 insertion hotspots in the first 300 bp of the tet gene, in addition to many low frequency insertion sites (Berg, Schmandt and Lowe, 1983, GENETICS, in press). Tn5 generates 9 bp duplications of target sequences, which are believed to reflect a 9 bp spacing of cleavages of the two target DNA strands during insertion. The only obvious feature common to each of the hotspots is a GC base pair at the first and ninth positions of the duplication. To test whether a GC cutting preference helps quide Tn5 to its hotspots, we used in vitro bisulfite mutagenesis to generate a GC→AT transition at pBR322 position 31 (position 1 of the hotspot I duplication). None of 62 insertions of Tn5 into the tet gene of this transition mutant were at hotspot I, in contrast to 21 of 75 in pBR322-wild type. Single base pair changes (CG→GC and CG→AT) at position 32 of pBR322 (position 2 of the hotspot I duplication) (from D. Shortle) do not significantly affect the frequency of insertion at hotspot I (5 of 33, and 3 of 27 insertions in the tet gene respectively). Tn5's transposase may search for each of several distinctive features in target DNA sequences in choosing insertion hotspots. Our inactivation of the major hotspot for Tn5 insertion in pBR322 by the GC→AT transition at position 31 argues that a GC cutting preference is one important factor in target site selection.

1190 MUTANTS OF λ::MINI-MU ALTERED IN MU-SPECIFIC INTEGRATION, REPLICATION, AND GROWTH INHIBITION PROPERTIES. Martha M. Howe, Janet L. Miller, Debra L. Lynn, Mark G. Obukowicz, Anna C. Glasgow and Richard P. Burlingame, Department of Bacteriology, University of Wisconsin, Madison, WI 53706.

λ::mini-Mu phages with the left EcoRl fragment and portions of the right EcoRl fragment of Mu DNA in their normal relative orientation carry out Mu-specific DNA replication, Mu-promoted integration of a mini-Mu --λ-- mini-Mu cointegrate, and Mu-promoted inhibition of growth of the λ::mini-Mu phage (EOP of 10^{-3}-10^{-5} in a Mu^S host). To define the processes causing growth inhibition we isolated and characterized two types of λ::mini-Mu mutants: 1) inhibition-defective mutants selected by growth on a Mu^S host, and 2) deletion mutants selected by growth in the presence of citrate. Each of 69 recessive inhibition-defective point mutants were defective in the mini-Mu A or B functions; the A-defective mutants integrated at a reduced frequency. Deletions removing the contiguous mini-Mu late genes M through U and gin had no effect on the integration or growth inhibition properties. Mutants with deletions extending into the mini-Mu A or B genes exhibited recessive defects in growth inhibition. Two classes of deletions removing markers near the mini-Mu ends were completely defective in integration and exhibited cis-dominant defects in growth inhibition. These mutants are postulated to be deleted for the left or right Mu attachment site. The properties of these mutants suggest that growth inhibition results from mini-Mu replication within the λ::mini-Mu phage. Sequence analysis of cis-dominant integration-deficient point and deletion mutants is being carried out to define the Mu DNA sequences required for integration and replication.

1191 ANALYSIS OF INHIBITORS OF SITE-SPECIFIC RECOMBINATION BY TN3 RESOLVASE, Michael A. Fennewald and Roland Saldanha, University of Notre Dame, Notre Dame, IN 46556.

We have isolated five inhibitors of site-specific recombination mediated by the Tn3 resolvase. These inhibitors will inhibit site-specific recombination both in vivo and in vitro. Two of these inhibitors, A1062 and A20832, will inhibit both site-specific recombination and the topoisomerase activities of the Tn3 resolvase. For the inhibitor A1062, we have tested a series of analogues of this compound to try and define the important structures for inhibition and to design an inhibitor of greater effectiveness. We have also tested the in vivo effects of these inhibitors. Because Tn3 resolvase performs site-specific recombination so quickly in vivo, it has been necessary to isolate temperature-sensitive mutations in the Tn3 resolvase. With these ts alleles, we measured inhibition of site-specific very effectively and are currently defining the dose-response and kinetics of inhibition as well as the effects on other site-specific recombination systems.

1192 SYNCHRONIZATION OF REPLICATIVE TRANSPOSITION OF BACTERIOPHAGE MU, Martin L. Pato, Claudia Reich and Barbara Waggoner, National Jewish Hospital & Research Center, Denver, Colorado 80206

Replicative transposition of bacteriophage Mu DNA was synchronized by induction of a Mu lysogen in the absence of DNA synthesis for a time sufficient to develop the potential for Mu DNA replication in all cells in the population. Release of the block on DNA synthesis in the presence of chloramphenicol (to inhibit resynthesis of the transposase, which is used stoichiometrically) is followed by a single replicative transposition event. We have used this system for studying the location and orientation of the two resulting prophage copies. We have also examined the kinetics of accumulation of replication potential following induction. Approximately 12 min are required for the population of cells to achieve replication potential; protein synthesis is required up to about 9 min, suggesting that 3 min are required for other events such as bringing together prophage ends, binding to the target DNA site, appropriate nicking and ligation events and attachment to host replication machinery.

1193 CHARACTERIZATION OF DNA SEQUENCES WHICH ARE REREPLICATED IN A SINGLE CELL CYCLE AFTER TREATMENT WITH AGENTS WHICH FACILITATE GENE AMPLIFICATION
T.D.Tlsty and R.T.Schimke

Our laboratory has been studying the molecular events which lead to the amplification of the dihydrofolate reductase (DHFR) gene in response to methotrexate (MTX) selection. We have found that agents ,such as UV radiation and hydroxyurea, which transiently inhibit DNA synthesis facilitate the emergence of MTX resistant colonies and that a large proportion of these colonies were found to have an increased gene copy number of the DHFR genes (i.e. gene amplification). Using the density label, bromodeoxyuridine, and the Fluorescence Activated Cell Sorter, recent work from this laboratory has identified the initial event of this process as a multiple replication of a set of DNA sequences within a single cell cycle. Analysis of the rereplicated sequences shows that the DHFR gene is not the only sequence to increase in gene copy number. In a synchronized population of CHO K1 cells the majority of sequences which are replicated in a semi-conservative manner early in the S phase, before the initiation of the DNA synthetic block, are rereplicated when the block is released. We are now in the process of characterizing the subset of DNA sequences which are rereplicated under these conditions in an effort to determine the criteria necessary to target gene amplification.

1194 CHARACTERIZATION OF CIRCULAR DNAS BEFORE AND AFTER HYDROXYUREA TREATMENT IN MOUSE CELLS RESISTANT TO METHOTREXATE

Anna B. Hill, David T. Smouse, Stephen M. Beverley, and Robert T. Schimke, Stanford University, Stanford, California 94305

The C3 subclone of the mouse lymphoma L5178Y has 800 copies of the dihydrofolate reductase (dhfr) gene stably localized in a large homogeneously staining region on chromosome 2. The appearance of extrachromosomal DNA has been observed in C3 metaphases following a 6 hour block of DNA synthesis by hydroxyurea (HU). To determine if this extrachromosomal DNA contains circular copies of the dhfr gene, DNA was isolated from control and HU treated cells, treated with Pol 1 and T4 DNA ligase to close nicks potentially induced by HU, and centrifuged through CsCl-EtBr. gradients. Fractions from the gradients were electrophoresed on 0.5% agarose gels; gels were dried and hybridized *in situ* with ^{32}P nicktranslated cDNA to dhfr (dll) and mouse mitochondrial DNA. Our results show that there are DNA sequences that hybridize to dll present in the portion of the gradient in which closed circular molecules migrate. Mitochondrial DNAs band in this portion of the gradient, but the mitochondrial DNA probe does not cross hybridize to the dhfr sequences, indicating that the dhfr containing circles are not due to mitochondrial DNA or plasmid contamination. In control cells, the extrachromosomal DNA is larger than 20kb, while in HU treated cells the bulk of the extrachromosomal DNA appears to be smaller than 16kb. These studies, while preliminary, suggest that HU treatment results in the production of small circular DNAs enriched for dhfr, and perhaps representing intermediates in the HU induced amplification of dhfr.

1195 OVERPRODUCTION OF HUMAN TISSUE-PLASMINOGEN ACTIVATOR IN MAMMALIAN CELLS BY GENE AMPLIFICATION

Randal J. Kaufman, Louise C. Wasley, Amelia J. Spiliotes, Stuart D. Gossels, Robert M. Kay, and Glenn R. Larsen, Genetics Institute, Boston, MA 02115

A cDNA for human tissue-type plasminogen activator (tPA) was cloned into an expression vector and introduced with a dihydrofolate reductase (DHFR) cDNA gene into Chinese hamster ovary DHFR$^-$ cells. Transformants isolated on the basis of growth in media lacking nucleosides contained low numbers of tPA genes and DHFR genes. Stepwise selection in increasing concentrations of methotrexate generated cells which amplified both DHFR genes and tPA genes over 100 fold. Different approaches taken to optimize amplification of the tPA gene involved altering the vector DNA in order to facilitate cotransfer of the unlinked genes, altering the ratio of tPA genes to the DHFR genes in the transfection, and modifying the scheme whereby cells are selected for high methotrexate resistance. Data concerning the nature of the amplified DNA, its chromosomal location, and stability upon growth in the absence of methotrexate selection will be presented. This approach has been used to isolate cell lines which express high levels of an active tPA which is glycosylated and secreted.

1196 NOVEL DNA REARRANGEMENTS ARE ASSOCIATED WITH DIHYDROFOLATE REDUCTASE GENE AMPLIFICATION.

Nancy A. Federspiel, Stephen M. Beverley, James W. Schilling, and Robert T. Schimke, Stanford University, Stanford, CA 94305

We have employed the technique of chromosome "walking" to determine the structure of 240 kb of amplified DNA surrounding the dihydrofolate reductase gene in the M500 mouse cell line. In this region, we have found five variant "junction" fragments which are due to amplification specific events and thus are not present in the parental S180 cell line. These variant fragments are specific to the M500 cell line and are not found in any other dihydrofolate reductase-amplified mouse cell lines which we have examined. In five independently cloned 3T6 cell lines which each contain 10-15 copies of the dihydrofolate reductase gene, both structural and quantitative variations in the amplified region were observed. Finally, significant changes in the amplified region were observed with continued propagation of amplified cells in culture; both structural and quantitative variations can occur over time. These studies indicate that there is no simple repetitive amplified "unit" in these cells; rather, a dynamic and complex arrangement of the amplified sequences exists which is continually being modified. With these considerations in mind, it would be advisable to study the processes of gene amplification at the earliest possible times during selection in order to be able to distinguish primary from secondary events.

1197 AMPLIFICATION OF A CHINESE HAMSTER CHROMOSOMAL REPLICON THAT CONTAINS THE GENE FOR DIHYDROFOLATE REDUCTASE, J. L. Hamlin, N. H. Heintz, J. D. Milbrandt, M. Montoya-Zavala, and J. E. Looney, University of Virginia, Charlottesville, VA 22908.
Chinese hamster cells that have been selected in increasingly-higher concentrations of the antifolate drug, methotrexate (MTX), invariably contain multiple copies of a sequence that includes the dihydrofolate reductase (DHFR) gene. The unit amplified sequence (amplicon) is approximately 135 kb in length, and therefore contains sequences in addition to the 25 kb DHFR gene itself. In a highly-MTX-resistant Chinese hamster ovary cell line (CHOC 400) developed in our laboratory, there are approximately 1,000 copies of this DHFR amplicon per cell, and the restriction pattern of the amplified sequence can be visualized in agarose gels stained with ethidium bromide. In pulse-labelling studies on synchronized cells, we have been able to show that DNA synthesis initiates within the DHFR domain at only one locus, and have therefore suggested that an amplicon may be equivalent to a chromosomal replicon. The amplification that occurs during the development of drug resistance would then be a result of multiple rounds of DNA synthesis of the DHFR replicon in a single cell cycle, with subsequent tandem integration of the extra copies of the replicon into the chromosome. This model predicts that independently-derived, MTX-resistant Chinese hamster cells should amplify the same DNA sequence (determined by the boundaries of the DHFR replicon in the sensitive, parental Chinese hamster cell). We are testing this possibility by using a collection of overlapping recombinant cosmids cloned from the amplicon in CHOC 400 to examine the structure of amplicons in other MTX-resistant cells. At least 120 kb of the 150 kb CHOC 400 amplicon is also amplified in four independently-derived MTX-resistant Chinese hamster cell lines.

1198 CHARACTERIZATION OF IMMUNOLOGICALLY ISOLATED DIHYDROFOLATE REDUCTASE MESSENGER RNA, Shoukat Dedhar and James Goldie, Cancer Control Agency of B.C., Vancouver, Canada
A methotrexate (MTX) -resistant L5178Y mouse leukemia cell line overproduces two antigenically distinct forms of dihydrofolate reductase (DHFR) which also differ markedly in the affinity for MTX, isoelectric points and heat stability. The overproduction is due to gene amplification as determined by Southern blot analysis of DNA from the MTX-sensitive and resistant cells. Highly enriched mRNA (greater than 95% pure) was prepared by polysome immunoprecipitation using rabbit serum antibodies directed towards each form of the enzyme. Northern blot analysis of total polysomal mRNA from MTX-sensitive and resistant cells demonstrated 8 distinct polysomal DHFR-mRNA species in the resistant cells and 5 in the sensitive cells. The mRNAS ranged from greater than 5Kb to 0.8Kb. The 2.2Kb and 1.05Kb mRNAs were clearly absent in the sensitive cells. Northern blot analysis of the polysomal immunoprecipitated mRNA from resistant cells consistently demonstrated three mRNA species; the 0.8Kb and two smaller molecular size messengers. None of the higher molecular size mRNAs were recognized by the antibodies. The cDNA used in these studies has a 0.9Kb 3' non-coding region in addition to the 0.6Kb coding region. It has been shown by others that the size heterogeneity of DHFR mRNAs is due to heterogeneity at the 3' ends corresponding to the 3' non-coding region of the cDNA, and that many of the high molecular weight mRNAs can code for DHFR in an in vitro translation system. Our results indicate however, that in vivo the high molecular weight species may be processed at the level of the polysomes and only the smaller mRNA species (corresponding to the coding region) translate into functional DHFR capable of binding the antibody.

1199 AMPLIFICATION OF EXTRACHROMOSOMAL DNA CIRCLES DURING SENESCENCE OF HUMAN DIPLOID CELLS. R.J. SHMOOKLER REIS, A. Srivastava, K.T. Riabowol and S. Goldstein, University of Arkansas for Medical Sciences and VA Medical Center, Little Rock, AR 72205.
We have utilized a moderately repetitive DNA sequence (2-30 copies/cell) to monitor DNA rearrangements near the highly repetitive Alu repeats (>600,000 copies/cell) with which they are associated in the human genome (Nature 301: 394-398, 1983). We examined DNA from serially passaged diploid human fibroblasts, in which we had previously demonstrated progressive loss of reiterated sequences (Cell 21: 739-749, 1980). While the expected chromosomally integrated copies of "Inter-Alu" sequence remained constant, additional homologous DNA species appeared or were amplified at late passage, in 4 of 6 strains examined. The amplified copies were extrachromosomal, and included covalently closed circles. A single size class (4.8 kb) of extrachromosomal DNA containing Inter-Alu was also amplified in normal lymphocytes, predominantly in B cells, isolated from 16 of 24 old donors (61-91 yr) but not from any of 18 young donors (21-31 yr). Both in vitro and in vivo aging are thus associated with the amplification of Inter-Alu circles. The genomic Inter-Alu sequence is highly methylated, and is rarely transcribed. However, its DNA sequence includes features of a promoter region, followed by an open reading frame. Restriction site mapping of various circular species containing Inter-Alu indicates that they are related to each other but markedly diverged from the corresponding chromosomal sequence. Considerable inter-clonal heterogeneity was observed within the cell population, with respect to number of Inter-Alu circles, and at least some of this heterogeneity appears to have arisen de novo in subclones.

1200 EXTRACHROMOSOMAL NUCLEAR cccDNA CLONES FROM HUMAN LYMPHOCYTES ARE HOMOLOGOUS TO THE PUTATIVLY MOBILE HIND III-KPN I REPETITIVE SEQUENCE FAMILY. K.T. Riabowol, S. Goldstein, and R.J. Shmookler Reis, University of Arkansas for Medical Sciences and VA Medical Center, Little Rock, AR 72205.

Total nuclear cccDNA has been isolated from human diploid cells and cloned into plasmid pBR322 to generate extrachromosomal cccDNA libraries, members of which contain the highly repetitive (~600,000 copies/cell) Alu sequence as well as sequences homologous to the Hind III-Kpn I repetitive (~10,000 copies/cell) family. While representation of Alu positive clones is as statistically predicted from their relative genomic abundance, Hind III-Kpn I homologous clones are over-represented by at least 5 fold. Recent evidence (PNAS 79: 1497-1500, 1982, Nuc. Acids Res. 11: 321-338, 1983) indicates that these repetitive families are mobile within the vertebrate genome which is supported by our results that they are present and in some cases overly abundant in extrachromosomal cccDNA species. We have previously demonstrated (Nature 301: 394-398, 1983) the age dependent amplification of an extrachromosomal 4.8 kb. sequence ("Inter-Alu") both in vitro (human diploid fibroblasts) and in vivo (human lymphocytes). Circular DNA species containing this moderatly repetitive DNA sequence (2-30 copies/cell) seem related when restriction mapped, but diverge markedly from the corresponding chromosomal sequence from which our probe was derived. Inter-Alu represents a DNA segment present once per genome located within a cluster of Alu repeats which may be responsible for its chromosomal excision and amplification. We are presently investigating whether clones derived from extrachromosomal cccDNA which contain these Alu/Hind III-Kpn I repeats a) amplify with age and b) are mobile within the genome.

1201 A COMPLEX SATELLITE DNA CONTAINS SEQUENCES CHARACTERISTIC OF RNA SPLICING, D. M. Skinner and R. F. Fowler, Biology Division and Univ. of Tenn.-Oak Ridge Grad. School Biomed. Sci., Oak Ridge National Laboratory, Oak Ridge, TN 37830

There are 1.6 x 10^4 copies of a complex G+C-rich (63%) satellite DNA in the genome of Bermuda land crab; the satellite has an average repeat unit of 2.07 kb. Of more than 20 cloned variants of the satellite that have been mapped or sequenced, no two are identical. Three cloned variants have been sequenced; they contain two distinct types of domains. Some domains as long as 594 bp are highly conserved; other specific domains are divergent. The divergent domains contain homopolymers of G or C, homocopolymers with strand bias either of purines or pyrimidines, inverted repeats, and alternating purines and pyrimidines. As revealed by computer analyses, the unusual sequence features of the three variants are in close association with potential RNA splice sites. Sequences highly homologous to the 5' end of U1 RNA are conserved in both strands of each variant; they may be donors in U1 RNA-mediated splicing events. In addition, sequences in perfect agreement with criteria characteristic of splice acceptors (i.e., homology to consensus sequence, 3' to a pyrimidine tract, and absence of AG within ~ 15 bp upstream) are present in each variant. These putative acceptors are conserved but are adjacent to the diverged regions in all three variants. Each of the three variants has its own unique S1 sensitive loci corresponding to differences in the divergent domains; this implies that each variant assumes a distinct conformation in the regions where divergence has occurred which in turn may possibly contribute to splicing specificity. (Research supported by NSF grant PCM78-23373 and USDOE under contract W-7405-eng-26 to UCC.)

1202 THE STRUCTURE AND OCCURRENCE OF MINUTE CHROMOSOMES IN MOUSE 3T3 CELLS, B.A. Hamkalo, University of California, Irvine, P.Brown and R.T. Schimke, Stanford University.

The Miller spreading procedure has been applied to an EM analysis of mouse 3T3 metaphase cells selected for high resistance to methotrexate (500 uM) and a concomitantly large number of double minutes (DMs). There is substantial variation in both the number and size of minutes in individual R500 cells and the smallest minutes would probably be undetected in standard light microscope analyses. These subchromosomal structures do not possess kinetochores, and appear singly or in pairs. They often lie close to chromosomal telomeres, arms or centromeres, suggesting a mechanism for their distribution during mitosis. Numerous chromatin fibers typically are seen between members of a pair of DMs, indicating that they may be topologically interlocked. Despite the differences noted, a general feature of minutes is their higher order organization. They are composed of nucleosomal chromatin folded into 25nm higher order fibers analogous to those seen in chromosomes; these 25nm fibers are, in turn, arranged as loops in a rosette-like conformation. Although the diameters of different minutes differ, the lengths of the loops in a given minute are rather uniform, implying an underlying repeating organization. Free ends are rarely observed in these structures, an observation which is consistent with, but does not prove, that the molecules are circular. A curious feature of the R500 line is its continued high level of production of dihydrofolate reductase after many passages in the absence of selection. There is no evidence for the appearance of an homogeneously staining region in revertant cells but they do possess numerous small minutes which are visible at the EM level. Since minutes are not seen in identical preparations of parental 3T3 cells, these data suggest induction of persistent minute production in the absence of selection.

1203 Evolution of a Second Major Primate Alu Family Repetitive DNA Sequence Prescott E. Deininger and Gary M. Daniels, Biochemistry, L S U Medical Center, 1901 Perdido St., New Orleans, LA 70112.

The major form of Alu family sequences in primate genomes is a 300 base sequence made up of two related, tandemly arranged sequences. The left half of this dimer carries an RNA polymerase III promoter and the right half has an as yet undefined function. This Alu family is present in hundreds of thousands of copies in all primate genomes. In studying the genome of a prosimian, Galago crassicaudatus, we have discovered that in addition to this typical Alu family (referred to as the Type I Alu family), there is an additional Alu family which is present in several-fold higher copy number. This new Alu family, termed Type II, is also 300 bases in length and its right half is almost identical to the right half of the normal, Type I, Alu family sequence. The left half is quite different, however. The Type II left half sequence also carries an RNA polymerase III promoter; one that fits the tRNA consensus promoter better than the Type I Alu family promoter. We also find a moderate copy number of sequences corresponding to the left half of the Type II Alu family, but without any attached Alu family right half sequences. These left half monomers are also active RNA polymerase III promoters. Our evidence indicates that the Type II Alu family arose through the fusion of a pre-existing family of repeated DNA sequences (the left-half monomers) with the right half of a Type I Alu family member. We have also demonstrated that the Type II family is much younger than the Type I family, yet the Type II is more prevalent in the galago genome than either of the sequences that it apparently arose from. The stronger promoter of the Type II could explain its advantage over the Type I in spreading itself through the genome, but we must also assume that the right half Alu family sequences also give the Type II a selective advantage relative to its left half sequences alone.

1204 C-Ha-RAS PROTO-ONCOGENE AMPLIFICATION AND EXPRESSION IN SENESCENT HUMAN DIPLOID FIBROBLASTS, Arun Srivastava, Robert J. Shmookler Reis and Samuel Goldstein, University of Arkansas for Medical Sciences and VA Medical Center, Little Rock, AR 72205.

Cellular oncogenes have recently been shown to be activated in certain malignant states and may also be involved in normal growth regulation. In order to identify the normal function(s) of cellular proto-oncogenes, we have undertaken studies to analyze genetic alterations in the normal homologue of the human bladder carcinoma oncogene, c-Ha-ras, in cultured human fibroblasts as a consequence of donor age ("aging in vivo") and as they traverse their limited lifespan ("aging in vitro"). Using Southern blot analyses, we have examined genomic DNA isolated from fibroblast strains derived from normal donors of varying chronological age, from mutant cell strains obtained from subjects with known genetic disorders of premature aging such as progeria and Werner's syndrome, and chromosomal breakage such as Bloom's syndrome and xeroderma pigmentosum. We have observed 5-10 fold amplification of the c-Ha-ras proto-oncogene in normal cell strains as well as in cell strains associated with genetic disorders. These studies have been extended to determine polymorphism, rearrangement and methylation pattern of this oncogene as a function of aging in vivo and in vitro. Northern blot analyses of total cellular and polyadenylated RNA have revealed expression of the c-Ha-ras proto-oncogene so far limited to normal cells aging in vitro. Studies to identify the gene product(s) are underway. Amplification and increased expression of cellular oncogenes during biological aging may reflect an adaptive response in cells with diminished replicative capacity, and predispose to the age-dependent rise in malignant disease.

1205 DNA SEQUENCE AMPLIFICATION AND TRANSPOSITION IN MULTIDRUG-RESISTANT CHINESE HAMSTER CELLS, I.B. Roninson[1], H.T. Abelson[2], P. Gros[1], D.E. Housman[1], N. Howell[2] and A. Varshavsky[1], M.I.T., Cambridge, MA 02139[1] & Dana-Farber Cancer Inst., Boston, MA 02115[2]

We have analyzed DNA rearrangements in Chinese hamster cells that are simultaneously resistant to several different cytotoxic drugs due to decreased intracellular accumulation of the drugs. We used adriamycin-resistant subclones of V79 cells, 77A and LZ, that are resistant to a 5-fold (77A) or a 3000-fold (LZ) higher concentration of adriamycin than the parental line. We also used C5, a colchicine-resistant subline of CHO cells, which is cross-resistant to a 150-fold relative concentration of adriamycin. DNA from these lines was assayed for the presence of amplified sequences by the in-gel renaturation technique (Nucl. Acids Res. 11, 5413-5431, 1983). Both LZ and C5 cells were shown to contain amplified DNA fragments. A subset of these fragments was amplified in both cell lines. The amplification of these DNA segments in two independently isolated lines suggests a common mechanism for drug-resistance in these cells. We have cloned one of the commonly amplified DNA fragments and shown that the degree of amplification of this fragment correlated with the degree of drug-resistance. This clone was also used as a probe to isolate several phage clones from a genomic library of LZ DNA. Transcription of these clones is being analyzed. In addition to DNA sequence amplification, the high degree of drug-resistance was also shown to correlate with transposition of a specific middle-repetitive sequence. This sequence appears at the same location in both LZ and C5 DNA, but is not found at this position in drug-sensitive cells or in cells with a low degree of drug-resistance, including the LZ cells that have reverted to a low-resistant phenotype after prolonged growth in the absence of drug selection.

1206 AMPLIFIED GENES ARE FOUND AS EXTRACHROMOSOMAL CIRCULAR MOLECULES IN METHOTREXATE-RESISTANT LEISHMANIA, Stephen M. Beverley, Dept. of Pharmacology, Harvard Medical School, Boston, Mass. 02115.

We have previously reported that MTX-resistant L. tropica (the R1000 line) overproduce a novel bifunctional dihydrofolate reductase-thymidylate synthetase and contain amplified DNA (Coderre et al., PNAS 80:2132-6). In recent work (Beverley, Coderre,Santi, Schimke, submitted) we have shown that two regions of wild-type DNA have been amplified separately in this line; one (the R region) encodes the bifunctional enzyme, while the coding potential of the other (the H region) is currently unknown. Both amplified DNAs are initially found as extrachromosomal circular molecules: the amplified R region consists of a unique 30 kb circle, while the amplified H DNA consists of an 80 kb circle containing two copies of a 35 kb region arranged in an inverted manner. These circular DNAs are unstable and lost in the absence of continued selection. With continued selection the amplified DNAs become stable; furthermore, these DNAs are now found as large tandem repetitive arrays located in chromosomal DNA. Present work concerns the role of genomic sequences in determining the occurrence, structure (i.e., direct vs. inverted repeats), and stabilization of amplified DNA.

There are clear parallels between unstable and stable gene amplification in vertebrate cells and Leishmania; specifically, between double minute chromosomes and expanded chromosomal regions in vertebrate cells and extrachromosomal circles and tandem repetitive arrays in Leishmania. In support of this, preliminary data (Hill et al., this symposia) indicates that amplified genes in mammalian cells may also exist as circular forms.

1207 GENE AMPLIFICATION IN COFORMYCIN RESISTANT VARIANTS OF CHINESE HAMSTER FIBROBLASTS. E. Petit-Koskas, M. Debatisse, B. Robert de Saint Vincent & G. Buttin, Unité de Génétique Somatique, Institut Pasteur, 28, rue du Dr. Roux, 75724 PARIS Cedex 15 (France)

Coformycin (cof), a well characterized inhibitor of adenosine deaminase, also inhibits AMP deaminase (AMPD). When Chinese hamster fibroblasts are exposed to a culture medium containing cof, adenine and azaserine, cells die of guanidylate starvation. The variants obtained by successive steps of selection with increasing cof concentrations, exhibit a level of AMPD up to 150 fold higher than wild type. SDS polyacrylamide gel electrophoresis of crude extracts from independant clones showed that these variants accumulate AMPD and additional unidentified proteins. These proteins as well as AMPD are present at the wild type level in revertant clones (Debatisse et al (1982) Molec. Gen. Genet. 2 : 1346). From one variant containing a level of AMPD 100 times higher than the wild type, we have prepared a cDNA library and isolated one cDNA clone corresponding to the mRNA coding for one of the coamplified proteins. This probe (H3) recognizes one mRNA species overproduced in all but one variants. In the latter mutant, the corresponding protein is not overaccumulated. The H3 probe hybridizes to DNA sequences which are amplified in all mutant lines. The mutant line which does not accumulate the H3 complementary mRNA, exhibits a specific DNA rearrangement at the genomic level when tested with this probe. Two other cDNA clones recognize different DNA sequences in all variants but the one containing the DNA rearrangement. We are currently using this set of probes to analyze the genomic rearrangements that take place during the early steps of gene amplification

1208 COORDINATE EXPRESSION OF AMPLIFIED METALLOTHIONEIN I AND II GENES IN CADMIUM RESISTANT CHINESE HAMSTER CELLS, Jeffrey K. Griffith, John L. Hanners and John L. Longmire, Genetics Group, Los Alamos National Laboratory, Los Alamos, NM. 87545

Recombinant DNA molecules encoding Chinese hamster metallothionein (MT) I and MT II mRNAs have been used to compare the concentrations of zinc-induced MT mRNA in five Chinese hamster cell lines possessing different numbers of amplified MT I and MT II genes and displaying different levels of resistance to cadmium. The cell lines CHO, Cd^r2C10, Cd^r20F4, Cd^r30F9 and Cd^r200T1 contain approximately 1 copy, 1 copy, 7 copies, 3 copies, and 14 copies of MT I and MT II genes respectively. Their respective thresholds to cadmium toxicity are 0.2uM, 2uM, 26uM, 40uM, and 145uM. The zinc-induced MT I mRNA concentrations in Cd^r2C10, Cd^r20F4, Cd^r30F9, and Cd^r200T1 were 75-fold, 75-fold, 215-fold and 1153-fold greater, respectively, than that measured in CHO. The comparable MT II mRNA concentrations were 182-fold, 182-fold, 500-fold and 2500-fold greater, respectively, than that measured in CHO. The measured MT II mRNA concentration was 5-6-fold lower than that of MT I mRNA in each of the cadmium resistant cell lines and 13-fold lower in the cadmium sensitive CHO. These results demonstrate that the coordination of the induction of MT I and MT II mRNA in the cadmium resistant cell lines is independent of the extents of either MT gene amplification or MT mRNA inducibility; and that the maximum inducible levels of MT I and MT II mRNA are not strictly correlated with either the levels of cellular cadmium resistance or MT gene amplification.

This work was performed under the auspices of the United States Department of Energy

1209 AMPLIFIED EXPRESSION OF THE Na^+,K^+-ATPase IN HeLa CELLS, J.F. Ash, R.M. Fineman, M. Morgan, P. Moore, T. Kalka and B. Wire, University of Utah, SLC, UT 84132

Ouabain was used in a step selection protocol to isolate clones of HeLa-S_3 cells which overproduce the sodium and potassium ion activated adenosinetriphosphatase 10 and 20 fold. This represents a content of 1-2 x 10^7 copies of this integral plasma membrane enzyme per cell. The increase was measured by specific ouabain binding and by quantitation of the relative levels of $^{32}PO_4$ labeled ATPase catalytic subunit in the wild type and selected cells. These ATPase amplified cells contain double minute chromosomes and other karyotype alterations. The ATPase amplified-ouabain resistant phenotype is not stable: when cells are grown in the absence of ouabain, revertant cells are isolated which contain normal levels of the ATPase and lack minute chromosomes. The loss of minutes is correllated with the loss of ouabain binding over the course of the reversion experiment. The ouabain resistant phenotype is associated with a variety of growth alterations, as well as metabolic and structural changes, which disappear in the revertant cells. These ATPase amplified cells provide new and powerful tools for the study of this membrane enzyme's structure and function, and, as the features of the selection and phenotype are consistent with gene amplification, it is also likely that these cells will facilitate the isolation of the ATPase gene.

1210 REGULATION AND AMPLIFICATION OF THE COPPER-INDUCIBLE CHELATIN GENE FROM YEAST, Tina Etcheverry, Joel Hayflick and Ron Hitzeman, Genentech, Inc., South San Francisco, CA 94080

The copper regulated chelatin gene from yeast has two remarkable properties. First, the copper sensitive promoter can be induced in the presence of copper. Second, the gene becomes amplified in tandem units along the chromosome when progressively higher copper stress is applied through several generations. Both these properties have been investigated, qualitatively and quantitatively, using the cloned chelatin gene.

This gene was cloned by complementation by transforming a copper sensitive strain with a YEp13 plasmid collection containing inserts of yeast chromosomal DNA. Several plasmids were obtained which conferred the copper resistant phenotype to the cells. These clones have similar restriction map patterns to those described by Fogel and Welch (PNAS 79, 5342, 1982).

We have subcloned portions of the promoter region and by doing so, defined the minimal 5' flanking DNA required for constitutive expression of the chelatin gene. Copper regulation was restored by reconstruction of upstream information.

The mechanism by which amplification occurs is a separate question. It is postulated that the process occurs by an unequal recombinational event. Whether or not the amplification occurs at specific junctions or at dispersed sites is being studied.

1211 LENGTH HOMOGENEITY OF THE BULK OF HUMAN rDNA, Frank P. Johnston, Laboratory of Genetics, University of Wisconsin, 445 Henry Mall, Madison, Wisconsin 53706.

Human rDNA occurs on the D and G Group chromosomes 13, 14, 15 and 21 and 22. The distribution of the complete approximately 44 kb rDNA repeat within and between these chromosomes has been examined, using non-transcribed spacer specific as well as rRNA-specific probes, in total genomic DNA and in somatic cell hybrids bearing a single or a subset of the NOR containing chromosomes. The organization and distribution of the repeats and observed variants suggests significant genetic exchange between the NOR's in man possibly associated with a high level of sequence correction.

1212 DISPROPORTIONATE REPLICATION OF THE HISTONE GENES DURING THE DEVELOPMENT OF DROSOPHILA MELANOGASTER. Linda D. Strausbaugh, Genetics and Cell Biology Section The University of Connecticut, Storrs, Ct. 06268.

The histone genes of Drosophila melanogaster are a tandemly repeated multigene family of approximately 100 repeats per haploid genome. These repeating units occur in two size classes of 4.8 and 5.0 kb., and each repeat contains one gene for each of the five histone protein subtypes. The vast majority of the histone repeats are clustered at a single site on the left arm of chromosome 2, at cytogenetic position 39DE. These tandemly repeated histone genes are shown to undergo a disproportionate replication when present in a single, rather than the ususal double, dose. This deficiency for the histone locus occurs in individuals which are constructed to be heterozygous for the chromosome Df(2L)161, a deficiency chromosome which has breakpoints at 38A6-B and 40A4-B1 on 2L, and therefore deletes the entire histone cluster. Quantitative dot hybridization and genomic blots with reconstruction controls reveal that there is a substantial increase in the histone repeats in DNA samples from adult deficiency heterozygotes. This amplification of histone DNA occurs during the ontogeny of the fly in a single generation. Results of nitrocellulose blots of fragments generated using a number of different restriction enzymes show that both major repeat types are included in the increased copies in adult DNA. A hemizygous genetic state is also shown to delay development time to clinbing larval stages and to delay adult eclosion times, suggesting that the combination of amplified copies and delayed development may compensate for a histone deficiency. Experiments are in progress to address tissue specificity.

1213 DNA rearrangement and amplification in the human beta- interferon gene family, Anurag D. Sagar and Pravinkumar B. Sehgal, Rockefeller University, New York, New York 10021

Recent experiments indicate that interferon (IFN) - beta related DNA rearrangement / amplification events can occur in bromodeoxyuridine treated Burkitt's lymphoma (Namalwa) cells. These alterations are detected in our experiments by the appearance of novel restriction fragments which hybridize with various beta-IFN specific probes. We have also detected the presence of interferon-beta related rearranged DNA in untreated senescent fibroblasts. These observations may have some implication for the evelution and dispersion of the interferon beta-related DNA in the human genome.

Poster Session II

1214 **GENE CONVERSION AT THE YEAST MITOCHONDRIAL 21S rDNA LOCUS. Alan P. Hudson, Ronald A. Butow and Andrew R. Zinn, Department of Biochemistry, University of Texas Health Science Center at Dallas, Dallas, Tx. 75235.**

The gene for the large (21S) mitochondrial rRNA contains an optional 1.1 kb intron called ω^+. In crosses between strains containing the ω^+ allele and those that lack it (ω^-), about 95% of the diploid progeny are ω^+. We have taken two approaches to study this process of asymmetric recombination or gene conversion. First, we have elucidated the kinetics of the conversion process. By taking advantage of allele-specific exon polymorphisms, we have detected non-parental forms of the 21S gene as early as 2.5 hours after the initiation of mating. This result is in sharp contrast to previous data on recombination of yeast mt DNA, which indicated that reciprocal recombination events are not evident until about 16 hours after mating. Preliminary data implicate a mating-specific double strand cut in the process of gene conversion. The second approach we have employed is to search for proteins involved in the gene conversion event. The various 21S alleles have been cloned into pBR322 and used in affinity column chromatography to purify mitochondrial DNA-binding proteins. We have detected two proteins of 48 kD and 59 kD that bind preferentially to ω^+ intron sequences and one protein of 41 kD that binds preferentially to 21S exon sequences. The function of these proteins in 21S recombination is presently under investigation. Supported by Grants from the NIH and the Robert A. Welch Foundation.

1215 DNA REPAIR GENES INVOLVED IN "CASSETTE SWITCHING" OF HETEROTHALLIC YEAST STRAINS, U.Wintersberger, R.Schiestl, University of Vienna, A-1090 Wien, Austria

The low frequency of mating type switching in heterothallic strains of yeast can be enhanced by treating the cells with DNA damaging agents (1). Being interested in the "enzyme overlap" between processes leading to genome rearrangements and those serving the maintenance of genome integrity we have started to test the influence of mutations in DNA repair genes on the spontaneous as well as the induced cassette switching frequency. Strains defective in the incision step of excision repair (rad3-2) have identical spontaneous as well as X-ray induced frequencies but are less inducible by UV than wild types. The mutation rad18-3 which has been shown to cause a "hyper-rec" phenotype (2) does not influence the switching frequency under any condition tested so far (spontaneous, X-ray, UV). Spontaneous as well as induced mating type interconversion is totally abolished in strains being deficient in DNA double strand break repair (rad52-1). Our data will be discussed in the light of the proposals (i) that cassette switching might be a model for the regulation of cell type during differentiation (3) and (ii) that genome rearrangements triggered in cells engaged in DNA repair might play a role in carcinogenesis (4).

(1) R.S., U.W. MGG 186, 512 (1982) and 191, 59 (1983); (2) W.Boram, H.Roman PNAS 73, 2828 (1976); (3) J.Strathern, J.Herskowitz Cell 17, 377 (1979); (4) U.W. Naturwiss. 69, 107 (1982).

1216 TY-MEDIATED ENHANCEMENT OF DUR1,2 GENE EXPRESSION. George Chisholm, and Terrance Cooper, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

Expression of the allantoin pathway genes in yeast is subject to induction by allophanate and nitrogen catabolite repression. In the past, we reported the isolation of several mutants (DUR80) in which one of the pathway genes, DUR1,2, was expressed constitutively. Other allophanate-inducible genes associated with allantoin degradation were completely unaffected. The DUR80 mutant phenotype, which was expressed both in haploid and MATa/MATα diploid strains, did not appear to be an alteration of the normal control system for this gene because its expression remained fully inducible and sensitive to nitrogen catabolite repression. Instead, much higher levels of DUR1,2 specific RNA were observed under all physiological conditions, i.e. the over-production trait was superimposed on normal regulation of the gene. To explain the enhancement of DUR1,2 gene expression, we cloned and sequenced portions of three independently isolated DUR80 alleles. All three mutant alleles were found to contain a Ty sequence inserted within 10 bp of one another (positions -643 to -652 from ATG). This Ty-mediated enhancement of DUR1,2 gene expression is similar in many respects to that generated by retroviruses in mammalian cells and may provide a useful model for its study. This work was supported by grants GM-19386 and GM-20693.

1217 RECOMBINATION OF FLANKING MARKERS DURING GENE CONVERSION IN YEAST, Makkuni Jayaram, Scripps Clinic and Research Foundation, La Jolla, CA 92037

I have used the yeast plasmid, 2 micron circle, as a convenient substrate to study the frequency of flanking marker recombination during gene conversion in yeast. The structural feature that enables me to do this is a pair of inverted repeats present on the plasmid. The plasmid substrate used is a 2 micron circle derivative in which the endogenous recombination system, which catalyses cross-over between the repeats to cause intramolecular inversion, has been eliminated. The process of gene conversion is initiated by introducing a gap in one of the repeats. The gap is repaired efficiently in yeast and the repair process occurs with or without intramolecular inversion with approximately equal probability. Similar experiments have also been done on a plasmid substrate that carries the 2 micron circle repeats in direct, rather than inverted, orientation.

1218 FLUCTUATIONS IN THE PATTERN OF EXPRESSION OF YEAST ACTIN-E.COLI LAC Z GENE FUSIONS

John J. Rossi and Yuzuru Iimura
Beckman Research Institute of the City of Hope
Department of Molecular Genetics, Duarte, California

Fusions between a eukaryotic gene (*S. cerevisiae* actin) and a defective *E. coli lac* Z gene have been constructed. Splicing of the yeast actin 309 base intervening sequence must take place for expression of the fusion actin-β-galactosidase message in yeast. Using these fusions we have deleted 274 of the 309 base pairs of this intron, completely removing the intron 5' donor signal as well as the internal control sequence located upstream of the 3' acceptor site. This mutant produces an unspliced mRNA which is translationally out of phase with the *lac* Z sequences. A β-gal expressing revertant of this mutant has been isolated and characterized. This mutant has at least two different types of *actin-lac* Z fusions. The original defective fusion and an *actin-lac* Z fusion harboring the complete actin intervening sequence, both integrated at the actin locus. These mutants lose and regain β-galactosidase activity at a high frequency (ca. 10^{-2} to 10^{-4}). Transcripts from mutants not expressing β-galactosidase contain the out-of-phase fusion message, but no properly spliced fusion mRNAs. Transcripts from the β-galactosidase expressing revertants contain small amounts of the out-of phase message, but larger quantities of properly spliced, fusion mRNAs.

Southern hybridization analyses of restricted DNAs from the original and revertant mutants demonstrate amplification and possible rearrangement of the integrated DNAs as the basis for the fluctuations in expression.

1219 THE MAL LOCI OF SACCHAROMYCES ARE A SMALL FAMILY OF DISPERSED REPEATED GENES, Corinne A. Michels, Maureen J. Charron and Richard B. Needleman

The fermentation of maltose is controlled by a series of 5 unlinked MAL loci (MAL1, MAL2, MAL3, MAL4 and MAL6) which have been identified in different isolates of yeast. Haploid maltose fermenting strains contain only a single functional copy of one of these MAL loci. In addition, each of the strains examined also contains one or more cryptic copies of the MAL genes which are related to the functional copy by nucleic acid homology. The cryptic copies are insufficient in themselves to permit maltose fermentation and they each usually fall into one of two complementation groups (called MALp and MALg) capable of complementing eachother to give a maltose positive phenotype.

The MAL6 locus has been isolated on a recombinant DNA plasmid and we have localized the MALp and MALg containing regions within the locus. Utilizing subclones of the MAL6 locus, we have begun to isolate and compare the organization of the other functional and cryptic MAL loci to MAL6. Most interesting is the fact that 3 of the 5 MAL loci are located near the telomere of their respective chromosomes. We are characterizing the environment of these loci in order to determine the mechanism of amplification of the MAL family.

1220 EFFECTS OF TEMPERATURE AND MATING COMPETENCE ON TRANSPOSITION OF YEAST TY ELEMENTS: Charlotte Paquin and Valerie M. Williamson, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin CA 94568

We have developed an assay for transposition of Ty elements in Saccharomyces cerevisiae based on the ability of Ty insertion to alter alcohol dehydrogenase (ADH) gene expression. This assay can be used to investigate the effects of physiological and environmental factors on transposition rates. We have shown that transposition rates are 100 fold higher (approximately $1x10^{-8}$ transpositions/cell/generation) in cells grown at 15°C than in cells grown at 30°C, the optimum growth temperature for yeast. This is particularly interesting because Tn3, IS1 and IS2 also have higher frequencies of transposition at lower than optimum growth temperatures of E. coli. There are reports that Ty RNA levels are 5 fold lower in a/α cells than in mating competent cells (a, α, a/a, α/α) grown on glucose medium and 10 to 20 fold lower in a/α strains grown on glycerol. It has been postulated that Ty RNA is an intermediate in the transposition process. However, we have found no significant differences in transposition rates among a, α, a/a, α/α, a/α isogenic strains grown at 15°C in glucose. Experiments to determine transposition rates for our isogenic strains after growth on glycerol are in progress.

Only about 30% of the mutants with altered ADH regulation which we have isolated from strains grown at low temperatures have Ty insertions at the ADH2 locus. Some of the other mutants have been shown by Southern analysis to carry a copy of Ty at a new location. We are investigating the possibility that the phenotype of these mutants is due to the insertion of a Ty element near or into an ADH2 regulatory gene.

1221 GENOME REARRANGEMENTS ASSOCIATED WITH TY1 ELEMENTS IN YEAST, S.W. Liebman, S. Picologlou, K. Downs and M. Dicig, University of Illinois, Chicago, Ill. 60680

The CYC1 region of some haploid strains contains 2 directly oriented Ty1 elements. These elements cause the frequent deletion (10^{-5}) of the 13 kb of bracketed DNA. The deletions differ in the number or orientation of Ty1's remaining at the junction point, suggesting that the δ elements at the ends of the Ty1's are preferred sites for the rearrangements and that multiple events are occurring. In diploids, mitotic recombination in this region is associated with an increase of greater than 1,000 fold in the deletion frequency. A third Ty1 found in these strains is inversely oriented relative to the other 2, about 6 kb distal, and is generally not involved in the deletions. However, haploid strains that contain an inversion of the 6 kb between the inversely oriented TY1's, give rise to deletions that are often (9 out of 39) accompanied by a reinversion of the 6 kb. Reinversion only occurs in these strains with a concurrent deletion. While the frequency of deletion events is unchanged by the rec- mutant, rad52, reinversions seem to be prevented (0 out of 26). One unusual haploid strain shows a phenomenally high frequency of multiple rearrangements which seem to involve tandem duplications and inversions bounded by the 3 Ty1 elements in the CYC1 region. Finally, deletions that occur in strains containing only one Ty1 element in the CYC1 region have been obtained. These deletions have one end at or within the Ty1 element, and extend for various distances in one direction. Some of these deletions involve additional complex events at the deletion junction point.

1222 ORGANIZATION AND REARRANGEMENT OF THE PLANT MITOCHONDRIAL GENOME, Maureen R. Hanson, Maury L. Boeshore, M. Rothenberg, H. Nivison, U. Virginia, Charlottesville, VA 22903

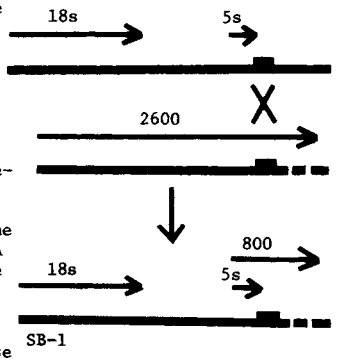
We are investigating the organization and rearrangement of the large (in Petunia species, more than 300 kb) higher plant mitochondrial (mt) genome, following somatic hybridization, long-term tissue culture, and during evolution.
We have analyzed the mt genomes of somatic hybrid plants regenerated after fusing cell of two different Petunia lines carrying cytoplasms from two closely related, sexually compatible species. The mtDNAs of the two parental lines exhibit multiple divergences according to restriction analysis. Each individual somatic hybrid analyzed carries a novel genome consisting of new combinations of DNA regions from both parents (Boeshore, M., I. Lifshitz, M.R. Hanson, S. Izhar, 1983 MGG 190:459-467).
In order to learn more about how the novel genomes were generated, we have cloned somatic hybrid mtDNA regions which carry restriction fragments characteristic of both parents, as well as a region carrying a novel fragment not present in either parent.
Changes in plant mt genomes can also be detected in long-term (2-3 year) tissue cultures. We have cloned probes which can detect alterations in mtDNAS present in initial vs. long-term cultures.
We have also detected divergence in genome organization of selection regions of the mtDNAs of the two closely related somatic hybrid parental lines. This contrasts to the sequence conservation of these two lines' chloroplast DNAs and the conserved organization of chloroplast genomes of rather distantly related species.

1223 INDUCIBLE EXPRESSION OF THE VIRULENCE GENES OF THE A6 AGROBACTERIUM TUMEFACIENS TI-PLASMID, Scott Stachel, Gynheung An, and Eugene Nester, University of Washington, Seattle, WA 98195

A. tumefaciens is able to transfer a specific segment of plasmid DNA from itself to plant cells. The transferred DNA becomes integrated into the plant nuclear genome and expressed, and this expression results in the phenotypic transformation of plant cells. The genes responsible for the transfer and integration steps are not contained on the DNA which is integrated, but are located on a separate thirty kilobase region of bacterial plasmid DNA designated the virulence (vir) region. We have analyzed the expression and regulation of twelve separate vir loci. LacZ fusions were randomly generated throughout the vir region by employing a Tn3:LacZ system, and the expression of these fusions was determined in Agrobacterium under a variety of growth conditions. Both actively growing dicot and monocot plant cells produce a small molecular weight factor that induces vir gene expression. A regulatory vir locus has been tentatively identified. We are currently characterizing the molecular changes that occur within induced bacteria as part of the virulence process.

1224 SOYBEAN MITOCHONDRIAL DNA REARRANGEMENT AND ALTERED RNA EXPRESSION, Elizabeth Grabau, Peter Morgens and Ray Gesteland, University of Utah, Salt Lake City, Utah 84112

We have been studying a region of the soybean mitochondrial genome adjacent to the 18s and 5s mt rRNA genes. A sequence from this region appears to be repeated elsewhere in the mitochondrial genome, not adjacent to the rRNA gene in its second environment. One soybean tissue culture line (SB-1) exhibits an altered DNA arrangement in the environment adjacent to the ribosomal genes. Restriction mapping data of these regions from the different soybean sources suggest that the novel tissue culture sequence may have arisen via homologous recombination. Associated with the DNA rearrangement is a change in RNA expression. An 800 nucleotide transcript is present in the SB-1 line that is not found in any other soybean mitochondria. This transcript contains the coding sequence for the 5s rRNA gene at its 5' end. The RNA data can also be explained by recombination resulting in the fusion of the gene for the 5s rRNA with a region coding for another abundant transcript (2600 nucleotides). We have recently cloned the soybean mitochondrial genome using the cosmid vector pHC79 and are in the process of examining these regions in greater detail.



Hypothetical Recombination

1225 EFFICIENT AGROBACTERIUM VECTORS FOR PLANT CELL TRANSFORMATION.
Michael Bevan, Plant Breeding Institute, Trumpington, Cambridge, CB2 2LQ, England.

The large plasmids of Agrobacterium tumefaciens confer on that bacterium the ability to incite tumours on a wide variety of plants. The molecular basis for the transformation is the insertion and expression of a large segment of Ti plasmid DNA in the host nuclear genome. Genes responsible for causing tumourous growth have been identified, and can be deleted without affecting the ability of Ti plasmid sequences to be transferred to the plant cell. Two types of transforming vector have been constructed that contain a chimaeric kanamycin resistance gene for selecting transformants, deleted oncogenes that allow for the regeneration of morphologically normal transformed plants, restriction sites for inserting passenger DNA, and a strong plant viral promoter for the expression of passenger DNA sequences. These vectors are easily used and transform plant protoplasts at a frequency of approximately 1-10%.

1226 ORGANIZATION AND EXPRESSION OF THE COMPLEX HOR 1 AND HOR 2 LOCI IN DEVELOPING BARLEY GRAIN. Brian G. Forde, Martin Kreis, Sadiq Rahman, Richard P. Fry, Martin S. Williamson, Hilary M. Lewis, Jacqueline Pywell, Peter R. Shewry and Benjamin J. Miflin, Biochemistry Department, Rothamsted Experimental Station, Harpenden, U.K.

Over 90% of the storage proteins ('hordeins') in the barley endosperm are encoded by two genetic loci (Hor 1 and Hor 2) about 8 centimorgans apart on the short arm of chromosome 5. Each locus consists of at least 10-15 genes and specifies a heterogeneous group of polypeptides. We have studied the expression of these gene families during seed development. The coordinated, tissue-specific expression of the two loci is modulated by temporal and nutritional factors and by a trans acting 'regulatory' gene. Each of these secondary controls have been found to act differentially on the two loci and even differentially on two sub-families of genes within the Hor 2 locus. Sequence analysis of cDNA clones has revealed the occurrence of short repeated sequences at both loci and similarities between these repeats at the two loci suggest the possibility of a common evolutionary origin for the two gene families.

1227 HERBICIDE RESISTANCE IN PLANTS: AN EXAMPLE OF GENE AMPLIFICATION, Howard M. Goodman, Edmund Tischer, and Guenter Donn, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

A variant line of alfalfa tissue culture cells has been isolated which are resistant to the experimental herbicide L-phosphinothricin, a mixed competitive inhibitor of glutamine synthetase (GS). The variant cell line contains approximately ten fold more GS activity than wild type cells. GS from the variant line has been purified to homogeneity and a partial amino acid sequence determined from a cyanogen bromide cleavage fragment. A cDNA clone coding for GS has also been isolated and its nucleotide sequence determined. Southern hybridization analysis indicates that herbicide resistance is due to the amplification of one of the structural genes for alfalfa GS.

1228 TRANSPOSITION OF TRYPANOSOME VSG GENES, John C. Boothroyd, David A. Campbell and Mark Van Bree, Stanford University Medical School, Stanford, CA 94305 USA.

The genes coding for variant surface glycoproteins (VSGs) of Trypanosoma brucei are activated by a process involving at least two, probably independent events. First, a VSG gene must be duplicated and transposed into an expression site located near the end of a chromosome and then that site must be activated. We have cloned the region of the genome which contains the 5'-end of an expressed VSG gene including 1400 base pairs of the upstream "barren" region. By comparison with the sequence of the silent basic copy of this gene, we have found that the 5'-limit of transposition occurs within the first (3'-most) of four unusual 81 bp repeats. We have also determined the structure of the barren region and find that it is composed of these same tandemly repeated 81 bp segments. These repeats are interrupted in one position within this barren region by a sequence of 270 bp consisting of TAA repeated 90 times with complete fidelity. The implications of these findings on the activation of VSG genes will be discussed.

1229 SEQUENCE ANALYSIS OF cDNA's FOR SURFACE GLYCOPROTEINS FROM METACYCLIC TRYPANOSOMES. Gregory Kelly, Michael J. Lenardo, Allison C. Rice-Ficht, Klaus M. Esser,[1] and John E. Donelson, University of Iowa, Iowa City, Iowa 52242, and Walter Reed Army Research Institute,[1] Washington, D.C., 20012.

African trypanosomes evade their mammalian host's immune system by the sequential expression of alternative surface glycoproteins. We have examined several cDNA sequences for the surface glycoproteins expressed on metacyclic trypanosomes, the final developmental stage in the tsetse fly. One of us (K.M.E.) has recently shown that metacyclic surface glycoproteins are still the predominant surface antigen of trypanosomes five days after infection in rats.

We have prepared a cDNA library using tyrpanosomes isolated from rat blood five days after infection. Clones containing sequences for metacyclic surface antigens have been identified using a plus/minus screening in conjunction with Grunstein colony hybridizations and RNA dot-blot experiments. Eight putative metacyclic surface glycoprotein cDNA sequences have been identified. In each of the clones sequenced to date the predicted amino acid sequence shows that the metacyclic surface glycoproteins can be classified into the same two C-terminal homology subsets established earlier for the bloodstream trypanosome's variable surface antigen. The expression of these metacyclic surface glycoproteins in trypanosomes will be discussed.

1230 GENOMIC MAPPING AND SEQUENCE ANALYSIS OF DNA FRAGMENTS CONTAINING THE CONSERVED 5' 35 NUCLEOTIDES OF TRYPANOSOME VARIABLE SURFACE GLYCOPROTEIN MESSENGER RNAS, David M. Dorfman and John E. Donelson, Department of Biochemistry, University of Iowa, Iowa City, IA 52242

Our laboratory and others have shown that the first 35 nucleotides of the mRNAs for different Trypanosoma brucei variable surface glycoproteins (VSGs) are identical. In each case this conserved sequence is not adjacent to the expressed copy of the VSG coding region in the genome. This suggests that different VSG genes are expressed from the same or similar genomic expression sites defined by the location of the 35 nucleotide sequence. To test this hypothesis we have synthesized a 21-mer oligonucleotide probe containing a portion of the 35 nucleotide sequence for genomic Southern analysis and to screen genomic bacteriophage libraries. Southern analysis confirms recent reports that the conserved 35 nucleotide sequence is present in many copies in the genome that are arranged in tandem repeat units of ∿1.4 kilobases. Using genomic subcloning and library screening we have isolated genomic restriction fragments containing copies of the 35 nucleotide sequence and flanking regions. DNA sequence analysis, genomic mapping, and other studies have been undertaken to further characterize and compare these repetitive regions and to ascertain their relationship to the VSG gene expression site(s). We are currently testing several models for VSG gene expression based on these findings.

1231 IDENTIFICATION AND CHARACTERIZATION OF TELOMERE-LINKED VSG GENES OF TRYPANOSOMES USING EXPRESSION SITE SPECIFIC PROBES, William J. Murphy and John E. Donelson, University of Iowa, Iowa City, Iowa 52242

We have previously described the cloning and sequence analysis of the IaTat 1.2 expression-linked extra copy (ELC) gene plus flanking sequences. Using a probe derived from the 5'-flanking region, we have identified and cloned another telomere-linked variable surface antigen gene which behaves, as analyzed by Southern blotting, similar to those genes expressed without an apparent duplication/transposition (non-duplication associated or NDA genes). The sequences flanking both the 5' and 3'-sides of this NDA gene are very similar to those of the ELC and suggest that an expressed, extra copy of this NDA gene may have preceeded the expression of the IaTat 1.2 gene. The NDA plus its basic copy (BC) gene appear to be partially modified at particular cytosine residues in all trypanosome clones tested, including cultured procyclic forms. This modification was detected by comparing the cleavage patterns generated by the isoschizomeric restriction enzymes Sau3A and MboI. By culturing procyclic trypanosomes in the presence of 5-azacytidine at concentrations above 20 μM, this modification is blocked. Using the same 5'-flanking sequence probe we have potentially identified the gene expressed directly before the antigenic switch to IaTat 1.2. The IaTat 1.2 trypanosome clone appears to have arisen, after antigenic switch, from a minor (heterologous) antigen type in the preceeding wave of parasitemia. Similarly, using the sequence flanking the 3'-side of the transposed ELC gene segment as probe, we have detected the extra copy of the gene expressed immediately following that of IaTat 1.2, in the subsequent wave of parasitemia.

1232 REGIONS OF HOMOLOGY IN THE CODING SEQUENCES AND THE 5'-FLANKING SEQUENCES FOR TRYPANOSOMA BRUCEI VARIANT SURFACE GLYCOPROTEIN (VSG) GENES.

Robert F. Aline Jr., Elizabeth E. Brown, Nina Agabian*, and Kenneth Stuart. Issaquah Health Research Institute, Issaquah, WA. 98027 and *University of Washington, Seattle, WA. 98195.

cDNA clones representing 6 different VSG genes each expressed by distinct variant antigenic types (IsTat 1.A, 1.1, 1.3, 1.5, 1.7, and 1.11) have been compared. Although the cDNAs each hybridize to distinct sets of genomic restiction fragments (sequence families) they were all found to contain a region of sequence homology. Unlike previously described homology observed at the 3' end of the VSG genes, this homology region was located between 200 and 500 nucleotides from the 5' end of the coding sequence.

Comparison of 5 genomic segments cloned in λ1059 containing one IsTat 1.A, two 1.3, and two 1.5 VSG genes respectively revealed segments of homology flanking the VSG coding sequences. One homology sequence was located 1.5Kb 5' to the 1.5-B, 1.5-C, and 1.3-B genes. It also occurred 1.3Kb 3' to the 3' terminus of the 1.A-B gene; this could represent a region 5' to another VSG gene The sequence did not occur in the 5' flanks of the 1.A-B or 1.3-C genes or other regions in the clones. This region of flanking homology does not hybridize to the 70bp repeat sequence which occurs 5' to other VSG genes (1.A-B, 1.3-C) in the IsTaR-1 serodeme and other serodemes. (Supported by NIH AI7375, DAMD17-92-2016, WHO and Murdock Charitable Trust (KS) and NIH AI17309 (NA) grants).

1233 GENE ACTIVATION AND TRANSCRIPTION OF A TRYPANOSOMA BRUCEI VARIANT SURFACE GLYCOPROTEIN

G. Matthyssens, F. Michiels, P. Kronenberger and R. Hamers, Vrije Universiteit Brussel, B-1640 St. Genesius-Rode, Belgium.

The expression of the Trypanosoma brucei variant surface glycoprotein AnTat 1.1 proceeds by a mechanism that transfers a duplicated gene copy into a new genomic environment, the so-called expression site, where it will be expressed. We have isolated a genomic fragment containing the region spanning the expression site-transposon junction, and the 5' half of the coding sequence. Comparing this DNA segment with its template copy (basic copy) allowed us to identify the exact breaking point and indicated a base sequence which could be involved in initiating the transposition event. The extreme 5' end of the mRNA is derived from a region in the expression site not immediately adjacent to the transposed DNA segment. This 35-bp mini-exon is found in about 250 copies per haploid genome, the majority of which is tandemly arranged on a 1.4 kb DNA segment. The significance of this exon is being investigated through DNA transformation experiments.

1234 EXPRESSION OF A PREDOMINANT T.BRUCEI VARIANT SURFACE GLYCOPROTEIN (VSG) GENE BY BOTH GENE DUPLICATIVE AND NON-DUPLICATIVE MECHANISMS,

Peter Myler, Richard G. Nelson,* Nina Agabian,* and Kenneth Stuart, Issaquah Health Research Institute, Issaquah, WA 98027, and *University of Washington, Seattle, WA 98195.

IsTat 1.A is the predominant variant antigen type (VAT) of the IsTaR 1 serodeme. The 1.A VSG genes were characterized in six independently-derived clones expressing 1.A VSG. In clones not expressing 1.A VSG there were two copies of the 1.A gene, one of which is located near a telomere. In two 1.A expressors (1.Ae and 1.A7) an extra telomeric copy of the 1.A gene was found, indicating that activation of the 1.A gene involved a duplication/transposition (ELC) mechanism. The telomeric 1.A gene appeared to act as the basic copy (BC). In the other four 1.A expressors activation of the 1.A gene occurred without gene duplication (NDA mechanism). Genomic southern analyses and DNase I studies indicated that the restriction maps 5' to the expressed copies of the 1.A gene in 1.Ae and 1.A7 were similar but not identical, suggesting that two different expression or acceptor sites were used. The restriction maps 5' to the BC in all clones examined were identical to the ELC in 1.A7. The ability of the 1.A gene to be activated by two different mechanisms and the similarity of the 5' environments of the BC gene to the expression sites may explain why it is the predominant VAT of the IsTaR 1 serodeme. (Supported by NIH AI7375, DAMD17-92-2016, WHO and Murdock Charitable Trust (KS) and NIH AI17309 (NA) grants).

1235 THE 5' SPLICED LEADER OF VARIANT ANTIGEN MRNAS IS ALSO SPLICED ONTO OTHER TRYPANOSOME MRNAS, Marilyn Parsons, Richard G. Nelson, Kenneth P. Watkins and Nina Agabian, University of Washington, Seattle, WA 98195

Antigenic variation in African trypanosomes is mediated by the sequential expression of individual genes encoding different variant surface glycoproteins (VSGs). Gene activation in many cases involves transposition of the active gene to a novel genomic location, which may differ for different activation events. All VSG mRNAs, however, have the same untranslated 35 nucleotides at their 5' termini which are not encoded adjacent to the expressed or silent VSG genes. Using a synthetic probe complementary to 22 nucleotides of this 35 nucleotide spliced leader (SL) we have found that the SL is highly reiterated in the genome. Each SL is located in a 1.4 kb unit. Most of these are directly and tandemly repeated to form a large cluster(s); a few are dispersed from the tandem array; and none are detectably linked to expressed VSG genes. Many clones which contained sequences homologous to the SL, but did not encode VSG, were isolated from cDNA libraries. In most cases, these cDNAs were homologous to single copy sequences in the genome. Like the SL, but unlike VSG genes, these sequences are transcribed during the procyclic stage of the trypanosome life cycle. Several of the single copy genes were isolated from a λ 1059 library of trypanosome DNA. None of these genomic clones contained SL sequences, although they contained up to 7 kb of DNA 5' to the structural gene. Thus the maturation of the mRNAs encoded by these genes also involves the addition or splicing of the SL sequence onto the structural gene transcripts. These results demonstrate that the transcription, splicing and utilization of SL sequences is not directly tied to VSG gene expression.

1236 TELOMERIC DNA REARRANGEMENTS AND ANTIGENIC VARIATION IN TRYPANOSOMES, Etienne Pays[+], Monique Laurent[+], Suzanne Van Assel[+], Nestor Van Meirvenne[°] and Maurice Steinert[+]. [+]University of Brussels, Rhode St. Genèse and [°] Institute for Tropical Medicine, Antwerp.

The transcriptional control of trypanosome surface antigen genes is effected by at least two types of DNA rearrangements, one of which is gene conversion. The extent of gene conversion is highly variable, and seems to depend on the homologies between recipient and donor sequences. Most of the antigen genes that we studied (in the T.b. brucei AnTAR 1 repertoire) are located in telomeres; according to their restriction maps, these telomeres can be divided in classes. When gene conversion takes place between telomeres of different classes, large telomeric sequences (more than 40 kb long) can be converted; this observation suggests the presence of homology blocks far upstream from the gene. Contrary to gene conversion, the alternative antigen gene activation mechanism does not involve duplication of the gene. This mechanism behaves as a reciprocal recombination between telomeres, since it leads to the conservation of the gene previously activated. Moreover, the gene expressed in this particular clone can be lost from the genome of the ensuing variant, if the antigen gene of the latter is activated by gene conversion. The hypothetical crossing-over point should be located far upstream from the gene, but downstream from the transcription promoter. Accordingly, so far the promoter could not be mapped within 40 kb upstream from the antigen gene.

1237 A NOVEL VARIABLE ANTIGEN SYSTEM IN PLASMODIUM FALCIPARUM, R.B. Saint, A.F. Cowman, D.J. Kemp, R.L. Coppel, K.R. Lingelbach, G.V. Brown and R.F. Anders, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3050, Australia.

The soluble heat-stable 'S'-antigens of the human malaria parasite Plasmodium falciparum, found in the sera of infected individuals and supernatants of in vitro cultures, are serologically diverse. We have studied genes encoding two serologically different members of this family and have revealed the following remarkable properties. First, both of these S-antigens are encoded in part by tandemlyrepeated sequences. There is, however, no sequence homology between the repeat units of different genes. The genes themselves are repeated within the genome of P.falciparum, but all isolates so far examined have one or other sequence type but never both. Restriction enzyme and sequence analyses have revealed similarities in the organisation of the two types of S-antigen genes. In particular, and as opposed to the repeat units of the coding regions, flanking non-coding DNA is highly homologous between the different types. Finally, no 'basic' or 'silent' copies of an S-antigen coding region have been detected in a strain not expressing that particular S-antigen gene. We conclude that the S-antigens comprise a novel variable antigen system.

1238 P-ELEMENT TRANSPOSITION - RELATED EVENTS IN DROSOPHILA MELANOGASTER CARRYING MR-h12, Jan C.J. Eeken and F.H. Sobels, State University of Leiden, The Netherlands

MR(Male Recombination)-h12, isolated and characterized by M.M. Green, is located between Tft and pr on the II-chromosome. MR phenomena are due to transposition of P-elements. An analysis of MR-h12 induced sex-linked lethals showed that they can arise at many sites and that most of them are correlated with the presence of a P-element. We determined the unstability of a particular MR-induced sn, sn*. This sn* is unstable, but only so when an active MR is also present. The reversionfrequency of sn* in the presence of MR-h12 is 1.6% (204/-12664). No reversions are found in the absence of MR (0/15069). In this experiment a second mutation at raspberry was isolated. The chromosome, that carries 2 unstable mutations, is designated sn* ras*. We studied secondary events that occur in this chromosome.
We measured the influence of several mutagens on the reversion frequency of sn* in males of the phenotype sn* ras*, MR-h12. The following results were obtained: Controle 1.1% (305/-26.803); ENU(3mM) 2.6% (154/5.803); MMS(5mM) 0.6% (40/6.185); formaldehyde (0.25%) 0.6% (79/13.050); irradiated spermatogonia (150/250R) 1.5% (11/747) and 1.6% (221/13.673). These agents have virtually no effect on the reversionfrequency whereas the effect on sex-linked lethals is profound (controle: 2.0%; ENU: 10.1%; MMS 5.8%; formaldehyde 4.2%), with the exeption of 150/250R; 2.1%. An analysis of lethals induced in the sn*ras* chromosome by MR-h12 and irradiation(sperm) showed that (1) in MR carrying males, lethals arise at ras and (2) many inversions with breaks preferentially at sn (P-element present) and at 14B (no P-element present) are found. sn* = snMR39B1 ras* = rasMR29A1.

Poster Session III

1239 GENOME REARRANGEMENTS DURING NORMAL AND ABNORMAL CELLULAR DIFFERENTIATION, Frederick W. Alt, George D. Yancopoulos, T. Keith Blackwell, Michael G. Reth, Nancy E. Kohl and Connie Gee, Columbia University, New York, NY 10032

We have derived null A-MuLV transformed pre-B cell lines which undergo the sequential assembly and expression of functional heavy and light chain genes in culture ultimately leading to the generation of daughter lines which express surface IgM molecules. We have extensively characterized both the assembly of the endogenous V_H and V_L gene segments in these lines as well as site specific recombination events between D and J_H segments that were introduced into the cells by DNA transformation procedures. The former studies have elucidated a biased usage of the V_H gene repertoire in pre-B cells while the latter studies have further defined mechanistic and regulatory aspects of the recombinase system. We have also cloned a human gene (N-myc) which derives from chromosome 2 and which undergoes transposition and amplification (up to 1000-fold) in most human neuroblastomas. We have shown that N-myc undergoes transposition events prior to amplification and that it has a tissue specific pattern of expression.

1240 IMMUNOGLOBULIN GENE REARRANGEMENT ON A DEFINED SUBSTRATE, Susanna Lewis and David Baltimore, M.I.T. Cambridge, MA. 02139.

The gene segments encoding the variable regions of immunoglobulins are assembled during the differentiation of a B cell through a series of site-specific recombinations. These involve widely separated segments of the heavy or light chain loci, and appear to occur in a tightly regulated fashion. An understanding of the mechanisms that control this rearrangement process, as well as the details of the recombination itself, would be facilitated by the development of a system whereby in-vivo gene recombination could be detected on defined, exogenously introduced sequences. To this end we designed a recombination substrate bearing regions of the kappa Ig locus, which can be introduced into an actively rearranging cell line in the form of a defective retrovirus. The substrate is constructed so that if it is rearranged, expression of a previously inactive selectable marker is achieved, and the recipient cell becomes drug resistant. By this means, we have isolated independant drug-resistant cell lines all of which appear to have recombined the introduced sequences. We have confirmed that authentic V_k region recombination occured by molecular cloning and DNA sequence analysis of the integrated substrate from the genome of one representative drug-resistant isolate. The design of the substrate enabled recovery not only of the V_kJ_k coding joint, but of the reciprocal recombination product as well. The reciprocal joint had a structure analogous to rearranged fragments which have been isolated from myelomas. This indicates that probably such fragments originate as direct by-products of V_kJ_k joining.

1241 SOMATIC RECOMBINATION OF IMMUNOGLOBULIN GENE SEGMENTS: PRESENCE OF A SITE-DIRECTED ENDONUCLEASE IN FETAL LIVER CELL EXTRACTS. G. D'Agostaro, E. Hevia, G.E. Wu and H. Murialdo, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The transcription units coding for immunoglobulin heavy and light chains are assembled from multiple gene segments by somatic recombination during the differentiation of B cells. In an attempt to detect rearrangements between V_K and J-C_K DNA sequences, we devised an in vitro assay based on the EDTA-resistance of phages λ carrying DNA deletions. The DNA from a phage λ containing the V_{K41} gene segment separated by 6 Kbp from the entire J-C_K sequence (λVJC_K) was incubated with partially purified whole-cell extracts derived from mouse fetal livers. After treatment, the λVJC_K DNA was packaged in vitro and the population of phages assembled in vitro was screened for the EDTA-resistance phenotype. A 20-fold increase in the frequency of EDTA-resistant phages has been observed upon treatment of λVJC_K DNA with extracts derived from fetal liver cells at 15 day of gestation. However, no phages carrying DNA deletions consistent with site-specific V-J recombination events have been detected. Moreover, upon treatment with fetal liver cell extracts, the packaging efficiency of λ$VJKC_K$ DNA was 100 to 1000 fold lower than that of phage λ DNA. The suggestion of site-directed endonucleolytic activity was reinforced by the observation that the regions of λVJC_K DNA containing the V and J gene segments were preferentially degraded. In order to detect the products of this site-directed endonucleolytic activity, a plasmid containing the 5 J gene segments was used as substrate and the products of the reaction were visualized by autoradiography in Southern blots. Double-stranded breaks were observed to occur at, or very near, the 5' end of each one of the 5 J gene segments. Experiments with cell extracts derived from mice deficient in the development of B cells are in progress.

1242 IMMUNOGLOBULIN GENE REARRANGEMENTS: TOWARDS A CELL-FREE ASSAY, Valerie Darby and Frederick R. Blattner, Laboratory of Genetics, University of Wisconsin, Madison Wisconsin, 53706

A cell-free system has been devised for rearrangement of Ig gene segments by DNA splicing. Mouse heavy chain D and J gene segments were used to construct a substrate in bacteriophage λ. Extracts made from murine leukemia virus - transformed lymphoid cell lines have been shown to promote various recombination events including homologous recombination, though no accurate D-J splices have yet been found.

1243 AN ABERRANT REARRANGEMENT OF THE MOUSE IMMUNOGLOBULIN κ LOCUS IS DUE TO A TRANSLOCATION BETWEEN CHROMOSOMES 6 AND 15, Marjorie Shapiro, Brian G. Van Ness and Martin Weigert, The Institute for Cancer Research, Philadelphia, PA 19111.

Functional antibody genes are created by a site specific recombination process. In the case of mouse κ light chains, a variable (V) gene segment and joining (J) gene segment are fused to form a complete V_K region gene. Such recombinations fall into 2 classes: κ^+ or a productive rearrangement and κ^- or a nonproductive rearrangement. Typical κ^- rearrangements have been shown to be the result of errors in recombination or the use of Ψ V or Ψ J genes.

We have focused on a subset of κ^- rearrangements common to plasmacytomas. Instead of being produced by the VJ recombination process, these κ^- rearrangemnts are produced by chromosome translocation. We have studied the plasmacytoma PC7183 which has undergone an aberrant rearrangement at the κ locus. This tumor contains a κ^- rearrangement and its reciprocal recombinaiton product. Further analysis show these products to be the result of a translocation between chromosome 6, which contains the κ locus, and chromosome 15. The myc oncogene, which is known to reside on chromosome 15 and to be rearranged in other plasmacytomas, is not involved in this process.

1244 AN UNUSUAL κ-ASSOCIATED DNA RECOMBINATION IS FREQUENTLY FOUND IN MOUSE λ-PRODUCING B LYMPHOCYTES, Mark W. Moore, Jeannine M. Durdik and Erik Selsing, Brandeis Univ., Waltham, MA 02254

Evidence indicates that recombination of κ and λ light chain genes are developmentally ordered events during the ontogeny of B cell precursors with κ gene recombination occurring prior to λ gene recombination.

Unusual DNA recombination events were detected in two λ-producing myelomas that involve non-functional recombined κ genes and a segment of DNA that appears to be downstream of the exon (λ associated recombining sequence or LARS DNA). LARS DNA recombinations are found in both κ and λ-producing plasmacytomas. Intriguingly, by analysis of hybridoma DNAs, LARS recombinations appear to be exclusively contributed by normal λ producing B cells (8/10) but not by κ-producing B cells (0/14).

Analysis of two LARS recombination sites suggests that Vκ genes might recombine directly with the LARS sequence. Most LARS DNA events do not involve V-J joined κ genes. The structures of Vκ-Jκ-LARS (and the putative Vκ-LARS) recombined sequences place LARS sequences downstream from potentially active Ig gene promoters. LARS DNA recombination could play an active role in the developmental switch from κ to λ gene recombination in maturing B cells.

Recombined LARS DNA were isolated and sequenced from CH2 and MOPC 315 cells to determine their structures. Our data show that the LARS DNA from the two cell lines are identical, and both recombine within the Jκ-Cκ intron. These joining sites are within 3 nucleotides of each other and adjacent to an immunoglobulin recognition site heptamer, thus LARS recombination may occur in a similar fashion to the V-J joining process.

1245 TRANSLOCATIONS THAT HIGHLIGHT CHROMOSOMAL REGIONS OF DIFFERENTIATED ACTIVITY. Gregory F. Hollis, Judith Brown, Cynthia Morton, Stanley Korsmeyer and Ilan R. Kirsch, NCI-Navy Medical Oncology Branch/DCT/NCI/NIH, Bethesda, MD 20814 and Medical College of Virginia and Harvard Medical School and Metabolism Branch, NCI.

The frequent translocation of the oncogene c-myc into the immunoglobulin loci in tumors of B-lymphocytes prompted us to ask whether disease associated chromosomal translocations in other cell types would also involve regions of the genome that encoded important differentiation specific products made by these cells. A cytogenetic analysis of two patients with erythroleukemia and a commonly available erythroleukemia cell line, K562 (late passages), show translocations within the regions where the genes that encode alpha and beta globin reside. The translocation in K562, not seen in early passages of these cells, has been shown to correlate with a change in globin inducibility. When cloned B-lymphocytes from a patient with ataxia-telangiectasia are analyzed, a translocation into the regions encoding the immunoglobulin light and heavy chain genes are observed in distinction to the translocation seen in T-lymphocytes from the same patient. Both kappa and lambda producing cells manifest this translocation. These examples provide insight into the mechanism of chromosomal translocation in both cancerous and noncancerous conditions and lead to the speculation that genomic activity is a necessary, if not sufficient, factor for the occurence of such translocations.

1246 **GENOMIC REARRANGEMENTS IN THE MOUSE MHC AS EXAMINED BY OLIGONUCLEOTIDE HYBRIDIZATION**, C.G. Miyada, C. Klofelt, A.A. Reyes, E. McLaughlin-Taylor and R.B. Wallace, Beckman Research Institute of the City of Hope, Duarte, CA 91010

The class I genes of the murine major histocompatibility complex (MHC) constitute a multigene family encoding cell-surface glycoproteins. Included within this set of genes are those coding for the classical transplantation antigens, K, D and L. These antigens show great diversity in that each is represented by a large number of alleles. The bml mutation of the $H\text{-}2K^b$ allele is the result of 7 base pair changes over a 13 base pair region. These 7 base changes result in 3 amino acid substitutions in the C1 domain of the protein product. The clustering of base pair changes suggests that a gene-conversion-like event generated the bml mutation and thus, a putative "donor gene" should be present in the wild-type genome. A 23-base oligonucleotide complimentary to the bml mutant gene in the region of the 7 nucleotide changes was used to probe chromosomal DNA digests of 4 haplotypes (b, d, k and q) as well as DNA of the bml mutant. Our results show that a potential donor gene for the bml mutation exists in the parental b haplotype as well as the d, k and q haplotypes. The potential donor gene shows no restriction-fragment-length polymorphism in *Bam*HI, *Eco*RI or *Pvu*II digests. Since another H-2 allele, $H\text{-}2L^d$, contains the exact nucleotide changes in the homologous region of the chromosome, gene-conversion-like events are a likely mechanism for creating diversity among the class I genes of the mouse MHC.

1247 REARRANGEMENT OF NON-EXPRESSED IMMUNOGLOBULIN GENES IN B CELLS AND MYC GENE ACTIVATION CAUSED BY RAV-1 INTEGRATION. Carol Nottenburg, Irving L. Weissman, Harold E. Varmus Stanford University, Stanford, CA and University of California, San Francisco, CA.

Immunoglobulin gene rearrangements in normal IgM, IgD expressing B cells occur on both the expressed and non-expressed chromosome. We have isolated and sequenced eight examples of non-expressed rearrangements from mouse and find three categories of rearrangements: i) D/J only rearrangements ii) V/D/J rearrnagements which may be functional and iii) deletions in the J gene region.

RAV-1 is an avian leukosis virus that causes bursal lymphomas. It integrates near and activates a cellular oncogene c-myc. We are investigation the myc transcript resulting from a viral integration that occurred downstream of the myc gene. We are also delineating the physical distance of gene activation that RAV-1 causes. Results of these experiments will be presented.

1248 RECOMBINANT RETROVIRAL DNA FOR PRODUCTION OF HEPATITIS B SURFACE ANTIGEN, Johannes Doehmer, Joanne Hughes, and Christian Stratowa, Max-Planck-Institut für Biochemie, D-8033, West-Germany

Transposable Elements might be used as vectors for introducing genes into the genome of a cell or organism in a more defined way in respect to organization and location of the transferred gene.

Retroviruses behave like transposable elements and are studied for their use as vectors. A gene which can be used to study the properties of a vector system is a part of the hepatitis B virus genome encoding the surface antigen (HBsAg), because this gene does not have a cellular counterpart, thus avoiding background problems and expression can easily be checked because the product is released into the culture medium.
We have established several cell lines using recombinant Moloney Mouse Sarcoma Virus DNA producing HBsAg (EMBO Journal 1, 1573-1578, 1982). The recombinant retroviral DNA alone is not sufficient for transposon like integration. Experiments are going on to achieve transposon like integration without depending on an infectious recombinant virus.

1249 "Pseudo-exon" flanked by a long inverted repeat sequence (IR) in the human γ fibrinogen gene. AJ Fornace Jr., J. Kant, & GR Crabtree, Lab. of Pathology, NIH, Bethesda, Md.

We have found that a portion of the seventh exon of the γ fibrinogen gene is duplicated 1 kbp away in the preceding intron. This duplicated sequence, which we have termed a "pseudo-exon", is a 150 bp imperfect copy of the true exon and is flanked on both sides by a 102 bp IR. The termini of the pseudo-exon do not correspond to the splice sites in the seventh exon, instead the 5' end of the pseudo-exon starts 30 bp after the 5' splice site. The frequencies of point substitutions between the true exon and the pseudo-exon and between the 2 sides of the IR were similiar 8.0 vs. 11.8% respectively; we calculated from these frequencies that the pseudo-exon and IR both arose approximately 10-20 million years ago. Although the general structure of this duplication resembles transposable sequences found in bacteria and two recently described in eukaryotes, genomic Southern analysis indicates that the pseudo-exon and IR have remained a single copy since their formation. Thus, it is likely that the pseudo-exon and IR do not function as a transposable element, but were created as a result of a perturbation of some process such as DNA replication. Up to 5% of the human and other eukaryotic genomes are reported to consist of IR; a substantial portion of these IR are poorly characterized sequences, many of which are non-repetitive. If other IR were produced in the same manner as in our case, this could represent a mechanism responsible for a significant portion of genetic duplications in eukaryotic evolution.

1250 ISOLATION OF COAT COLOUR GENES OF MOUSE. Ian J. Jackson, MRC Mammalian Development Unit, Wolfson House, 4, Stephenson Way, London NW1 2HE. England.

Genes affecting coat colouration in the mouse have been studied for many years. Mutations at these loci are readily detected and many have been preserved in mouse stocks.

A number of alleles at several loci show position effect variegation; suggesting that these mutations may be due to some form of genome rearrangement or unstable DNA insertion. DNA probes for coat colour genes are being isolated to examine the structure of these alleles.

Many coat colour genes have been shown to be melanocyte-autonomous; indicating that the genes are expressed in the melanocyte. The B16 melanoma cell line can be grown in vitro under conditions where no melanin is produced. Melanin synthesis can be activated by increasing the pH of the medium, and by adding tyrosine. This apparent differentiation provides a system in which, by differential screening of a melanised cell cDNA library, those genes activated during melanisation can be isolated.

It is known that tyrosinase (the product of the albino gene) is newly synthesised after induction of differentiation. Preliminary results from this approach towards isolating albino and other coat colour genes will be presented.

1251 DNA STRAND BREAK FORMATION AND REJOINING DURING CELLULAR DIFFERENTIATION
Farzin Farzaneh, Sylvie Féon and Sydney Shall, Cell & Molecular Biology laboratory, University of Sussex, Brighton, BN1 9QG, U.K.

We have previously demonstrated that during the spontaneous differentiation of primary avian skeletal myoblasts in culture single-strand DNA breaks appear. We also detect the formation of DNA strand breaks during the induced differentiation of a human pro-myelocytic cell line, HL-60. A number of studies have suggested a reduction in the ability to repair DNA damage in post-mitotic terminally differentiating cells as compared to their proliferating precursors. We shall present evidence that there is no detectable reduction in the rate of removal of DNA lesions induced by either dimethyl sulphate or γ-radiation following either the spontaneous differentiation of primary myoblasts or the induced differentiation of HL-60 cells. Inhibitors of nuclear adenosine diphosphoribosyl transferase (ADPRT), an enzyme involved in DNA excision repair, block the repair of DNA damage in both muscle and HL-60 cells. The observation that ADPRT inhibition blocks the differentiation of these and a number of other cells suggests that DNA strand break formation and removal, and hence ADPRT activity, may be required in a general process involved in cellular differentiation. Such a process may be gene amplifications, DNA transpositions and/or regional chromatin relaxations by topoisomerases; these events would require DNA strand break formation and rejoining.

1252 RETROVIRAL DNA INTEGRATION. Alexander, F., Roche Institute of Molecular Biology, Nutley, NJ 07110.

The ability of DNA to transpose, amplify and rearrange results in a eucaryotic genome which is far more dynamic, particulary in an evolutionary sense, than previously suspected. To better understand how such events occur, it is important to identify some of the enzymes responsible for mediating DNA movement. Our lab has been examining the molecular mechanisms of DNA integration and recombination in avian retroviral systems. From our studies, it has become apparent that the viral enzyme reverse transcriptase plays a major role in recombination. Since this enzyme contains a variety of enzymatic activities, including a site specific endonuclease, it is likely to also participate in the integration of retroviral proviruses. In addition, the origin of pseudogenes which structurally resemble the mRNA transcripts of their normal genes has remained a mystery. To facilitate studying whether viral reverse transcriptase mediates DNA integration of proviruses and other eucaryotic DNA sequences, we have cloned the pol gene of Avian sarcoma virus for expression in a procaryotic expression vector. We are presently cloning it for expression in eucaryotic cells, to have a well regulated intracellular supply of the enzyme, which is not encased within the virion. We intend to examine, both in vivo and in vitro, whether reverse transcriptase plays a role in DNA integration.

1253 INTRACISTERNAL A-PARTICLE GENES: A FAMILY OF COPIA-LIKE TRANSPOSABLE ELEMENTS IN MUS MUSCULUS, Robert G. Hawley, Marc J. Shulman and Nobumichi Hozumi, The Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Toronto, Canada.

We have characterized two functionally defective mouse immunoglobulin kappa (κ) chain genes that arose spontaneously during the growth of the Sp6 hybridoma cell line in culture. By comparing molecular clones of the mutant κ chain genes to the cloned wild-type κ chain gene, we have been able to demonstrate directly that the defect in the expression of each of the mutant κ chain genes is due to the insertion of a different member of the intracisternal A-particle (IAP) family of repetitive DNA elements. These results indicate, therefore, that the IAP genes of the mouse, which have previously been shown to be similar in structure to the copia-like transposable elements of lower eucaryotes, also share with these elements the ability to move within their resident genome. In contrast to what is observed at the insertion sites of these other transposable elements, however, insertion of IAP genes does not always result in the duplication of short sequences of target DNA. Furthermore, we have found that the long terminal repeats (LTRs) of the IAP gene that did not generate the target DNA repeat are not identical. The significance of these findings will be discussed in the context of the possible modes of transposition of IAP genes. We are also investigating the manner in which these IAP gene insertions are affecting the expression of the mutant κ chain genes. Preliminary results, if confirmed, will provide definitive evidence for a role of conformation in the splicing of eucaryotic mRNA.

1254 INSTABILITY OF ENDOGENOUS ECOTROPIC PROVIRUSES OF RF/J-DERIVED HYBRID MICE, Neal G. Copeland and Nancy A. Jenkins, University of Cincinnati College of Medicine, Cincinnati, OH 45267

RF/J mice carry three endogenous ecotropic proviruses designated Emv-1, Emv-16, and Emv-17 (endogenous ecotropic murine leukemia viral locus 1, 16, and 17; respectively). These proviral loci are not spontaneously expressed in RF/J mice due to both genetic and non-genetic factors and the endogenous (germline) ecotropic proviral DNA content of RF/J mice is stable. Genetic and molecular analysis of the endogenous ecotropic proviral DNA content of SWR/J X (SWR/J X RF/J) hybrid mice showed that Emv-16 and Emv-17 are very tightly linked ($\leq$0.9 cM), but are not tandemly duplicated. Interestingly, unlike RF/J mice, RF/J-SWR/J-derived hybrid mice are highly viremic and their endogenous ecotropic proviral content is not static. Both excision and amplification of proviral sequences occurred. In fact, more than 40 new ecotropic proviruses were detected in the germline of only 120 RF/J-derived hybrid mice analyzed. As many as 50% of the progeny of some litters contained amplified proviruses and as many as six new proviruses were acquired by a single animal. These sequences were acquired early in development, at different developmental stages, and were always present in the germline.

RF/J-derived hybrid mice represent a potentially valuable experimental system for generating virally induced mutations in mice and for identifying and characterizing, at the molecular level, genes important in normal mammalian development. In addition, analysis of the amplified proviruses in RF/J-derived hybrid mice may yield important insights into the interactions of viruses and their host backgrounds.

1255 SEQUENCE-SPECIFIC BINDING OF GLUCOCORTICOID RECEPTOR TO MTV DNA AND ANALYSIS OF GLUCOCORTICOID INDUCED GENE EXPRESSION USING A TRANSIENT ASSAY, Donald DeFranco, Monika Lusky* and Keith R. Yamamoto, University of California, San Francisco, CA 94143 and *University of California, Berkeley, CA 94720

Nuclease footprinting assays have been performed to identify murine mammary tumor virus (MTV) DNA sequences which interact with purified glucocorticoid receptor. Five footprinted sequences have been identified upstream of the MTV transcription initiation site and four others within transcribed sequences. The footprinted sequences lack extensive sequence homology although a family of related octanucleotides ($AGA\overset{A}{\underset{A}{}}CAG\overset{A}{\underset{A}{}}$), can be discerned which occurs at least once in each footprinted sequence. A restriction fragment containing four footprint sequences from one of the regions has previously been shown to act in vivo as a receptor-dependent transcriptional enhancer element, implying that the binding sites detected in vitro may be biologically functional.

The activity of E. coli chloramphenicol acetyltransferase (CAT) can be monitored following the transfection of its gene into eukaryotic cells. A number of CAT vectors have been constructed which contain various glucocorticoid receptor binding regions of MTV DNA fused to different eukaryotic promoters. Hormone mediated induction of CAT activity has been observed using both the MTV and herpes simplex virus thymidine kinase gene promoters which is increased if additional receptor-independent transcriptional enhancer sequences are present in these vectors. Thus two distinct regulatory elements appear to function in concert resulting in an additive affect of their enhancement properties.

1256 CHARACTERIZATION OF A METALLOTHIONEIN MULTIGENE FAMILY FROM RAT, Robert D. Andersen, Bruce W. Birren, Susan J. Taplitz and Harvey R. Herschman, Lab of Biomedical and Environmental Sciences, Univ. of California, Los Angeles, CA 90024

The metallothionein-1 (MT-1) structural gene and two related MT-1 pseudogenes have been isolated from a rat genomic library. The sequences of these genes indicates that the structural gene contains two intervening sequences and the pseudogenes are processed retroposons with characteristic polyadenylation terminators. However, one of these pseudogenes preserves the upstream promoter sequence of the MT-1 structural gene and shows almost exact homology with the MT-1 cDNA. This pseudogene also carries an additional T residue between Ser_{28} and Cys_{29} and, if transcribed, would direct the synthesis of a 63 amino acid peptide which terminates at a TGA stop just beyond the original TGA stop codon in the MT-1 gene (TGAAGTGA). This peptide would differ from MT-1 in its C-terminal half. We propose that this pseudogene may have arisen by two distinct events in which a cDNA transcript was reinserted into the genome followed by, or simultaneous with, a recombination between this sequence and the MT-1 structural gene. The other MT-1 pseudogene shows the accumulation of numerous mutations and deletions and is flanked by direct repeats in which 13 out of 16 bases match. The lack of any upstream homology with the MT-1 structural gene promoter indicates that this pseudogene is probably not expressed. Data concerning the organization of the rat MT-2 genes will also be presented.

1257 DIFFERENTIAL TISSUE-EXPRESSION DURING MOUSE EMBRYOGENESIS OF TRANSPOSON-LIKE REPETITIVE DNA SEQUENCE. Philippe Brûlet, Service de Génétique cellulaire du Collège de France et de l'Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15.

A family of long, moderately repetitive and dispersed DNA sequence in the mouse genome was recently described (Brûlet et al., PNAS, 80, 5641, 1983). They present structural analogies with integrated retroviral sequences. A 6 kb RNA transcript is detected in undifferentiated embryonal carcinoma cell lines but not in any differentiated cell lines tested. By in situ hybridization, the RNA is also differentially detected in the tissue of early mouse embryo. The inner cell mass and the embryonic ectoderm at day 6 and 7 expressed the RNA but preimplantation embryos, trophectoderm and endoderm do not at a detectable level.

Data to correlate the tissue specific expression with the DNA sequence will be presented as available.

1258 DNA SEQUENCES INVOLVED IN CELL SPECIFIC EXPRESSION OF EXOCRINE PANCREATIC GENES, Anne M. Boulet and William J. Rutter, Dept. of Biochem. and Biophys. and Hormone Research Laboratory, Univ. of Calif., San Francisco, CA 94143

DNA sequences within the 5' flanking portion of the rat chymotrypsin b gene are capable of eliciting preferential expression of this gene in exocrine pancreatic cells: A construction linking this region to coding sequences for chloramphenicol acetyl transferase (CAT) directs expression of a higher level of CAT enzyme activity in a pancreatic cell line, in which the chymotrypsin gene is actively transcribed, than in fibroblast cell lines. The sequences responsible for cell specific expression are located within a region 125 to 210 base pairs upstream of the mRNA cap site and can function independently of the chymotrypsin promoter. This cell specific element resembles viral enhancers in that it can increase expression from heterologous promoters, though only in the pancreatic cell line, and in that this activity is manifested when the element is placed upstream in either the normal or inverted orientation with respect to the direction of transcription. The amylase gene and other exocrine pancreatic genes are being examined for the presence of cell specific elements in order to determine whether common control sequences are involved in coordinate expression of these genes in the rat pancreas.

1259 RELATIONSHIP BETWEEN LARGE DELETIONS IN THE HUMAN β-GLOBIN GENE CLUSTER F.Grosveld, E.Vanin, D.Kioussis, O.Smithies, R.Taramelli, S.Wright MRC Inst., Mill Hill London, U.K and Univ. of Wisconsin, Wisconsin 53706

Two independent γδβ-thalassaemias are each associated with large deletions. We show, by comparing the DNA sequences across the breakpoints of the two deletions with the equivalent regions of normal DNA, that the deletions are due to non-homologous DNA exchanges. We also show that their 5' breakpoints are located approximately the same distance apart and in the same order along the DNA as are their respective 3' breakpoints. These results suggest that the deletions were generated by a common mechanism. Perhaps the 5' and 3' breakpoints are physically close in the nucleus, although far apart on the linear DNA. In addition we show that the β-globin gene present in a Dutch case of γδβ thalassaemia is identical to the normal β-globon gene with respect to DNA sequence and its transcription in Hela cells. DNaseI sensitivity and methylation experiments show that the affected β-globin gene is present in an inactive configuration in vivo. This is the result of a translocation of a normally inactive locus next to the β-globin gene on the affected chromosome, or the deletion of sequences which are normally required for the maintenance of the active state.

1260 A TRANSPOSON FROM DICTYOSTELIUM ENCODING HEAT SHOCK AND DEVELOPMENTALLY REGULATED TRANSCRIPTS, Elliot Rosen, Annegrethe Sivertsen and Richard A. Firtel. Univ. of Calif. San Diego, La Jolla, CA 92093.

We have isolated and characterized a transposable element from Dictyostelium denoted Tdd-1. There are ~50 complete copies of the element as well as 150 partial elements per genome. Analysis of Southern blots of DNA from different Dictyostelium strains shows that Tdd-1 is a mobile element. Interestingly we have found short sequences homologous to Tdd-1 in regions surrounding the transposon. Tdd-1 is 4.9kb long and contains 313 bp inverted repeats. The repeats lie near the termini but unlike other transposable elements one end of Tdd-1 extends 36 base pairs past the repeat. The Tdd-1 element hybridizes to two sets of transcripts. One set consists of a series of developmentally regulated transcripts all with the same polarity. Although present in vegetative cells, these transcripts dramatically increase in abundance after 10 hours of development. With the exception of the inverted repeats all subfragments of Tdd-1 that have been examined hybridize to these developmental transcripts. In addition we have identified a 0.9kb transcript that is induced by heat shock. This message is transcribed off the opposite strand as the developmentally regulated set of transcripts. In order to study the heat shock induced transcript from a single element, we have cloned parts of Tdd-1 in yeast. We have identified a heat shock inducible RNA in yeast transformants that has the same polarity as the heat shock inducible message hybridizing to Tdd-1 in Dictyostelium.

1261 CHARACTERIZATION OF THE INVERTED TERMINAL REPEATS OF THE DICTYOSTELIUM TRANSPOSON, DIRS-1. Joe Cappello, Charles Zuker and Harvey F. Lodish. Massachusetts Institute of Technology, Cambridge, MA 02139

The 4.7 kb Dictyostelium discoideum transposable element, DIRS-1 (Dictyostelium Intermediate Repetitive Sequence 1) contains 332 and 360 nucleotide inverted terminal repeats. We have determined the nucleotide sequence of nine of these repeats both left and right from six different elements and have found that the repeats are highly conserved (< 10% overall divergence) and that the final 100 bases are almost completely invariant. Left repeats and right repeats can be distinguished by their terminal residues. All left repeats terminate with a 32 nucleotide sequence composed almost entirely of A + T. All right repeats are extended by 27 bases of a common A + T sequence. The endpoints of the repeats are identical in all cases allowing precise definition of the flanking genomic sequences. There is no evidence for either sequence specificity or duplication of sequences at the insertion site. Sequence analysis of genomic clone SB41 reveals that it contains an intact DIRS-1 element which has inserted into a pre-existing DIRS-1 related sequence. Therefore the nucleotide sequence flanking the repeats of the intact element can be aligned exactly with an internal sequence found within the element. This alignment confirms that there is no duplication of the preinsertion site sequence greater than possibly a single base pair.

1262 Dispersed, Repetitious Sequence Families in the Xenopus Genome, James Garrett and Dana Carroll, University of Utah, Salt Lake City, UT 84112

We have isolated and characterized two different families of mobile genetic elements in the Xenopus laevis genome. The PTR element is present in about 750 copies/haploid genome. The element averages 7Kbp in length, with the main body of the element composed of tandem repeat clusters of two different 400bp sequences. Individual elements vary in the number of tandem repeats, but otherwise the family is highly homogeneous. Sequence analysis has shown PTR elements to be bounded by 4bp direct repeats and 24bp inverted repeats. We have shown that PTR elements can vary in specific location in different individual frogs.

The TX2 element was isolated as a 6Kbp insertion in a cloned PTR element. The TX2 element generated a 23bp target site duplication upon insertion, but the element itself does not have direct or inverted terminal repeats. There are 100-200 TX2 elements/haploid genome.

In situ hybridization shows both elements to be randomly dispersed throughout the Xenopus laevis genome. Both elements are present in highly conserved form in the species X.borealis and X.mulleri.

1263 TU-ELEMENT-LIKE SEQUENCES IN THE MAMMALIAN GENOME, David M. Gilbert, Barbara Hoffman-Liebermann, Dan Liebermann, Larry Kedes, and Stanley N. Cohen, Stanford University Medical Center, Stanford, CA 94305

TU-elements are a heterogeneous family of transposable elements found originally in the genome of the sea urchin S. purpuratus. Members of this family are similar in structure but not sequence to the foldback elements of Drosophila. They contain long terminal inverted repeats (IVR's) with an outer domain of tandem direct repeats 15 bp in length and a non-repeating inner domain. One of the most striking features of this family of transposons is their remarkable evolutionary conservation. We have found sequences highly homologous to the outer domain of the TU-IVR's in a wide variety of unrelated species including the mammalian genome. In humans, both the sequence and periodicity of the IVR have been found to be similar to that of the sea urchin. In addition, a non-contiguous sequence, located in the middle region of the sea urchin transposon has also been found to reside in the mammalian genome. This sequence is present in a much higher copy number than the IVR in all species examined. We are currently studying the sequences and structures of these elements in both mouse and human and are testing their mobility using a variety of genetic and molecular approaches.

1264 DNA REARRANGEMENTS DURING MACRONUCLEAR MATURATION IN EUPLOTES CRASSUS, M. Roth and D. Prescott, Department of MCD Biology, University of Colorado, Boulder Co. 80309

During vegetative growth, hypotrichous ciliates contain two types of nuclei: a) the micronucleus which contains typical eukaryotic chromosomes, divides by mitosis, and is transcriptionally inactive and b) the macronucleus which consists of small gene-sized DNA molecules derived from the micronucleus, divides by amitosis, and is the source of all nuclear transcripts. Each macronuclear sequence is of discrete size and is bounded by inverted terminal repeats of the sequence 5'CCCCAAAA3'. These telomere sequences are not found at the ends of macronuclear sequences as they exist in the micronucleus. After conjugation the macronucleus is destroyed and a new one is constructed from a micronucleus. Cytologically this process can be divided into two parts; first, the polytenization of the chromosomes and second, the vesiclization of the polytene chromosomes. In order to understand how macronuclear sequences are specifically excised from the chromosomes, and how telomeres are added to them we have developed techniques for obtaining large numbers of Euplotes crassus at different stages of macronuclear development. We have purified DNA from maturing macronuclei and used cloned macronuclear sequences as probes to study the specific structural and temporal nature of this process. The results of this study reveal that excision of macronuclear sequences from the chromosomes is a multi-step process. The first step involves excision of the presumptive macronuclear sequence along with some flanking DNA from the chromosome. This flanking DNA is then removed and larger than normal telomeres are added to the ends of the genes. At the end of macronuclear development these extended telomeres are reduced to there macronuclear size.

1265 ANALYSIS OF MICRONUCLEAR $(CCCCAA)_n$ BLOCKS IN THE CILIATED PROTOZOAN Tetrahymena. J. Michael Cherry and Elizabeth H. Blackburn, Univ. of Calif., Berkeley, CA 94720

The ciliated protozoan Tetrahymena thermophila contains a 2N gametic nucleus, the micronucleus and a 45C somatic nucleus derived from the micronucleus, the macronucleus. Fragments of micronuclear DNA containing the macronuclear telomeric repeat $5'(CCCCAA)_n3'$ were cloned and analysed. This analysis included DNA sequencing and fine structure fate mapping using subcloned fragments against isolated nuclear DNA. We find three striking similarities between these cloned sequences: 1) All micronuclear $(CCCCAA)_n$ blocks, referred to as mic-$(CCCCAA)_n$, are surrounded by AT rich regions, 85-90% A+T. These AT rich regions are similar to AT rich regions found adjacent to the telomeric $(CCCCAA)_n$ in macronuclear DNA. 2) In all but one cloned mic$(CCCCAA)_n$ block a high percentage of degenerate CCCCAA repeats are found. The exception contains 40 perfect CCCCAA repeats. Only perfect CCCCAA repeats have been found on macronuclear telomeres. 3) A highly conserved sequence is found 3' of all mic-$(CCCCAA)_n$ repeats. In two cases this conserved region includes a 13bp palindromic sequence. We have subcloned a variety of $(CCCCAA)_n$ flanking sequences and used these subcloned fragments as probes in a Southern blot restriction enzyme analysis of micronuclear and macronuclear DNA. Our results, in agreement with previous work, show that the majority of these flanking domains are middle-repetitive in the micronucleus and eliminated from the macronucleus. However, in contrast to previous results we find a sequence flanking one mic-$(CCCCAA)_n$ maintained in the macronucleus within what appears to be terminal restriction fragments. This flanking sequence has homology to the terminal AT rich region of the rDNA.

1266 IS THERE REORGANIZATION OF TELOMERIC SEQUENCES IN HYPOTRICHOUS CILIATED PROTOZOA?
Carolyn L. Jahn and David M. Prescott, University of Colorado, Boulder, CO 80309

In hypotrichous ciliated protozoa, the genes are converted from their micronuclear, chromosomal location to transcriptionally active, "gene-sized" linear DNA molecules in the macronucleus. These macronuclear DNA molecules have a defined telomeric structure consisting of repeats of the sequence CCCCAAAA (C4A4). Determining how these telomeres are generated is one approach to studying this genomic reorganization. We have begun by analyzing the distribution of C4A4 repeats in the micronucleus. The C4A4 repeats do not occur at the ends of macronuclear-destined sequences as they reside in the micronuclear genome. However, quantitation of the amount of C4A4 homologous sequences in the micronuclear genome indicates that sufficient copies of C4A4 exist in the micronucleus to provide all of the termini found in the macronucleus. The micronuclear copies of C4A4 are not digestible to sizes smaller than 10 KB by any restriction enzymes, alone or in combination. In addition, a micronuclear genomic library yields C4A4 repeat containing clones at only 1% of the frequency expected. Moreover, the clones that are obtained from the library contain only short blocks (<100 BP) of C4A4 repeats. These data suggest that most of the micronuclear C4A4 repeats exist as very large (>10 KB) tandem arrays. An additional possibility is that the C4A4 repeats occur at the telomeres of micronuclear chromosomes. We are investigating these possibilities by: a) determining whether C4A4 repeats in the micronucleus are sensitive to Bal 31 nuclease, b) using alternative methods to clone C4A4 repeat containing DNA, and c) examining the chromosomal distribution of C4A4 repeats by in situ hybridization.

1267 GENOME REARRANGEMENT IN A HYPOTRICHOUS CILIATED PROTOZOAN, Lawrence A. Klobutcher, Carolyn L. Jahn and David M. Prescott, University of Colorado, Boulder, COL 80309

In the hypotrichous ciliated protozoan Oxytricha nova all of the DNA in the transcriptionally active macronucleus is in the form of low molecular weight, linear, gene-sized molecules with an average size of 2,200 base pairs (bp). These macronuclear DNA molecules, referred to as macronuclear genes, are derived from a copy of the chromosomal micronuclear genome by a complex series of genomic changes following each sexual cycle. In order to characterize in greater detail the genomic rearrangement events that occur during macronuclear development, we have cloned and sequenced an 810bp macronuclear gene as well as the region of the micronuclear genome that gives rise to it during development. The micronuclear version of this gene differs from the mature macronuclear sequence in two ways. First, it lacks all of the $5'C_4A_4 3'$ terminal repeats present on this and all other macronuclear genes. Second, the micronuclear version of the gene contains three short blocks of sequence (two 49bp blocks and one 32bp block) that are absent in the mature macronuclear gene. Although these three blocks of internal eliminated sequences (IES) differ from each other in primary sequence, they are similar to each other in possessing a short direct repeat at their ends and an immediately adjacent internal inverted repeat. These results indicate that the following three types of DNA rearrangement events must occur during macronuclear development: 1)macronuclear genes must be excised from the micronuclear chromosome, 2)terminal $5'C_4A_4 3'$ repeats must be added to the ends of macronuclear genes, and 3)IES sequences must be excised from macronuclear genes, suggesting the existence of a nucleic acid splicing process.

1268 MACRONUCLEAR TELOMERE FORMATION IN TETRAHYMENA THERMOPHILA, Elizabeth Spangler, Thecla Ryan and Elizabeth H. Blackburn, Univ. of Calif., Berkeley, CA 94720

The ciliated protozoan Tetrahymena thermophila contains two nuclei: a diploid micronucleus and a polyploid macronucleus which is derived from the micronucleus during conjugation. Formation of the macronucleus involves extensive genomic rearrangements as well as sequence elimination, and amplification of the genes encoding the ribosomal RNA's. An essential aspect of this process is the generation of new telomeres at the ends of macronuclear DNA molecules.

It has been shown that the repeated hexanucleotide $5'(CCCCAA)_n 3'$ is present near macronuclear DNA ends. (1) In order to obtain clones of macronuclear telomeric sequences we treated isolated macronuclear DNA with nuclease S1, which should leave a blunt end, and followed this by digestion with a restriction endonuclease. The resultant fragments were then ligated to a fragment of pBr322 having a blunt end and the appropriate sticky end. Using the repeated hexanucleotide as a probe, we isolated several macronuclear DNA sequences which contain C_4A_2 repeats. We find that these C_4A_2 associated sequences are quickly digested upon treatment of isolated macronuclear DNA with the exonuclease Bal-31, indicating that they are indeed telomeric. We find no evident sequence homology between these several telomere-associated sequences.

1. Yao, M.-C. & Yao, C.-H., Proc.Nat.Acad.Sci. (1982) 78, 7436-7439

1269 ALTERNATE JUSTAPOSITION OF MACRONUCLEAR SEQUENCES IN THE HYPOTRICHOUS CILIATE OXYTRICHA FALLAX, Glenn Herrick and Sam Cartinhour, University of Utah, Salt Lake City, UT 84132

The three members of the cross-hybridizing pMA81 family of macronuclear minichromosomes (4890, 2780, 1640 bp) from the protozoan Oxytricha fallax have in common a conserved sequence block 1300-1550 bp long. Adjacent to the common block in the two larger DNAs are sequences which are unique to them, while the smallest DNA contains few if any additional sequences. All members of the family reappear, and with the same stoichimetry, when the macronucleus is replaced following conjugation, and can be detected in another O. fallax subspecies. In a random collection of cloned macronuclear DNAs, 6 or 15 hybridize to macronuclear DNA families. This high frequency suggests that families sharing common sequence blocks have an important role in macronuclear function. The genesis of the pMA81 family following conjugation has been investigated by studying these sequences in micronuclear DNA. At least two different loci appear competent to give rise to all three family members; to do so each must suffer removal of micronucleus-limited sequences embedded within the macronuclear family-destined sequences and must be alternately processed to give rise separately to each of the family members. The genesis of the C_4A_4 telomeres of each family member must be intimately related to this alternate processing mechanism.

1270 SITE-SPECIFIC EXCISION AMPLIFICATION OF MITOCHONDRIAL "PLASMIDS". Donald J. Cummings. University of Colorado Health Sciences Center, Denver, CO 80262

Senescence in the filamentous fungus Podospora anserina is maternally inherited. Several specific regions of the mitochondrial genome are excised and amplified. These self-replicating "plasmids" are isolated as multimeric sets. We have cloned the monomer unit of three of these "plasmids" as well as the genomic DNA from which they are derived. The excision sites have been determined for two of these plasmids, α- and β-senDNA.

		GTTATATAAC		
Hae23	5'	GGCCAAGTGTTCAATATATTGCAG	GTGCGCCGTTTAACGTGCGTTTTAAGTCCGG	3'
α		CTATATAGACTAAGGACTGGCTGCTTATCCTAC	GTGCGCCGTTTAACGTGCGTTTTAAGTCCGG	3'
			TAATATATTA	
Hae14		CTATATAGACTAAGGACTGGCTGCTTATCCTAC	ATAACCAATTATATAATAGCATCATTCAG	3'
PstI0	5'	CTGCAGCCACGTCATACCACACC	GCACCACACTCACTTTATTTT	3'
β		TTTGTACTATATAATCTATAAGTTATTGATGTT	GCACCACACTCACTTTATTTT	3'
EcoI1		TTTGTACTATATAATCTATAAGTTATTGATGTT	GCACCACACGAAAGAAGAAGT	3'

α β

The excision sites are different for each "plasmid". Certain features bear resemblance to known mobile elements.

1271 EXPRESSED VSG GENES CAN BE ON DIFFERENT BUT RELATED TELOMERES. Janette Allison, Peter Myler, George Newport*, Nina Agabian* and Kenneth Stuart, Issaquah Health Research Institute, Issaquah, WA. 98027 and University of Washington, Seattle, WA. 98195.

Of the 7-11 genes that comprise the IsTat 1.3 VSG gene family one has a telomeric location on a "minichromosome". A single restriction site difference between this gene and the ELC present in variant antigenic type (VAT) 1.3 has been found near the 3' end of the coding sequence. This implies that it is not the basic copy (BC) of the ELC although the BC has not been identified despite isolation of two 1.3 genes from genomic libraries.

The 1.3 ELC is retained in a DNase I insensitive state in a later VAT, 1.7, which has a 1.7 ELC. It is also retained in procyclic forms derived from these VATs. When both 1.3 and 1.7 relapsed to the predominant 1.A VAT, they again retained the 1.3 ELC. However, the relapse from 1.7 ($1.A^7$) contained a 1.A ELC while the relapse from 1.3 ($1.A^3$) did not. The 1.3 ELC has an identical restriction map for over 30 kb 5' to the coding sequence in all these cases. The 1.3 ELC is located on a telomere which resembles that occupied by the ELC of a preceding VAT (1.Ae). This telomere differs from that occupied by the 1.7 (or $1.A^7$) ELC. While these two telomeres (1.3 versus 1.7) are different, they appear related. (Supported by NIH AI7375, DAMD17-92-2016, WHO and Murdock Charitable Trust (KS) and NIH AI17309 (NA) grants).

1272 SINGLE-STRANDED REGIONS IN THE 5' FLANKING DNA OF THE DROSOPHILA HSP 70 GENE. Ronald L. Seale, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The hsp 70 gene of Drosophila nuclei was cleaved at two positions by single-strand endonucleases. The cleavage sites were in the 5' flanking region and were not identical to either the DNase I or micrococcal nuclease hypersensitive sites. Plasmids containing the entire coding region plus 1.5 kb of 5' flanking DNA were not cleaved at the positions recognized in chromatin; this was true of supercoiled plasmids, linear plasmids, and DNA incubated under conditions that induce Z-DNA formation. When nuclei were digested in progressive increments of ionic strength, the cleavage sites disappeared between 200 and 300 mM NaCl. Thus, presence of single-strand regions in the control region of hsp 70 is attributed to chromatin structure, possibly due to non-histone proteins, or to higher order configurations.

1273 INTERACTIONS BETWEEN DROSOPHILA GENES, THE MOBILE ELEMENT GYPSY, AND THE SUPPRESSOR OF HAIRY WING, Mark Peifer and Welcome Bender, Harvard Medical School, Boston MA 02115.

Extragenic suppressors have long been studied in Drosophila melanogaster, and among them the best characterized is the suppressor of Hairy wing (su(Hw)). A recessive lesion in this locus will, in combination with selected alleles of a variety of different genes, suppress their mutant phenotype. Recent molecular studies undertaken in our lab have demonstrated that most of the mutant alleles which are suppressed by su(Hw) are lesions caused by the insertion of a particular copia-like mobile element called gypsy. The insertion of this element acts in some manner to inactivate nearby genes; this inactivation depends on the presence of the wild type product of su(Hw). A number of experiments are in progress to elucidate both of these phenomena. These include (1) examination of supressible alleles and their revertants at a molecular level, (2) studies of the structure of the gypsy element and it's various intracellular forms, (3) characterization of gypsy transcription and its possible control by su(Hw), and finally (4) constructions placing a gypsy element at different positions around well-characterized genes, upstream, within introns, and downstream. We are reintroducing these constructions into the fly genome via P element-mediated transformation, and examing their phenotype and suppressibility.

1274 PRESENCE AND VARIATION OF EXTRACHROMOSOMAL DNA FORMS HYBRIDISING TO COPIA-LIKE TRANSPOSABLE ELEMENTS AND MIDDLE REPETITIVE DNA SEQUENCES IN DROSOPHILA MELANOGASTER CULTURED CELLS AND EMBRYOS. Kevin G. Mossie[1], Michael W. Young[2], and Harold E. Varmus[1,3]. Division of Genetics, Dept. of Biochemistry[1] and Biophysics, and the Dept. Microbiology and Immunology[3], University of California, San Francisco, CA 94143, and Rockefeller University[2], New York, NY 10021.

The transposable element (TE) copia of Drosophila melanogaster resembles vertebrate retroviruses by several criteria: (i) cultured Drosophila cells contain virus-like particles with reverse transcriptase activity and copia RNA (1,2), (ii) the structure and functionally-important sequences of copia and proviral DNA are homologous (3,4), and (iii) both copia and retroviral DNAs assume unintegrated circular forms with one or two copies of the long terminal repeat (5,6). We have extended these comparisons to other copia-like TEs and dispersed middle repetitive DNA elements by seeking unintegrated DNA forms in embryos as well as cultured Drosophila cells. We find closed circular DNA related to several copia-like elements (copia, 297, 412, mdg3, gypsy) and to most (8/12) of the middle repetitive DNA elements tested. The abundance of the unintegrated DNA varies as much as 20-fold between the cultured Drosophila cells and embryos. These variations do not correlate with either the abundance of RNA transcripts of the elements or the number of elements integrated in the genome.

1. Heine etal. (1980) J. Gen. Micro. 49, 385-395. 2. Shiba & Siago (1983) Nature 302, 119-124. 3. Rubin (1983) Chapter 8 in Mobile Genetic Elements, Academic Press. 4. Varmus, Chapter 10 in Mobile Genetic Elements. 5/6. Flavell & Ish-Horowicz (1981) Nature 292, and (1983) Cell 34.

1275 A NEW COPIA-LIKE TRANSPOSABLE ELEMENT FOUND IN AN rDNA GENE UNIT, Jeffrey R. Bell, Thomas Schmidt, Alicia M. Bogardus and Maria Pellegrini, University of Southern California, Los Angeles, CA 90089-1481

We have discovered a member of a new family of copia-like transposable elements inserted into the non-transcribed spacer between two ribosomal genes (rDNA). This family, which we call 3S18, consists of approximately 15 elements which are scattered throughout the Drosophila melanogaster genome. The elements of this family are approximately 6.5 kb long and have 0.5 kb terminal direct repeats. The elements are homogeneous as there are no changes in their restriction pattern. All of these properties are very similar to those of the copia-like families of transposons such as Copia, 412 or 297.

We found 3S18 in a λ clone containing rDNA. The inserted element is located in the non-transcribed spacer approximately 1 kb 3' from a 28S gene containing a type I IVS. On the other side of 3S18 is the rest of the non-transcribed spacer and the start of the next rDNA gene, including the 5' end of the 18S gene. Preliminary "in situ" hybridization results show no evidence of 3S18 in the nucleolus suggesting that either the rDNA unit in which we found 3S18 is non-nucleolar or else the presence of 3S18 is the result of a fairly recent transposition (the "in situ's" were performed on a different strain of flies than the strain from which 3S18 was isolated).

1276 CHROMOSOME REARRANGEMENTS INDUCED BY P-M HYBRID DYSGENESIS IN DROSOPHILA MELANOGASTER. Kevin O'Hare, Imperial College, London SW7 2AZ, England.

Chromosome rearrangements generated during P-M hybrid dysgenesis occur at positions where P elements appear to reside. The rate for a particular site seems to be correlated with the structure of the resident P element. Thus, intact 2.9kb elements are found at two hotspots for rearrangement while defective small elements are found elsewhere. Using P element sequences as probes, the structure of rearranged chromosomes has been investigated. In particular, the structure of rearrangements between heldup (hdp) and singed (sn) have been analysed by cloning. The precise structures of the rearranged chromosomes shows that there can be a net loss of P element sequences (as indicated by the in situ hybridisation studies of W.R.Engels and C.Preston, University of Wisconsin-Madison - personal communication). The implications of these findings for the mechanism of chromosomal rearrangement will be discussed.