DEVELOPMENTAL BIOLOGY USING PURIFIED GENES

Donald D. Brown, Organizer

March 15–March 20

Plenary Sessions

Poster Sessions

Specialized Genes

1024 PREPROINSULIN AND PREPROINSULIN-LIKE GENES. Argiris Efstratiadis, Cara Berman, Cecilia Lo, Francine Perler, Nadia Rosenthal, and Scott Zeitlin. Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.

Knowledge of the structure of preproinsulin genes (1-5) provides the necessary background information and the specific probes to study their expression. For example, by using RNA blot-hybridization, we asked the question whether glucose (the physiological stimulus for insulin biosynthesis and release) induces transcription of preproinsulin sequences in isolated pancreatic islets and in cultured cells derived from a transplantable rat insulinoma.

To assay the expression of in vitro-modified genes, without depending on the availability of selectable markers, we have begun introducing genes into cultured cells by microinjection, using iontophoresis. Our results indicate that the method could be succesfully applied for the introduction of any gene into any cell. Such transformed cells contain the injected DNA sequences stably integrated into chromosomal DNA. The integration frequency is high.

Comparisons of the structure of the preproinsulin gene between different organisms have offered some new insights into the evolutionary process (5). This study has now been extended. The preproinsulin gene belongs to a superfamily that includes the genes encoding insulin-related polypeptides (nerve growth factor, insulin-like growth factors and relaxin). We will describe our studies on insulin-like sequences isolated from a human chromosomal DNA library and from a human fetal liver cDNA library.

1. Lomedico,P., Rosenthal,N., Efstratiadis,A., Gilbert,W., Kolodner,R., and Tizard,R. (1979). Cell 18, 545.
2. Cordell,B., Bell,G., DeNoto,F., Tischer,E., Ullrich,A., Pictet,R., Rutter,W.J. and Goodman,H.M. (1979). Cell 18, 533.
3. Bell,G., Pictet,R., Rutter,W.J., Cordell,B., Tischer,E., and Goodman,H.M. (1980). Nature 284, 26.
4. Ullrich,A., Dull,T.J., Gray,A., Brosius,J., and Sures,I. (1980). Science 209, 612.
5. Perler,F., Efstratiadis,A., Lomedico,P., Gilbert,W., Kolodner,R., and Dodgson,J. (1980). Cell 20, 555.

1025 THE COLLAGEN GENE, Benoit de Crombrugghe, Gabriel Vogeli, Hiroaki Ohkubo, Yoshihiko Yamada, Enrico Avvedimento, Mark Sobel, Maria Mudryj and Ira Pastan, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD

The collagens belong to a family of proteins which constitute the principal component of the extracellular matrix of animal tissues. We have used chick embryo fibroblasts as a model system to study the regulation of type I collagen synthesis. Type I collagen, the major collagen species synthesized by these cells, is composed of two alpha 1 subunits and one alpha 2 subunit. When p60 ^{src}, the transformation protein encoded by Rous sarcoma virus, is present the synthesis of collagen is severely reduced in these cells. Recent evidence strongly suggests that this regulation occurs at the level of transcription.

We have isolated a series of overlapping clones which span the entire gene for chick alpha 2 (type I) plus its 5' and 3' flanking sequences, by successive screenings of a library of genomic DNA fragments. The alpha 2 collagen gene has a length of about 38 kilobases whereas the corresponding mRNA is 5,000 nucleotides long. The coding information of the gene is subdivided into more than 50 exons which are interrupted by introns of various sizes.

We have determined the size and sequence of nine exons which come fron three different segments of the gene encoding the helical portion of the protein. Eight of these exons have a length of 54 bp, the ninth has a length of 99 bp. The sequences within these exons vary except for the glycine codons which occur every third triplet. Each exon begins with a glycine codon and ends with a triplet which precedes a glycine codon. The size and the sequences of the introns do not show any homology except at their ends. Of the eight introns examined the first six bases at the 5' end of 6 introns are identical. The sequences at the 3' end of the introns also show similarities. Our results indicate that most exons of this gene have an identical length of 54 bp and hence imply that the ancestral gene for collagen arose by multiple duplications of a single genetic unit containing a 54 bp coding segment. During evolution the size of the exons has been strongly conserved although the sequences within these exons drifted by successive point mutations and in some cases by additions or deletions of 9 bp or multiples of 9 bp. Nine bp encode the basic gly-x-y repeat of collagen. Only additions or deletions of 9 bp or multiples thereof were tolerated. Other collagen genes probably arose by duplication of a first completed gene.

1026 THE DOPA DECARBOXYLASE GENE LOCUS OF DROSOPHILA MELANOGASTER. J. Hirsh, D. Gilbert, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

We are utilizing chromosomal DNA clones containing the Drosphila dopa decarboxylase (DDC) gene to study two aspects of this locus. 1) Regulation of the DDC gene. At one time in development, DDC is induced as a rapid response to the steroid molting hormone β-ecdysone (1), but at another developmental stage, DDC activity appears when β-ecdysone titers are at basal levels (2). Thus, it is likely that the gene is being induced by more than one regulatory signal. Our studies show that DDC RNA levels vary through development as a direct function of DDC levels, indicating that some pre-translational step is limiting for DDC induction at both of these developmental stages. We are examining that nature of precursor and mature RNA species at both of the developmental stages at which DDC is induced. 2) Genes near DDC. At least 12 other genes are located near the DDC gene (T. Wright, personal communication). At least two of these genes appear to have developmental relations to the DDC gene (3), suggesting a clustering of developmentally related genes. We have identified at least four transcribed regions apart from the DDC gene in some 80 kb of adjacent cloned sequences. We are examining the developmental expression of the transcripts, and in collaboration with Wright, attempting to correlate the transcribed regions with the identified genes.

(1) Kraminsky, G.P., Clark, W.C., Estelle, M.A., Gietz, R.D., Sage, B.A., O'Connor, J.D. and Hodgetts, R.B. (1980) Proc. Nat. Acad. Sci. USA 77, 4175-4179.
(2) Hodgetts, R.B., Sage, B., O'Connor, J.D. (1977) Dev. Biol. 60, 310-317.
(3) Wright, T.R.F., Hodgetts, R.B. and Sherald, A.F. (1976) Genetics 84, 267-285.

1027 THE STRUCTURE AND TRANSCRIPTION OF NORMAL AND ABNORMAL HUMAN GLOBIN GENES. Nicholas Proudfoot, Monica Shander, Susan Vande Woude and Tom Maniatis. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Each member of the human α-like and β-like globin gene families has been isolated and extensively characterized using molecular cloning procedures (see 1 for review). A comparative analysis of different globin gene sequences has identified putative transcription and mRNA processing signals, provided interesting information regarding globin gene evolution (2) and demonstrated the existence of human globin pseudogenes (3). The nucleotide sequence of one pseudogene (Ψα1) differs substantially from its functional counterpart. However, one gene which was originally thought to be an embryonic α-like globin gene (ζ1) contains only one alteration of its sequence which is incompatible with normal globin gene function; a UAG terminator at the codon for amino acid 6. Thus, ζ1 appears to be a pseudogene of very recent evolutionary origin.

As one means of correlating sequence organization and gene function we previously demonstrated the feasibility of studying the in vitro transcription of human globin genes (4). Recently we have used a SV40 vector system to study globin gene transcription and mRNA processing in vivo. Our in vitro transcription results to date are summarized as follows: 1) The δ-globin gene which is expressed less efficiently than the β-globin gene in adult erythroid cells is also poorly transcribed in vitro; 2) The α-globin pseudogene Ψα1 is transcribed in vitro but less efficiently than the normal α-globin gene; 3) β-globin genes were isolated from the DNA of seven different individuals with β^{o}-thalassemia, a genetic disease which is characterized by the complete absence of β-globin polypeptide. All of these genes are transcribed in vitro with the same efficiency as that of the normal β-globin gene. Comparison of the nucleotide sequence of one of the β^{o}-globin genes with the sequence of the normal β-globin gene (5) revealed the presence of a G to A transition within the GT sequence located at the 5' end of the large intervening sequence. Since the GT is an invariant feature of all globin gene introns it is possible that a transcript from this β^{o} gene would not be spliced. In vivo experiments are in progress to test this possibility.

(1) Maniatis, T., Fritsch, E. F., Lauer, J., and Lawn, R. M. (1980) Ann. Rev. Genetics 14, 145-178.
(2) Efstratiadis et al., (1980) Cell 21, 653-668.
(3) Proudfoot, N. J. and Maniatis, T. (1980) Cell 21, 537-544.
(4) Proudfoot, N. J., Shander, M. H. M., Manley, J. L., Gefter, M. L., and Maniatis, T. (1980)Science 209, 1329-1336.
(5) Lawn, R. M., Efstratiadis, A., O'Connell, C. O., and Maniatis, T. (1980) Cell 21, 647-651.

Transposable Genes

1028 HUMAN IMMUNOGLOBULIN GENES: DIVERSITY GENERATED THROUGH THE SHUFFLING OF DNA AND RNA SEQUENCES. P. Leder*, P. Hieter*, K. Kelly*, I. Kirsch*, E. Max*, J. Ravetch*, S. Korsmeyer+ and T. Waldmann+, *NICHD and +NCI, NIH, Bethesda, Md. 20205

We have used partial gene fragment purification together with probes derived from the mouse to clone the immunoglobulin light and heavy chain genes of man. The mouse and human genes, which arose from common ancestral sequences approximately 70 million years ago, have undergone considerable divergence, but have conserved regions of partial homology in coding sequences and complete homology in the short sequences thought to be involved in V/J somatic recombination, the central event involved in generating antibody diversity and in formation of an active immunoglobulin gene. In addition, detailed analysis of the λ light chain locus suggests that it encodes at least four λ constant region genes presumably corresponding to the four non-allelic human light chains. We have also detected several polymorphisms in the restriction sites surrounding these λ genes that should provide valuable human gene markers. We are in the process of determining the arrangement and nature of the joining sites (J-regions) that must occur within this complex locus. With respect to the human heavy chain genes, we have detected several extensive regions of homology between mouse and human sequences that may play a role in the recombination events that accomplish the heavy chain class switch and that provide for alternative RNA splicing pathways for membrane-bound and secreted heavy chains.

1029 **TRANSPOSABLE ELEMENTS IN DROSOPHILA**, Gerald M. Rubin, Mary Collins, Roger Karess, and Robert Levis, Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210

412, **copia**, and **297** are examples of families of dispersed repeated DNA sequences in which the elements are closely conserved, terminally redundant, transcribed, and transposable (1-3). We refer to that subset of the dispersed repeated DNA sequences in the Drosophila genome which shares all of these properties as "**copia**-like" elements. Studies on the structure of **copia**-like elements and their insertion sites (4, 5, E. Young and G. Rubin, unpublished) as well as data on the structure (A. Flavell, R. Levis and G. Rubin, unpublished) and translation (6) of **copia** transcripts will be briefly reviewed.

A strategy which employs **copia**-like elements to isolate genetic loci will be described. This strategy has been used to isolate the **white** locus (P. Bingham, R. Levis, and G. Rubin, unpublished). Preliminary data on the molecular nature of several unstable alleles of the **white** locus will be presented.

1. Finnegan, D. J., Rubin, G. M., Young, M. W. and Hogness, D. S. (1978) Cold Spring Harbor Symp. Quant. Biol. **42**, 1053-1063.
2. Potter, S. S., Brorein, W. J., Jr., Dunsmuir, P. and Rubin, G. M. (1979) Cell **17**, 415-427.
3. Strobel, E., Dunsmuir, P. and Rubin, G. M. (1979) Cell **17**, 429-439.
4. Dunsmuir, P., Brorein, W. J., Jr., Simon, M. A. and Rubin, G. M. (1980) Cell **21**, 575-579.
5. Levis, R., Dunsmuir, P. and Rubin, G. M. (1980) Cell **21**, 581-588.
6. Flavell, A. J., Ruby, S. W., Toole, J. J., Roberts, B. E. and Rubin, G. M. (1980) Proc. Nat. Acad. Sci. USA, in press.

1030 SOMATIC DNA REARRANGEMENT AND DIFFERENTIAL RNA PROCESSING IN DEVELOPMENT OF B LYMPHOCYTES, Susumu Tonegawa, Yoshikazu Kurosawa, Richard Maki, and Histoshi Sakano, Basel Institute for Immunology, Postfach, 4005 Basel 5, Switzerland

The variable region of an immunoglobulin chain is encoded in multiple DNA segments that are scattered along a chromosome of a germ-line genome. These DNA segments are assembled into a continuous stretch with concomitant deletion of the spacer sequences during differentiation of B lymphocytes. The somatic rearrangement of DNA sequences is considered to play key roles both in somatic amplification of antibody diversity and in determination of the monospecificity of lymphocyte clones. In the mouse light chains of both λ and κ types, two DNA segments, V and J, are separate in the germ-line genome and are joined in the lymphocytes which express these DNA segments. The V and J segments code for about 95 residues at the NH_2 terminal and about 13 residues at the CO_2H terminal ends of the V region, respectively. In contrast, the heavy chains are encoded in three separate DNA segments, V, D and J, on the germ-line genome, where the D DNA segment encodes the third hypervariable region which determines in part the shape and the size of the antigen-combining sites. Somatic joinings of the three DNA segments are necessary to generate a complete heavy-chain V gene.

Recently we have identified a series of germ-line D DNA segments and characterized the structure and organization of these DNA segments. We will discuss roles of these DNA segments and the V-D-J joining with respect to somatic amplification of antibody repertoire.

While DNA rearrangements play key roles in the control of gene expression in development of B lymphocytes, another mechanism - namely, differential processing of primary RNA transcripts - is also important in some of the developmental pathways stages. We will describe two such examples in the differentiation of B cells.

Multigene Systems

1031 THE DEVELOPMENTALLY REGULATED CHORION GENE FAMILIES, Fotis C. Kafatos, Harvard Biological Laboratories, 16 Divinity Avenue, Cambridge, MA 02138

The silkmoth eggshell (chorion) proteins are encoded in several families of genes. The families have been generated during evolution by gene duplication and diversification; they are highly regulated during development, so that nearly 200 distinct chorion proteins are produced during specific stages in choriogenesis. Chorion genes are clustered in a single chromosome, and in the aggregate account for a significant portion of that chromosome. They have been cloned and their structure and organization have been studied by recombinant DNA techniques. I shall summarize the information currently available, and relate it to the processes of gene evolution and regulation of gene expression during development.

1032 INTERNECINE HISTONE GENE FAMILIES, Larry Kedes, Geoffrey Childs, Rob Maxson and Ronald H. Cohn, Department of Medicine and Howard Hughes Medical Institute Laboratory, Stanford Medical School, Stanford, CA 94304.

We report the discovery of dispersed, solitary members of tandem multigene families. We refer to these expatriate genetic elements that have been ostracized by their families as orphons. We have isolated orphons by null restriction cloning which entails cleavage of genomic DNA with restriction enzymes known not to cut the repetitive gene family. The intact tandemly linked gene clusters are too large to be cloned in plasmid vectors. Any family members that are flanked on both sides by the enzymatic recognition sequences are smaller and are easily cloned. For our initial analysis we chose the histone gene family of the sea urchin Lytechinus pictus. Surprisingly, blot hybridization analysis of genomic DNA reveals that there are a large number (>50) of histone gene orphons in each genome. Each of the five histone gene coding regions has a number of orphons (5-20) in addition to the several hundred copies in the clusters. Most such orphons appear to contain only one coding region. Nearly all sea urchin histone gene orphon loci are polymorphic in the population and no two individuals have the same sets of histone orphons for any coding region.

An H3 coding orphon was isolated and examined in detail. The H3 region homologous to the histone gene clusters is 1200 bases long and includes the complete H3 gene and some surrounding histone DNA. The orphon is flanked by at least 1.7 kb of 5' and 3 kb of 3' non-histone DNA. These flanking sequences are each moderately repetitive in the genome and are not homologous to each other. DNA sequence analysis of the junctions between the H3 region and the immediate flanking non-histone DNA reveals no evidence of repetitive or palindromic sequences.

Orphons arise from both protein-coding and non protein-coding structural gene families including those of histone and ribosomal genes, and occur in sea urchins (L. pictus), Drosophila melanogaster, and yeast (Saccharomyces cerevisiae). We have conducted Southern transfer hybridization experiments on DNA prepared from these organisms and digested with enzymes that do not cut the major repeat unit of the family of genes of interest. Probing such DNA blots with the appropriate gene probe reveals ribosomal gene orphons in yeast and ribosomal and H3 histone gene orphons in Drosophila. The function and generation of orphons will be discussed.

1033 CHORION GENE AMPLIFICATION DURING THE DEVELOPMENT OF DROSOPHILA FOLLICLE CELLS Allan C. Spradling, The Carnegie Institution of Washington, Baltimore, MD 21210

The Drorophila chorion (eggshell) is syntheiszed by the ovarian follicle cells during the final five hours of egg chamber development. Genes for the major chorion structural proteins are expressed at high levels in these cells during this period. Four chorion genes have been cloned and mapped in clusters located on the X and 3erd chromosomes (1). About ten hours prior to the onset of chorion protein synthesis, the DNA in both clusters begins a process of amplification which is probably necessary for the achievement of the extraordinary rates of protein production per germ line gene which occur in these cells (2). Studies of the mechanism of chorion gene amplification have shown that:
1)Large contiguous regions surrounding both gene clusters are amplified
2)The sequences on both homologues contribute equally to the amplified DNA
3)The extent of amplification decreases monotonically with distance along the chromosome from the coding sequences over a span of at least 40 kb of DNA

The pleiotropic mutation ocelliless acts in cis to reduce the production of at least two chorion genes mapping in the X chromosome cluster (3). One breakpoint of this chromosome inversion has been mapped within the amplified region. Evidence will be presented demonstrating that the inversion alters the pattern and extent of amplification. Alterations in DNA replication may be a common mechanism by which sequence rearrangements cause the changes in gene expression which have been termed "position effects".

1. Spradling, A.C., Digan, M.E., Mahowald, A.P., Scott, M.W. and Craig, E.A.(1980). Cell 19, 905-914.
2. Spradling, A.C. and Mahowald, A.P.(1980). Proc. Nat. Acad. Sci., USA 77, 1096-1100.
3. Spradling, A.C.,Waring, G.W. and Mahowald, A.P.(1979). Cell 16, 609-616.

Genes with Complex Functions

1034 MOLECULAR ORGANIZATION AND EXPRESSION IN THE BITHORAX GENE COMPLEX OF DROSOPHILA. David S. Hogness, Welcome W. Bender*, Michael E. Akam, Robert B. Saint, Pierre Spierer+. Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

"Walking" and "jumping" methods have been used to obtain a set of overlapping cloned DNA segments (Dm segments) that cover 145 kb of the bithorax gene complex in Drosophila melanogaster. This cluster of genes determines the developmental pathways for thoracic and abdominal segments and is located within the polytene chromosome bands 89E1-4. We jumped into the bithorax complex from another region by means of an inversion ($In(3R)Cbx^{+R1}$; T. Kaufman) with one breakpoint in band 87E1,2, within this region, and the other in 89E1,2, within the complex. The breakpoint in 87E1,2 was located within a Dm segment belonging to a set of overlapping segments covering some 300 kb of genomic DNA that extends from band 87D11 through band 87E5. We used the DNA sequences in this segment as a probe to screen a library of cloned Dm segments obtained from the inversion stock for a segment in which DNA sequences from 87E1,2 were fused to sequences of the bithorax complex in 89E1,2. The 89E1,2 sequences in the fusion segment were then used to initiate the 145-kb walk within the wild-type bithorax complex. We are currently using the Dm segments resulting from this walk for two purposes. One is to map and determine the nature of the DNA sequence changes associated with mutations that have been used to define the genes in the bithorax complex. (See E. B. Lewis, Nature 276, 565-570, 1980 and the abstract of his talk at this symposium for these definitions.) The other is to map and define the nature of the RNA transcripts derived from the bithorax complex. Two sorts of mutations have been examined and located on the molecular map: chromosomal rearrangements of sufficient extent to produce detectable changes in the polytene band pattern, and "pseudo-point" mutations. Pseudo-point mutations are those that were originally classified as point mutations on the basis of normal polytene chtology and genetic tests, but which we have shown by molecular analysis to involve changes of hundreds or thousands of base pairs, rather than of one or a few base pairs. Of particular interest are the spontaneous mutations in the bithorax complex because most are pseudo-point mutations characterized by the insertion of DNA elements which are several kb long and appear to be transposable. Mutants of the complex that are suppressed by the suppressor of Hairy wing, $su(Hw)^2$, belong to this class. We have used both chromosomal rearrangements and pseudo-point mutations to construct a molecular map of the abx, bx, Cbx, Ubx, bxd and pbx loci in the complex. We have also detected and begun to define and map certain RNA transcripts derived from the complex.

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1035 GENE REGULATION AND THE BITHORAX GENE COMPLEX, E. B. Lewis, California Institute of Technology, Pasadena, CA 91125

The bithorax gene complex (BX-C) in Drosophila consists of at least eight recessive loss-of-function (LOF) mutants in the proximal-distal order: abx, bx, Ubx, bxd, pbx, iab-2, iab-5 (M. Crosby) and iab-8 (first found in the laboratory of J. Kiger in the Uab chromosome; iab-2 [D. Woods] and another iab-8 mutant were found in a tumorous-head-3 strain in the laboratory of D. Kuhn); and five different types of dominant gain-of-function (GOF) mutants: Cbx-like* (M. Akam), Cbx, Hab, Uab, and Mcp where * symbolizes a visible chromosomal rearrangement. With the exception of pbx, the more proximal a LOF locus is in BX-C the more anterior is the region of the body where its effect is manifested, commencing with anterobithorax (abx), a newly discovered locus mapping closely proximal to bx and affecting the extreme anterior portion of the metathorax, and proceeding to the as yet most distally mapped mutant of the complex, iab-8, whose effects appear to be manifested in, but not necessarily restricted to, the 8th abdominal segment. The GOF loci define signal regions which, in the two cases subjected to recombinational analysis, are immediately proximal to the gene(s) they regulate. A homozygous deficient embryo for the Polycomb gene appears to have most if not all of BX-C derepressed and the Pc mutant strongly enhances the GOF mutants: hence Pc^+ is believed to produce a major repressor of the complex. An additional LOF locus, iab-3, has been inferred from comparative studies of embryonic cuticle of homozygotes for overlapping BX-C deficiencies (Nature, 276: 565-570, 1978). Evidence mounts for still more functional units within BX-C; e.g., although a thoracic-like ventral setal band (VSB) on the first abdominal (AB1) segment and presence on AB1 (and following) segments of a pair of ventral pits (VP) in embryonic and larval stages had occurred in hemizygotes for all moderate to extreme bxd mutants, a new mutant, bxd^{111} (translocation of 89E3-4 to 90B2, inclusive, to section 4 of X) has an extreme adult bxd transformation; yet, the VSB is abdominal-like on AB1. Another rearrangement, Uab^5, lacks any trace of adult bxd transformation in trans to bxd mutants or bxd deficiencies; yet, such genotypes show presence of VP on AB1 (and following segments). A collaborative molecular analysis with W. Bender and D. Hogness of representative spontaneous and induced (X-ray and EMS) BX-C mutants is expected to throw light on the way in which BX-C is organized and regulated.

1036 GENETIC DISSECTION OF EMBRYOGENESIS IN CAENORHABDITIS ELEGANS. Günter von Ehrenstein, Randall Cassada, Edoardo Isnenghi, Kenneth Denich, Khosro Radnia, Einhard Schierenberg and Kenneth Smith. Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, 3400 Göttingen, Federal Republic of Germany.

The complex process of embryogenesis in the simple nematode Caenorhabditis elegans is invariant from animal to animal. Cell lineages have been studied by direct observation of individual cells in living embryos using Nomarski differential-interference-contrast microscopy (1). To genetically dissect events involved in embryogenesis, we have isolated a set of 55 recessive temperature-sensitive (ts) mutants in at least 35 separate emb-genes, which cause arrest of embryonic development (2). The fraction of emb mutants amongst total ts lethals and the recurrence frequency (second alleles) allowed two independent estimates of 200-600 genes essential for embryogenesis (out of a total of about 2000 essential genes). So far 54 emb genes have been detected (2,3,4), still far from genetic saturation. We have tested the mode of expression (the necessity and/or sufficiency for normal embryogenesis) of the wild-type alleles of these 35 genes in the parents and zygote by performing genetic crosses in which a wild-type allele appears in various configurations, and then determining at the restrictive temperature (25° C) the effect on the viability of the resulting progeny genotypes (3,4). Our results agree with those on two other sets of mutants (3,4) that in C. elegans a majority of the emb genes are of the maternal-expression-necessary class (22 of 31 genes studied). We have also found 1 zygotic-necessary-and-sufficient gene and 2 genes for which paternal expression is partially sufficient. The remaining 6 are of the parental-or-zygotic-expression-sufficient class. We have ordered the ts mutants sequentially in development by temperature shift experiments and according to their arrest stage (terminal phenotypes). Their cellular and subcellular properties are being studied with the goal of identifying the cellular processes defective in the mutants. By inference we are learning about the genetic control of cell behavior in embryogenesis. In another set of mutants, we already have described a variety of defects in early cell lineages, particularly in the timing of embryonic cell divisions (5).

(1) Deppe, U., Schierenberg, E., Cole, T., Krieg, C., Schmitt, D., Yoder, B., von Ehrenstein, G. (1978) Proc.Nat.Acad.Sci.USA. 75, 376-380
(2) Cassada, R., Isnenghi, E., Culotti, M, von Ehrenstein, G. (1981) Develop.Biol., in press.
(3) Wood, W.B., Hecht, R., Carr, S., Vanderslice, R., Wolf, N., Hirsh, D. (1980) Develop.Biol. 74, 446-469.
(4) Miwa, J., Schierenberg, E., Miwa, S., von Ehrenstein, G. (1980) Develop.Biol. 76,160-174.
(5) Schierenberg, E., Miwa, J., von Ehrenstein, G. (1980) Develop.Biol. 76, 141-159.

Methods

1037 DNA SEQUENCING, RESTRICTION ENDONUCLEASES AND COMPUTERS. R.J. Roberts, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

More than 250 Type II restriction endonucleases have been characterized and at least 69 different specificities are known to occur. These enzymes have been used as reagents in a variety of biochemical approaches to study genome structure and function. Among the many approaches, the present rapid techniques for nucleic acid sequence determination have yielded enormous quantities of data-so much, in fact, that computer assisted methods have become essential for both collecting and analyzing the data.

Current methods for determining DNA sequences will be compared and techniques for evaluating the biological significance of these sequences will be presented. Although the discussion will center around techniques using restriction endonucleases, particular attention will be given to both the theoretical and practical roles that the computer can play.

1038 SITE-SPECIFIC MUTAGENESIS USING SYNTHETIC OLIGODEOXYRIBONUCLEOTIDES. Michael Smith, Department of Biochemistry, Faculty of Medicine, University of British Columbia, 2075 Wesbrook Mall, Vancouver, B.C., Canada, V6T 1W5.

The precise assignment of genetic functions to DNA sequences usually requires a mutant in the function of interest. Often standard genetic techniques do not provide the desired mutant. This has led to the development of procedures for constructing mutants in vitro by modification of DNA followed by observations on the biological or biochemical properties of the modified DNAs. Constructed deletions (1) or insertions (2) can be used to define the positions and boundaries of genetic functions. Localized point mutants can be constructed (3,4) and used to define the role of individual nucleotides. The most precise method of constructing point mutants is to use synthetic oligodeoxyribonucleotides, containing the desired change, as primers for E. coli DNA polymerase I (Klenow fragment) on circular wild-type DNA templates (5,6). Ligation, using DNA ligase, results in complete integration of the synthetic oligodeoxyribonucleotide into covalently closed circular DNA. After transfection of host cells, the mutant can be isolated. This can be achieved by screening or selecting phenotypically. In the absence of a phenotype, the mutant can be detected in suitable cases where it generates or destroys a restriction endonuclease recognition sequence. The mutating synthetic oligodeoxyribonucleotide can be used to screen for mutant DNA or, more conveniently, to select for the DNA. These techniques have been used to define the genetic functions of specific nucleotides in DNA sequences.

1. Lai, C.J. and Nathans, D. (1974). J. Mol. Biol. 89, 179-193.
2. Heffron, F., So, M. and McCarthy, B.J. (1978). Proc. Natl. Acad. Sci. USA 77, 2796-2800.
3. Shortle, D., Pipas, J., Lazarowitz, S., DiMaio, D. and Nathans, D. (1979). Genetic Engineering. Principles and Methods. Vol. I (Setlow, J.K. and Hollaender, A., eds.), pp. 73-92. Plenum Press, New York, N.Y.
4. Weissmann, C., Nagata, S., Taniguchi, T., Weber, H. and Meyer, F. (1979). Genetic Engineering. Principles and Methods. Vol. I (Setlow, J.K. and Hollaender, A., eds.), pp. 133-150. Plenum Press, New York, N.Y.
5. Hutchison, C.A. III, Phillips, S., Edgell, M., Gillam, S., Jahnke, P. and Smith, M. (1978). J. Biol. Chem. 253, 6551-6560.
6. Smith, M. and Gillam, S. (1980). Genetic Engineering. Principles and Methods, Vol. III (Setlow, J.K. and Hollaender, A., eds.). In press. Plenum Press, New York, N.Y.

1039 PROBING PROTEIN STRUCTURE AND INTERACTIONS WITH MONOCLONAL ANTIBODIES. D. P. Lane, Department of Biochemistry, Imperial College, London SW7, England.

Monoclonal antibodies have been raised to the Large T antigen of SV40 virus as tools for the analysis of this transforming proteins activity. A novel assay was devised that allowed very large numbers of samples to be screened for anti T antibody in a matter of hours without expensive equipment. The location of the binding sites of the monoclonal antibodies on the linear sequence of the protein was deduced by examining their reaction with Adeno SV40 viruses that make fragments of large T. Two of the antibodies were found to bind within 100 amino acids of each other by this method. That these antibodies did not compete for each others binding to the native molecule was shown by purifying and iodinating the antibodies and performing competition radioimmunoassays. These sandwich radioimmunoassays have made possible the quantitation of T antigen in crude cell lysates, the measurement of the association between large T and a host protein of 53,000 molecular weight (1) and the measurement of the other SV40 viral tumour antigen, small t.

One of the anti-T monoclonals was found to cross react with a cellular protein of 68,000 molecular weight (2). This cross reacting species is present only in actively dividing cells and disappears from resting cells. It may in some way represent a cellular equivalent to the viral transforming gene but in any case clearly illustrates how monoclonal antibodies may reveal new relationships between proteins at the level of their detailed surface topology.

1. Lane, D.P. and Crawford, L.V. (1979). T-antigen is bound to a host protein in SV40 transformed cells. Nature (London) 278, 261-263.
2. Lane, D.P. and Hoeffler, W.K. (1980). SV40 large T shares an antigenic determinant with a cellular protein of molecular weight 68,000. Nature (London) In Press.

Chromatin Structure

1040 CHROMOSOME STRUCTURE OF GLOBIN GENES, Harold Weintraub, Department of Genetics, Fred Hutchinson Cancer Research Center, Seattle, WA 98104

We have analyzed the chromosome structure of both the α and β globin gene cluster in 3 cell types present at various stages of erythroid differentiation in developing chick embryos. These 3 cell types are: (1) embryonic erythroblasts synthesizing embryonic α and β globin chains (2) adult erythroblasts synthesizing adult α and β globin chains and (3) precursor red cells not synthesizing globin mRNA. We show, using 3 assays of chromosome structure (DNase I sensitivity of active genes; in vitro run off transcription; and DNA undermethylation), that the globin genes are inactive in precursor cells and during differentiation to the embryonic lineage, the embryonic, but not the adult genes become active while during differentiation to the adult erythroid lineage, the adult genes, but not the embryonic genes become activated. These results are discussed in terms of a model where domains of chromosome structure determine which globin gene is active. Finally, we have also focused on the biochemical basis for the DNase I sensitivity of active genes. We have shown that two proteins, HMG 14 and 17, are responsible for inducing a DNase I sensitivity upon active nucleosomes. Since these proteins have a preferential affinity for active nucleosomes we have coupled them to an affinity column and show that active nucleosomes can then be purified by virtue of their binding to the HMG 14/17 column.

1041 THE ROLE OF DNA TOPOSIOMERASES IN THE FOLDING OF EUKARYOTIC CHROMOSOMES, Douglas L. Brutlag, Tao-Shih Hsieh, Timothy Nelson, John Weiss, and Roger C. Wiegand, Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305

Drosophila embryos are capable of very rapid DNA replication and assembly of newly synthesized DNA into chromosomes. Extracts of these embryos are a rich source of DNA topoisomerase I (nicking-closing enzyme), DNA topoisomerase II (an ATP-dependent topoisomerase), and chromatin assembly activity. We have found that a highly purified topoisomerase I from these extracts is not sufficient to mediate chromatin assembly in vitro. By fractionating the chromatin assembly activity from the embryos we have found that the activity requires both the DNA topoisomerase I and a polyanionic fraction which we demonstrate to be RNA. Exogenous natural and homopolymer RNAs can also facilitate assembly. These polyanions are required in amounts stoichiometric with histones while the DNA topoisomerase is required in catalytic amounts. Physiological numbers of superhelical turns can be formed in circular DNA using a highly purified DNA topoisomerase, pure RNAs and core histones.

We have shown that the ATP-dependent DNA topoisomerase is capable of catenating and decatenating covalently closed circular DNAs as well as relaxing supercoiled DNA in the presence of ATP. We have shown that this topoisomerase uses a mechanism which allows one double-strand segment segment of DNA to pass through another. Circular DNAs can be catenated into large networks in the presence of the ATP-dependent DNA topoisomerase, ATP, and specific cations that condense DNA. The most efficient cations that mediate the catenation reaction are spermine, histone H1, and a histone H1-like protein purified from early embryos. In the absence of such cations, catenated networks can be resolved back into monomer circles. The ATP-dependent DNA topoisomerase cannot replace DNA topoisomerase I in the chromatin assembly reaction. Thus in Drosophila, as in other organisms, there appear to be two types of DNA topoisomerases one of which relieves torsional strain within a DNA helix (type I) and another which allows DNA helices to pass through each other (type II). The reactions mediated by each suggest intimate roles for these enzymes in the folding of eukaryotic chromosomes.

1042 MODULATION OF THE STRUCTURE OF THE CANONICAL NUCLEOSOME CORE PARTICLE, Robert T. Simpson, L. William Bergman and Peter Kunzler, Developmental Biochemistry Section, NIAMDD, National Institutes of Health, Bethesda, MD 20205

The core particle of chromatin is currently modeled as a squat cylinder containing an inner octamer of histones H2A, H2B, H3 and H4 wrapped with 1.75 turns of DNA in a helical path about 80 bp in circumference. The particle likely has a dyad symmetry. Characteristic sites of accessability or lack of same to nucleases reflect both the periodicity of the DNA helix and interactions of histones with the DNA. The particle undergoes two defined conformational changes: unfolding at low ionic strength and a thermally induced, reversible unwinding of 20-25 bp at each end of the DNA segment from association with the histone octamer.

We and others have recently detailed alterations in the structure of this canonical core particle resulting from presence of H1, chemically modified inner histones, variant forms of the inner histones, differing DNA sequences, and nonhistone proteins. Several of these may relate to the availability of nucleosomal DNA for transcription.

When H1 is present, the resultant particle, a chromatosome, contains 10 additional bp DNA at each end of the core particle segment, completing two full turns of nucleic acid around the histone core, as shown by linking number experiments. Certain highly accessible DNase cutting sites in the core particle are blocked by H1. Both conformational changes characteristic of the core particle are absent for the chromatosome. Highly acetylated H3 and H4 lead to a decrease in melting temperature for chromatin and the core particle and an increased susceptibility to DNase cutting of the ends and center of the core particle DNA segment. In contast, the variant H2A and H2B of sea urchin sperm lead to a marked stabilization of the core particle to melting and to blocking of some normally highly susceptible DNase cutting sites. The "early" histones of sea urchin blastula have similar but less marked effects on core particle structure. HMG 14/17 bind stoichiometrically to isolated nucleosomes (two per particle), interacting with DNA near the ends of the segment and altering the thermal denaturation profile for the particle (1). DNA sequence effects on core particle organization are apparent in comparisons of DNase cutting site frequency for semisynthetic core particles containing poly-(dA-dT) to those for particles containing poly(dG-dC).

In contrast to the view of the general constancy of structure of the core particle, the results obtained in the studies described here demonstrate that variations in histones, nonhistones and DNA can all modulate the structure of chromatin, even at this most basic level. Possible implications of these structural modulations in transcriptional activity of chromatin will be discussed.

(1) G. Sandeen, W.I. Wood and G. Felsenfeld, Nucleic Acids Res. 8:3757-3778 (1980)

1043 CHROMOSOME STRUCTURAL STUDIES, Ulrich K. Laemmli, Catherine D. Lewis and Jane S. Lebkowski, University of Geneva, 1211 Geneva 4, Switzerland.

Considerable evidence suggest that both metaphase chromosomes and nuclei contain a residual skeleton (scaffold) which can maintain the DNA in a compact, looped arrangement following removal of the histones and many non-histone proteins. This skeleton is observed in chromosomes and nuclei purified by a variety of methods using both high or low ionic strength procedures. Electron microscopy studies (thin sections and Miller-spreads) of intact, unextracted chromosomes are also consistent with a scaffold structure. Although it is difficult to prove that the scaffold has a genuine organizational role for the nucleoprotein fiber of chromosomes and nuclei, available evidence indicates that it is not a simple artefact of manipulation.

We have studied and compared in some detail the protein composition of the nuclear and chromosomal scaffolds. We can distinguish 2 types of nuclear scaffolds. Type I scaffolds results from treatment of nuclei with 2M Nacl (sedimentation coefficient with DNA of about 7500s) and are composed of the three lamina proteins (60-70.000 dalton), a protein of 35.000 dalton and a few characteristic proteins of high molecular weight. Type II scaffolds have a much simple protein pattern (sedimentation coefficient with DNA about 3300s) consisting mainly of the 3 nuclear lamina proteins. Type II scaffolds are obtained by treatment with β-mercaptoethanol or o-phenanthroline, the latter of which suggests an involvement of metalloprotein interactions. About 12 proteins of type I scaffolds and 4 proteins of type II scaffold bind DNA including the 3 major lamina proteins. This suggests that the peripheral lamina is involved in the long-range organization of the nuclear DNA.

DNA Transformation

1044 THE INTEGRATION AND EXPRESSION OF TRANSFORMING GENES, Richard Axel, Institute of Cancer Research, Columbia University, New York, N.Y. 10032

Transformation results in the integration of heterologous DNA into the chromosome of the transformed host. We have used hybridization *in situ* to demonstrate the linkage and insertion of most, if not all, cotransformed sequences into the recipient cell chromosome. Insertion is not restricted to a unique chromosome or chromosomal region, but frequently occurs at a site of gross chromosomal rearrangements. Genomic blot hybridization along with hybridizations *in situ* now permit us to correlate chromosomal and molecular events associated with insertion and excision of DNA in the chromosome. Expression of transformed genes provides an assay for the functional role of DNA sequence organization about specific genes. We are currently employing *in vitro* mutagenesis in concert with transformation to define a block of nucleotides essential for the expression of the viral tk gene, the hamster aprt gene, and the major *Drosophila* heat shock gene integrated in the chromosome of mouse fibroblasts.

1045 DNA TRANSFORMATION OF *SACCHAROMYCES CEREVISIAE*, Ronald W. Davis, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305

Saccharomyces cerevisiae (baker's yeast) can be transformed with DNA using the procedure of Hinnen, Hicks and Fink. The cell wall is enzymatically removed and DNA is taken up by the cell upon addition of calcium and polyethylene glycol. No carrier DNA is used. Four general types of yeast vectors have been developed. They are double vectors containing sequences that allow selection and maintenance of DNA sequences in both *E. coli* and yeast. Selection and maintenance in *E. coli* is achieved by use of the *E. coli* vector pBR322 which contains a Col EI replicator, and ampicillin and tetracycline resistance genes. Selection in yeast is generally achieved by incorporating into the vector one or more yeast genes in one of the amino acid, purine or pyramidine biosynthetic pathways. The major variable in these vectors is the manner in which they are maintained in a yeast cell. YIp vectors are maintained in yeast cells by integration into a yeast chromosome by homologous recombination. The recombination can occur in the selectable marker or in the inserted yeast sequences. YEp vectors contain sections of the yeast plasmid Scpl which allow autonomous replication in a yeast cell. We have transformable yeast strains which are devoid of the endogenous yeast plasmid and upon transformation with a YEp vector, the yeast contains about 30 copies of the vector sequences per haploid genome. YRp vectors are maintained in a yeast cell as an autonomously replicating sequence by incorporating chromosomal DNA sequences called *ars*'s (autonomously replicating sequences). These vectors can also integrate into a yeast chromosome which is not deleterious and does not result in rearrangements in the DNA sequence. YCp vectors contain sections of DNA which have centromere function. We have isolated a DNA segment on chromosome IV near the TRP1 gene, which behaves, during meiosis and mitosis, as a yeast centromere. These vectors are apparently maintained stably in the yeast cell in a single copy state. Transformation of yeast cells normally results in the addition of genetic information. A recently developed technique, however, allows the replacement of sequences following transformation. This process has been termed transplacement. Two vectors have been developed for transplacements, termed YRp14 and YRp15. Integration by homologous recombination can be stimulated, thus targeted by cleaving the targeting DNA sequence on the vector. These linear molecules are found to integrate at a much higher frequency by homologous recombination. The integration results in complete repair of the cleaved DNA sequence. These four vector systems are now being used to study gene structure, chromosome structure and the regulation of gene expression. They can be used to manipulate DNA sequences on an autonomously replicating vector or on a yeast chromosome.

1046 EXPRESSION OF CLONED GENES IN ANIMAL CELLS, C. Weissmann, P. Dierks, A. van Ooyen, U. Weidle and N. Mantei, Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland.

A potent tool for the elucidation of DNA structure-function relationships in eukaryotic cells is based on the introduction, into appropriate cells, of cloned DNA segments which have been modified in vitro. Introduction of DNA may result in transient or stable cell transformation. Transient transformation is the major event when the transforming DNA is joined to a viral vector, such as polyoma or SV40, and introduced into cell cultures either by viral particles or directly, in the presence of Ca_2PO_4. Stable transformation is frequent when cells are transformed with DNA containing a selectable marker, and grown under selective conditions. Transient transformation may give expression at relatively high levels for hours or days, whereas stable transformation mostly results in low level expression for indefinite periods. In neither case has expression so far been observed to be subject to normal control in cells of higher eukaryotes.

We have investigated the effect on expression of modifying the promoter of a β-globin gene. Mouse TK^- L cells were transformed with concatenates of cloned HSVI TK DNA and a set of rabbit β-globin DNAs, in which the globin genes were preceded by native flanking sequences of various lengths. Selection for TK^+ cells led to lines producing 5 to 1500 mature rabbit β-globin RNA strands per cell. Their 5' termini mapped to (a) the "cap" nucleotide, (b) positions 42 to 48 nucleotides downstream from the cap site, or (c) positions in the vector DNA. The gene with only 14 bp of 5' flanking sequence gave rise to a high level of rabbit β-globin RNA; however, most transcripts originated in the vector moiety. With 66 bp of 5' flanking sequence, 5% of the termini were correct. The region between 14 and 66 bp preceding the cap site contains the Hogness box and appears to be essential for correct initiation of transcription. The region between 66 bp and 76 bp before the cap site contains a variant of the canonical sequence GG$\overset{C}{\underset{T}{}}$CAATCT preceding many genes at a similar location, and may modulate the efficiency of transcription.

We have also used a polyoma-pBR322 vector to introduce a cloned human chromosomal interferon α-1 gene into mouse cells. Transient excretion of interferon α-1 occurred 24-36 hours after transformation.

Vectors for DNA Transformation into Eucaryotes

1047 INTRODUCING GENES INTO MAMMALIAN CELLS, Paul Berg, Department of Biochemistry, Stanford University Medical School, Stanford, California 94305.

A group of DNA vectors containing selectable genetic markers has been developed for introducing cloned genes into mammalian cells. Our goal is to use such vectors to introduce a variety of genes, particularly those expressed in a developmentally regulated or differentiation specific fashion, into cultured, mammaliam cells and to study structural and other parameters that govern their expression. Examples of such experimental approaches and some current findings will be presented.

1048 THE DEVELOPMENT OF HOST VECTORS FOR DIRECTED GENE-TRANSFER IN PLANTS. J. Schell*°, M. Van Montagu°, J. Leemans°, H. De Greve°, J.P. Hernalsteens, C. Shaw*, L. Willmitzer*, L. Otten*. * Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, W. Germany; ° Laboratorium voor Genetika, RUG, Gent, Belgium.

The Ti-plasmids of Agrobacterium tumefaciens are natural gene-vectors for plants. A strategy was developed to make use of Ti-plasmids as general vectors to introduce cloned genes into plants by looking for answers to the following questions: 1° Which segment of the Ti-plasmid is transferred and stably maintained in transformed plant cells? Southern blotting hybridization experiments demonstrated that a well defined region (the T-region) of Ti-plasmids is transferred. The Ti-plasmid derived DNA in transformed plant cells (the so-called T-DNA) is localized in the nucleus and is co-linear with the T-region. 2° Which segments of the T-DNA are transcribed? A mapping of the transcripts indicated that different parts of the T-DNA are reproducibly transcribed to different extents, thus indicating a defined transcription pattern. This transcription appears to be due to the plant RNA polymerase II. The transcription pattern can be correlated with the known genetic functions of the T-DNA. 3° Can one introduce "foreign" DNA in the transcribed segments of the T-region? Is this "foreign" DNA transferred to the nucleus of transformed plant cells and is it expressed? Tn7, harbouring a gene coding for a methothrexate resistant DHFR, was introduced, in vivo, into the T-region of a Ti-plasmid. Plant tumours induced with this plasmid were shown to contain Tn7 DNA in their nucleus and to be methotrexate resistent. 4° Can one inactivate the tumour functions of the T-DNA and obtain "normal" transformed plants? By inactivating a specific locus in the T-DNA via insertion, a mutant Ti-plasmid was obtained. This plasmid yields transformed plant calli which regenerate "normal" fertile plants. All tissues (leaves, stems, roots) of these plants were shown to contain and express T-DNA segments. 5° How can one introduce isolated genes into a specific site of the T-region of Ti-plasmids? "Intermediate vectors" were developed consisting of a single fragment of the T-region cloned in a small plasmid that replicates in A.tumefaciens. Isolated genes can be cloned in such an intermediate vector and introduced at a specific site of the T-region of a Ti-plasmid via an in vivo substitution recombination between the intermediate vector and the Ti-plasmid.

1049 REGULATION OF ADENOVIRUS VA RNA GENE EXPRESSION. Thomas Shenk[1], Dana Fowlkes[2], Cary Weinberger[1], Bayar Thimmappaya[1], [1]Department of Microbiology, Health Sciences Center, State University of New York, Stony Brook, NY 11794, [2]Laboratory of Pathology, NCI, National Institutes of Health, Bethesda, MD 20205.

The adenovirus genome encodes two RNAs transcribed by RNA polymerase III. These are called VAI and VAII, and are synthesized in very large amounts within infected cells at late times after infection. By constructing deletion mutations in cloned adenovirus types 2 and 5 VAI genes and measuring the ability of altered templates to direct transcription of VAI RNA in HeLa cell extracts, we have located two transcriptional control regions. The first is an intragenic region located between positions +9 and +72 relative to the 5' end of the VAI (A) RNA. Those deletions examined within these sequences abolished the transcription of mutant templates in HeLa cell extracts. The second control region includes 5' flanking sequences which abut the VAI coding region. Mutations here can reduce the efficiency with which the VAI gene is transcribed. Nucleotide sequence similarities were noted on comparison of the VAI intragenic control region to tRNA sequences which lead us to speculate that the transcriptional regulation of these two types of genes may be quite similar, and the adenovirus VA genes may even have evolved from a tRNA gene(s). To further evaluate the function of the VA RNAs and their control regions, altered VA genes have been "rebuilt" into the adenovirus chromosome, and their expression studied within infected HeLa cells.

1050 RETROVIRUSES AS CLONING VEHICLES, G. F. Vande Woude, D. G. Blair*, W. L. McClements, T. G. Wood, and M. Oskarsson, National Institutes of Health, National Cancer Institute, Laboratory of Molecular Virology, *Laboratory of Viral Carcinogenesis, Bethesda, Maryland.

The acute transforming retroviruses possess specific sequences that share homology with normal host cellular DNA sequences (1). These sequences appear to have been transduced from the host cell into rescuable viral genomes and their presence in the virus confers a specific transformed phenotype to the infected host cell. In essence retroviruses have acted as cloning vehicles for host cellular genes with transforming potential. Understanding the acquisition process of such sequences and determining how viral and cellular sequences interact to effect a transforming phenotype may allow us to identify a variety of normal cell genes with transforming potential. In this regard, we have used the Moloney sarcoma virus (MSV) as a model to determine the molecular elements essential for expressing a transformed phenotype. In a direct DNA transfection-transformation assay, we have demonstrated the long terminal repeat (LTR) of the MSV enhances the transforming activity of V-mos (the acquired cellular sequence in MSV) (2) and activates the transforming potential of the homologous cellular sequence, C-mos (3,4). Experiments demonstrating LTR enhancement or activation of mos transformation are: I. Subgenomic MSV DNA fragments containing a single LTR plus MSV sequences at the 5' end of V-mos (2); II. Subgenomic MSV fragments with one LTR plus MSV sequences placed at the 3' end of V-mos (2); III. Cotransfection of unlinked V-mos and LTR sequences (2,4); IV. Recombinants with MSV sequences (including an LTR) placed at the 5' end of C-mos (3); and V. Hybrid recombinants containing LTR at variable distances upstream from C-mos (4). These studies suggest that other cellular sequences with transforming potential might be capable of activation in the same manner. By linking in vitro, the appropriate proviral sequences (including an LTR) to fragments of a cellular genome, transforming sequences should be activated and in some cases new transforming retrovirus generated.

References

1. P. H. Duesberg, Cold Spring Harbor Symp. Quant. Biol. 44: 13-29 (1979).
2. D. G. Blair, et. al., Proc. Nat. Acad. Sci. USA, 77: 3504-3508 (1980).
3. M. Oskarsson, et. al., Science, 207: 1222-1224 (1980).
4. W. L. McClements, et. al., Cold Spring Harbor Symp. Quant. Biol. 45: in press (1980).

Accurate Transcription in Vitro

1051 **TRANSCRIPTION OF 5S RNA GENES,** H.R.B. Pelham, D.F. Bogenhagen, S. Sakonju, M. Wormington & D.D. Brown, Carnegie Institution of Washington, Department of Embryology, Baltimore, MD 21210.

There are two types of 5S RNA genes in Xenopus: somatic genes are transcribed in all tissues while oocyte-type genes are only expressed in oocytes. Single cloned genes of both types are faithfully transcribed in nuclear extracts of oocytes. Deletion analysis of one such cloned gene has shown that specific initiation is directed by an intragenic control region which spans residues 50-83 of the gene (1,2). Similar analysis indicates that termination requires a minimum of four T residues, but the efficiency of termination is influenced by the adjacent nucleotide sequence.

Accurate transcription requires polymerase III and at least three other factors. One of these is specific for the 5S RNA genes. It has been purified and shown to bind to the intragenic control region (3,4). We have devised a sensitive assay in which the relative strength of altered 5S genes is estimated from their ability to compete a standard gene in the transcription system. In general, there is a good correlation between the competitive strength of altered genes and their ability to bind the transcription factor, as measured by "footprinting". However, sequences flanking the 5' side of the genes also contribute to their competitive strength. This may reflect the interaction of polymerase III or another transcription factor with this region. Cloned oocyte genes are weaker competitors than somatic genes. By constructing hybrid genes we have shown that the weakness results from base changes in the control region, rather than from the extensive sequence differences in the 5' flanking region.

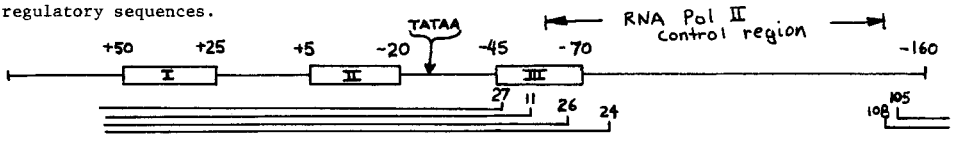
In immature oocytes, the 5S transcription factor has an additional function: it binds to 5S RNA to form an abundant cytoplasmic storage particle (4,5). Conversely, added 5S RNA binds to the factor in the oocyte cell-free system and specifically inhibits transcription of 5S RNA genes. A similar effect is found in cell-free systems derived from somatic cells. These cells contain a protein which is similar to, but distinct from the oocyte factor. Thus it is possible that 5S RNA controls its own synthesis in somatic cells by interacting with this protein and inhibiting transcription.

1. Sakonju, S., Bogenhagen, D.F. & Brown, D.D. (1980) Cell 19, 13-25.
2. Bogenhagen, D.F., Sakonju, S. & Brown, D.D. (1980) Cell 19, 27-35.
3. Engelke, D.R. et al. (1980) Cell 19, 717-728.
4. Pelham, H.R.B. & Brown, D.D. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 4170-4174.
5. Picard, B. & Wegnez, M. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 241-245.

1052 DEFINITION OF THE SV40 EARLY TRANSCRIPTIONAL CONTROL REGION AND ITS RELATION TO THE T ANTIGEN BINDING SITES, Robert Tjian and Donald C. Rio, Department of Biochemistry, University of California, Berkeley, CA 94720

The transcription of Simian Virus 40 (SV40) in productively infected monkey cells is a temporally regulated process which is in part modulated by the viral A gene product (T antigen). We have recently shown that SV40 T antigen is capable of repressing SV40 early transcription *in vitro*. Under conditions of the transcription assay, T antigen is able to bind specifically to the T antigen binding sites, which are located at +50 to +25 bp (site I), +5 to -20 bp (site II), -45 to -70 bp (site III) from the major *in vivo* early RNA cap site (+1). Here we report the use of *in vitro* mutagenesis and recombinant DNA techniques to define the limits of the SV40 early transcriptional control region.

A cell-free RNA synthesizing system was used to assay the ability of cloned mutant viral DNA templates to initiate RNA polymerase II transcription. Run-off RNA products of discrete length are efficiently synthesized when transcription is directed by DNA restriction fragments containing wild-type SV40 early promoter sequences. Deletion mutants DL 11 and DL 27, generated *in vitro* by Bal 31 nuclease, remove the TATAA (Hogness) box at -25 to -30, yet exhibit an unaltered level of *in vitro* RNA synthesis, suggesting that this sequence is not essential for early transcription initiation. However, deletion mutants DL 24, 26, 105, and 108 show markedly reduced ability to direct the synthesis of discrete run-off RNA products. These deletion mutants serve to define the 5' and 3' borders of the SV40 early transcriptional control region at sequences located from -60 to -160 bp. The region at -60 to -80 bp, defined by mutants DL 24 and 26, lies within or directly adjacent to the third SV40 T antigen binding site (-45 to -70 bp). Thus it is probable that the T antigen mediated repression of early SV40 transcription is due to a direct competition between the RNA polymerase II transcription complex and T antigen for adjacent overlapping binding sites on SV40 DNA. To test further this idea, we have constructed a hybrid plasmid containing the SV40 T antigen binding sites placed 200 bp distal to the major adenovirus 2 (Ad2) late promoter. Transcription from this Ad2 late promoter was not inhibited by saturating levels of T antigen in the *in vitro* assay. Thus, these findings are consistent with the idea that repression of transcription by the SV40 T antigen involves a direct steric blockage at the RNA polymerase binding site due to overlapping regulatory sequences.



Specialized and Transposable Genes

1053 MACRONUCLEAR GENES OF HYPOTRICHOUS CILIATES ARE SPLIT PALINDROMES, Lawrence A. Klobutcher, Marshal T. Swanton, Pierluigi Donini, Robert E. Boswell and David M. Prescott, University of Colorado, Boulder, Colorado 80309

DNA from the macronuclei of hypotrichous ciliates exists as low molecular weight gene size DNA pieces, ranging in size from 400 to 20,000 base pairs (bp). These sequences are excised from the complex and high molecular weight micronuclear genome during the development of a new macronucleus following each sexual cycle. Previously, macronuclear genes were found to contain identical inverted terminal repeat sequences. We have determined the DNA sequences of the inverted terminal repeats of Oxytricha sp., Stylonychia pustulata, and Euplotes aediculatus. Each of these organisms possessed a similar terminal repeat sequence, based on repetitions of the sequence 5'(CCCCAAAA)$_n$3', but varying in the number of repeats. In addition, the 3' ends were found to protrude up to 16 bases past the 5' termini. In the case of Oxytricha sp., the terminal sequence was confirmed by analysis of specific cloned macronuclear DNA segments. The presence of an identical terminal sequence on each macronuclear DNA molecule, and the sequence conservation between genera, suggest that this sequence is important in some fundemental property of the cell, such as the developmental excision or replication of macronuclear DNA sequences.

1054 INTRACHROMOSOMAL GENE CONVERSION: A NEW TYPE OF GENETIC EXCHANGE INVOLVED IN MAINTAINING SEQUENCE HOMOGENEITY, Hannah L. Klein and Thomas D. Petes, The University of Chicago, Chicago, Illinois 60637

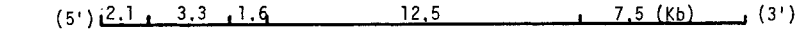
The yeast Saccharomyces cerevisiae has a mechanism by which information from one gene can be transferred non-reciprocally to a repeated copy of the gene on the same chromosome. We have called this event intrachromosomal gene conversion. To demonstrate this genetic mechanism, we transformed a yeast strain that had a mutation at the LEU2 locus with a recombinant plasmid that contained a wild type LEU2 gene. The recombinant plasmid integrated by reciprocal recombination into the host genome. The resulting chromosome, therefore, contained a wild type LEU2 gene separated from a mutant leu2 gene by bacterial plasmid sequences. We found that during meiosis the mutation in the mutant leu2 gene was transferred to the wild type LEU2 gene but was maintained in the mutant leu2 gene. This conversion event occured in non-selected tetrads at a frequency of 2%. If conversion from mutant type occurs at the same frequency, the overall of intrachromosomal gene conversion is estimated to be 4%. We propose that similar type of conversion may operate to maintain sequence homogeneity within families of repeated eukaryotic genes.

1055 DNA SEQUENCE ANALYSIS OF α-FETOPROTEIN AND SERUM ALBUMIN GENES, A. Dugaiczyk, S. Law, O.E. Dennison and J.W. Hawkins, Baylor College of Med., Houston, Tx. 77030.

In order to understand the basic molecular biology of the α-fetoprotein (AFP) serum albumin genes, it is essential that an accurate sequence of the mRNAs and the structure of the genes can be established. To this end, we have established the complete nucleotide sequence of mouse AFP mRNA as determined from two overlapping cDNA clones. It is comprised of a 5'-untranslated region (41 nucleotides), a prepeptide sequence (57 nucleotides), the translated portion (1,758 nucleotides), and a 3'-untranslated sequence of 153 nucleotides excluding poly(A). The amino acid sequence of 586 residues, deduced from the above analysis, confirms the C-terminal valine but our sequence at the N-terminus shows some discrepancy with published amino acid sequence.

Similarly, the nucleotide sequence has been established for the mRNAs for mouse and human serum albumin. The sequence reveals that the positions of the cystein residues are the same within the AFP and albumin polypeptide chains, suggesting the formation of identical -S-S- bonds in the secondary structure. The two sequences show in addition extensive stretches of amino acid sequence homology. It is deduced that α-fetoprotein and serum albumin are evolutionary related.

From analysis of a Hae III/Alu I human gene library, the albumin gene has been localized on five Eco RI fragments, ordered as follows:



1056 MOLECULAR ORGANIZATION OF DROSOPHILA ALCOHOL DEHYDROGENASE GENE. Cheeptip Benyajati, Cancer Biology Program, Frederick Cancer Research Center, P.O. Box B, Frederick, MD 21701

Alcohol dehydrogenase (ADH) in Drosophila melanogaster is a single copy gene that appears to be regulated during development and displays tissue-specific expression. I have isolated ADH genomic clones from a bacteriophage lambda library containing Drosophila DNA using an ADH-cDNA plasmid as a probe, and I have studied the molecular organization of the gene and the flanking regions by DNA sequencing. Two intervening sequences exist within the coding sequence for the ADH enzyme (monomer = 255 aa). The first, 65 nucleotides in length, occurs between amino acids 32 and 33, while the second, 70 bases, lies between residues 167 and 168. Both contain the 5'GT and 3'AG dinucleotides characteristic of those at the intervening sequence boundaries of eucaryotic genes. Interesting features common to other eucaryotic genes include the octanucleotide TATAAATA in the 5' flanking region, 94 bases from the initiation codon, and the hexanucleotide AATAAA in the 3' untranslated region, 46 bases from the putative polyadenylation site. I am currently analyzing the homozygous viable ADH-negative flies (isolated by W. Sofer, Rutgers University) by Southern blotting and DNA sequencing. Preliminary results suggest that some of the mutants that do not possess stable mRNA may be deleted in the 5' region covering the sequence necessary for gene expression. Research supported by Contract No. NOI-CO-75380 with Litton Bionetics, Inc.

1057 Isolation and Characterization of the Drosophila Tropomyosin Gene, Robert V. Storti*, Victoria Bautch*, Dietmar Mischke† and Mary Lou Pardue†, *University of Illinois Medical Center, Chicago, Illinois, †Massachusetts Institute of Technology, Cambridge, Massachusetts.

A ^{32}P-cDNA probe, made from oligo dt fractionated cytoplasmic RNA of differentiated Drosophila myotube cells and depleted of non-muscle nucleotide sequences by hybridization to non-muscle Schneider cell RNA, was used to screen a recombinant genomic DNA library from Drosophila melanogaster. Approximately 300 clones hybridized to the ^{32}P-cDNA probe. These clones were further identified by a positive hybridization selection procedure followed by in vitro translation and two dimensional gel electrophoresis. One clone (λDm85) encodes an abundant protein of myotube cells. This protein co-migrates with chick tropomyosin on two dimensional gels and partial proteolysis with chymotrypsin shows it to have homology with chick tropomyosin. Moreover, the Drosophila tropomyosin undergoes the apparent relative molecular weight increase when electrophoresed in urea-SDS gels characteristic of vertebrate tropomyosins. In situ hybridization of λDm85 DNA to polytene chromosomes results in hybridization to a single site at chromosome position 88F2-5.

1058 HUMAN GLYCOPROTEIN HORMONES GENES, John C. Fiddes, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724 and Howard M. Goodman, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

The three pituitary glycoprotein hormones, LH, FSH and TSH, and the placental glycoprotein hormone, CG, are all dimeric proteins consisting of dissilimar α and β subunits. The β subunits are unique to each hormone and confer biological specificity, while the α subunit appears to be very similar, or identical, for all four hormones. Using the cloned cDNA for the α subunit of CG as a hybridization probe, a single isolate of an α subunit chromosomal gene has been obtained. This gene spans a total of 9.4kb and contains three intervening sequences of 6.4, 1.7 and 0.4 kb, the precise locations of which have been established by DNA sequencing. The 6.4kb intervening sequence is located within the 5' untranslated region. Restriction enzyme analysis of total human DNA obtained from different individuals reveals at least three different polymorphic types. Comparison of these restriction enzyme digestion patterns with the restriction enzyme map of the cloned gene implies that there is a single α subunit gene, which is expressed in the pituitary and the placenta for all four hormones.

1059 CLONING AND SEQUENCE ANALYSIS OF A MUTATION IN THE LEADER REGION OF THE YEAST ISO-1-CYTOCHROME c mRNA, John I. Stiles[1], Jack W. Szostak[2], Ray Wu[3] and Fred Sherman[4], Indiana State University, Terre Haute IN[1], Sidney Farber Cancer Institute, Boston MA[2], Cornell University, Ithaca NY[3] and University of Rochester, Rochester NY[4].

Yeast can be transformed by integration of the transforming vector into the chromosomal DNA via homologous recombination between a sequence on the vector and a sequence in the chromosome. We have taken advantage of this to design a transformation vehicle which will selectively integrate adjacent to the CYC1 locus which codes for iso-1-cytochrome c. This vector consists of pBR322, the yeast URA3 gene, which serves as a selectable marker, and a segment of DNA which lies adjacent to the CYC1 locus. The CYC1 gene can be easily recovered by digestion of total DNA from the transformed strain with BglII, an enzyme which does not cut the vector or the CYC1 gene, followed by ligation and transformation of E. coli. The only clones recovered are those containing the CYC1 locus. This approach has been used to clone a mutant defective in translation. Genetic mapping indicated that the mutation in cyc1-362 was 5' to the initiation site. DNA sequence analysis of this mutant indicates that it resulted from two distinct base changes in the leader region of the mRNA. One change resulted in the creation of a new AUG 5' to the normal initiation codon and out of phase with the regular reading frame of the protein. The second base change resulted in the formation of a sequence in front of the mutant initiation codon which is identical in sequence and similar in position to a sequence preceding the wild type initiation codon. This sequence, CACACA, appears in the leader region of several other yeast mRNAs, but is not present in all yeast messages, and may be involved in the initiation of translation. Revertants of this mutant are currently being analyzed.

1060 COORDINATED GENE EXPRESSION IN A MAMMALIAN CELL-LINE, Amy S. Lee, Debra J. Scharff and Angelo Delegeane, Department of Biochemistry, University of Southern California, School of Medicine, 2025 Zonal Avenue, Los Angeles, CA 90033

A temperature-sensitive mutant, K12, isolated from an established Chinese hamster fibroblast cell-line, WglA, is used to study the coordinated expression of three specific genes. When K12 cells are incubated at the nonpermissive temperature (40.5° C), the synthesis of three specific proteins of M.W. 94K, 78K and 58K daltons is greatly enhanced, as compared to non-mutant cells grown at the same temperature (see figure). We have identified these proteins as glucose-regulated proteins similar to those observed in chick embryo fibroblasts when the cells are starved of glucose. In particular, the 78K dalton proteins isolated from the hamster K12 cell-line and chick embryo fibroblasts have identical electrophoretic mobilities in two-dimensional isoelectric focusing gels and near identical peptide maps. However, these proteins are different from heat-shock proteins previously described for animal cells. We have constructed 500 cDNA clones using the RNA extracted from the hamster K12 cells incubated at 40.5° C. Clones which hybridize preferentially with cDNA made from RNA at 40.5° C are selected. Using the hybrid-selection technique, followed by in vitro translation, a cDNA clone containing a 2500 nucleotide insert coding for the K12 78K dalton protein has been identified. The cloned gene probe is used to determine the organization of this gene as well as the size of its transcripts in both hamster and chick embryo cells. (Supported by grants from NIH, CA27607, and ACS, JFRA24, to A.S.L.). 1 2

1061 INTERVENING SEQUENCE MUTATION IN A CLONED HUMAN β^+-THALASSEMIC GLOBIN GENE, Richard A. Spritz, Pudur Jagadeeswaran, Prabhakara V. Choudary, P. Andrew Biro, James T. Elder, Jon K. deRiel, James L. Manley, Malcolm L. Gefter, Bernard G. Forget and Sherman M. Weissamn, Yale University, New Haven, CT 06510 and Massachusetts Institute of Technology, Cambridge, MA

β^+-thalassemia (β^+-thal.) is an inherited anemia characterized by decreased synthesis of β globin mRNA and hence β globin chains in the erythroid cells of affected individuals. In order to facilitate the investigation of the molecular basis of this disorder, we have cloned β globin gene fragments from a Greek Cypriot patient with typical homozygous β^+-thal. Detailed restriction endonuclease mapping of the cloned gene fragments revealed no deletions or other rearrangements, and transcription of the β^+-thal. gene by RNA polymerase II appeared normal in vitro. We have determined the complete nucleotide sequence of the β^+-thal. gene, and comparison with the DNA sequence of a normal β globin gene reveals only a single divergent nucleotide, within the body of the small intervening sequence. This base change creates a sequence which is homologous to that of the 3' splice site of the small intervening sequence, and is also complementary to the 5' portion of the small nuclear RNA, U-1. We infer that the single base substitution in the small intervening sequence may be the primary molecular defect in β^+-thal., and suggest possible models for anomalous splicing of β globin mRNA precursor molecules. Alternatively, the β^+-thal. gene may be defective at a more distant site than has been analyzed. We are presently testing these models by investigating processing of the β^+-thal. mRNA precursor in vitro in order to determine whether splicing does occur at the site which we have identified.

1062 CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF CENTROMERE DNA FROM YEAST CHROMOSOMES III AND XI, Molly Fitzgerald-Hayes, Louise Clarke, Jean-Marie Buhler and John Carbon, Department Biological Sciences, University of California, Santa Barbara, CA 93106

We have cloned segments of yeast DNA that contain centromere sequences from several different yeast chromosomes. When present on a plasmid vector capable of DNA replication in yeast, the centromere DNA enables the plasmid to function as a normal chromosome. Genetic markers on these minichromosomes are stably maintained through mitosis and segregate in meiosis as centromere-linked genes. A fine structure analysis of centromere DNA from chromosomes III and XI is in progress. The centromere (CEN3) from chromosome III was originally isolated on a 8 kbp DNA segment that also contained the yeast CDC10 gene, on plasmid pYe(CDC10)1 (Clarke & Carbon, Nature 287, 504 (1980)). The MET14 gene is tightly linked to the centromere on chromosome XI. This centromere (CEN11) was isolated by selecting for complementation of yeast trp1 met14 mutations by segments of yeast DNA cloned in plasmid YRp7. One plasmid, pYe(MET14)2, obtained in this manner, apparently carries both the MET14 gene and CEN11 on a 5.2 kbp DNA segment. Restriction mapping has failed to demonstrate large DNA fragments common to both CEN3 and CEN11. The functional CEN3 region has been recloned on a 550 bp restriction fragment (pYe(CEN3)30), and the CEN11 activity has been recloned on a 1.5 kbp fragment (pYe(CEN11)12). Preliminary nucleotide sequence analysis of the 550 bp CEN3 fragment reveals interesting features including relatively long stretches of A+T rich DNA, and regions where purines are clustered in one strand.

1063 ISOLATION AND STRUCTURE OF HUMAN HISTOCOMPATIBILITY cDNA CLONE, Ashwani Sood, Dennis Pereira and Sherman M. Weissman, Yale University New Haven, CT 06510

We have isolated a cDNA clone for one of the HLA-B locus allo-antigens by hybridisation with a 30-nucleotide long DNA probe. This probe was isolated from a reverse transcriptase catalysed cDNA synthesis reaction on poly(A) mRNA where an oligonucleotide (32p) dCTTCTCCACAToH served as a primer and where dideoxynucleoside triphosphates were used to reduce the size and hetergeneity of cDNA products. This approach is extremely sensitive and may be used to clone those genes for which the corresponding mRNAs constitute approximately 0.01% of poly(A) mRNA. The nucleotide sequence of the cDNA clone will also be presented .

1064 THE STRUCTURE AND EVOLUTION OF RAT SERUM PROTEINS: ALBUMIN AND ALPHA-FETOPROTEIN, Linda L. Jagodzinski, Thomas D. Sargent, Maria Yang, and James Bonner, California Institute of Technology, Pasadena, CA 91125

We have determined the nucleotide sequence of the rat serum albumin (RSA) mRNA, all of the exons of the RSA gene, and part of the rat alpha-fetoprotein (RAFP) mRNA. By comparing these sets of sequence data we have inferred the exact location of all of the fourteen introns in the albumin gene. Each of the three domains of albumin are encoded by a set of four exons. These sets, or subgenes, are partially homologous in nucleotide sequence, exhibit very similar patterns of interruptions by introns, and probably arose from a common ancestor by intragenic duplication. The leader exon was apparently attached to the 5' end of the albumin gene by a rearrangement or "shuffling" mechanism. The RAFP mRNA sequence is homologous to the RSA mRNA, suggesting that these two genes arose by intergenic duplication. Furthermore, there is evidence that the exons comprising the albumin subgenes arose by duplication. Therefore, it is likely that the albumin-AFP gene family originated as a very simple precursor gene, possibly equivalent to a single small exon which underwent a complex series of intergenic and intragenic duplications followed by mutational divergence.

1065 THERE ARE INTERSPERSED REPETITIVE SEQUENCES IN HUMAN DNA, STRUCTURALLY ANALOGOUS TO TRANSPOSABLE ELEMENTS IN LOWER EUKARYOTES, Pudur Jagadeeswaran, P. Andrew Biro, Dorothy Tuan, Bernard G. Forget, and S. M. Weissman, Yale University School of Medicine, New Haven, CT. 06510.

We have analyzed and compared the nucleotide sequences of the intergenic DNA preceding the human fetal globin and adult delta globin genes. We have at least two categories of reiterated sequence in this globin DNA. One category consists of inverted 300 nucleotide long repetitive "Alu" family DNA sequences. The DNA between the closely spaced paired inverted repeats of Alu sequences may also be repetitive.

The second category of repetitive DNA is a sequence of over five kilobases present between the embryonic and fetal globin genes and pairs are also found downstream from the beta chain genes. The sequence across one end of this repeat shows a stretch of approximately 600 nucleotides bounded by a terminal imperfect inverted repeat of 17 nucleotides beginning with the dinucleotide TG. We suggest that this element could be an analogue in human genome to movable DNA elements such as a TY1 element of yeast and copia of Drosophila. Similar sequences are repeated of the order of 1,000 times throughout the genome.

1066 MOLECULAR DETAILS OF THE DIRECTED DNA REARRANGEMENT THAT DIFFERENTIATES CELL TYPE IN YEAST, Jeffrey N. Strathern, James B. Hicks, Amar J.S. Klar and Kim Nasmyth, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Cell type in Saccharomyces is determined by two partially nonhomologous alleles of the mating type locus, MATa and MATα. Changes in cell type reflect substitutions of DNA at MAT that interconvert these two alleles. The sources of the incoming DNAs are two loci (HML and HMR) at which a complete, unexpressed copy of the a or α genes exist. For example, HMLα MATa HMRa yeast (a cell type) can switch to the α cell type by replacing the 642 base pair a specific sequence at MAT with a replica of the 747 base pair α specific sequence stored at HMLα. This switch occurs as often as every generation in homothallic (HO) yeast. We will focus on two areas of this regulatory mechanism: 1) We have detected switching intermediates in DNA from homothallic cells. Further, we have demonstrated that MAT on an autonomously replicating plasmid can switch and that HML on a plasmid can function as a cassette donor. Thus, we can define sites required for switching and the molecular details of the switch. 2) The unexpressed copies of the a and α genes at HML and HMR are turned off at the transcriptional level by the MAR/SIR genes (4 loci). This is particularly intriguing because HMLα HMRα and MATα sequences are identical for hundreds of bases upstream of the mature messages. We have identified sites required for this genetic position effect both by mutagenesis and by subcloning a portion of HML that titrates the MAR/SIR regulators.

1067 MOLECULAR BASIS OF CELL TYPE DETERMINATION BY THE YEAST MATING TYPE LOCUS, George F. Sprague, Jr., Rob Jensen, and Ira Herskowitz, Institute of Molecular Biology and Department of Biology, University of Oregon, Eugene, OR 97403.

The three yeast cell types (a, α, and a/α) are determined by alleles of the mating type locus, MATa and MATα. a and α cells mate and undergo efficient mating type interconversion (if they carry the HO gene); a/α cells do not mate or undergo mating type interconversion. It has been proposed that MATa and MATα are regulatory loci that control expression of unlinked genes required for mating, sporulation, and mating type interconversion. MATα is proposed to code for two functions--α1, a positive regulator of unlinked α-specific genes, and α2, a negative regulator of unlinked a-specific genes. Several genes which might be controlled by the mating type locus have been identified based on genetic analysis in our laboratory and elsewhere: two α-specific genes, STE3 and STE13; an a-specific gene, BAR1; and the a/α-inhibited gene, HO. We have cloned STE3, STE13, and HO in order to determine whether transcription of these genes is controlled by the mating type locus. For at least one of these genes, STE3, an RNA species complementary to the cloned DNA is produced in MATα cells but not in MATa or MATa/MATα cells. This species is produced by MATα1$^+$matα2$^-$ cells but not by matα1$^-$MATα2$^+$ cells. These results indicate that expression of the STE3 gene is controlled at the level of stable RNA production and support the view that MATα1 codes for a positive regulator of α-specific genes.

1068 STRUCTURE OF HUMAN β GLOBIN GENES IN β^+ THALASSEMIA, Robert G. Pergolizzi, A. Lee Burns, Sally Spence, Kathe Kosche, Carl Miller, Helen Schreiner, and Arthur Bank, Columbia Univ., Depts. of Medicine, and Human Genetics and Development, 701 West 168th Street, New York, New York 10032

A clone of human DNA containing the β globin structural gene and its 3' and 5' flanking sequences has been isolated from a patient with β^+ thalassemia. Two subclones have been characterized by restriction endonuclease analysis and by direct DNA sequencing. These subclones in pBR 322 include: 1) The large intervening sequences (IVS), and 2) The 5' flanking region and 5' end of the gene, up to the large IVS. The fragments generated from these subclones by restriction enzymes are identical to those from similar subclones from normal β globin genes. The DNA sequence of the entire small IVS and part of the large IVS, including the two intron-exon junctions, and the structural sequence between them, is identical to the published sequence of a normal β globin gene. This suggests that there is considerable conservation of nucleotide sequences in these regions in different individuals. Although studies of RNA metabolism have indicated a possible defect in RNA processing in β^+ thalassemia, the defect in this patient does not reside in the small IVS sequence. (Supported by NIH, March of Dimes Birth Defects Foundation, and Cooley's Anemia Foundation.)

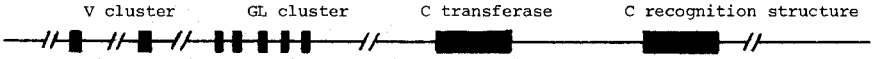
1069 EXTENSIVE POLYMORPHISM AND EVOLUTION IN ZETA GLOBIN GENES, Barbara S. Chapman, Karen A. Vincent and Allan C. Wilson, Dept. of Biochemistry, University of California, Berkeley

ζ globin genes, like their adult α globin counterparts, are present in human chromosomal DNA as tandem duplicates. Although closely linked to the adult α genes, ζ genes are expressed only in early stages of embryonic development, have large intervening sequences and are separated by a large intergenic distance. We have examined ζ gene arrangement in filter-bound DNA from 70 human individuals and several ape species, using ^{32}P-labeled ζ gene fragments as probes. There is a startling level of polymorphism in both the distance between paired ζ genes, and in the distance from the 3' ζ gene to the center of the adult α gene pair. Restriction endonuclease Bgl II digestion reveals at least 3 discrete fragment sizes for the distance between ζ genes, and at least 3 fragment sizes representing the 3' ζ to 3' α distance. 20% of the individuals sampled have 3 or 4 different ζ gene-containing fragments. This variation is probably not related to gene rearrangements before expression: DNA from K562 cells shows the same cleavage pattern with 6 restriction enzymes whether or not embryonic hemoglobin synthesis is induced with hemin. Alternatively, the polymorphisms in human ζ gene arrangement may reflect evolutionary processes. Since our results indicate that ζ genes are duplicated in chimpanzees, gorillas and gibbons, we have speculated that ζ genes evolve in concert as do adult α genes, maintaining homogeneity of sequence within a species by frequent loss and reduplication events. The larger ζ intergenic distance and large introns may be clues that the mechanism of their concerted evolution is different from that operating in adult α genes. Extensive mapping of ζ gene fragment variants in humans and apes is being used to define the molecular basis of this phenomenon.

1070 GENOMIC STRUCTURE OF THE MAJOR HISTOCOMPATIBILITY GENES, Barry E. Rothenberg, Salk Institute, Tumor Virology Laboratory, San Diego, California 92138

It has been suggested that some genes of the major histocompatibility complex code for a bimolecular system whose function is mediated through the complementary interaction of protein and carbohydrate (Rothenberg, B.E., Devel. & Comp. Immun. 2, 23, 1978). In support of this hypothesis Higgins, O'Neill and Parish, using monoclonal antibodies have reproducibly shown that H-2K^k and Ia antigens exist in two forms glycoprotein and glycolipid, where the protein and carbohydrate moieties determine the alloantigens, respectively.

Because of the extensive polymorphism and high acceptable mutation rate of the major histocompatibility genes and the fact that they are responsible for determining the specificity of carbohydrate and protein defined alloantigens, suggests that these genes are not colinear with their products, but are encoded by a series of discrete transposable gene segments, consisting of multiple variable (V), five glycosidic linkage (GL) (GL is comparable to the J region in immunoglobulin genes), and two constant (C) region genes.



The demonstration of oligosaccharide determined alloantigens is compatible with the "Self-Recognition Concept" where restrictive recognition is germ-line encoded, but is incompatible with the accepted dogma that restriction is learned. Evidence for the nature of the MHC genomic structure and its application to a general model for differentiation and cell-cell recognition will be presented.

1071 THE STRUCTURE AND TRANSCRIPTION OF HUMAN GLOBIN GENES. N. J. Proudfoot, M. H. M. Shander, C. O'Connell and T. Maniatis.

Detailed structural analyses of the human α-like and β-like globin gene clusters has revealed the presence of five α-like genes, ζ2-ζ1-Ψα1-α2-α1 (Lauer et al., 1980) and seven β-like genes, ε-Ψβ2-$^{G}\gamma$-$^{A}\gamma$-Ψβ1-δ-β (Fritsch et al., 1980). In addition to the genes which encode normal globin polypeptide chains, a number of sequences (designated by the prefix Ψ) could not be identified with any known globin polypeptides. The complete nucleotide sequence of Ψα1 revealed numerous sequence alterations which are incompatible with normal globin gene function (Proudfoot and Maniatis, 1980). Ψα1 is therefore a pseudoglobin gene. Preliminary nucleotide and amino acid sequence data suggested ζ1 was a functional gene. However, when the complete nucleotide sequence of this gene was determined an in-phase termination codon was found at amino acid 6. No other features of the ζ1 gene appear nonfunctional. Surprisingly, the two introns of ζ1 are large: 1.2 kb and 300 bp, 10 and 2.5 times larger than those of the normal α-globin genes, and contain extensive regions of simple repeat sequence.

We recently reported a preliminary in-vitro transcription study of human globin genes (Proudfoot et al., 1981). We find that the δ-globin gene which is expressed less efficiently than the β-globin gene in normal adult erythroid cells is transcribed less efficiently in-vitro. In addition, Ψα1 is transcribed poorly in-vitro when compared to the normal α-globin gene. Work is in progress to correlate the in-vitro transcription of normal and pseudoglobin genes with transcription in-vivo using a new SV40 mammalian vector system. Fritsch, E. F., Lawn, R. M., and Maniatis, T. (1980) Cell 19, 959-972. Lauer, J., Shen, C.-K. J., and Maniatis, T. (1980) Cell 20, 119-130. Proudfoot, N. J. and Maniatis, T. (1980) Cell 21, 537-544. Proudfoot, N. J., Shander, M. H. M., Manley, J. L., Gefter, M. L. and Maniatis, T. (1980) Science 209, 1329-1336.

1072 DUCK GLOBIN GENE SEQUENCES

Gary V. Paddock, Jim Gaubatz, Robert Frankis, and Fu-Kuen Lin; Dept. of Basic and Clinical Immunology and Microbiology, Medical University of South Carolina 29403

The duck globin genes present an interesting model system, because when the animals are made anemic, there is a shift in the globins produced. We have determined the nucleotide sequence of a recombinant cDNA for a duck alpha globin gene which indicates a gene with a novel evolutionary history. The duck globin S1 treated double stranded cDNA was inserted into the Pst I site of pBR322. This enabled us to design a rapid nucleotide sequencing protocol which takes advantage of the pBR322 restriction endonuclease sites so that only one gel purification step is required to prepare the labeled fragments for sequencing. Analysis of our recombinant revealed that it contains the translated region and all of the 3' untranslated for an alpha globin gene. This duck globin has sequences related to the chicken alpha A globin for the first 100 codons, whereas in the terminal regions it is more clearly related to alpha globins found in anemic chickens. The data are thus suggestive of a possible fusion alpha globin gene. We have also identified an alpha D globin gene via partial sequence analysis. The recombinant cDNA for this gene was constructed using the new floppy loop recombinant cDNA procedures developed in this laboratory. The presence of alpha D globin in anemic ducks implies that the duck globin shift is different from that observed for chickens, where alpha D is not produced.

1073 ISOLATION AND CHARACTERIZATION OF SOMATOSTATIN GENES FROM ANGLERFISH. R. Crawford, P. Hobart, L. Shen, R. Pictet & W. Rutter. Dept. of Biochemistry & Biophysics, University of California, San Francisco, Ca 94143

Somatostatin plays a key role in hormone homeostasis by modulating the secretion of several peptide hormones. It may also be involved in neurotransmission. Recently we identified two mRNA sequences from the anglerfish endocrine pancreas that encode two distinct preprosomatostatins. This finding suggests that a family of somatostatin genes may be involved in the regulatory hormone activities attributed to somatostatin. We aim to compare the structural organization and the regulatory sequences of somatostatin genes in several vertebrates. Accordingly we have constructed an anglerfish genome library, using Charon 28 vector and anglerfish DNA partially digested with MboI. We have isolated several clones that hybridize to the somatostatin cDNAs. One of these clones hybridizes with both somatostatin cDNAs. Restriction analysis of this clone demonstrates that the DNA sequence hybridizing to one of the somatostatin cDNAs is separated from the DNA hybridizing to the other somatostatin cDNA, by no more than five kilobases.

1074 STRUCTURE AND FUNCTION CORRELATES OF THE 5'-FLANKING AND VARIABLE REGION NUCLEOTIDE SEQUENCES OF FUNCTIONAL AND NON-FUNCTIONAL MOUSE IMMUNOGLOBULIN HEAVY CHAIN GENES FOR HYBRIDOMA ANTIBODIES DIRECTED AGAINST THE HEMAGGLUTININ A OF INFLUENZA VIRUS, Louise Showe, Roland G. Kallen, Dept. Biochem. & Biophys., and Walter Gerhard, Univ. of Pa. Sch. of Medicine and the Wistar Institute, Phila, Pa. 19104.

Screening of gene libraries by appropriate constant region probes has provided a series of functional and non-functional genes from mouse hybridomas which secrete antibodies directed against the hemagglutinin A of influenza virus. The relatedness of combining sites for the functional genes has been assessed by an extensive reactivity type analysis employing a bank of influenza viral variants. The nucleotide sequences of the combining-site related heavy chain variable regions and the 5'-flanking sequences are being compared among the functional genes and with the non-functional genes present in the same hybridomas. The transcriptional activities of these various genes will be assessed in collaboration with Dr. Pierre Chambon.

1075 CHARACTERIZATION OF THE CHICK VIMENTIN GENE, Zendra E. Zehner and Bruce Paterson, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Intermediate filaments are ubiquitous constituents of the cytoskeleton of higher eukaryotic cells. These proteins are thought to provide a stable cytoskeletal framework within the cell and to be involved in such functions as maintenance of cell shape, intracellular transport, organelle attachment, and cell locomotion in cultured cells. Biochemical and immunofluorescent techniques have established 5 major classes of intermediate filaments, each class composed of a number of heterogeneous subunits. Marked similarities in the ultrastructure and biochemistry of the intermediate filaments have suggested a general model of a 3 polypeptide subunit containing 2 discrete coiled coil α-helical segments of similar size interspersed with non α-helical regions of variable size. It has been suggested that the intermediate filament subunit is composed of constant and variable domains analogous to the heavy and light chains of the IgG molecule. The likelihood of such an arrangement would merit an investigation into the organization of this complex family of proteins at the DNA level. In order to study the genetic arrangement of this gene family, we have chosen to focus on the fibroblastic intermediate filament protein, vimentin. Several genomic clones have been isolated from a chick DNA library. Coding sequences were confirmed by mRNA selection followed by identification of the translation product by isoelectric focusing. Preliminary characterization of the vimentin genomic clones indicates that the coding information is present in multiple copies in the chick genome interspersed with repetative DNA sequences present at many copies in the genome. Hybridization with a vimentin cDNA clone against different eukaryotic DNA's indicates that the vimentin gene is well-conserved throughout evolution.

1076 MULTIPLE HUMAN GROWTH HORMONE RELATED GENES, David Moore, Frances DeNoto, Robert Hallewell, John Fiddes, and Howard Goodman, Department of Biochemistry, University of California San Francisco, San Francisco, California 94143

We have isolated seven different human growth hormone (hGH) related genes from human genomic DNA. Only two proteins, hGH and chorionic somatommamotropin (hCS), are known to be encoded by this gene family. The cloned genes are quite closely related to each other. This is demonstrated by the conservation of the locations of restriction sites and Alu family repeat sequences in an around the genes, and by comparisons of the T_m of heteroduplexes between the genes and hGH or hCS cDNAs. Two cloned segments each contain two hGH-related genes.

The member of the family which encodes hGH has been sequenced. Comparison of this sequence with the cDNA sequence shows that the gene is interrupted by four intervening sequences. Fragments from the sequenced gene and cDNA have been used to analyse pituitary hGH mRNA using S1 mapping. Two different Hogness box sequences separated by 54 base pairs give rise to at least two different 5' ends for hGH messages. Each message would be expected to initiate translation of hGH at the same AUG. At least one alternative splicing site which generates a shorter mRNA has been located. An hGH peptide with exactly the expected internal deletion is observed in normal pituitaries. Thus, there is diversity in the hGH mRNA from a single gene in addition to the potential diversity from the multiple related genes.

1077 ENDOCRINE PANCREAS SPECIFIC GENES IN VERTEBRATES AND INVERTEBRATES, Lu Ping Shen, Peter Hobart, Robert Crawford, Raymond Pictet, and William J. Rutter, University of San Francico, San Francisco, Calif. 94143

We are attempting to isolate and analyze DNA sequences in vertebrates and invertebrates which hybridize to genes specifically expressed by the vertebrate endocrine pancreas. Recently, we reported the cloning and analysis of two cDNA sequences encoding the precursor peptides containing distinct somatostatin hormones (termed somatostatin I and II). The cDNA molecules, (non-cross-hybridizing)were derived from mRNAs which are both expressed in the anglerfish (Lophius americanus) endocrine pancreas. The somatostatin I probe (which encodes a peptide hormone with the same amino acidsequence of that in mammals) was used to analyze (using Southern blots) genome DNA from vertebrates (human and fish) and from invertebrates (tunicate, sea urchin, and Drosophila). This probe hybridized to at least seven EcoRI fragments in fish (8, 6.4, 4.2, 2.9, 2.3, 2.1, and 1.5kb) and to six EcoRI fragments in the human genome (14, 10.8, 6.4, 4.2, 2.5, and 2.3kb). The combined size of these fragments indicate either there are many large introns in the somatostatin I gene or there are multiple somatostatin I-type genes. Further analysis of cloned fragments from a library of fish DNA supports the latter. No hybridization signal is detected in invertebrate genomes with the somatostatin I probe. A similar analysis using the somatostatin II cDNA probe resolves two EcoRI fragments in fish (9.4 and 4.4kb). In addition, the somatostatin II probe hybridizes to a 4.8kb EcoRI genome fragment in sea urchins and to a 7.0, 5.0 and 3.1kb fragments in Drosophila. Neither probe hybridized to tunicate DNA. Further analysis of these invertebrate DNA fragments is being investigated using cloned genomic fragments in lambda phage.

1078 THE CONTROL OF CELL TYPE BY THE MATING TYPE LOCUS IN YEAST, Kim Nasmyth and Kelly Tatchell*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; University of Washington, Seattle, WA 98195

In heterothallic cells, MAT**a** and MATα are stable Mendelian "alleleos", which, when hemizygous or homozygous (as in **a** or α haploids or **a**/**a** or α/α diploids), confer an ability to mate with cells of the opposite mating type and, when heterozygous (as in **a**/α diploids), turn off mating functions and allow meiosis and sporulation. MAT**a** and MATα, which contain nonhomologous blocks of DNA, have been proposed to code for regulatory proteins that control unlinked **a** or α specific genes. MATα contains two genes: α1 which is necessary for the expression of α specific mating genes and α2 which represses **a** specific genes. MAT**a** contains a single gene, MAT**a**1, which in conjunction with MATα2, specifies the **a**/α diploid cell type. One of the means of which the latter is performed is through the repression of α1 transcription. The results of RNA mapping, in vitro mutagenesis, and DNA sequencing have located the exact positions of all three MAT genes on recombinant plasmids. In order to study their mode of action and control we have constructed various chimeras between MAT genes and that from β-galactosidase from E. coli. These will be used for (1) identifying MAT proteins by their production in E. coli using the β-gal promoter and (2) analysing the regulation in yeast of a hybrid α1- β-gal gene.

1079 ESTROGEN RESPONSIVE GENES OF AVIAN LIVER, Roger G. Deeley, Robert Wiskocil, Paula Goldman and Sinbee Han, Queen's University, Kingston, ONTARIO K7L 3N6

In oviparous vertebrates, egg-yolk proteins are synthesized in the liver of mature females. Males normally do not synthesize these proteins but can be induced to do so by the administration of estrogenic steroids. We have cloned three genes expressed at high rates in hen liver. They specify serum albumin; vitellogenin, precursor of the egg-yolk phosphoproteins and apoVLDLII a major serum and egg-yolk apolipoprotein. Hybridization analyses indicate that both apoVLDLII and vitellogenin genes are normally dormant in roosters. However, they respond coordinately to induction by estrogen. In addition to its effects on transcription of these genes, estrogen also stabiizes both vitellogenin and apoVLDLII mRNA. The half-lives of these mRNA species in its presence are approx. 24hrs and in its absence are less 3hrs. The stability of serum albumin shows no depenednce on the estrogenic state of the rooster.

Transcriptional initiation regions of both apoVLDLII and serum albumin genes have been subcloned into plasmids and used for in vitro transcription studies. Their primary structures are being determined. Both genes are characterized by a short, 'leader', exon, followed by a relatively long intron.

We are examining the possibility that hormonally dependent shifts in the preferred processing pathway of apoVLDLII and vitellogenin mRNA may influence their stability in the cytoplasm. Data have been obtained indicating that cytpolasmic apoVLDLII mRNA, produced early during induction contains sequences absent from that produced later. The effects of these additional sequences on mRNA secondary structure are being investigated by electron microscopy and nuclease mapping.

1080 CLONING OF MITOCHONDRIAL DNA FROM PODOSPORA ANSERINA: DELETIONS AND REARRANGEMENTS, Donald J. Cummings, Jane L. Laping, Richard M. Wright, and Mark A. Horrum, University of Colorado School of Medicine, Denver, Colo. 80262

Podospora anserina, like most ascomycetes, undergoes vegetative death or senescence, and various studies have shown that this senescence is maternally inherited. Previous work from our laboratory has revealed that during senescence, a multimeric set of tandemly repeated circular DNA molecules are generated in the mitochondrion. This occurrence is similar in many respects to the rho-petite mutation in yeast. To better understand this unusual process, we have cloned several EcoRI and Hae III endonuclease fragments from young (wild-type; wt) mitochondrial (mt) DNA as well as mt DNA isolated from senescent cultures. Hybridization studies between cloned fragments of wt and senescent mt DNA have shown that the tandem repeats arose from fragments of DNA of higher molecular weight. The relationships between senescent fragments and wt mt DNA are being explored. In addition, those fragments containing rRNA genes have been identified.

1081 EXPRESSION OF GLOBIN GENES DURING RABBIT DEVELOPMENT, Ross C. Hardison, The Pennsylvania State University, University Park, PA 16802.

Globin genes are differentially expressed during rabbit embryonic development. In order to study the molecular basis for this regulated gene expression, a group of four β-like globin genes was isolated as a set of overlapping clones from a rabbit recombinant DNA library. The gene cluster is arranged in the order 5'-β4-8kb-β3-5kb-β2-7kb-β1-3'. Gene β1 encodes the adult β-globin polypeptide, β2 is a nonexpressed pseudogene, and β3 and β4 encode embryonic β-like polypeptides. All four genes contain two intervening sequences in identical places. Numerous repeated elements, some of which are transcribed, are interspersed throughout the gene cluster.

Rabbit embryos gradually switch from production of nucleated, primitive erythrocytes in the blood islands to production of anucleated erythrocytes in the fetal liver about half way through gestation. Two embryonic β-like globins (ε-globins) are produced in the primitive erythrocytes, although one is turned on before the other. Both ε-globins are minor compared to β in fetal erythrocytes. We are currently measuring levels of globin precursor RNA, mRNA and polypetide during this period of development to establish the major points of control in differential hemoglobin synthesis.

Nucleotide sequence analysis of all four β-like genes is almost complete. An examination of the flanking, untranslated, and intervening sequences shows that rabbit β3 and human γ (fetal) are strikingly similar, as are β4 and human ε (embryonic). A comparison of the sequences and times of expression of both the rabbit and human β-like genes will provide important information about the evolution and perhaps the regulation of these two gene families.

1082 TRANSCRIPTION OF YOLK PROTEIN GENES DURING DROSOPHILA VITELLOGENESIS. Thomas Barnett, Rosenstiel Center, Brandeis University, Waltham, Mass. 02254.

The preservation of a species like Drosophila melanogaster is enhanced by the laying of large numbers of eggs. At eclosion, the major biosynthetic activities of Drosophila female adults are associated with a number of developmental programs involving the synthesis and regulation of the various components comprising a dipteran egg. One such program, vitellogenesis, is primarily responsible for the temporal production of the major food storage proteins of the egg, the three yolk proteins (YPs). The synthesis of yolk proteins in a newly eclosed female is negligible compared to the subsequent 24 hour period when the YPs can become as much as 5-10% of the total fly body weight. This dramatic increase prompted us to initiate investigations into the organization of the yolk protein genes (Barnett et al., Cell 21, 729-738, 1980) and more recently, to examine the possible in vivo mechanisms responsible for this increased activityover such a short time span. Using cloned yolk protein genes as hybridization probes, our results indicate that unlike another major developmental program of oogenesis, chorionogenesis, the yolk protein genes are not specifically amplified in the cells which synthesize these proteins. The transcription and accumulation of YP mRNAs as a function of vitellogenesis was determined by taking advantage of our ability to distinguish among the individual YP mRNA classes. Such transcription studies indicate that there is coordinate appearance of RNAs from two of the linked and divergently transcribed genes, YP1 and YP2, but the manner of their increase is not straightforward. In addition, one gene (YP2) is apparently transcribed into two different-sized poly A^+ RNAs. The translational capabilities of each are being determined.

1083 MOLECULAR ORGANIZATION AND EXPRESSION OF A CLONED FRENCH BEAN PHASEOLIN GENE, Prabhakara V. Choudary and Timothy C. Hall, University of Wisconsin, Madison, WI 53706

A gene coding for the major storage protein (phaseolin, Gl-globulin) was purified from a recombinant phage library constructed by packaging 8-22 kb fragments selected from a partial $EcoR_1$-digest of French bean genomic DNA in λ Charon 24A. Partial characterization of the clone revealed the presence of at least 3 intervening sequences, and an amino acid sequence of 185 amino acids was deduced from the nucleotide sequence; this represents about 40% of a phaseolin polypeptide (Sun et al., 1980, Nature, in press).

Detailed structural analysis, of this first plant nuclear gene to have been cloned, in terms of nucleotide sequence and heteroduplex mapping reveals similarities with animal and viral genes by the presence of IVS_s, and even the common splicing frames (except in one case). Further screening of the library in this laboratory has yielded 20 more clones exhibiting varying degrees of homology as evidenced by the differing intensities of plaques on hybridization with labeled probe. These clones are currently being further characterized.

We are currently studying the expression of this purified gene that codes for an economically important protein. Attempts are underway to engineer a suitable composite host vector (using yeast, plant and bacterial plasmids) that will facilitate directed transfer of this gene in various lengths and forms into plants as well as protoplasts. All these and related results will be discussed in detail.

1084 STRUCTURAL STUDIES OF MOUSE TRANSPLANTATION ANTIGEN GENES, Michael Steinmetz, John G. Frelinger, Douglas A. Fisher and Leroy E. Hood, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Transplantation antigens are cell-surface glycoproteins encoded by the major histocompatibility complex (H-2 complex in the mouse). They have attracted considerable interest because of their involvement in a variety of immunological phenomena and their exceptionally high degree of polymorphism. In order to investigate the organization and diversification of this multigene complex, we have isolated cDNA clones from two different cell lines known to overproduce transplantation antigens. The nature of the cDNA clones was verified by DNA sequence determination. The structure of the cDNAs and experiments using the cDNA clones to investigate the organization of the chromosomal genes will be presented.

1085 AN UNUSUAL SYMMETRIC RECOMBINANT (SYREC) BETWEEN ADENOVIRUS TYPE 12 DNA AND HUMAN CELL DNA, Walter Doerfler[1], Renate Deuring[1] and Günther Klotz[2], Institute of Genetics[1], University of Cologne, Cologne, Germany, and Dept. of Microbiology[2], University of Ulm, Ulm, Germany.

Upon purification of human adenovirus type 12 (Ad12) in CsCl density gradients, two bands of particles, Ad12-3 and Ad12-3a, were observed. The particles from band Ad12-3a contain a recombinant between human host cell DNA and Ad12 DNA. The human cell DNA sequences are of the repetitive type occurring about 200- to 500-times in cellular DNA. Ad12 DNA and the recombinant genomes exhibit the same or very similar lengths. Upon cleavage of KB cellular DNA with EcoRI, BamHI, HinfI, MspI, MboI, PstI, and BglII, the [^{32}P]-labeled cellular DNA from Ad12-3a particles hybridizes on Southern blots to distinct bands of KB DNA and to heterogeneously sized DNA. The cellular DNA from Ad12-3a particles is not methylated, whereas the same sequences in KB DNA appear to be extensively methylated. Upon denaturation and renaturation, the recombinant DNA molecules are converted to molecules of half the length of Ad12 DNA as determined by gel electrophoresis and by electron microscopy. The data are consistent with a model in which 500 to 1000 base pairs from the left terminus of Ad12 DNA are linked to repetitive host cell DNA, and this structure is symmetrically duplicated as a large inverted repeat. The recombinants appear to be stable upon serial passage of the virus preparation for many years. These SYREC molecules may suggest a way of how to use adenovirus DNA as an eukaryotic vector.

1086 STRUCTURES OF THE PROLACTIN AND GROWTH HORMONE GENES OF HOMOZYGOUS DWARF AND NORMAL MICE,

Leslie M. Hoffman, Mary B. Slabaugh and Jack Gorski, Dept. of Biochemistry, University of Wisconsin, Madison, WI 53706

Dwarf mice of the Snell (dw/dw) and Ames (df/df) strains display a marked retardation of growth correlated with a complete lack of detectable pituitary growth hormone (GH) or prolactin (Prl) synthesis when compared with their homozygous normal counterparts (Dw/Dw and Df/Df). We have investigated the molecular basis of the lack of GH and Prl gene expression by genomic Southern hybridization and molecular cloning of the respective genes in lambda bacteriophage. The GH gene appears to be present in all 4 genotypes and resides in a 5.6 Kb Bam HI restriction fragment. Likewise, the Prl genes of both dwarf and normal mice are located in restriction fragments of identical size. Thus, no gross deletions or rearrangements at either gene locus was detectable. Studies are in progress in which size-fractionated Bam HI-digested DNA from each of the 4 mouse genotypes will be ligated with lambda Charon 27 vector arms, packaged into viable phage *in vitro* and screened with a plasmid containing bovine GH cDNA. Recombinant phages containing the GH genes will be analyzed by extensive restriction mapping, heteroduplex formation and DNA sequencing. If no abnormalities in the dwarf genes such as nonsense mutations, altered splicing signals or mutations in the flanking regions are identified, the observed defect in GH synthesis must be ascribed to regulatory molecules or to posttranscriptional events. (Supported in part by NIH Grant CA18110.)

1087 GENES FOR HUMAN U1 SMALL NUCLEAR RNA AND $tRNA^{ASN}$ ON THE SAME Eco RI DNA FRAGMENT, Elsebet Lund, James E. Dahlberg, Richard Buckland+ and Howard Cooke+, University of Wisconsin, Madison, Wisconsin 53706 and +MRC Mammalian Genome Unit, Edinburgh University, Edinburgh, Scotland

A 14 kb Eco RI fragment of human DNA has been isolated which contains sequences hybridizing both to U1 small nuclear RNA and to $tRNA^{Asn}$. Restriction enzyme analysis of the cloned DNA shows that it contains only one copy of each sequence and that these sequences are separated by at least 3.4 kb.

When probed with a mixture of 4-6S human or mouse cell RNA, under stringent conditions, the clone hybridizes only to U1 snRNA and $tRNA^{Asn}$. Also, since U1 snRNA hybridizes to a single 200 b.p. long Hha I fragment, we conclude that snRNA genes are not tandemly arranged, at least in this clone. Likewise, the $tRNA^{Asn}$ gene does not appear to be clustered with other tRNA genes. At lower stringency conditions, 7S RNA also hybridizes, indicating that the cloned fragment contains one or more "Alu" middle repeat sequences.

Analysis of the U1 DNA sequence indicates that it is a gene rather than a pseudogene; transcription studies are in progress to test this conclusion.

The clone was originally detected by its ability to hybridize to a cloned cluster of X. laevis tRNA genes. Using this same screening procedure we have isolated another 14 kb partial RI fragment containing the sequence for $tRNA^{Asn}$. This DNA fragment has a restriction endonuclease pattern different from the fragment described above and it contains no sequences complementary to any other tRNA or to snRNA U1.

1088 GENOMIC ARRANGEMENT OF VARIANT ANTIGEN AND TUBULIN GENE FAMILIES IN TRYPANOSOMA BRUCEI, Nina Agabian, Linda Thomashow, Kenneth Stuart and Michael Milhausen, University of Washington, Seattle, WA 98195.

African trypanosomes are able to modulate their antigenic character by varying the molecular composition of their surface coat during infection of the mammalian host. Cloned cDNA sequences for two variant antigen genes have been obtained from sequential relapse of a chronically infected host. These cDNA sequences have been used to study the genomic arrangement of the variant antigen genes in the context of variant antigen expression and in six variants expressing immunologically different surface glycoproteins. The genomic context of the variant antigen gene sequences varies even among trypanosome populations expressing other, different antigens. In contrast, the arrangement of the other cloned DNA sequence appears unchanged with antigenic variation.

In addition to the surface glycoprotein genes, the tubulin genes of T. brucei have been cloned and their expression during the developmental cycle of T. brucei in mammalian and insect forms has been studied. The α and β tubulin genes are found in tandem in the genome, an arrangement not previously reported, and the possibility that these genes are differentially transcribed during trypanosome development is presently under investigation.

Multigene Systems and Genes with Complex Functions

1089 ACTIN GENE EXPRESSION IN DEVELOPING SEA URCHIN EMBRYOS, William R. Crain, Jr., David S. Durica, and Kevin Van Doren, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

We are examining the expression of the actin multigene family during early sea urchin development. In vivo labeling of newly synthesized proteins and in vitro translation of polysomal and cytoplasmic RNA at several developmental stages indicates that the synthesis of actin increases dramatically between 8 and 24 hr of development and that this increase is due to an increase in abundance of actin mRNA. To determine actin mRNA sizes and assay their relative abundance directly, polysomal and cytoplasmic RNA from egg and 5 stages of development (2,5,8,13 and 18 hr) was examined by RNA blot analysis using a cloned sea urchin actin sequence as probe. Two actin mRNA size classes (2.1 and 1.7 kb) are present in egg and developing embryos. During the first 8 hr of development the abundance per embryo of these actin mRNA classes remains similar to that in the unfertilized egg. After 8 hr their abundance begins to increase so that by 18 hr the cytoplasmic abundance of these actin message classes has increased 9 and 25 fold, respectively. While the abundance of each size class increases over the same time interval, suggesting a coordinate "turn on", their abundance relative to each other shifts nearly 3 fold during this time suggesting differential accumulation of these actin mRNAs.

1090 CELL SPECIFIC GENE EXPRESSION IN THE NEMATODE CAENORHABDITIS ELEGANS, Michael R. Klass, Biology Department, University of Houston, Houston, Texas 77004

We have recently discovered a major sperm-specific protein in the nematode C. elegans. This 15,000 molecular weight protein has an isoelectric point of 8.6, a molecular weight of 30,000 under nondenaturing conditions, and accounts for approximately 15% of the total sperm protein. Its synthesis begins at the early stages of spermotogenesis (39-42 hrs post hatch at 20° c) in a specific region of the male gonad. This 15k protein is only detectable in the male gonad and in mature sperm of both the male and hermaphrodite by immunocytochemical techniques. Ammino acid analysis and immunocytochemistry indicates that 15k is not a histone or a nuclear binding protein. Poly A mRNA coding for 15k is first detected at 39-40 hrs corresponding to the time of synthesis of 15k. Inhibitor studies with actinomycin-D and α-amanitin suggest the gene for 15k is regulated at the transcriptional level. Spermatogenesis defective mutants are being screened by two dimensional gel electrophoresis for alterations in 15k synthesis.

The genomic sequence coding for 15k has recently been cloned in a lambda charon 10 vehicle. We are now using this clone as a probe to determine when and in what tissue RNA for 15k is synthesised and to determine the presence of intervening sequences.

1091 CHANGES IN KERATIN GENE EXPRESSION DURING TERMINAL DIFFERENTIATION IN MAMMALIAN EPITHELIAL CELLS, Elaine Fuchs, The University of Chicago, Chicago, IL 60637, and Howard Green, MIT, Cambridge, MA 02139.

Mammalian keratins comprise a family of proteins (MW 40-70K daltons) that form 80 Å intermediate filaments in the cytoplasm of most epithelial cells. In the epidermis, the keratins are the major differentiation product and form the bulk of the outer, dead stratum corneum layer. The pattern of keratins produced by an epidermal cell changes during its course of terminal differentiation. Cells of the inner layers of the epidermis contain only small keratins (46-58K). As these cells migrate to the outer layers, they produce large keratins in addition to small ones. The early changes that take place within each differentiating cell result largely from changes in synthesis and are reflected at the level of translatable mRNA. Changes occurring late in this differentiation process probably involve post-translational processing. We are presently preparing cloned keratin cDNA probes to investigate the early changes at the level of transcription and hnRNA processing. Finally, we have shown that in stratified squamous epithelia of internal organs, the pattern of keratins is different from that of epidermis, implying that several routes of keratin gene expression are possible in human epithelial cells.

1092 THE HUMAN LEUKOCYTE INTERFERON GENE FAMILY: STRUCTURE AND EXPRESSION IN MAMMALIAN CELLS, Axel Ullrich, David V. Goeddel, Alane M. Gray, Thomas J. Dull, John P. Adelman, Mitchell Gross, Peter H. Seeburg and Richard M. Lawn, Genentech, Inc., 460 Point San Bruno Blvd., So. San Francisco, CA 94080

Recent studies, including cDNA cloning, genome analysis and gene isolation from a human genomic DNA library, reveal the existence of a family of 10-20 closely related leukocyte interferon genes. Several of these genes are clustered in the genome. The genes characterized thus far do not contain any intervening sequences. Flanking and intergenic sequences will be compared and discussed. Attempts to express the interferon protein in mammalian cells will be described.

1093 DIFFERENTIAL EXPRESSION OF THE ACTIN MULTIGENE FAMILY, Robert J. Schwartz and Katrina N. Rothblum, Baylor College of Medicine, Cell Biology, Houston, Texas 77030

We described the construction of an α actin cDNA clone, pAC269 (Schwartz et al., Biochemistry, 1980), that was used as a hybridization probe in the current investigation to examine the induction of actin mRNA during myogenesis. A Tm difference of 10°C-13°C between skeletal muscle α actin and non-muscle β and γ actin mRNAs and pAC269 allowed us to establish the highly stringent hybridization conditions necessary to measure separately the content of α actin mRNA and βγ actin mRNAs during muscle development in culture. We observed low levels of α actin mRNA (50 molecules per cell) in replicating prefusion myoblasts. The vast majority of actin mRNA (2000 molecules per cell) present at this stage was accounted for by β and γ actin mRNA. Beginning at myoblast fusion, α actin mRNA was induced and within 50 hours reached a level 700-fold greater than that observed in the undifferentiated state. At 95 hours in culture when myotube formation was completed, α actin content was at its peak (36,000 molecules/nucleus). Conversely, β and γ actins' mRNA content began to decline at the beginning of fusion and by the end of myotube formation they were undetectable by our techniques. The switching of actin mRNA species was inhibited in myoblasts treated with BudR. The induction of α actin mRNA and the repression of β and γ actin mRNA was observed following the reversal of the BudR block, and coincident with the onset of myoblast fusion. We found that the expression of actin genes within the actin multigene family is switched in myogenesis through a strict developmental pattern.

1094 GENES CODING FOR THE KERATIN PROTEINS OF SHEEP'S WOOL, Merilyn J. Sleigh, Kevin A. Ward[1], Barry C. Powell[2] and George E. Rogers[2], C.S.I.R.O. Molecular and Cellular Biology Unit, P.O. Box 184, North Ryde, N.S.W. 2113, Australia, [1]C.S.I.R.O. Division of Animal Production, P.O. Box 239, Blacktown, N.S.W. 2148 and [2]Department of Biochemistry, University of Adelaide, G.P.O. Box 498, South Australia. 5001.

Wool fibres are composed of at least three families of proteins, the low sulphur, high sulphur and high tyrosine groups, which may be coordinately expressed in wool follicle cells. Within families, many of the proteins show a considerable degree of amino acid homology. This raises interesting questions about the way in which the genes coding for these groups of related proteins are arranged on the chromosome, and how their expression is regulated.

We have prepared a library of cDNA clones from keratin mRNA isolated from wool follicle cells. The clones were grouped by hybridisation analysis, and the groups identified with particular keratin proteins or protein families by in vitro translation of hybrid-selected mRNA. Further identification was made by comparing nucleotide sequences of cDNA clones and available amino acid sequences for keratin proteins.

Characterised cDNA clones were used to investigate the chromosomal arrangement of keratin genes by Southern blotting, or to identify by hybridisation keratin gene-containing clones from a sheep genome library. Analysis of these genome fragments is in progress.

1095 SEQUENCE DIVERGENCE AND EVOLUTION OF THE PROTAMINE GENE FAMILY IN RAINBOW TROUT.

Michael A. Wosnick, Lashitew Gedamu, Kostas Iatrou, Wayne Connor, Judd Aiken and Gordon H. Dixon, Division of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada, and the Biological Laboratories, Harvard University, Cambridge, Mass.

The developing rainbow trout testis provides an excellent opportunity to study a developmentally regulated multi-gene system, namely the protamine gene family. One facet of our studies on the trout protamine genes has been the cloning of the family of double-stranded cDNAs synthesized on a template of purified protamine mRNAs. Complete nucleotide sequences have been determined for selected sequence components. Our data confirms the existence of several different protamine components, which share an extremely high degree of homology, but which predict slightly different amino acid sequences. Surprisingly, the 3'-untranslated portion of these sequences shows a degree of conservation comparable to that seen in the coding portion. Further analysis of these sequences, especially when added to other existing sequence information, suggests that the genes coding for these protamine components have arisen through a series of gene and/or chromosomal duplication events.

These studies are currently being extended through an examination of trout genomic fragments, cloned in lambda Charon 4A, in an attempt to determine the exact number, sequence organization, and evolutionary history within this multi-gene family.

1096 CHARACTERISATION OF MAMMALIAN ACTIN GENES, Stephen E. Humphries, Adrian J. Minty, Margaret E. Buckingham and Robert Williamson, St. Mary's Hospital Medical School, London and Pasteur Institut, Paris.

The actins represent a multi-gene family of which the different iso-forms can be separated by iso-electric focussing, though they are largely (90%) homologous in amino acid sequence and are very conserved among mammals and higher eukaryotes. We have used a recombinant plasmid molecule containing the majority (80%) of the mouse skeletal muscle actin mRNA sequence, to detect actin gene sequences in the mouse and human genome. When the hybridised Southern blot filters were washed at low stringency (1 x SSC) 15 - 20 bands of different intensity, ranging in size from 2 - 20 kb were observed in EcoRI, Hind III and BglII digests. Following a high stringency wash (0.1 x SSC) a simpler pattern was observed with two major bands in the EcoRI and Hind III digests and one major band in the BglII digest of human DNA. We interpret this result as indicating that there may be 15 - 20 different actin genes in the mammalian genome. This is a considerably higher number than the reported values for Drosophila (6 genes) and chicken (4 - 7 genes).

We have subsequently screened a human λ genomic library and of nine recombinants picked, eight contained different sized RI fragments that hybridise with an actin probe. This is not unexpected if indeed there are 15 - 20 different actin genes. Preliminary work on a genomic DNA fragment containing a mouse muscle actin gene indicates the presence of at least two introns. This is another difference compared with the actin genes of Drosophila and chicken where only one intron has been observed.

1097 STRUCTURE AND EXPRESSION OF THE YEAST ACTIN GENE, Dieter Gallwitz, Wolfgang Nellen and Marion Moos, Physiologisch-Chemisches Institut I, Lahnberge, Universität Marburg, D-3550 Marburg/Lahn

The actin gene from yeast Saccharomyces cerevisiae has been isolated and its DNA sequence determined (1,2). There is only one actin gene in yeast and it is interrupted by an 309 bp intron.
By using yeast transformation with in vitro mutated actin genes answers to questions concerning actin function, structural requirements for actin gene expression and mRNA splicing can be expected. The expression and effects of some mutant genes constructed in vitro will be discussed.
A structural comparison of the yeast actin gene with actin genes which we have isolated from other eukaryotes will also be presented.

1. D.Gallwitz and I.Sures (1980) Proc.Natl.Acad.Sci.U.S.A. 77, 2546-2550
2. R.Ng and J.Abelson (1980) Proc.Natl.Acad.Sci.U.S.A. 77, 3912-3916

1098 COLLAGEN GENES: SEQUENCE AND STRUCTURE ANALYSIS AND EVOLUTIONARY IMPLICATIONS, Forrest H. Fuller, John M. Wozney, Douglas A. Hanahan, Helga Boedtker and Paul M. Doty, Harvard University, Cambridge, MA 02138

DNA sequence analysis of 5 cDNA clones corresponding to the 3' one third of two procollagen mRNAs [chicken pro α1(I) and pro α2(I)] indicates a limited degree of sequence homology given that the mRNAs code for peptides with about 65% homology. The pro α1 mRNA is much more GC rich than the pro α2 mRNA and the dinucleotide pCG occurs more frequently in the former. Sequence differences are due mostly to differences in codon choices: both mRNAs strongly prefer pyrimidines and pro α1 strongly prefers C while pro α2 prefers U in codon third positions. The calculated sequence divergence of the two mRNAs is virtually identical in the triple helical (repeating Gly-X-Y) coding regions as compared to the C-terminal (globular) coding regions for both silent and replacement sites.

The specified amino acid sequence of the C-terminal globular domains indicates clustering of functionally active residues and implies the presence of 3 or 4 functional domains. Accordingly we find 1) a high degree of nucleotide sequence divergence, 2) a lack of protein "secondary structure" as determined by Chou and Fassman parameters, and 3) the presence of introns in the boundaries between implied functional domains. Evolutionary shuffeling of these domains could, in part, account for differences in functionalities in C-terminal regions of other collagen types and in collagen like peptides (e.g. complement component C1q).

Analysis of pro α2(I) genome clones indicates numerous (~40) small triple helical coding segments interrupted by introns of various size. The sequences of 15 5' and 15 3' intron/exon junctions in the helical coding region have been determined. Coding segments reflect the repeating Gly-X-Y nature of collagens being always multiples of 9 nucleotides and always beginning with a glycine codon. Exons are of two size classes; 54 or 45 and 108 or 99 nucleotides. These are found to alternate regularly throughout most of the genomic sequence. Introns are not found within the regions coding for "peptide linkers" which connect globular domains with helical domains; however, introns surround these sequences and thus segregate functional domains which contain cleavage sites for specific endopeptidases.

1099 THE HUMAN INTERFERON-α GENE FAMILY. STRUCTURAL RELATIONSHIPS BETWEEN SIXTEEN CLONED CHROMOSOMAL GENE-CONTAINING FRAGMENTS. Ch. Brack,* S. Nagata, N. Mantei and C. Weissmann, *Institute for Immunology,4058 Basel and Institut für Molekularbiologie I, 8093 Zürich,Switzerland.
Sixteen cloned DNA fragments containing human interferon-α-type genes (Nagata et al., Nature 287,401,1980) were characterized by restriction mapping and electron microscopy. The coding regions were accurately mapped by R-loop formation with total polyA$^+$ mRNA from interferon producing human leukocytes. A partial linkage map was obtained by heteroduplex analysis. The distances between three linked IFN-α like genes are 5kb, 14kb, and 16kb, respectively. A combination of R-loop and heteroduplex technique (R-hybrid) allowed us to establish complex sequence relationships between the flanking regions of different genes. Several fragments contain a block of homology of 4-5kb surrounding the gene. This homology block is flanked by inverted repeat sequences that are revealed as foldback structures in the electron microscope.

1100 TISSUE-SPECIFIC EXPRESSION OF MOUSE α-AMYLASE GENES, Richard A. Young, Otto Hagenbuchle Ueli Schibler, Mario Tosi, and Peter Wellauer,Swiss Institute for Experimental Cancer Research,1066 Epalinges, Switzerland.
The molecular features of mouse α-amylase genes and their products are being examined in an effort to define what governs the differential activity of these genes in several tissues. The nucleotide sequences of the α-amylase mRNAs which accumulate in the pancreas, salivary gland and liver have been elucidated. An intriguing sequence relationship exists between the salivary gland and liver mRNA species; the two share identical coding and 3' noncoding residues but differ in length and sequence in their 5' noncoding regions. The DNA which specifies these mRNAs has been purified using recombinant DNA techniques. This DNA sequence (Amy-1^A), present once per haploid genome, specifies both of the two different 5' terminal mRNA leaders as well as the common body of the salivary gland and liver α-amylase mRNAs. No gross rearrangement of this DNA is detected in the salivary gland or liver. Thus, salivary gland and liver α-amylase mRNAs are differentially transcribed and/or processed from identical DNA sequences in different tissues. To investigate the molecular mechanisms involved in the tissue-specific expression of Amy-1^A, we are currently examining α-amylase mRNA precursors, chromatin architecture, and the products of transcription of α-amylase genes *in vitro*.

1101 MOLECULAR ANALYSIS OF THE PROTAMINE GENE FAMILY IN THE DEVELOPING RAINBOW TROUT TESTES. Lashitew Gedamu, Michael A Wosnick, *Kostas Iatrou and Gordon H Dixon, Division of Medical Biochemistry, University of Calgary, Calgary, Alberta, Canada and *The Biological Laboratories, Harvard University, Cambridge, Mass., USA.
Protamines comprise a family of (3-4) basic proteins synthesized at the spermatid stage of development during the terminal differentiation of rainbow trout testes. Previous studies indicated that the mRNAs coding for each protamine component cannot easily be separated from one another. Therefore, in order to understand the control of expression, sequence organization and evolution of the protamine genes, the corresponding family of protamine double-stranded cDNAs has been cloned and single clones which contain sequentially pure DNAs corresponding to individual members of the protamine gene family have been isolated. The protamine components encoded by various clones were identified by hybrid-selected translation assay. Furthermore the complete nucleotide sequences of selected clones have been determined.
Our data supports the existence of several different protamine mRNA components with a high degree of homology. However, the predicted amino acid sequences are not entirely consistent with previously published amino acid sequences determined directly from the polypeptides. Our studies further indicated that the genes coding for the protamine components may be grouped into two major families with further sub-division of these two families on the basis of sequence variation at a genetic "hot-spot" in the coding regions. (Supported by MRC of Canada and NSF of the USA).

1102 HISTONES OF CAENORHABDITIS ELEGANS. Ulrich Certa, Randall Cassada and Günter von Ehrenstein. Department of Molecular Biology, Max-Planck-Institute for Experimental Medicine, 3400 Göttingen, Federal Republic of Germany.

We have proposed histone involvement in regulating timing of chromosome replication in embryogenesis[1]. To discover possible developmental variants of histones in C.elegans, we have analyzed histones from different developmental stages of the wild-type. To test whether histones are altered in embryonic arrest (emb) mutants, the wild-type histones are compared with those of a number of emb mutants[2] with timing defects, similar to those already described for a small selected set of emb mutants[3]. Acid-extracted histones were separated into H1, H2a, H2b, H3 and H4 by HPLC on Zorbax C8. This procedure is fast and sensitive (80 min runs with 5 µg protein)[4] separating according to amino acid composition, with single amino acid changes detectable[4]. To increase sensitivity, we have digested each purified histone with Staph. aureus V8 protease and compared the resulting peptides in the same HPLC system. The histone genes have been identified in a wild-type C.elegans gene bank cloned in a cosmid vector. We are determining the linkage of histone plasmids to the six chromosomes of C.elegans as proposed in 5. Histone genes may be among already defined emb genes, e.g., the 10 emb genes that map in the middle of chromosome III[6].

1 von Ehrenstein, G. et al. (1979) In: N. LeDouarin (ed.), Cell Lineages, Stem Cells and Cell Determination, North Holland, Amsterdam, pp. 49-58. 2 Cassada, R. et al. (1981) Develop.Biol., in press. 3 Schierenberg, E. et al. (1980) Develop.Biol. 76, 141-159. 4 Meek, J.L. (1980) Proc.Nat.Acad.Sci.USA 77, 1632-1636. 6 Hirsh, D. et al. (1979) In: Axel, R., Maniatis, T., Fox, C.F., (eds.) Eukaryotic Gene Regulation, Academic Press, New York.

1103 ORGANIZATION AND EXPRESSION OF SOYBEAN LECTIN GENES. R.E. Swiderski, G. Hoschek, L.O. Vodkin, R.L. Fischer, and R.B. Goldberg. Department of Biology, UCLA, Los Angeles, CA, 90024. Lectin gene organization and expression were investigated in two soybean lines, one which accumulates seed lectin (Li+) and one which does not (Li-). The ultimate aim of this work is to use these lines in order to identify DNA sequences essential for lectin gene expression. An enriched lectin mRNA population was used to select a lectin cDNA clone (designated L-9) from a pBR322 library of Li+ embryo mRNAs. Hybridization of labeled L-9 DNA with excess Li+ and Li- DNAs demonstrated that both lines possessed 2-3 lectin genes. In addition, hybridization of ^{32}P-L-9 with Li+ and Li- DNAs which were digested with EcoRI, electrophoresed, and bound to nitrocellulose strips failed to detect any gross differences in lectin gene arrangement in the Li+ and Li- lines. To investigate the intragenic arrangement of lectin genes, lectin genomic clones (designated λL-9) were selected from a Charon 4 library of Li+ DNA sequences. Visualization of R-loops formed between lectin mRNA and λL-9 DNA failed to reveal any introns at the EM level. Lectin gene expression was compared in Li+ and Li- embryos by titrating a single-stranded, ^{3}H-L-9 probe with nuclear, cytoplasmic, and polysomal RNAs. The findings demonstrated that lectin mRNA comprised 0.6% of the Li+ mRNA but only 6 x 10^{-5}% of the Li- mRNA. No additional lectin gene transcripts were found in other Li- cellular compartments, suggesting that the Li- line has a transcriptional defect in lectin gene expression.

1104 MOLECULAR CLONING OF MUSCLE-REGULATED GENE SETS, Charles P. Ordahl, Temple University Medical School, Philadelphia, PA 19140.

The research in my laboratory involves using embryonic limb muscle development as a model system to study the role that differential gene expression plays in embryonic cell differentiation. Using recombinant DNA techniques we have isolated cDNA clones corresponding to distinct sets of genes which are developmentally regulated during muscle differentiation (muscle-regulated genes). One set (class A genes) is comprised of the familiar muscle-specific genes which code for muscle-specific proteins such as a-actin, muscle-creatine kinase, myoglobin, etc. The second set (class B genes) is comprised of genes regulated in an "embryo-specific" pattern in that they are transcribed at early stages of myogenesis when myoblasts predominate but are repressed in fully differentiated muscle fibers. Using these cDNA clones as hybridization probes, we have analyzed the appearance and accumulation of these muscle-regulated mRNA sequences during the course of differentiation of mature muscle from a population of pluripotent stem cells. The polypeptides encoded by several of the cloned mRNA sequences has been identified by positive selection/translation and included among the cloned messages are the sequences for a-actin and muscle-creatine kinase. The expression of the class A and class B genes appears to be under coordinate regulation in the embryonic muscle cell. In order to determine the molecular basis for such regulation, we are analyzing the sequence structure and organization of several class A and class B genes which have been isolated from a cloned genomic library. Data will be presented regarding the analysis of the mRNAs using the cDNA clones as well as the structure of the cloned genomic DNA segments.

1105 A "Heat Shock" Phenomenon in Higher Plants. Joe L. Key, F. Schöffl, C. Y. Lin and Y. M. Chen. University of Georgia, Athens, Georgia 30602. We are investigating the influence of several stress agents on the patterns of transcription/translation in plants. We report here on the influence of temperature shifts on RNA/protein synthesis in soybean seedlings. When seedling tissue is transferred from 28° to 40°, there is a rapid dissociation of polysomes into monosomes (15 min) with a gradual recovery of a low level of polysomes (10-15%). The synthesis of many 28° proteins is greatly depressed and a set of new proteins, as viewed on SDS gels, is induced during the first hr at 40°. A detailed analysis of the in vivo patterns of protein synthesis to a range of temperature regimes has been made. In vitro translation of poly(A)RNA isolated from 28° and 40° tissue indicates that the new proteins made in vivo are translated from mRNAs which accumulate during "heat shock" and that many 28° mRNAs persist at 40° while not being significantly translated in vivo. cDNA cloning and replica plate screening of the clones with 28° and 40° RNA confirms that the appearance of the new proteins at 40° results from the accumulation of their mRNAs during heat shock. These cloned cDNAs are being used to assess the kinetics of appearance and disappearance of "heat shock" mRNAs under a variety of physiological states, to study the organization of homologous genomic DNAs (soybean gene bank already available), and to assess whether other environmental stress agents induce common mRNAs for synthesis of a set of "stress proteins". The results reflect a phenomenon in plants analagous to the heat shock response in Drosophila and indicate that both transcriptional and translational controls function in effecting the new pattern of protein synthesis.

1106 DIFFERING SEQUENCE ORGANISATION AT THE BOUNDARIES OF THE TWO TYPES OF D. MELANOGASTER rDNA INSERTION. J. Ross Miller, Heli Roiha, Lesley Woods and David M. Glover, Cancer Research Campaign Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College, London SW7.
The two major types of D. melanogaster rDNA insertion occur at sites separated by 47 nucleotides in the 28S gene. The type I sequence insertion is found both in the X chromosome rDNA and within chromocentral heterochromatin, where we know it can occur in tandem arrays. We have sequenced the junctions of type I insertions in rDNA and the junctions of tandemly arrayed type I units. We conclude that a 9 base pair fragment is removed from an uninterrupted 28S gene and replaced by a type I insertion sequence, to generate a type I interrupted rDNA unit. Each type I insertion element in tandem array was found to have a small segment of rDNA sequence at each end, the left end differing from the right. These sequences are identical to those flanking the 9 base pair segment in the uninterrupted 28S gene. This suggests recombinational mechanisms are involved in this process. rDNA genes containing type II insertions are found on both the X and Y chromosomes. The type II insertions have a different arrangement at their junctions with rDNA. As a consequence of the insertion, a six base pair repeat is generated at the left hand junction. This sequence is not found at the right hand junction but instead a tract of 19dA residues occurs. These differences in the sequence arrangements of the two insertion types are reflected by different transcriptional patterns, implying a difference in function of the two types of interrupted rDNA unit.

1107 CHARACTERIZATION OF HUMAN ACTIN GENES, Joanne Engel, Peter Gunning, and Larry Kedes, Howard Hughes Medical Institute at Stanford Medical School, Stanford, CA. 94304.

We have isolated 12 different human genomic actin clones. Each of these clones is homologous to human actin mRNA as judged by its ability to hybridize specifically to a human mRNA, which, when translated in a reticulocyte lysate assay, directs the synthesis of a 42,000 dalton protein. This protein has been shown to be identical with actin by co-electrophoresis with authentic actin on 1D and 2D gels as well as by peptide mapping. Restriction enzyme analysis of these 12 clones demonstrates that they are derived from different, non-overlapping regions of the genome. Mapping of their coding regions suggests that several of the clones contain intervening sequences. Using these clones as probes to Southern blots of human DNA cleaved with various infrequently cutting restriction enzymes, we estimate that the human genome contains 20-30 actin coding fragments. No polymorphisms in actin gene containing fragments were detected when Southern blots of human DNA isolated from several different individuals were hybridized to these probes. Four of these clones have been further identified as coding for a beta-actin protein and 2 others for a gamma-actin protein by differential melting of alpha-, beta-, and gamma-actin mRNA from each of the human actin clones. Recently, we have prepared cDNA clones to alpha-, beta-, and gamma-actin mRNA and are using these probes to study the regulation of actin gene transcription during the switch from beta- and gamma-actin to alpha-actin synthesis when normal or dystrophic human muscle cells differentiate in vitro.

1108 MOLECULAR STRUCTURE OF A GLUE PROTEIN LOCUS IN DROSOPHILA, Steven K. Beckendorf, William McGinnis and Antony Shermoen, University of California, Berkeley, CA 94720

We have examined the size of the DNA region required for expression of a Drosophila gene. This region, termed the unit of expression, includes not only the structural gene but also any cis-acting sequences that modulate its activity. The locus we have chosen, Sgs-4, codes for one of the glue proteins secreted by larval Drosophila salivary glands. Cytological deficiencies have been identified that eliminate sequences on one side or the other of Sgs-4 without affecting its expression. The ends of these deficiences have been localized accurately with respect to restriction endonuclease sites in and near the locus. These endpoints limit the Sgs-4 structural gene and essential flanking sequences to a 16- to 19-kilobase region of the X chromosome.

Within this region, we have located three DNase I hypersensitive sites that are present in both embryonic and larval salivary gland nuclei and a fourth that is present only in the salivary gland nuclei. In some strains we find repetitive sequence elements at either side of the Sgs-4 region. These elements are involved in several of the chromosomal rearrangements that occur near Sgs-4.

1109 GENETIC AND MOLECULAR ANALYSIS OF A QUANTITATIVE VARIANT FOR A YOLK POLYPEPTIDE IN DROSOPHILA, Paul D. Shirk[1], Robert Kaschnitz[2] and John H. Postlethwait[1], [1]Department of Biology, University of Oregon, Eugene, OR 97403 and [2]Institute for Molecular Biology, Salzburg, Austria.

In adult female Drosophila melanogaster, synthesis of the three yolk polypeptides (YPs) by the fat body can be hormonally stimulated by either juvenile hormone or 20-hydroxyecdysone. The structural genes map to the X chromosome at 20.5 for Yp1 and Yp2 and 44 for Yp3. We have found several cis-acting variants which show quantitative changes for each of the YPs and are closely linked to the structural gene. These are therefore candidates for mutations at regulatory sites. D. melanogaster strain RI was found to lack YP3 and yet had normal quantities of YP1 and YP2. The RI quantitative variant has been analyzed to determine the level at which the mutation is manifested. Peptide digests of YP1 and YP2 from RI flies showed no fragments characteristic of YP3 which would suggest that YP3 RI is not a modification of YP3 causing it to co-migrate with YP1 or YP2. In vitro translation of total RI RNA in a reticulocyte lysate followed by precipitation with YP antiserum did not produce the 46,000 dalton precursor to YP3 but did produce the 47,000 dalton precursors for YP1 and YP2. Southern transfers of restricted DNA from RI, Oregon R and Canton-S probed with labeled cloned Yp3 showed all three strains to contain similar sized restriction fragments complimentary to Yp3, indicating that the majority, if not all, of the YP3 structural gene to be present in the RI genome. Northern transfers of poly(A) RNA extracted from RI also showed the presence of RNAs complimentary to Yp3 probe. All of these data suggest that the mutation in RI does not allow for the translation of the Yp3 transcriptional products.

1110 INTEGRATION AND EXPRESSION OF MODIFIED ALCOHOL DEHYDROGENASE GENES IN THE YEAST SACCHAROMYCES CEREVISIA, Ted Young, Department of Biochemistry, University of Washington, Seattle, WA 98195

Yeast contain multiple forms of the enzyme alcohol dehydrogenase. These homologous enzymes are encoded by nuclear genes which are regulated transcriptionally. In order to distinguish the mRNAs transcribed from the two homologous genes coding for the major isozymes, we have used the previously cloned strucutural genes to construct mutant genes containing internal deletions and insertions. The mutant genes have been introduced into yeast by transformation using the integrating yeast vector YIp5.

1111 MULTIPLE CHROMOSOMAL GENES FOR HUMAN LEUKOCYTE INTERFERON, Shuichiro Maeda, Warren P. Levy, Russell McCandliss, and Sidney Pestka, Roche Institute of Molecular Biology, Nutley, NJ 07110

The human interferons produced in leukocytes (HuIFL) are a class of homologous proteins with potent antiviral activity. They are produced when leukocytes are exposed to interferon inducers such as viruses. In order to study the molecular mechanism of interferon biosynthesis, we isolated a bacterial plasmid containing HuIFL cDNA sequence derived from a human myeloblastoid cell line (S. Maeda et al. (1980) Proc. Natl. Acad. Sci. U.S.A., in press). The cDNA contains most of the structural gene of HuIFL and was used successfully as a probe for finding a full-length copy of the HuIFL mRNA sequence for production of HuIFL in E. coli (D.V. Goeddel et al. (1980) Nature 287, 411). With the use of cDNA as a probe, we screened a human gene bank (R.M. Lawn et al. (1978) Cell 15, 1157) for the presence of chromosomal HuIFL gene(s) by in situ plaque hybridization. Twelve IFL cDNA-related sequences were isolated from 500,000 plaques. Preliminary restriction endonuclease mapping of the twelve genomic clones showed that two inserts are identical but the others are distinct. Genomic DNA fragments of two of the clones have been partially sequenced. One clone contains a single IFL-related sequence, while the other contains two IFL-related sequences. The three homologous genomic sequences do not appear to have intervening sequences, and are distinct from one another. The cDNA sequence used as the probe for screening is also distinct from the three genomic sequences. The data suggest the presence of multiple chromosomal genes for HuIFL. The sequences of the other IFL-related genomic DNAs are currently being analyzed.

1112 MOLECULAR ORGANIZATION OF THE 5S rRNA GENES OF MAIZE. Irwin Rubenstein, Peter Mascia and Daniel E. Geraghty, University of Minnesota, St.Paul, MN. 55108

The organization of the 5S rRNA genes of maize has been investigated by restriction enzyme analysis, genome cloning and nucleotide sequence analysis. The enzyme Taq I defines the repeat, generating a fragment of about 320 bases. The enzyme Bam HI has a frequent site in the 5S genes. Complete Bam HI digests exhibit a ladder of bands starting at 320 nucleotides. Multimers up to 20 times this size, however, are clearly present. The enzyme Mbo I, which recognizes the internal tetranucleotide of the Bam HI site, has two sites in the 5S repeat. The enzymes Hpa II and Msp I, which recognize the sequence CCGG, are inhibited by methylation at the internal and external C, respectively. Hpa II does not seem to have a site in the 5S region. Msp I, on the other hand, generates a ladder of bands, initiated at the dimer level, in register with Bam HI ladder. These results suggest that the internal C is highly modified and that some modification is present that inhibits recognition at a large number of Msp I sites. Genomic segments representing the 5S rRNA genes have been isolated from a lambda charion 4 library of Bam HI fragmented maize DNA. The cloned genomic segments fall into different classes as characterized by the number of repeats that are in the clone and the availability of restriction sites. Most of the clones contain more than one 320 bp repeat. Some of these clones contain multiple repeats cleavable by Bam HI; others contain repeats not cleavable by Bam HI. Nucleotide sequence analysis has shown that the Bam HI site, when present, occurs within the structural region of the gene. These results suggest that the DNA sequence of the 5S repeat contains sites at which both nucleotide sequence divergence and base modification has occurred.

1113 CLONING AND MAPPING THE COORDINATELY ACTIVATED MUSCLE GENES OF DROSOPHILA, S.I. Bernstein, A.W. Glenn and C.P. Emerson, Jr., Biology Dept., Univ. of Virginia, Charlottesville, VA 22901. and J. J. Donady, Biology Dept., Wesleyan Univ. Middletown, CT 06457.

Drosophila muscle cells, at the fusion stage of myogenesis, coordinately accumulate translatable mRNAs which code for muscle specific polypeptides (Dev. Biol. 79: 388, 1980). We are isolating the genes that code for these proteins in order to compare their structure and determine if they are clustered in the genome. Our strategy involves: 1) analyzing cDNA clones (prepared from mature muscle poly(A)+RNA) by mRNA-selection hybridization and subsequent in vitro translation and 2) probing a Drosophila genomic library using identified cDNA clones and heterologous contractile protein gene probes. cDNA clone VG8 selects mRNA which codes for two fusion-stage specific muscle proteins. Three Drosophila genomic clones homologous to VG8 also hybridize to mRNA coding for both of these proteins, while a fourth only hybridizes to mRNA coding for one of the two proteins. In situ hybridization mapping indicates that these genes are located at one site in the 87 region of chromosome 3 and thus appear to form a cluster of structurally related genes which are coordinately expressed during myogenesis. Since two actin genes map near the site of VG8 (Cell 19:365, 1980), we are interested in determing if this chromosomal region contains many coordinately activated muscle genes. We are therefore isolating and mapping Drosophila genes coding for troponin-I, troponin-C and myosin heavy chain using heterologous probes which crossreact with Drosophila genomic DNA.

1114 cDNA CLONING OF mRNAs COORDINATELY REGULATED DURING MYOGENESIS, Kenneth E.M. Hastings and Charles P. Emerson Jr. Univ. of Virginia, Charlottesville, VA, 22901.

We are interested in how eucaryotic cells coordinate the expression of functional sets of structurally unrelated proteins. A good model system is presented by the coordinate activation of contractile protein synthesis in differentiating cultures of embryonic quail myoblasts. Taking the recombinant DNA approach we have prepared a shotgun cDNA clone library from polyA$^+$ RNA extracted from differentiated myofiber cultures. From this library we selected in a colony hybridization screen 28 clones containing sequences more abundant in myofiber RNA than in myoblast RNA. Among this set we identified by hybridization-translation and DNA sequencing cloned copies of mRNAs encoding the contractile proteins α actin, α tropomyosin, myosin light chain 2, troponin C, and troponin I. These cDNA clones were used as labeled sequence probes in RNA gel transfer hybridization analyses. The results directly demonstrate a ten-fold or greater increase in the relative abundance of this set of mRNAs during myogenesis. This coordinate accumulation of contractile protein mRNAs is presumably the immediate cause of the coordinate activation of contractile protein synthesis during myogenesis. Other results from this laboratory indicate that contractile protein mRNA accumulation is regulated at the nuclear, rather than cytoplasmic, level. Whether this nuclear regulation consists of transcriptional control, or involves processing of nuclear RNA transcripts, is not known. We are currently using the cDNA clones as sequence probes in experiments designed to answer this question.

1115 AN INTERVENING SEQUENCE WITH TRANSPOSON-LIKE PROPERTIES WITHIN A SEA URCHIN HISTONE GENE ORPHON, Rob Maxson, Geoff Childs, Joel Weinthal and Larry Kedes, Howard Hughes Medical Institute at Stanford Medical School, Stanford, CA 94305

We have discovered a remarkable sequence interruption in a protein coding sea urchin gene that shares features with both an intervening sequence (intron) and a transposable genetic element. Animal genomes contain a number of solitary, dispersed members of clustered, tandemly repetitive gene families. We have cloned several such elements, termed orphons, from the sea urchin histone multi gene family and have examined their sequence organization. One clone contains a single H2B histone gene which, surprisingly, is interrupted in its protein coding portion by a 2.9 kilobase intervening sequence. The gene is otherwise very similar in sequence to its congener in the major repeat unit. This is the first known instance of an IVS in a histone gene. DNA sequencing at the IVS-protein coding junctions has shown that splicing could occur at one of several sites without shifting the reading frame of the protein. While there is no obvious structural feature of the junctions that would preclude the processing of a primary transcript and the proper translation of the resultant mRNA, we have no direct evidence as yet that this particular gene is expressed in vivo. DNA sequencing has shown the intervening structure is remarkly similar to a bacterial transposon. The termini consist of 300 bp long direct repeat sequences. The termini are themselves 150 bp long inverted repeats. There are 8 bp direct repeats in the recipient DNA at the insertion site. Genomic Southern transfer experiments have shown that the IVS is a member of a repetitive sequence family containing at least 200 members which exhibit extensive sequence polymorphism in 6 individuals examined. The IVS may therefore be a member of a family of transposable elements.

1116 THE ACTIN GENES OF DROSOPHILA: PROTEIN CODING REGIONS ARE HIGHLY CONSERVED BUT INTRON POSITIONS ARE NOT. Eric A. Fyrberg, Beverley J. Bond, N. Davis Hershey, Katharine S. Mixter and Norman Davidson, California Institute of Technology, Pasadena, California, 91125

We have isolated the entire set of six closely related Drosophila actin genes using recombinant DNA methodology and characterized the structures of the respective protein coding regions by gene mapping techniques and by nucleotide sequencing of selected portions. Structural comparisons of these genes have resulted in several unexpected findings. Most striking is the nonconservation of the positions of intervening sequences within the protein encoding regions of these genes. One of the Drosophila actin genes, DmA4, is split within a glycine codon at position 13; none of the remaining five genes is interrupted in the analogous position. Another gene, DmA6, is split within a glycine codon at position 307; at least two of the Drosophila actin genes are not split in the analogous position. Additionally, none of the Drosophila actin genes is split within codon four, where the yeast actin gene is interrupted. The six Drosophila actin genes encode several different proteins, but the amino acid sequence of each is very similar to that of vertebrate cytoplasmic actins. None of the genes encodes a protein comparable in primary sequence to vertebrate skeletal muscle actin. Surprisingly, in each of these derived actin amino acid sequences the initiator methionine is directly followed by a cysteine residue, which in turn precedes the string of three acidic amono acids characteristic of the amino termini of vertebrate actins. Preliminary studies of the tissue specific expression of the several actin genes will be presented.

1117 A Structural Comparison of the Heat Shock Genes of Drosophila, Elizabeth A. Craig and Thomas D. Ingolia, Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706

Brief exposure of Drosophila melanogaster larvae or tissue culture cells to elevated temperature or a variety of drugs results in the activation of a small number of genes (1). We have studied the structure of the genes encoding five heat shock proteins, the major heat shock protein, hsp 70, (which is encoded a two chromosomal locations, 87A and 87C) and the genes for the four smaller heat shock proteins, hsp 27, 26, 23 and 22, (found at 67B). The primary sequence of the 5' flanking regions of these five genes have been determined. One novel feature of these five genes includes a region of homology beginning near the proposed site of initiation of transcription and extending about 18 bp into the 5' noncoding region of the genes. Also, the leaders of these five heat shock genes are long, from 111 to 253 bases in length, as well as unusually A rich, from 46% to 51%A. In addition, 195+12 bp from the proposed site of initiation of transcription, each of the four smaller genes contains the sequence ACTTTNA. A related sequence AATTTCT is found 182 bp from the proposed transcription initiation point of the hsp70 gene. There are multiple copies of the hsp70 gene in the haploid genome. Two types of hsp70 genes have been identified by restriction enzyme and primary sequence analysis (1); one gene type is found at 87A, the other at 87C. We have identified a third type of hsp70 gene which differs significantly from genes previously identified. This gene resides at 87A, and is more closely related to the previously identified 87A type, than the 87C type.

1. For review see Ashburner and Bonner (1980) Cell 17, 241-254

1118 TOWARDS A MOLECULAR ANALYSIS OF A LOCUS INVOLVED IN DEVELOPMENTAL COMPARTMENTALIZATION, Douglass J. Forbes, Jerry M. Kuner, Mikiye Nakanishi, Tom Kornberg, and Patrick H. O'Farrell, University of California at San Francisco, S.F., CA 94143

The engrailed locus of Drosophila is involved in partioning the embryo into developmentally autonomous units or compartments. We have obtained DNA fragments derived from the engrailed locus, the analysis of which will hopefully lead to a more detailed understanding of the processes of developmental compartmentalization. The engrailed locus, originally defined by the en^1 mutation, has been localized to band 48A of the cytological map (Kornberg,1980). Using met_2 tRNA (provided by Robert Elder) as a probe, we isolated from a bank of Drosophila clones several carrying met_2 tRNA genes. In situ hybridization localized two of these clones to 48B. Southern analysis showed that one of the tRNA-containing clones overlaps a deletion of the engrailed locus, en^{x31}(Kornberg, 1980). A bank of cloned DNA fragments was constructed from this mutant strain and a clone carrying DNA sequences from both sides of the deletion isolated. Since the en^{x31} deletion fails to complement engrailed mutations, the engrailed gene should lie within the several hundred kilobases of DNA removed by the deletion. We have isolated a series of overlapping clones from both phage and cosmid banks (provided to us by S. Artavanis-Tsakonas). At this time, we have isolated 100kb of cloned sequences extending from 48B toward 48A and 150kb extending into 48A from the opposite side of the band. We are using Southern analysis to locate within this region the positions of chromosomal breakpoints associated with mutation of the engrailed locus. Analysis of the cloned DNA fragments containing the engrailed gene should allow identification of the gene product and can be used to test models predicting specific spatial and temporal patterns of expression.

1119 HUMAN RIBOSOMAL RNA GENE SPACER SEQUENCES ARE FOUND INTERSPERSED ELSEWHERE IN THE GENOME, Russell G. Higuchi, Howard D. Stang, Jeffrey K. Browne and Winston A. Salser, University of Calif.at Los Angeles, Los Angeles, Ca. 90024.

19.4 kb of the approximately 43 kb human rDNA repeat extending from the 18s rRNA gene into the non-transcribed spacer was obtained in cloned form and mapped with restriction endonucleases. A 7 kb region of the non-transcribed spacer DNA shared in common between 5 independently isolated clones was subjected to comparative restriction digests in order to assess the level of sequence homology between different repeats of the spacer. It was estimated that sequences among the 5 different spacer isolates varied, if all the observed differences are assumed due to point mutation, by no more than 1.0%. Analysis of the Hae II restriction fragments from within this same 7 kb region gave evidence of sequences carried not only within the tandem repeats of the gene cluster but interspersed elsewhere in the genome as well.

1120 THE ACTIN GENES OF CAENORHABDITIS ELEGANS, James Files, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309

We have cloned the actin genes of C. elegans and are investigating their structure and organization. Southern blots of C. elegans DNA, probed with a Dictyostelium cDNA actin clone, show four actin-coding or actin-like sequences. All four sequences have been obtained as recombinant DNA's from a lambda Charon 10 clone bank of C. elegans DNA. Three of the four actin sequences are clustered within a 12,000 base pair region. We have obtained this entire cluster on several single lambda clones. Restriction site differences among the four actin sequences indicate that they are not identical. Further, multiple actins have been observed on two-dimensional gels of ^{35}S-labeled C. elegans proteins. DNA sequencing of the 5' end of each sequence is in progress to demonstrate differences among them, and to identify muscle verses cytoplasmic actin sequences. At least one actin sequence contains an intervening sequence, near the 5' end of the coding region.

We are mapping the actin genes genetically to be able to accompany biochemical analyses with a genetic analysis of actin expression. Mapping is possible because two interbreeding strains of C. elegans contain restriction site differences due to a 1600 base pair "insertion" adjacent to the actin gene cluster in one of the strains. We have mapped this difference to linkage group five, and more detailed mapping is in progress. Restriction site differences between strains provides a general method in C. elegans for mapping genes for which mutants do not exist.

1121 A GENETIC AND PHYSICAL ANALYSIS OF THE CLONED QA GENE CLUSTER FROM NEUROSPORA CRASSA. Sidney R. Kushner, Michael Schweizer, Mary E. Case, Virginia Patel, Christine C. Dykstra, and Norman H. Giles, University of Georgia, Athens, Georgia 30602

The catabolism of quinic acid in Neurospora crassa is a positively regulated pathway consisting of at least three enzymes(qa-2, catabolic dehydroquinase; qa-4, dehydroshikimate dehydratase; and qa-3, quinate dehydrogenase) and a regulatory protein(qa-1). This tightly linked gene cluster offers unique opportunities for the study of gene regulation at the molecular level. Follwoing earlier identification of the qa-2 gene by means of its functional expression in E. coli, the entire qa gene cluster(qa-2, qa-4, qa-3, qa-1) has been cloned as a 36 kb series of contiguous EcoRI fragments in the cosmid pHC79. The presence of the qa-4, qa-3 and qa-1 loci have been detected by means of transformation back into N. crassa. Preliminary evidence suggests that the genes are physically in very close proximity. Additionally, analysis of N. crassa mRNA with radioactively labeled DNA probes indicates the presence of 5 distinct quinic acid inducible coding sequences. Several constitutively synthesized genes are also present on the cloned fragments. Work is currently in progress to determine the precise location of the various coding sequences.(This work was supported in part by grants from the National Science Foundation and the National Institutes of General Medical Sciences.)

1122 STRUCTURE AND REGULATION OF DROSOPHILA TRNA GENES. Melvin Silberklang, Hans A. Hosbach & Brian J. McCarthy. Department of Biochemistry & Biophysics, University of California, San Francisco, Ca 94143

A large number of Drosophila tRNA gene clones was isolated from several plasmid clone banks by screening with crude tRNA probe; from these, 13 distinct clones have been characterized by restriction mapping. Two are <2 kb in size, while the others are 9 kb or larger. By the criterion of in situ hybridization of complete clones to larval polytene chromosomes, the tRNA genes fall into 3 categories: 8 are homologous to unique sites; 2 have several sites of homology and 3 carry repeated sequence elements with multiple (>30) homologous loci. On the other hand, use of short tRNA gene region-specific probes, prepared from some larger clones, reveals additional homologous loci for these genes in Southern blots of restricted genomic DNA. For one of the small clones, TR13, we have isolated overlapping clones that extend in opposite directions and together encompass a cluster of 5 identical glutamate tRNA genes, as determined by DNA sequence analysis (1). Further DNA sequence studies are in progress with other clones to investigate the general features of Drosophila tRNA gene structure and organization. In addition one primary focus of this project is to use the phenomenon of DNase I hypersensitivity of transcriptionally active genes and other techniques to address the complex question of possible differential developmental regulation of tRNA genes at the transcriptional level.

1. Hosbach, H., Silberklang, M. & McCarthy, B.J. (1980). Evolution of a Drosophila melanogaster glutamate tRNA gene cluster. Cell 21, 169-178.

1123 THE LARVAL CUTICLE GENES OF DROSOPHILA. Michael Snyder, Michael Hunkapiller, Leroy Hood, and Norman Davidson, California Institute of Technology, Pasadena, California, 91125.

We are studying the sequence organization and expression of the larval cuticle genes of Drosophila. Five major cuticle proteins are synthesized and secreted by late larvae in order to provide a protective pupal coat. Using recombinant DNA techniques a 39 kb DNA segment of the Drosophila genome which codes for several larval cuticle genes has been isolated. This segment has been localized to 44D and encodes five genes all of which are expressed in the same tissue and time of Drosophila development. Four of the genes are clustered within 7.9 kb of DNA and are abundantly expressed in late third instar poly A^+ RNA. A fifth gene lies 8 kb away from this gene cluster and is expressed at a much lower level. Three of the four abundantly expressed genes have been shown to code for larval cuticle proteins by positive selection and translation of RNA, two-dimensional gel analysis and immunoprecipitations of translated polypeptides. Protein and DNA sequencing studies thus far have confirmed these results for two of these genes. Moreover, these sequence comparisons have shown that the three cuticle proteins studied are greater than 50% homologous in amino acid sequence over the portions sequenced. Thus, the cuticle genes encoded at 44D represent members of a family of genes of common ancestry which share the same pattern of developmental expression and reside in a small segment of the Drosophila genome.

1124 A PUTATIVE LATE HISTONE GENE IN THE SEA URCHIN STRONGYLOCENTROTUS PURPURATUS, A.R. Lohe and M. Grunstein, Molecular Biology Institute, ULCA, Los Angeles, CA 90024.

Soon after fertilization in the sea urchin S. purpuratus, the main early group of histone genes (300-500 copies) is activated to produce H1, H2A, H2B, H3 and H4 mRNAs. All five genes are closely linked on a 6.5 kb DNA repeat unit. At mid-blastula (12 hours of development) a minor, late class of histone mRNAs appears on polysomes. Our objective is to isolate recombinant DNA clones containing the late histone genes and compare their structure to that of the early class.

A putative late clone was isolated from a lambda Charon 4 library of S. purpuratus DNA. This clone forms RNAase stable hybrids only with late histone H4 mRNA. A 4.5 kb subclone contains the H4 gene as well as highly repeated DNA sequences. These data show that the putative late histone H4 gene is not closely linked to the H1, H2A, H2B or H3 late genes, and its organization is unlike that of the early histone genes.

1125 STRUCTURE AND EXPRESSION OF A CHICK GENE CODING FOR U1 RNA. Dennis R. Roop, Paula Kristo, William E. Stumph, Ming-Jer Tsai and Bert O'Malley. Baylor College of Medicine, Houston, Texas, 77030.

Recently, several laboratories have proposed that small nuclear RNAs may be involved in the splicing of intervening sequences from messenger RNA precursors. Some of these proposals are based on the observation that there is considerable sequence homology between the 5' end of rat U1a RNA, a major small nuclear RNA, and the consensus sequence of a number of splice junctions. Our laboratory has isolated and sequenced the genes of two chicken egg-white proteins, ovalbumin and ovomucoid. Both of these genes contain seven intervening sequences. Therefore, we were interested in comparing the sequence of these splice junctions with that of chicken U1 RNA. We were also interested in determining the structure and organization of the U1 genomic gene and studying its expression since this might provide information concerning the function of this RNA in the cell. We have prepared a cDNA clone to chick U1 RNA and determined its sequence. Using the U1 DNA probe, we have isolated a genomic fragment containing sequences complementary to chicken U1 RNA. The sequence of this genomic U1 gene is completely homologous with and colinear with that of chicken U1 RNA. Therefore, this gene does not contain intervening sequences. This gene is part of a multigene family ($\sim$10 copies) and these loci do not appear to be clustered. Sequences complementary to other small nuclear RNAs are not present within the genomic fragment containing the U1 gene. We have determined that U1 RNA is synthesized by polymerase II, however, a "Hogness box" is not present up stream from its "cap site". Also, the synthesis of U1 RNA in oviduct nuclei during different states of hormonal induction appears to be constitutive.

1126 A GENETIC ANALYSIS OF HISTONE H2B PROTEINS IN THE YEAST SACCHAROMYCES CEREVISIAE, Rykowski, M., Wallis, J., Choe, J. and Grunstein, M., Molecular Biology Institute, UCLA, Los Angeles, CA 90024. Histone variants are developmentally controlled in the sea urchin. In order to investigate the possible function of histone variants we turned to a genetic approach utilizing Saccharomyces cerevisiae. Yeast, like other eukaryotes investigated, has multiple histone genes. S. cerevisiae has two unlinked copies of the H2B gene. We have sequenced both of these genes and show that they code for different amino acid sequences. These differences, which represent four amino acid changes, are all present at the amino terminus.

A second aspect of our work has involved creating in vitro frameshift mutations in cloned copies of each of these genes. This was done by filling in HindIII sticky ends in sequences coding for the conserved portions of the H2B proteins. These mutated DNA molecules were separately used to transform yeast cells and then used to replace the wild type homologous copy of each gene.

Our results to date show that the mutation of one of these genes (H2B 2) has no effect on either vegetative growth or sporulation. A similar mutation in the H2B 1 gene also results in a viable cell which grows at a somewhat slower rate than normal. Experiments are currently in progress to determine a) the effect on sporulation of the h2B 1 mutation and b) the effect of the total lack of H2B protein (resulting from the h2B 1, h2B 2 mutation) on cell viability.

Despite the ubiquitous nature of histones in eukaryotes, the function of these proteins is still not known. These experiments provide an approach for the genetic dissection of chromatin function.

1127 THE USE OF CLONED DNA PROBES FOR THE ANALYSIS OF EXPRESSION OF SPECIFIC GENES DURING MYOGENESIS, U. Nudel, M. Shani, D. Katcoff, D. Zevin-Sonkin, Y. Carmon and D. Yaffe. Department of Cell Biology, Weizmann Institute of Science, Rehovot, Israel

Recombinant plasmids containing cDNA sequences of rat myosin heavy chain, myosin light chain 2 and skeletal muscle actin were contructed. The plasmids were used for investigations along the following lines:

1. Isolation from a rat genomic DNA library, of recombinant phages containing the corresponding genes. The structure of these genes was studied using the techniques of restriction enzymes analysis, DNA sequencing, heteroduplex mapping and R-looping analysis. Clones containing DNA sequences of eight different actin genes, 3 myosin heavy chain genes and 1 myosin light chain 2 gene were identified and partially characterized.
2. Measurement of activation of specific genes and accumulation of their mRNAs during myogenesis.
3. Comparison of sequence homology of the coding part versus the 3'- untranslated region of various actin mRNAs.

1128 CHROMOSOMAL "WALKING" IN THE CHORION GENE LOCUS OF BOMBYX MORI, Thomas H. Eickbush and Fotis C. Kafatos, Harvard University, Cambridge, MA 02138

As a terminal step in their differentiation, the follicle cells surrounding each oocyte of the silkmoth, Bombyx mori, synthesize and secrete over 100 chorion (eggshell) proteins. Genetic studies have indicated that the structural genes encoding these proteins are localized in a single chromosome within a few map units (Goldsmith,M.R. and Clermont-Rattner,E. [1979] Genetics 92:1173). In view of the clustered arrangement of the chorion genes we have undertaken a chromosomal DNA "walk" at this locus in order to determine the detailed organization and evolution of the chorion multigene family. Facilitated by the location of the chorion genes at less than 15 kb. intervals; the difficulties of finding overlapping clones in a genome with widely dispersed repetitive DNA ("Xenopus pattern"), and the time-consuming process of screening large numbers of clones at each "step", were largely circumvented by using for the walk genomic sublibraries enriched for chorion genes. Such sublibraries were generated by screening total genomic libraries with either total or specific chorion mRNA probes and the isolation of all positive clones. As an initial goal in the walk all 14 genes have been isolated which correspond to a set of homologous proteins, termed the 1911 subfamily. Eleven of these 1911 genes are localized within a 115 kb. DNA region while the remaining 3 are clustered in a separate 25kb. segment. Each of the 1911 genes was found to be adjacent to a copy of a second non-homologous chorion gene, 2574. Together the 2574 genes comprise a second chorion gene subfamily. Analysis of the DNA throughout these two regions suggests that the 1911 and 2574 genes have evolved together as part of a larger unit, and that copies of this unit are positioned at 4-14 kb. intervals in a continuum of repetitive DNA.

1129 ORGANIZATION OF EARLY AND LATE EMBRYO HISTONE GENES IN SEA URCHIN, D. Holmes, C. Bush and B. Orris, SUNY Albany, N.Y. 12222.

Genomic DNA enriched for histone genes, plasmids pSp2, pSp17 and various subclones containing the 5 standard early histone genes, and several charon and plasmid clones containing variant histone repeats have been hybridized with early embryo (8 hr) purified histone mRNA and late embryo (40 hr) 9-14S RNA and visualized in the electron microscope after R-looping or gene-32 spreading. In addition Southern and Northern blots of several of the above components have been carried out. Four conclusions will be documented. (1) There are several different but related minor families of contiguous early histone repeat. (2) There are at least 2 classes of the early H2B gene that share homology in the 3' two thirds of the coding sequence but have diverged in the 5' one third of the coding sequence. This may be related to the different functions of the C and N terminals of the H2B protein in the nucleosome. (3) Some late embryo histone gene transcripts are considerably larger than their early embryo counterparts and in some cases the location of the extra RNA has been mapped relative to the coding sequence. (4) There are major regions of conserved homology between the early and late embryo histone genes that may reflect conservation of functional domains between the two classes of histone proteins.

1130 ACTIN GENES OF DROSOPHILA MELANOGASTER, Ursula Rdest, Federico Sánchez, Brian J. McCarthy, University of California, San Francisco, CA 94143, and Sara L. Tobin, University of California, Berkeley, CA 94720.

The actin genes of Drosophila melanogaster are the members of a dispersed multigene family. There appear to be six genes, one at each of six chromosomal loci: 5C (X); 42A and 57B (2R); 79B (3L); and 87E and 88F (3R). We are studying Canton S genome recombinant plasmids which represent each of these genes. The cytological locus of each of these plasmids has been established by in situ hybridization to D. melanogaster polytene chromosomes. Physical maps localizing the putative protein coding region of each gene within the D. melanogaster DNA insert have been constructed. We are studying the molecular weight of the mRNA transcript of each of these genes and the isoelectric form of actin (actin I,II or III) which is synthesized by each. We are emphasizing the sequence analysis of the coding region of two of the plasmids (79B and 88F) and we can show that both genes carry an AvaI restriction endonucease site at amino acid 100. In both cases an intervening sequence starts at amino acid 307 which is 362 base pairs long in 79B and 60 base pairs in 88F. We have evidence that both genes code for the larval muscle specific actin (actin I).

1131 ACTIN GENES IN DICTYOSTELIUM, M. McKeown and R. A. Firtel, Dept. of Biology, B-022, University of California, San Diego, La Jolla, CA 92093

We have been examining the 17-member multigene family for actin in Dictyostelium. We have cloned and analyzed 10 of these genes and cDNA representing at least two more genes. We have been able to determine the 5' end of RNAs derived from different genes and the relative level of these RNAs during development by S_1 nuclease mapping. Four of five cloned genes examined are represented at levels of >1% of actin RNA. At least 2-3 other genes are represented as cDNA clones and are therefore also expressed. Not all of the genes are expressed at equal levels. The level of RNA from some of those genes expressed as >20% of the actin RNA in vegetative cells changes substantially during development. The one gene not shown to be expressed is believed to be a pseudogene based on our failure to detect its mRNA and its unusual nucleotide sequence. None of the genes examined contain intervening sequences. The 5' ends of the expressed genes are not alike but do contain TATAA boxes, T rich regions, and the start of transcription in similar places. Two different sizes of mRNA are known, differing by about 100 b at the 3' end. Three families of 3' ends have been found. One gene is now known which codes for the shorter mRNA. Its 3' untranslated region is 43-45 b long and there is no transcribed poly(A). The two other classes represent the longer mRNA and contain a transcribed poly(A) plus the post transcriptionally added poly(A). Of the genes known to be transcribed, only one, represented at <5% of actin RNA, has a variant amino acid sequence.

1132 A 5S rRNA CLONE FROM Balb/C MOUSE DNA, Babette D. Coté, Elsebet Lund and James E. Dahlberg, University of Wisconsin, Madison, Wisconsin 53706

We have isolated a 13.2 kb Eco RI fragment of Balb/C mouse DNA which is capable of specifically hybridizing 5S rRNA. The fragment was isolated from a genomic library of partial Eco R1 fragments cloned into λ ch 4A.

Only 5S rRNA and 7S RNA hybridize to this fragment, when probed with a mixture of mouse small RNAs. The 7S RNA presumably hybridizes to middle repetitive sequences on the DNA (such as B-1 or Alu-type sequences).

Restriction mapping of the 13.2 kb Eco R1 fragment indicates that the 5S sequence is on a 1.6 kb Kpn fragment separated by at least 4.9 kb from any other 5S or tRNA hybridizing sequences. This result shows that the 5S sequence is not highly repeated, but we cannot rule out the possibility of a short tandem repeat within the 1.6 kb fragment.

We are currently doing transcription and sequencing studies on this DNA to determine whether it is an authentic 5S rRNA gene.

Methods, Chromatin and Transcription

1133 ISOLATION OF HUMAN α1-ANTITRYPSIN mRNA FROM LIVER TISSUE FOR MOLECULAR CLONING, John T. Wilson, Dan P. Kestler, Jim Travis, Fred Garver, Lois B. Wilson, Medical College of Georgia, Augusta, Georgia 30912

Studies aimed towards the isolation of unique genes known to be involved in any one of the various inborn errors of metabolism have both scientific and clinical importance. Scientifically, those studies should provide additional information on gene structure and regulation as well as provide reagents necessory for gene transfer. Clinically, such studies should facilitate replacement therapy. Here we will provide evidence for having isolated (enriched for) human α1-antitrypsin (α1-AT) mRNA through immunoprecipitation of polysomes. These procedures have allowed us to isolate an mRNA fraction which produces a single major new protein product following in vitro translation. However, that product is slightly larger in molecular weight than normal α1-AT yet has the antigenic properties of α1-AT. We interpret these results as evidence toward the existence of a preprotein in that system. In addition, restriction enzyme digest of cDNA produced from our isolated mRNA fraction provides us with a cleavage pattern which can be utilized as a means for colony selection following molecular cloning. Since α1-AT mRNA probably represents a minor component of total liver mRNA, these studies demonstrate the feasibility of using such techniques for the isolation of many unique genes.

1134 DEFINITION OF THE 5' FLANKING SEQUENCES REQUIRED FOR SPECIFIC INITIATION OF OVALBUMIN GENE TRANSCRIPTION IN VITRO. M.-J. Tsai, S.Y. Tsai, T. Schulz, S. Woo and B.W. O'Malley. Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

An in vitro system has been used to study the initiation of transcription of the ovalbumin gene. The DNA template was a cloned ovalbumin gene fragment that contained 5' flanking sequences, and 393 nucleotides of gene sequence. A HeLa cell crude extract was used as the source of RNA polymerase and initiation factors. Correct initiation was judged by the size of RNA product, by S1 mapping and by the sizes of transcription products generated from ovalbumin DNA templates truncated at 3' end. A series of deletion mutants were constructed by trimming 5' flanking sequences of the ovalbumin DNA template using exonuclease III and S1 nuclease. The DNAs generated were then recloned in pBR322 and used as templates to determine which sequences were necessary for initiation of transcription. Specific initiation of the ovalbumin gene was unaffected by deletion of all but 61 nucleotides of the 5' flanking sequences. However, specific initiation was abolished by deletion of all but 26 nucleotides of the 5' flanking sequences. Thus, a region between 61 and 26 nucleotides upstream from the cap site, which includes the Hogness box (TATATAT) at position 32-26, is essential for the correct initiation of the ovalbumin gene. Nevertheless, natural DNA fragments containing false Hogness boxes which are not normally located in its immediate 5' flanking region of ovalbumin gene did not serve as promoters for initiation of transcription. Recently, we have generated 3' deletion mutant and mutants with single base change at the TATA box. Analysis of these mutants will be discussed.

1135 INVERTED REPEATS ADOPT HAIRPIN LOOP STRUCTURES UNDER TORTIONAL CONSTRAINT, David M.J. Lilley, Searle Research Laboratories, Lane End Road, High Wycombe, U.K.

Five inverted repeats have been identified (Proc. Nat. Acad. Sci. USA, In Press) which adopt hairpin loop conformations in underwound closed circles. The single strand specific nuclease S1 cleaves each repeat at the center, and fine mapping of the cleavages indicates maximal attack at the loop center, flanked by minor cleavages at one nucleotide on either side. The inverted repeats vary in size between stem sizes of 13 to 9 nucleotides with loops of between 2 to 6 nucleotides. ColE1 contains a powerful S1 site of sequence

A Sau3AI, EcoRI fragment containing this repeat has been cloned into pBR322 whereupon it readopts its hairpin conformation, and overpowers the smaller repeats present in pBR322. Thus the property of hairpin formation is local, transmissible, hierarchical and cis-dominant. Direct competition between repeats in natural and cloned DNA enables the following conformational deductions: Limited A-C, G-T base pairing is permissible but either A-G, C-T or loop-outs are highly destabilising.

This is the first demonstration of hairpin loop (or possible 4-stranded DNA) formation in ds-DNA. The sequences could clearly affect the conformation of nearby genes, if they constitute a single supercoiled unit or domain, and are of proven recognisability by at least one enzyme. The studies have been extended to functional regions of papova virus genomes.

1136 HIGH RESOLUTION TWO-DIMENSIONAL RESTRICTION ANALYSIS OF COMPLEX GENOMES, Steven S. Smith and C.A. Thomas, Jr., Scripps Clinic and Research Foundation, La Jolla, CA 92037

The two-dimensional display of end-labelled restriction segments from complex genomes such as Drosophila, mouse, etc. reveals a significant abundance of multicopy segments. These segments, which are seen as hot spots and hot lines in the two-dimensional displays, could arise from long complex sequences that are interspersed or arranged tandemly in clusters in the genome. In mouse, the pattern of hot spots and hot lines obtained with BamHl and EcoRl shows tissue-specific differences. Studies with HpaII and MspI have shown that the DNA of Drosphila is not modified at any of the several thousand CCGG sites which can be inspected by the two-dimensional display. In contrast, the multicopy segments which are observed in mouse DNA show a complex pattern of modification at the CCGG site. This pattern also shows tissue-specific differences, suggesting that a tissue-specific pattern of modification at the CCGG site is present in the multicopy DNA of mouse.

1137 HISTONE MODIFICATION AND CHROMATIN ASSEMBLY IN DROSOPHILA MELANOGASTER, Roger C. Wiegand and Douglas L. Brutlag, Department of Biochemistry, Stanford University, Stanford, CA 94305

We have purified a histone acetylase from extracts of Drosophila melanogaster embryos about 1,200-fold. The enzyme, which is still heterogeneous, is completely specific for histone H4. All of the acetate incorporated into histone H4 is located in the very basic amino terminus, in amino acids 4-17. This peptide contains the four internal lysine residues which are acetylated in vivo. The reaction is inhibited by DNA in amounts stoichiometric with the substrate histone. The acetylase will not act on chromatin.

All newly synthesized H4 is acetylated in the cytoplasm, and this is likely to be the role of the enzyme. We have proposed that chromatin assembly is promoted by agents which prevent the rapid, random aggregation of histones and DNA and permit a slower, more orderly assembly to occur. Acetylation, by eliminating the charge of lysines in the exposed, highly basic N-terminus of H4, might be a physiological mechanism by which the nonspecific attraction of histones for DNA might be limited.

Pre-blastoderm embryos of Drosophila contain a pool of highly unusual histones. These histones, when assembled into chromatin in vivo are demodified. Chromatin assembly using these endogeneous histones in vitro yields chromatin with properly spaced nucleosomes, unlike assembly reactions done using histones purified from chromatin.

1138 THE REACTIVATION OF DEVELOPMENTALLY INERT 5S GENES IN SOMATIC NUCLEI INJECTED INTO XENOPUS OOCYTES, Laurence J. Korn and John B. Gurdon, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

In the frog Xenopus one class of 5S genes, the somatic-type, is active in all cells; the other, oocyte-type, is active in oocytes but inactive in somatic cells. When somatic nuclei are injected into oocytes, the products of both types of 5S genes can be detected, using a new method of native gel electrophoresis which resolves RNAs of the same length but different sequence. Under certain conditions, the developmentally inactive oocyte-type 5S genes in somatic nuclei can be reactivated. Under other conditions, these genes remain inactive, thus showing they have been transferred to another cell in such a way as to conserve their regulated condition.

1139 ACTIVATION OF THE MAJOR DROSOPHILA HEAT SHOCK GENES IN VITRO, Brian L. Craine, Department of Biochemistry and Biophysics, Univ. of Calif., San Francisco, CA 94143.

The major Drosophila heat shock gene (coding for the 70K protein) has been shown to be preferentially transcribed by E. coli RNA polymerase when in an active state. Transcriptional mapping has revealed an extraordinary preference for a region of DNA just 5' distal to the active structural gene. The inactive heat shock genes were not transcribed in this fashion. The change in chromatin structure, resulting in this increased accesibility to the exogenous polymerase, can be induced in nuclei isolated from non-heat shocked cells by exposure to cytoplasmic extracts prepared from heat shocked tissue culture cells in vitro. The activation is specific for the heat shock genes since extract prepared from non-heat shocked cells had no effect and the heat shock extract had no effect on the transcription of the histone genes, which are also normally transcribed by RNA polymerase II. The factor responsible for this activation has been partially purified and characterized. The factor has been shown to contain a protein component. The in vitro activation is DNA dependent, dependent on the concentration of extract, displays measurable kinetics and occurs in histone H1 depleted nuclei. It has also been demonstrated that endogenous RNA polymerase II activity is not required to effect the alteration in chromatin structure. This suggests that the events being monitored in this in vitro system occur before transcription, therefore, raising the possibility that the alterations in chromatin structure may be occurring in a regulatory fashion, rather than an effect of transcription.

1140 MOLECULAR GENETIC ANALYSIS OF X-CHROMOSOME INACTIVATION, R. Michael Liskay, Yale University School of Medicine, New Haven, Ct., 06510

The basic features of X-chromosome inactivation in placental mammals, its initial randomness, subsequent stability and chromosome-wide nature, have, in general, been convincingly demonstrated by genetic and cytologic experiments. The molecular nature of this process has, in contrast, remained undefined. Recently, we have reported on studies that strongly suggest that purified inactive X chromosome DNA from two different mammalian female cell lines is not functional in transformation for the X-linked gene coding for hypoxanthine phosphoribosyl transferase (HPRT) (PNAS 77:4895). These results suggest that a form of DNA modification, e.g. methylation, plays a role in X chromosome inactivation. Using DNA-mediated transformation techniques we are currently attempting to isolate the mouse HPRT gene to provide a probe for directly asking whether DNA alterations occur in the vicinity of the HPRT gene. Furthermore, current experiments are being performed to ask whether DNA's from 1) the inactive X of a mouse somatic tissue that shows only paternal inactivation, and 2) the X chromosome of primary mouse spermatocytes, are capable of HPRT transformation.

1141 IDENTIFICATION OF A GENETICALLY-DEFINED EUCARYOTIC REGULATORY PROTEIN, George A. Marzluf and Gregory Grove, Ohio State University, Columbus, Ohio 43210 A major regulatory gene of Neurospora crassa, designated nit-2, is responsible for turning on the expression of a set of unlinked genes which specify nitrogen catabolic enzymes. Previous results suggest that control of this nitrogen circuit occurs at the transcriptional level and that glutamine is the metabolite responsible for nitrogen catabolite repression. We have looked directly for the nit-2 gene product, predicting that it might be a nuclear localized, DNA-binding protein with an affinity for glutamine. After first removing DNA and histones, nuclear proteins were ^{3}H-labeled in vitro by reductive methylation and applied to DNA-cellulose. After washing well, the column was specifically eluted with glutamine and the protein peak obtained was analyzed by gel electrophoresis and fluorography. Nuclei from wild-type possess a single DNA-binding protein which is eluted from DNA cellulose by glutamine but not asparagine. Two nit-2 control mutants have greatly reduced amounts of this protein. A revertant of one of these nit-2 mutants has regained almost a wild-type level of this same nuclear protein. These results tentatively identify the nit-2 regulatory gene product as a DNA-binding protein whose affinity for DNA is substantially reduced by glutamine.

1142 INVESTIGATING ACTIVE GENE STRUCTURE BY HMG 14 AND 17 CHROMATOGRAPHY, Stu Weisbrod, D. Rhodes and J.B. Gurdon, MRC Lab. Mol. Biol., Hills Road, Cambridge CB22QH England. Previously it has been shown that chicken HMGs 14 and 17 when coupled to a solid support, either agarose or glycosylated controlled pore glass beads, can be used to isolate chicken DNase I sensitive or "potentially" active genes (Weisbrod, Kane and Weintraub, Cell (1981) in press). We have shown that a chicken HMG 14 and 17 column is also capable of isolating the active genes, as either nucleosomes or lightly sheared chromatin, from other species, e.g. Xenopus and bovine. We are using this fractionation technique to provide material to characterize the structure of the active nucleosome monomer by: (1) mapping the DNase I cutting sites within the active nucleosome core, and (2) using electron microscopy and image reconstruction to produce a low resolution three dimensional density map of the active histone octamer, similar to that produced for bulk nucleosomes by Klug et al. (Nature 287 (1980) p.509). We are also attempting to use the HMG column to follow the potential activity of particular genes (e.g. globin and vitellogenin) during the early development of Xenopus laevis.

1143 POST-TRANSCRIPTIONAL PROCESSING REGULATION OF THE CALCITONIN GENE, Ronald M. Evans, Estelita Ong, Susan Amara¶ and Michael G. Rosenfeld¶, The Salk Institute, San Diego, CA 92138 and ¶ University of California, San Diego, La Jolla, CA 92093.

Nearly full length cDNA copies of calcitonin mRNA from rat medullary thyroid carcinomas (MTC) have been cloned. The cellular concentration size and structure of the calcitonin mRNA has been determined. DNA sequence analysis reveals that calcitonin is a component of a larger polypeptide precursor which may harbor other cryptic peptides of unknown biological function. RNA blot analysis indicates that calcitonin mRNA is 1,050 nucleotides in length and is generated from a series of 6 larger nuclear precursors. Tumors showing altered calcitonin expression synthesize calcitonin-like or pseudo (Ψ) mRNAs with unique primary structures. Several of the Ψ mRNAs are found on polysomes. One Ψ mRNA is shown to encode a unique polypeptide unrelated to calcitonin. The production of the Ψ mRNAs is associated with specific changes in the nuclear precursor profile. Although multiple related genes may exist, analysis of nuclear precursors suggests that a splicing-choice mechanism specific to particular tumor stages may play a role in generating these multiple mRNA products.

1144 THE CONTROL REGION FOR ADENOVIRUS VA TRANSCRIPTION, Roberto Weinmann and Richard Guilfoyle, The Wistar Institute, Phila., PA 19104

Cloned fragments of adenovirus 2 DNA containing the genes for virus associated (VA) RNA are faithfully transcribed in vitro by a crude RNA polymerase III prepared as described by Wu (Proc. Nat. Acad. Sci. USA 75: 2175-2179). Using the precessive nuclease BAL 31 we have constructed a series of deleted VA_I genes, which have sequences of the viral DNA substituted by plasmid sequences from nucleotide -25 up to +55 counting from the first G of the VA_I RNA gene product. The deletions up to and including nucleotide +10 inside the VA_I gene support the synthesis of a product of a size similar to VA_I RNA. However, deletions that go up to +14, +22 and +55 completely eliminate VA_I transcription while VA_{II} RNA transcription continues unabated. Deletion of sequences from the 3' end eliminates the VA_{II} RNA gene and the termination signal for VA_I RNA transcription, when nucleotides downstream of +75 are substituted with plasmid sequences. These variants show termination of transcription at sequences downstream, as detected by the different size RNA transcription products. Retention of the respective parental 5' or 3' end sequences of the RNA products was determined by fingerprint analysis. Therefore an internal control region for RNA polymerase transcription initiation for this particular gene is located between nucleotides +11 to +75 inside the VA_I gene, as has been previously reported for the 5S genes of Xenopus transcribed by RNA polymerase III (Sakonju, S., Bogenhagen, D.F. and Brown, D. D., Cell 19: 13-26, 1980).

1145 NUCLEOSOMES CONTAINING HYPERACETYLATED HISTONE H4 EXIST IN CLUSTERS IN TROUT TESTIS CHROMATIN, Mark Christensen and Gordon Dixon, Univ. of Calgary, Calgary, Canada T2N1N4

Trout testis chromatin, which exhibits a high degree of histone H4 hyperacetylation (3-4 acetyl groups per molecule), was digested very briefly with micrococcal nuclease (0.3-0.6% acid-solubility) to generate a mixture of oligonucleosomes of which less than 10% are mononucleosomes. The mixture was further fractionated on the basis of solubility at physiological ionic strength. There is a strong correlation between solubility in 0.1 M NaCl and the presence of hyperacetylated histone H4, even in oligomers containing 10-15 nucleosomes. That is, the stoichiometry of the hyperacetylated H4 relative to the other core histones is roughly the same in the oligomers as in the monomers and dimers. This suggests that hyperacetylated histone H4-containing nucleosomes are present in clusters in trout testis chromatin. The fragments insoluble in 0.1 M NaCl appear to completely lack hyperacetylated H4, at least up to a length of 15 nucleosomes. Furthermore, in order for such a clean fractionation of the hyperacetylated from the non-hyperacetylated fragments to be possible, the hyperacetylated regions are likely to be significantly longer than the 10-15 nucleosome oligomers isolated thus far, unless the nuclease is able to cleave preferentially at the borders of the acetylated and non-acetylated regions. Clustering of histone H4 hyperacetylation suggests that entire stretches of chromatin may be altered with respect to conformation, consistent with the proposed role of acetylation in opening up regions active in transcription. We are currently using a cloned probe to analyze the distribution of protamine gene sequences in hyperacetylated versus non-hyperacetylated chromatin. (Supported by M.R.C. of Canada and N.I.H.)

1146 INFLUENCE OF 5' FLANKING SEQUENCES ON tRNA TRANSCRIPTION IN VITRO, Stuart G. Clarkson, Raymond A. Koski, Janine Corlet and Robert A. Hipskind, University of Geneva Medical School, Geneva, Switzerland.

Two X.laevis methionyl-tRNA genes are transcribed with very different efficiencies in a homologous cell-free system. The two genes differ at a single position intragenically and at several positions in their flanking regions. In vitro transcription of hybrid genes clearly demonstrates that the 5' flanking regions are responsible for the different transcription efficiencies of the two genes. One interpretation of these experiments is that a sequence somehow inhibitory for transcription precedes the relatively inactive gene. We are investigating a possible candidate for such a sequence - an alternating purine-pyrimidine tract that conceivably could locally alter the DNA conformation.

1147 DIFFERENTIAL HORMONAL REGULATION OF THE RAT MILK PROTEIN GENE FAMILY - Andrew A. Hobbs, Prabhakar Gupta, Donald J. Kessler and Jeffrey M. Rosen, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

The mechanism by which peptide and steroid hormones regulate the expression of a family of milk protein genes is being studied in rat mammary gland organ culture. The effects of prolactin (M) and hydrocortisone (F) on accumulation of mRNAs for the major milk proteins have been quantitated using cloned cDNA probes. During the initial 48 hour incubation period with insulin (I) and F alone, all four mRNAs (α, β, & γ-caseins and α-lactalbumin) in the mid-pregnant tissue decreased to steady state levels (1.3%, 0.7%, 0.07% and 2.1% respectively of the levels present in 8-day lactating tissue). Addition of M then resulted in the rapid induction of the casein mRNAs reaching levels 13-,27-and 200-fold greater than with IF alone after 24 hours. In contrast, the level of α-lactalbumin mRNA decreased for 12 hours before increasing 4.5 fold by 24 hours. The kinetics of induction suggest that accumulation of each of the mRNAs is independently controlled. F potentiated the effects of M, since omission of F during both the initial incubation and the induction period resulted in a 70% reduction in mRNA levels, although the fold induction for each remained unchanged. Previous results using a combined cDNA probe for the α- and β-casein mRNAs suggested that these hormones exert their effects at both transcriptional and post-transcriptional levels (Cell 17:1013, 1979). Similar studies are now being carried out using individual cloned probes to elucidate the mechanisms of the apparent non-coordinated accumulation of these mRNAs. Differential regulation of casein gene expression was unexpected, since the three casein genes recently have been localized to a single mouse chromosome. (Supported by NIH-CA16303).

1148 ORGANIZATION, STRUCTURE AND TRANSCRIPTION OF NON-ALLELIC HUMAN INITIATOR tRNA-met GENES. Tomas Santos and Michael Zasloff, Genetics and Biochemistry Branch, NIAMDD, NIH, Bethesda, MD. 20205

In the human genome the $tRNA_i^{met}$ loci comprise a multigene family of about 12 dispersed members. Four fragments of human fetal liver DNA ranging in size from 11 to 18 Kb, each containing a single $tRNA_i^{met}$ gene were cloned from a recombinant phage library. On the basis of restriction site mapping, electronmicroscopic analysis of heteroduplex structures, the pattern of repetitive sequence interspersion and the maps of sequences transcribed in vivo, the fragments were shown to represent at least two different loci with homologies limited to several dispersed repetitive sequences within each of the chromosomal neighborhoods. Detailed structural analysis of the tRNA regions revealed several blocks of homology at the proximal flanking sequences of two non-allelic genes one of which differed from the common vertebrate $tRNA_i^{met}$ sequence by one base change at position 56 (loop IV) with a T in place of a G. Both genes were transcribed in vitro in a Xenopus germinal vesicle extract with about the same efficiency but the product of the variant gene was unable to be processed into mature $tRNA_i^{met}$

1149 AN UPSTREAM CONTROL REGION REQUIRED FOR SILKWORM tRNA TRANSCRIPTION, Drena D. Larson and Karen U. Sprague, University of Oregon, Eugene, OR 97403

We are using in vitro mutagenesis to examine the role of 5' flanking sequences in the transcription of silkworm alanine tRNA genes. We have shown that sequences upstream from the initiation site are required for the in vitro transcription of a cloned $tRNA^{ala}$ gene in homologous transcription extracts derived from several silkworm tissues.[2] 250 nucleotides of silkworm 5' flanking sequences are sufficient for activity, whereas deletion of all but 11 nucleotides of this DNA eliminates transcription. In addition to the 5' flanking region there is at least one other region which regulates the expression of this gene in vitro.[1] This second site, probably located within the tRNA coding sequence, appears to interact with a factor required for specificity of RNA polymerase III transcription and may be analogous to the internal control region in Xenopus 5S genes.[2,3] We are constructing a series of deletions in vitro to localize the regions necessary for accurate homologous transcription of this gene and to determine their regulatory functions.

[1]Sprague, Larson and Morton (1980) Cell 22, 171-178.
[2]Sakonju, Bogenhagen and Brown (1980) Cell 19, 13-25.
[3]Engelke, Ng, Shastry and Roeder (1980) Cell 19, 717-728.

1150 NUCLEOSOME PHASING ON tRNA GENES OF XENOPUS LAEVIS, Philip N. Bryan and Max L. Birnstiel, University of Zürich, Zürich, Switzerland
We have mapped the positioning of nucleosomes on a cluster of 8 tRNA genes from Xenopus laevis. This cluster is 3.2 Kb in length and is repeated about 100 times per haploid genome. By mapping distances from known restriction enzyme sites to micrococcal nuclease cutting sites (micrococcal nuclease cuts preferencially in nucleosome spacer regions) we have been able to determine the position of nucleosomes throughout this DNA repeat. Nucleosomes are phased in nuclei from both mature erythrocytes and cultured kidney cells, although they occupy a different set of positions in each tissue. Since the specific position of nucleosomes with respect to a tRNA gene probably influences the binding of regulatory molecules to control regions of DNA, the different phases of nucleosomes may be partly responsible for differences in transcriptional activity. PNB is a Fellow in Cancer Research supported by Grant DRG - 371 - F of the Daymon Runyon - Walter Winchell Cancer Fund.

1151 A REGULATORY FACTOR FOR THE TRANSCRIPTION OF MOUSE RIBOSOMAL GENES, Ingrid Grummt and Gert Pflugfelder, Institut für Biochemie, Universität Würzburg, 8700 Würzburg, Germany.
The transcription of ribosomal RNA correlates with the proliferation rate of the cells. In order to investigate the molecular mechanisms underlying the switch-on and switch-off of rDNA expression a cell-free system for the transcription of ribosomal genes was established. Extracts derived from cultured mouse cells contain the factor(s) required for the accurate initiation of RNA polymerase I on cloned mouse rDNA. After nutritional shift-down the ability of the cell extracts to promote specific transcription is lost. The purification of the factor(s) was achieved by complementation of inactive extracts with fractions isolated from proliferating cells by ion exchange and affinity chromatography.

1152 IN SITU HYBRIDIZATION AT THE ELECTRON MICROSCOPE LEVEL. Barbara Hamkalo and Nancy Hutchison. Departments of Developmental and Cell Biology and Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

In situ hybridization has been used to map the chromosomal locations of many repeated DNA sequences. The small size of many chromosomes often makes this a difficult task, and the light microscope does not provide the resolution necessary for precise mapping. Therefore we have modified the hybridization procedure of Pardue and Gall (1975) for use on whole mount metaphase chromosome preparations to detect in situ hybrids at the EM level. In our test system, ^{3}H-cRNA to mouse satellite DNA was hybridized to mouse L929 metaphase chromosomes. The preparations were exposed to Ilford L4 emulsion for times up to 13 days and developed with Microdol X. Examination in the EM showed heavy labelling of the centromere regions of the chromosomes and clusters of label over interphase nuclei, consistent with numerous light microscope observations. The satellite sequences are distributed throughout the centromere regions. This observation coupled with the fact that satellite DNA represents approximately 50% of centromere DNA argues that these sequences are interspersed with non-satellite DNA. These results illustrate the feasibility of mapping genes to chromosomes at a higher resolution than previously obtained. In addition, DNA sequences can be mapped in relation to features of chromosome ultrastructure. These methods are being extended in order to perform hybridization to RNA transcripts associated with specific active genes using cloned DNA sequences and hybrid detection by both EM autoradiography and non-radioactive electron-dense tags.
Research supported by PHS GM 23241.

1153

A RAPID AND SENSITIVE IMMUNOLOGICAL METHOD FOR IN SITU GENE MAPPING. Pennina R. Langer and David C. Ward, Department of Human Genetics, Yale University, New Haven, CT 06510
A method for in situ localization of specific DNA sequences has been developed which exploits the interaction between modified nucleotides and antibodies directed agianst the modification. Cloned, unique sequences of drosophila DNA are nick translated in vitro in the presence of E. Coli DNA Polymerase I and an analogue of dUTP which contains a biotin molecule covalently linked to the C_5 position of the pyrimidine ring. The nick translated probe containing approximately 0.25% biotin substituted nucleotides, is hybridized in situ, according to standard protocols, to drosophila salivary gland chromosomes. After hybridization, the slides are incubated with monospecific Rabbit antibiotin followed by FITC-Goat anti-rabbit IgG. After counterstaining with Evan's Blue, a single yellow-green fluorescent band, corresponding to the map location of the cloned DNA is seen against a red fluorescent background of the salivary gland chromosomes. Current work is aimed at further refining the system for use in localization of unique sequences on mammalian metaphase chromosomes. FITC-Avidin and histochemical reagents are under investigation as substitutes for the indirect immunofluorescent localization of hybridized regions. Other potential applications of biotinyl polynucleotides as affinity reagents will also be presented.

1154 DEVELOPMENTAL REGULATION OF SEA URCHIN HISTONE GENES IN XENOPUS EGGS AND OOCYTES, Laurence Etkin, Dept. of Zoology, University of Tennessee, Knoxville, TN 37916
Sea urchin histone genes H1, H4, and H2B, contained in the recombinant plasmid pSp102, were microinjected into either the germinal vesicle of oocytes or the cytoplasm of fertilized eggs of Xenopus laevis. Recipient oocytes were treated with progesterone, to induce maturation, and incubated in ^3H lysine to label newly synthesized histones. Histone proteins were extracted with 10% GuCl-40% ETOH and analyzed by two dimensional gel electrophoresis and fluorography. Newly synthesized sea urchin H1, and H2B histones were detected in recipient oocytes cultured without progesterone. These proteins are not observed, however, in recipient oocytes which have matured in response to progesterone. This suggests that the sea urchin histone genes are not expressed in oocytes undergoing maturation. When the plasmid pSp102 is injected into fertilized eggs shortly after artificial insemination, sea urchin histones are not detected until the late gastrula- early neurula stage of development, and continue to be expressed as late as the tailbud stage. These results indicate that the microinjected sea urchin histone genes are expressed concurrently with endogenous Xenopus histone genes in growing oocytes, matured oocytes, and embryos, suggesting that the sea urchin genes are able to recognize and respond to endogenous Xenopus gene regulatory signals.

1155 USE OF RECOMBINANT DNA MOLECULES TO INVESTIGATE REGULATORY SIGNALS FOR THE INITIATION OF TRANSCRIPTION, Peter Gruss and George Khoury, National Institutes of Health, Bethesda, MD 20205
Nucleotide signals required for the initiation of transcription for the early region of the SV40 genome have been identified. This has been achieved by deleting portions of the genome between 0.69 and 0.71 map unit. A 72 bp repeat has been found to harbor a cis essential function, which cannot be provided in trans by either the early tsA28 or the late tsB4 mutant. Further studies have shown that this mutant is incapable of inducing early viral RNA as examined by S1 nuclease analysis, or the early gene product, T-antigen. Studies are in progress to determine whether this mutant can replicate its DNA in the presence of exogenously provided T-antigen. Further attempts to characterize this repetitive element involve replacing the SV40 72 bp repeats by a related repeat unit derived from Moloney sarcoma virus. Results of these experiments will be discussed in detail.

1156 CONTROL OF IMMUNOGLOBULIN GENE EXPRESSION, U. Storb, R. Wilson, E. Selsing, and B. Arp, Department of Microbiology, University of Washington, Seattle, WA 98195

The rearrangement of a variable (V) and a constant (C) gene precedes immunoglobulin gene expression. Multiple different rearranged κ genes were found in many myelomas which nevertheless show "allelic exclusion" of κ genes. To determine which role the chromatin conformation plays in immunoglobulin gene expression we have made use of the preferential digestion of potentially active genes by DNaseI. The DNaseI sensitivity of κ, λ and H-chain genes was determined in myelomas, B cell lymphomas, normal and malignant T cells, fetal liver pre-B cell hybridomas and nonlymphoid cells. It was found that all C_κ genes and their flanking sequences (including a V gene, if rearranged) are DNase sensitive in cells which produce κ chains regardless of whether the particular C_κ gene is functionally or nonfunctionally rearranged or in germline context. V_κ genes in germline context on the same chromosome are insensitive. Kappa genes in nonlymphoid cells are insensitive as well as λ genes in lymphoid cells which do not produce lambda chains. In T cells certain immunoglobulin C genes, but not all, are DNase sensitive. In pre B cell hybridomas which have rearranged μ genes, but germline κ genes, both μ and κ genes are DNase I sensitive. DNase I hypersensitive sites related to immunoglobulin genes are now being determined in different cell types by the method of Wu (Nature 286(1980)854). It appears that functional rearrangement is not a prerequisite for a DNase I sensitive chromatin conformation, but rather that cells active in or committed to the production of a certain immunoglobulin have all C genes for that immunoglobulin in an "active" chromatin conformation.

1157 HETEROGENEOUS INITIATION SITES FOR TRANSCRIPTION OF THE CHICKEN OVOMUCOID GENE, Eugene C. Lai, Dennis R. Roop, Ming-Jer Tsai, Savio L.C. Woo and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

The cloning of the entire ovomucoid gene from the chicken genome has allowed us to establish the detailed molecular structure of this hormone-regulated gene. It is 5.6 Kilobase pairs in length that includes seven intervening sequences of various sizes, and codes for a mature mRNA of 821 nucleotides. With this cloned gene as probe, we have identified ovomucoid precursor RNAs containing intervening sequences which are subsequently removed in a preferred order to generate the mature mRNA. Furthermore, we have carried our S1-nuclease mapping experiments to localize precisely the 5' terminus of ovomucoid transcripts synthesized in vivo. Using three different DNA fragments purified from the 5' region of the ovomucoid gene as probe, we have detected heterogeneity in the 5' terminal sequence of ovomucoid transcripts isolated from estrogen stimulated chick oviduct. Most of the transcripts (95-97%) are initiated at a preferred start site which is positioned at 30 basepairs downstream from a "TATATAT" sequence. However, there is another start site accounting for about 3-5% of the ovomucoid transcripts. It is situated about 80 basepairs upstream from the major site. Twenty-nine basepairs upstream from this minor site, there is also an A-T rich region resembling a "TATA" box sequence. The structure and location of control regions for the initiationof eucaryotic gene transcription is still not well understood. Our finding of heterogeneity in eucaryotic gene transcription adds one more piece of information to this yet unsolved puzzle. Experiments are now being conducted to examine whether these two start sites play any role in the hormone-regulated expression of the ovomucoid gene.

1158 SYNTHETIC OLIGONUCLEOTIDES AS HYBRIDIZATION PROBES FOR SPECIFIC GENES, Sidney Suggs, R. Bruce Wallace, Tadaaki Hirose, Eric H. Kawashima and Keiichi Itakura, City of Hope Research Institute, Duarte, California 91010.

We have demonstrated that a mixture of chemically synthesized oligodeoxyribonucleotides may be used as hybridization probes in the isolation of specific cDNA clones in pBR322. A cDNA clone for human β2-microglobulin (β2-m) was obtained using mixtures of pentadecanucleotides. Because of the redundancy of the genetic code, the five amino acid residues at the carboxy-terminus of β2-m can be coded for by 24 possible sequences in the mRNA. We synthesized 2 mixtures of 15-mers complementary to all 24 possible coding sequences. One mixture contained a family of 8 sequences; the other mixture contained a family of 16 sequences and was shown to include the sequence for human β2-m. We used the oligonucleotide mixtures for screening both in Southern blot and colony screening techniques. By comparing results with the 2 mixtures, we determined that under our conditions, oligonucleotide:cDNA duplexes with a single base mismatch are sufficiently less stable than perfectly matched duplexes to allow discrimination.

1159 ACCURATE INITIATION OF TRANSCRIPTION BY PURIFIED HUMAN RNA POLYMERASE II, Todd A. Leff and Stefan Surzycki, Indiana University, Bloomington, In. 47405

RNA polymerase II purified from human placenta accurately initiates transcription at the Adenovirus 2 (Ad2) major late promoter and the promoter for the human Aγ hemoglobin gene in an in vitro system without the addition of a cell extract. We have found accurate transcription to be highly dependent on salt concentration and the two promoters to have strikingly different salt optima. The accuracy of transcription was measured by gel electrophoresis of RNA synthesized from purified DNA templates which had been truncated at various distances downstream from the in vivo transcription initiation site. When RNA was synthesized from a 2000 base pair fragment of the Ad2 chromosome containing the major late promoter 198 base pairs from one end, in the presence of 20 mM ammonium sulfate, a transcript was observed which had a mobility of 200±20 nucleotides. At salt concentrations above 50 mM the transcript was not observed. When a 1700 base pair fragment of the globin gene was transcribed in the presence of 80 mM ammonium sulfate, an RNA product of the predicted size was observed. At 20 mM salt this transcript was not synthesized. When either template was shortened by restriction enzyme digestion the size of the specific transcript decreased by the predicted amount. RNA polymerase II enzymes purified from calf thymus and wheat germ accurately transcribe the hemoglobin fragment, although the specific transcript made by the wheat germ enzyme is only a small fraction of the total RNA synthesized.

1160 IN VITRO SYNTHESIS OF VACCINIA VIRUS RNA, S. Venkatesan, B. M. Baroudy, and B. Moss, National Institutes of Health, Bethesda, Maryland 20205

Vaccinia virus provides a unique system for studying transcription of a DNA genome: enzymes required for synthesis, processing, capping, methylation, and polyadenylylation of mRNA are packaged within the virus core. The fidelity of the in vitro system was demonstrated by determining the map coordinates and translational products of the 3 immediate early mRNAs encoded within the 9,000 base pair terminal segment. The 5' ends of the mRNAs were labeled by a unique procedure involving chemical removal of the original cap, followed by enzymatic recapping. Their 5' ends then mapped by gel electrophoresis of nuclease resistant RNA:DNA hybrids and by hybridization of the end labeled RNA to immobilized DNA restriction fragments. Both $m^7G(5')pppA^m$ and $m^7G(5')ppp G^m$ cap structures were identified at the end of each mRNA made in vivo or in vitro, indicating some terminal heterogeneity. Furthermore, by synthesizing RNA in the presence of [α-^{32}P]GTP, it could be shown that cap sites correspond to sites of RNA initiation. The DNA sequence around one promoter site was determined and the end of the RNA was mapped within several nucleotides by S1 nuclease protection experiments. In addition, 150 nucleotides of a reverse transcript of the mRNA was sequenced by the dideoxynucleotide method. These data indicated that a 40 base pair DNA sequence upstream of the major RNA initiation site is 86% A-T rich and that the first AUG codon lies about 50 base pairs down stream. Analysis of additional early and late genes will be necessary to determine a concensus promoter sequence for the vaccinia virus RNA polymerase.

1161 A NOVEL METHOD FOR PROBING DNA SEQUENCE DISTRIBUTION IN NUCLEOSOMES. Tim. L. Reudelhuber and William T. Garrard, Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Nucleosomes can be separated by electrophoresis into various classes which differ in protein composition. To study the distribution of specific DNA sequences in different electrophoretic forms of nucleosomes, we have developed a procedure termed the Southwestern Blot which allows the transfer of the DNA components of nucleosomes from gels to diazobenzyloxymethyl cellulose (DBM-paper). Nucleosomes separated on a one-dimensional gel are electrophoretically dehistonized leaving DNA fixed within the gel as the cetyltrimethylammonium salt. The DNA is then converted to the sodium salt, denatured and transferred electrophoretically to DBM-paper. Use of chromatin samples prepared from ^{3}H-thymidine labeled cells permits the visualization of the pattern of transferred DNA by fluorography. Comparison of this pattern with the autoradiograph obtained after hybridizing the same blot with ^{32}P-labeled probe permits precise quantitation of the distribution of a specific sequence among different electrophoretic forms of nucleosomes. Because DNA is covalently coupled, repeated hybridizations with different radioactive probes can readily be performed. Procedures have also been developed for electrophoretic transfer of DNA from two-dimensional gels which employ electrophoresis of nucleosomes in the first dimension followed by electrophoresis of either native or denatured DNA in the second dimension. Using cloned ribosomal and satellite DNA sequences as hybridization probes, we illustrate the above techniques and demonstrate that specific sequences are non-randomly distributed in electrophoretic displays of cultured mouse cell nucleosomes. (Supported by NIH Grant GM22201-06)

1162 COMPARISON OF TRANSCRIPTION OF UNREARRANGED AND REARRANGED V_κ GENES, Elizabeth L. Mather and Robert P. Perry, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA 19111

During the differentiation of B lymphocytes from stem cells, functional immunoglobulin genes are created by DNA rearrangements that bring V, D, J and C genes together. Lenhard-Schuller et al. have shown that 5' to a rearranged V kappa chain gene as much as 8 kb of germline DNA is retained (1). Although the exact location of initiation of transcription is not known, the largest κ chain mRNA precursor observed in a number of myelomas is on the order of 5 to 6 kb (2). Thus a V gene promotor is flanked by the same sequences in both the unrearranged (germline) and rearranged configuration. As a step toward understanding factors important in the regulation of immunoglobulin transcription, the transcriptional activity of a V_κ gene in its germline configuration was compared with the transcriptional activity of a V_κ gene in a productively rearranged configuration. Solution hybridization of appropriate V_κ gene probes and total nuclear RNA from a myeloma tumor showed that the steady state level of transcripts from the germline V_κ gene is 1/500-1/1000 the level of nuclear transcripts from the rearranged V_κ gene. In contrast, nuclear transcripts of germline C_κ genes have been shown to be present in amounts similar to the amounts of nuclear precursors for the authentic κ chain mRNA (2). These results support the idea that translocation of a V gene to the C gene locus greatly enhances its transcriptional activity.

(1) Lenhard-Schuller, R. et al. (1978) Proc. Natl. Acad. Sci. USA 75: 4709-4713.
(2) Perry, R. P. et al. (1980) Proc. Natl. Acad. Sci. USA 77: 1937-1941.

1163 IN VITRO TRANSCRIPTION OF CLONED SILK FIBROIN DNA, Raymond E. Zielinski and Paul M. Lizardi, Rockefeller University, New York, N.Y. 10021

We are using the HeLa cell-free transcription system (Manley et al., 1980. PNAS 77, 3855.) and a genomic sub-clone (pFS1) containing nearly all of the fibroin structural gene plus about 1kb of 5'-flanking sequences in order to study expression of the silk fibroin gene from Bombyx mori. Run off templates designed to test the accuracy of transcriptional initiation were generated by cleaving pFS1 DNA 188 bp and 514 bp 3'-distal to the presumed site of initiation (Tsuda et al., 1979. PNAS 76, 4872). By using these templates in the HeLa extract, we observe major radioactive products corresponding to the predicted sizes, as judged by electrophoresis of formaldehyde- or glyoxal-treated transcripts using rRNAs and Adenovirus (Ad2) run off transcripts as markers. Transcription is dependent upon addition of fibroin DNA sequences and is optimal at a total DNA concentration of 50 μg/ml (promotor concentration 6 nM); it is completely abolished by 1 μg/ml α-amanitin, indicating that it is directed by RNA polymerase II. The 188-nucleotide transcript has been characterized by T1 RNase fingerprinting. Initiation of fibroin transcription in the mammalian extract is about 10-20 fold lower than that of Ad2 major late DNA in the same system and approximately half as efficient as mouse β-globin DNA transcription in a purified RNA polymerase II system (Luse and Roeder, 1980. Cell 20, 691). Our results suggest that the structural features in insect DNA responsible for transcriptional initiation by RNA polymerase II are similar to those of mammalian genes.

1164 THE AUTONOMOUS PARVOVIRUS H-I CONSISTS OF TWO OVERLAPPING TRANSCRIPTION UNITS WITH INDEPENDENT PROMOTER SITES, Russell M. Lebovitz and Robert G. Roeder, Department of Biological Chemistry, Washington University School of Medicine, St. Louis, MO 63110

H-1 is a mammalian parvovirus which contains a (simple) linear single-stranded DNA genome of 5 kb. The virus replicates in rapidly dividing cells via the formation of linear double stranded RF structures, which presumably also serve as transcription templates. Polyadenylated viral RNAs of 3.0 and 2.8 kb (1:5 ratio) have been identified in the cytoplasm of infected cells by nuclease S1 mapping. The identification of a nucleus-specific polyadenylated viral transcript of 4.7 kb originally suggested that the cytoplasmic RNAs might be derived by differential splicing of a single large (near genomic length) primary transcript, by analogy with the adenovirus major late transcription unit. However, while the cytoplasmic RNAs contain a common 2.6 kb segment at the 3' ends, the demonstration of distinct 5' leader segments suggested that these transcripts might also result from distinct initiation sites. By analyzing the transcription of viral DNA fragments in our RNA polymerase II-dependent cell-free systems, we have identified two in vitro promoter sites at map positions 4 and 40 on the genome. The primary DNA sequences in these regions show considerable homology with other known RNA polymerase II promoter sites. Moreover, high resolution mapping has shown that the 5' terminii of the respective in vivo and in vitro transcripts are identical, suggesting that both promoters function in vivo (albeit at differential rates). Since the parvoviruses appear to encode only their (2) capsid proteins and are therefore completely dependent on host transcription factors for expression, they provide simple model systems for analysis of the regulation of normal cellular genes (especially those which may contain overlapping transcription units).

1165 THE NUCLEAR EFFECTS OF THE POLYPEPTIDE HORMONE, TRH, ON PROLACTIN GENE EXPRESSION, Michael G. Rosenfeld, Ellen Potter, Kathryn Nicolaisen and Ronald M. Evans, University of California, San Diego, La Jolla, CA 92093

Addition of TRH to cultured GH_4 cells results in the accumulation of prolactin cytoplasmic mRNA sequences. The accumulation of these RNA sequences appears to result from a rapid TRH-induced accumulation of a series of larger nuclear prolactin precursor mRNAs. The concentrations of nuclear prolactin cDNA-reactive poly(A)-rich RNA species of 6,400, 4,200 and 3,800 nucleotides are increased by TRH with a $T_{\frac{1}{2}}$ to maximal concentration of 30-40 min. The nuclear effects of TRH, in the absence of apparent alterations in cytoplasmic mRNA stability, appear to account for its action to increase prolactin biosynthesis. At 24 h after addition of TRH, the concentrations of the 10,400 4,200 and 3,800 nucleotide putative nuclear RNA precursors, while still increased, are considerably decreased compared to the maximal concentrations at 3 h after TRH addition. The concentration of one nuclear prolactin cDNA-reactive RNA species 1,800 nucleotides in length, is increased more than 8-fold by TRH, but with a kinetics of accumulation similar to that observed for the mature 1,000 nucleotide prolactin mRNA species. This result could reflect either a non-precursor relationship, a feed-back regulation of prolactin mRNA processing, or altered transcription of the prolactin gene or a related gene. This 1,800 nucleotide nuclear RNA species is present in normal pituitary cells. In addition, using a DNA-excess hybridization technique, a second polypeptide hormone, epidermal growth factor, was shown to exert an additive effect on prolactin mRNA concentration. This polypeptide hormone also exerts its effects at a nuclear level.

1166 TRANSCRIPTION OF A YEAST TYROSINE tRNA GENE in vivo AND in vitro. Rodney Rothstein, Cathy Betzel and Carolyn Levy, New Jersey Medical School, Newark, NJ 07103 and Doug Melton, MRC Cambridge, England.

A cloned fragment of one of the eight yeast tyrosine tRNA genes has been examined for both expression in vivo and transcription in Xenopus oocytes. We have cloned a 1700 base pair fragment containing the sequence for a yeast tyrosine amber suppressor. The cloned fragment exhibits suppressor function when assayed by yeast transformation. The same cloned fragment injected into Xenopus oocytes directs the transcription and processing of a tyrosine tRNA sized gene product. A small 125 nucleotide long fragment was subcloned in pBR322. This fragment includes the entire mature tRNA coding sequence (89 nucleotides), 3 nucleotides to the 5' end of the processed gene and 33 nucleotides at the 3' end. This cloned fragment when assayed in yeast does not express amber suppressor function. However, the same cloned fragment is actively transcribed and processed in Xenopus oocytes. The 125 nucleotide long fragment can be made to function in yeast by cloning two 125 nucleotide fragments in opposition (5' end to 5' end). This fragment is also transcribed and processed in Xenopus. Thus, all the information necessary to make a functional suppressor in yeast is contained within the 125 nucleotide fragment. We conclude that the configuration of the gene within the plasmid directly affects the expression of the gene in yeast.

1167 *IN VITRO* TRANSCRIPTION ANALYSIS OF GLOBIN GENE SWITCHING; ISOLATION AND FINE MAPPING OF THE ENTIRE β GLOBIN GENE REGION OF CHICKEN, Bryant Villeponteau, Greg Landes and Harold Martinson, Division of Biochemistry, Department of Chemistry, and the Molecular Biology Institute, U.C.L.A., Los Angeles, CA 90024

Chicken red blood cells (RBCs) from five-day embryos synthesize only embryonic β-like globin while 12-day RBCs synthesize adult β-globin. Using *in vitro* transcription, we have found that 0.02% of the labeled transcripts from 12-day embryonic RBC nuclei hybridize to filters bearing the adult β-globin structural gene probe pHB 1001. A significantly lower percentage of transcripts from 5-day embryonic RBC nuclei hybridize to pHB 1001. The low extent of hybridization we observe with transcripts of 5-day erythroid nuclei is consistent with the known ability of adult β structural gene probes to cross-hybridize with embryonic gene sequences. In order to obtain non-cross-hybridizing intervening and flanking genomic sequence probes, we have used pHB 1001 to screen a bacteriophage λ library derived from chicken chromosomal DNA. Many overlapping λ clones were isolated which delineate for the first time the complete 35 Kb stretch of chicken DNA containing the contiguous set of all four β-like globin genes. A detailed restriction map of the regions in and around the four β-like globin genes was then obtained using the restriction enzymes Hae III, Hinf I, and Hpa II. Various restriction fragments are being subcloned into pBR 322 and will be used as probes to monitor specific changes in chromatin structure and transcription as RBCs switch from embryonic to adult β-globin synthesis.

1168 SPECIFIC IN VITRO INITIATION OF TRANSCRIPTION ON KAPPA LIGHT CHAIN IMMUNOGLOBULIN GENES. Linda K. Clayton, Edmund Choi and Randolph Wall. University of California at Los Angeles, Los Angeles, California 90024

We have analyzed cloned immunoglobulin kappa (κ) gene segments to identify regions involved in transcription of light chain mRNAs. In these studies we have utilized a whole cell lysate prepared from Hela cells (1) to obtain transcription on a cloned mouse immunoglobulin gene. The template for transcription is a 2.1 kb restriction endonuclease fragment of a κ light chain gene cloned from a myeloma cell line, MPC 11. This DNA template has been shown to contain the origin of transcription by RNA mapping and includes the first 195 nucleotides of the V region as well as approximately 1.9 kb of the 5' flanking region. In vitro transcription on this template produces an RNA product approximately 650 nucleotides long thus mapping the site of initiation of transcription at about 450 nucleotides to the 5' end of the V gene. Characterization of the products of in vitro transcription by using restriction fragments of different sizes containing the purported promoter as templates or by hybridizing the in vitro synthesized RNA to nick-translated DNA fragments further delineates the sequences transcribed in vitro.

Similar in vitro studies are underway to investigate the specificity of initiation and mechanism of termination on a second cloned κ immunoglobulin gene template which contains the entire MPC 11 κ gene including the site for poly A addition used in vivo.

1. Manley, J.L., Fire, A., Cano, A., Sharp, P.A. and Gefter, M.L. PNAS 77:3855 (1980).

1169 LOCATION OF HIGH MOBILITY GROUP PROTEINS IN POLYTENE CHROMOSOMES. John C. Wooley, Jongsang Park, Melissa McCoy and Su-yun Chung, Princeton University, NJ 08544.

HMG 14 and 17 appear to modulate nucleosome structure in regions of active chromatin. To study the role of HMGs, we have prepared antisera specific for HMG 14 and 17 (isolated from calf thymus). Using immunofluorescence, we find that the antisera binds strongly to active and potential active transcriptional (puff) sites on Drosophila melanogaster polytene chromosomes. The antisera also binds at a lower level along entire chromosomes. Although active chromatin sites are thought to be enriched, HMG 14 and 17 are probably more uniformly distributed in chromatin than suggested by the relative distribution pattern we observe. Thus, the polypeptides recognized by the anti-HMG sera might be more accessible in regions of active chromatin and/or are in an altered configuration exposing a different antigenic determinant. We are using the anti-sera to determine the Drosophila polypeptides homologous to HMG 14 and 17 of mammals. We have already identified a number of Drosophila polypeptides which appear to be HMGs based on standard criteria. These putative HMGs include 5 unique low molecular weight polypeptides in addition to D1 (65 K), previously characterized by Alfageme et al. (Chromosoma 78, 1, 1980). We term these P4 (19K), P5 (18.3K), P6 (16K), P7 (14.5K) and P8 (13.5K). Antisera to P6 show a distribution pattern very different from HMG antisera. That is, antisera to P6 react at a limited number of non-puff sites, which differ from those observed for D1 by Alfageme et al. (loc. cit.). We are now characterizing the chromosomal location of P4, P5, P7 and P8 using the immunofluorescent technique. Research supported by NIH GM26332 and ACS CD-15.

1170 SPECIFIC TRANSCRIPTION OF SV40 DNA in vitro, Jay D. Gralla, D.W. Chandler, L.J. Poljak and J.B. Watson, UCLA, Los Angeles, CA 90024

The preferred sites for transcription initiation in vitro by two mammalian RNA polymerase II's occur within the SV40 DNA region specifying the 5' ends of in vivo m-RNA's. A human whole cell extract catalyzes principally 2 early transcripts (from .65 and .66 map units) and 2 late transcripts (from .68 and .72 map units). These assignments are based principally on the sizes of unique RNA's transcribed from purified SV40 DNA cut uniquely with the enzymes Taq 1 and Eco R_I. Purified RNA polymerase II from calf thymus exhibits a dissimilar transcription pattern using the same assay. Highly preferred transcription of SV40 DNA is detectable using DNA cut with Pvu II as evidenced by the appearance of a (240) base long band. This RNA probably initiates near 0.67 map units and terminates at the Pvu II site at .715 map units. Purified RNA polymerase II from wheat germ fails to catalyze any unique transcripts using these assays. Thus the mammalian enzymes produce a spectrum of RNA's in vitro which mimic, at least superficially, RNA's produced in vivo. However, the low efficiency of transcription in vitro suggests the systems are far from optimally reconstructed.

1171 TRANSCRIPTION OF ADENOVIRUS VA GENES IN A SV40 HYBRID VIRUS, Randal J. Kaufman, and Phillip A. Sharp, Mass. Institute of Technology, Cambridge, MA 02174

Adenovirus directs the synthesis of two species of low molecular weight RNAs, the 'virus-associated' RNAs, which have been designated as VAI and VAII. The VA genes are located adjacent to eachother between 29 and 30 map units on the adenovirus genome. They are transcribed by RNA polymerase III and late in infection represent a large fraction of newly synthesized RNA molecules. In adenovirus infected cells, the two RNAs appear with different kinetics. VAII appears early and then levels off whereas VAI becomes increasingly predominant late after infection. The function of these RNAs is unknown. It has been proposed that they may be involved in splicing of RNA or transport of RNA late in adenovirus infection. In order to analyze the transcriptional control and function of these RNAs, we have inserted the VA genes (DNA from the adenovirus genome spanning 27.9 to 31.5 map units) into the late region of SV40. Upon infection of cells with these hybrid virions, VAI is transcribed at levels similar to that found in adenovirus late infected cells. These results indicate that transcription of VAI is not dependent upon any other adenoviral gene product. Thus far we have detected no qualitative of quantitative change in the level of host and adenoviral specific protein synthesis in cells infected with the SV40 hybrid and then 30 - 40 hrs later infected with adenovirus. Experiments are presently in progress to determine whether VA RNA alters transcription, processing, or utilization of RNA expressed from the major late adenovirus promoter.

1172 IN VITRO TRANSCRIPTION OF MURINE TYPE C RETROVIRUSES, Michael Ostrowski and Gordon Hager, Lab of Tumor Virus Genetics, Natl. Cancer Inst., N.I.H., Bethesda, Md. 20205

Crude extracts prepared from HeLa cells were capable of initiating specific RNA polymerase II transcripts from cloned murine type C proviral templates. The cloned DNA utilized was derived from either a full length integrated copy of AKR murine leukemia virus (MuLV) or an unintegrated permuted copy of Harvey sarcoma virus (HSV) that contains only one long terminal redundancy (LTR). MuLV specific transcripts were promoted with equal efficiency from the two LTR's present at either end of the provirus, confirming the prediction from stuctural considerations that a copy of the retroviral promoter should exist at both ends. The RNA chains were initiated about 130 bases upstream from the 3' ends of the LTR's. The transcription start point in each LTR lies 25-30 bases downstream from a Hogness box. Pol II transcripts from the HSV clone were also initiated within the LTR 25-30 base pairs downstream from a Hogness box. Specific promotion of RNA by HSV is abolished if the template is fragmented with Tha I, a restriction enzyme having a single recognition site in the LTR located 50 bases upstream from the Pol II initiation point, in contrast with the results described for in vitro transcription of the chicken conalbumin gene [B. Wasylyk, C. Kedinger, J. Corden, O. Brison and P. Chambon, Nature 285:367 (1980)]. These observations indicate that, in vitro, sufficient information is present in the C-type LTR to promote specific initiation of viral RNA independent of host information. In addition to the Pol II promoter, HSV contains a Pol III promoter that is coincident with the 5' end of the viral sarcoma gene. The in vitro Pol III transcript is terminated 380 bases from its 5' end.

1173 MUTATIONS AT THE YEAST SUP4 $tRNA^{Tyr}$ LOCUS: FINE STRUCTURE MAPPING AND IN VITRO TRANSCRIPTION, R.A. Koski, J. Kurjan, M. Worthington, P. Shalit, B.D. Hall, S.G. Clarkson, S. Gillam and M. Smith, University of Washington, Seattle, WA; University of Geneva, Geneva, Switzerland; and University of British Columbia, Vancouver, B.C.

123 mutations that inactivate yeast suppressor $tRNA^{Tyr}$ function in vivo have been mapped to the SUP4 locus. Genetic fine structure mapping revealed that spontaneous mutations are distributed throughout the coding region of the $tRNA^{Tyr}$ gene, whereas most mutations induced by the acridine mustard ICR-170 are located within a GC-rich region located in the 3' half of the gene. DNA sequence analyses of 40 different cloned mutant genes identified 34 different mutations. Most of the mutations in the cloned SUP4 genes do not significantly affect transcription of the $tRNA^{Tyr}$ gene by RNA polymerase III in vitro. Two mutations create new T clusters within the gene and cause premature transcription termination. Two mutations significantly enhance transcription and two mutations which alter the invariant C within the TΨCG sequence dramatically reduce SUP4-o gene transcription. The regions of the SUP4-o gene that surround these mutations are partially homologous to intragenic sequences in many other eucaryotic tRNA and 5S RNA genes. We hypothesize that these homologous sequences are recognized as promoter regions during RNA polymerase III transcription initiation.

1174 KILO-SEQUENCING: A STRATEGY FOR RAPID ACQUISITION OF DNA SEQUENCE DATA FOR LARGE SEQUENCING PROJECTS. Wayne M. Barnes, Washington University, St. Louis, MO 63110.
DNA sequencing has become so rapid that others have chosen to sequence large regions of DNA essentially randomly, 200-300 nucleotides per experiment, with reliance on computer assistance to determine overlaps, and with reliance on luck to cover all of the desired sequence in a reasonable number of experiments. In contrast, I present a strategy which allows the directed acquisition of sequence data from large (5-10 kb) targets of DNA sequence analysis. This strategy retains the conveniences of M13 template, commercially available primer and the dideoxy method, while allowing convenient and continuously variable localization of sequencing efforts across a target sequence.

The basis of the method is the in vitro generation of a continuous range of deletions starting near the primer and extending for various distances (ideal increment 250 b.p., or whatever one can read from a single sequencing experiment, less 50 b.p. for overlap) across the target DNA.

M13 cloning vectors constructed for use with the method are asymmetric; that is, they contain several unique restriction sites that allow the cloning of passenger DNA with two different restriction site ends. In general the target DNA must be available with one end from the following list, and the other end any different enzyme, whether from this list or not: EcoRI, HindIII, PstI, MstI, KpnI, BstEII, XmaIII, Sph.

The method has been applied to the histidine operon of Salmonella typhimurium.

1175 POSITIONS OF HISTONES WITHIN NUCLEOSOMES. Michael Beer, Christian Stoeckert, Wendell Wiggins and Rex J. Hjelm, Johns Hopkins University, Baltimore, Maryland 21218
The positions of the histone molecules in nucleosomes are being determined by electron microscopy of particles in which some of the histones carry heavy atom labels. Chicken erythrocyte chromatin was labeled with methyl (methylthio) acetimidate. The histones were separated into H3-H4 and H2A-H2B pairs. Each pair of histones was combined with the complementary pair of unlabeled histones and in the presence of DNA reconstituted to form nucleosomes. The nuclease digestion patterns of nucleosomes with imidated histones were similar to those obtained with unmodified nucleosomes. These were reacted with Pt (gly-l-met) Cl to give a nucleosome in which one pair of histones carried 15-30 heavy atoms and the other pair only 6-8 Pt atoms. Examination with a high resolution Scanning Transmission Electron Microscope revealed differences presumed to be heavy atoms which indicate the positions of the labeled pairs of histones.

1176 THE HISTONE 2A CLASS-ITS CHARACTERIZATION AND EVOLUTION, Michael H. P. West, Panagiotis Pantazis, Roy S. Wu and William M. Bonner. Lab. of Molecular Pharmacology, NCI, NIH, Bethesda, MD 20205
The Histone 2A class is comprised (in mouse L1210 cells) of 2 homeomorphous variants differing by four amino acid substitutions, and 2 heteromorphous variants. Using a fingerprinting technique developed in our laboratory, we have shown that the heteromorphous variant X has 10 tryptic peptides larger than dipeptides identical to those in H2A.1; two from H2A.1 which through 1 substitution are joined into one, and 6 which appear rather different. We have also shown that the heteromorphous variant Z only has two peptides in common with H2A.1 and appears considerably different in the remainder of its sequence. H2A.X and Z occur not only in mammals, but also in lower eukaryotes such as sea urchin. Yeast contains H2A.Z but apparently no H2A.X. We show how the various members of the H2A class have been conserved between these organisms in a way which suggests the existence of two types of selection pressures, one of which has acted to conserve the H2A.2 sequence and the second of which has conserved the other H2A variants.

1177 M13 SHOTGUN DNA SEQUENCING. Joachim Messing, Roberto Crea* and Peter Seeburg*, Univ. of Minnesota, St. Paul, MN 55108 and *Genentech, Inc., So. San Francisco, CA 94080

The dideoxy sequencing technique (1) adopted for the M13 cloning system (2) requires to keep the primer invariant and the template highly variable. Consequently a small restriction fragment next to the cloning site of M13mp2 (3) which (-) strand can serve as a master primer has been conserved as an EcoRI fragment in an amplifiable plasmid (4). To enhance the changes to break down DNA in small pieces which can be cloned into M13mp2 directly an array of synthetic cloning sites have been introduced into the EcoRI site of M13mp2. The new phage M13mp7 is directly susceptible to fragments created by a number of enzymes including the 4 nucleotides cutters like Sau 3A, EcoRI*, HpaII, TaqI, AluI, RsaI, ThaI, HaeIII. Individual recombinants are identified as colorless plaques and characterized by a single sequencing reaction. From the sequence data of randomly chosen templates the entire primary sequence can be reconstructed by aligning the nucleotide sequences by overlaps and complementarities.

(1) Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-67.
(2) Messing, J., Gronenborn, B., Muller-Hill, B. and Hofschneider, P. H. (1977) Proc. Natl. Acad. Sci. USA 74, 3642-46.
(3) Gronenborn, B. and Messing, J. (1978) Nature 272, 375-77.
(4) Heidecker, G., Messing, J. and Gronenborn, B. (1980) Gene 10, 69-73.

1178 RNA PROCESSING IN XENOPUS OOCYTES MICROINJECTED WITH CLONED YEAST $tRNA^{Tyr}$ GENES, Kazuko Nishikura*and Eddy M. DeRobertis, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England

Microinjected tRNA genes are transcribed, and then the transcripts are processed in a precise, sequential order by frog oocytes (DeRobertis and Olson 1979, Nature 278, 137-143) :

104 precuesor —(trimming of 5' leader and 3' trailer, and CCA addition)→ 92 precursor —(splicing)→ 78 mature $tRNA^{Tyr}$

In this study, we have investigated further details of the RNA processing of transcripts copied from yeast $tRNA^{Tyr}$ genes following microinjection into Xenopus oocytes. (1)We analyzed localization of the splicing enzymes in frog oocytes by manual separation of the nuclear envelope, the nuclear contents, and the cytoplasm and by in vitro analyzing system for splicing using these cell components. All the splicing activity were found to be located in the nuclear contents and not associated with the nuclear menbrane. (2)We studied the effect of 18 single nucleotide changes of the processing of tRNA gene transcripts in frog oocytes using a collection of point mutations isolated from an ochre supressor $tRNA^{Tyr}$. A single nucleotide change by mutation affects or sometimes blocks the RNA processing pathway. Especially mutations that affect splicing of the 92 precursor are mainly located in the anticodon loop and the intervening sequence. (3)We analyzed base modifications of ^{32}P-labelled 104 and 92 precursors and 78 mature $tRNA^{Tyr}$ separately and found that all base modifications occur in a strict order which precisely correlates with the size alteration of the $tRNA^{Tyr}$ precursor.
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DNA Transformation and Vectors for DNA Transformation into Eucaryotes

1179 EFFECT OF METHOTREXATE TREATMENT ON PERSISTENCE OF THYMIDINE KINASE-POSITIVE TRANSFORMED PHENOTYPE OF MOUSE HEMATOPOIETIC CELLS IN LIVING ANIMALS.
K.E. Mercola, M.J. Cline, H.D. Stang
From the Department of Medicine, University of California, Los Angeles, CA

Our laboratory has developed a technique for the incorporation of new genetic information into the hematopoietic cells of living mice, one strain involves the use of syngeneic CBA mice, one strain of which has a marker chromosome to allow monitoring the fate of transformed cells. We have compared purified herpes virus thymidine kinase gene in a variety of forms, in E. coli plasmid, free gene, and ligated gene as a transforming vector in experiments where calcium microprecipitates of viral DNA were used to transform mouse hematopoietic cells. These transformed cells were injected into recipient animals which had been lethally irradiated and allowed to reconstitute their marrows. Animals were treated with methotrexate in order to assess the effect of selective pressure on the proliferation of transformed marrow cells containing the viral enzyme. A total of 120 animals were examined. In both control and methotrexate treated animals, 11%-15% of animals maintained the transformed phenotype over periods as long as 200 days. Animals receiving ligated thymidine kinase gene had the highest persistence of transformed phenotype after 60 days even in the absence of drug selection, while animals receiving thymidine kinase in plasmid form tended to lose the transformed phenotype in 30-60 days despite methotrexate treatment. The herpes virus thymidine kinase enzyme has a greater affinity for its substrate and is a less specific pyrimidine kinase than the mammalian enzyme and this in itself may confer an advantage to cells which express the viral gene.

1180 ERYTHROCYTES AS CARRIERS FOR RECOMBINANT DNA: POTENTIAL FOR GENE TRANSFER AFTER CELL FUSION, Garret M. Ihler, John D. Humphreys and Thomas D. Edlind, Texas A&M University, College Station, Texas 77843.

Erythrocytes have been used as carriers for various substances including drugs, enzymes and other macromolecules which can enter holes that reversibly form in the erythrocyte membrane under hypotonic conditions. After addition of 0.9% NaCl, the holes reseal, trapping the substances to be loaded in the erythrocyte. We have recently devised conditions whereby the erythrocyte can serve as a carrier for DNA, including recombinant DNA. Since erythrocytes can readily be fused with other cell types in culture, it should be possible to introduce multiple copies of recombinant DNA into the recipient cell with high efficiency.

1181 CHANGES TO DNA SEQUENCE AND CHROMOSOME ORGANISATION DURING GENE AMPLIFICATION IN METHOTREXATE-RESISTANT MOUSE LYMPHOMA CELLS. Christopher Bostock and Christopher Tyler-Smith, M.R.C. Mammalian Genome Unit, King's Buildings, West Mains Road, Edinburgh, Scotland, U.K.

We have studied the mechanism of dihydrofolate reductase (DHFR) gene amplification in four independently derived methotrexate (MTX)-resistant lines of mouse T-cell derived lymphoma EL/4. Each line is resistant to 10^{-3} M MTX, has approximately 4 per cent of its protein as structurally unaltered DHFR and has about 1000-fold amplification of the DHFR gene. In three of the lines amplification has resulted in rearrangement of the DNA sequences near the 5' and/or 3' ends of the gene. Other unique and repeated sequences have also been amplified. Some repeated sequences are common to all four lines, some are present in only three, others are common to only two, and still others are unique to each cell line. Three of the four cell lines lose resistance rapidly when grown in the absence of MTX. This is associated with the presence of acentric chromosome fragments and circular chromosomes, which stain "homogeneously". Resistance in the fourth cell line is stable. In this cell there are 6 - 8 large homogeneously staining chromosomes which contain a primary constriction. Studies on a stable derivative of one of the unstable lines suggests that homogeneously staining chromosomes are derived from "double minutes", which become circularised, increase in size and eventually fragment.

1182 AMPLIFICATION OF THE METALLOTHIONEIN-I GENE IN CADMIUM-RESISTANT MOUSE CELLS, Larry R. Beach and Richard D. Palmiter, Biochemistry Department, University of Washington, Seattle, WA 98195

Friend leukeumia cells were selected that are resistant to cadmium toxicity. Over 70% of total cysteine incorporation in these cells is into the metal-binding protein, metallothionein. We used cDNA and genomic DNA clones containing the metallothionein-I gene to measure the concentration of this mRNA, its rate of transcription and the number of genes. On a per cell basis, optimally-induced, cadmium-resistant cells have 14-fold more metallothionein-I mRNA, 6-fold higher rate of metallothionein-I mRNA transcription, and 6-fold more metallothionein-I genes than nonresistant cells. Metaphase spreads reveal that the resistant cells are nearly tetraploid and contain on the average 3 double-miunute chromosomes, whereas the nonresistant cells are near diploid and contain no double-minutes.

1183 HIGH EFFICIENCY DNA TRANSFECTION AND RAPID DETECTION OF TRANSFECTED DNA EXPRESSION Gregory Milman, The Johns Hopkins University, 615 N. Wolfe St., Baltimore, MD 21205

DEAE dextran mediated DNA transfection in suspension routinely gives gene expression in 0.1% to 1% of the transfected cells. Transfection occurs with almost equal efficiency in human diploid skin fibroblasts, monkey BSC cells, mouse L or 3T6 cells and rat cells. Unlike calcium phosphate mediated transfection which yields stably transformed cell lines, DEAE dextran mediated transfection appears to produce cells which only transiently express DNA analogous to the abortive transformation described for polyoma and SV40 viruses. However, the high efficiency of DEAE dextran mediated transfection allows one to screen individual cells during the one to seven days of transient gene expression. Transfection with a bacterial plasmid containing the genes for Herpes thymidine kinase and polyoma T-antigen was detected by an in situ autoradiographic assay for thymidine kinase and/or an in situ immunofluorescence assay for T-antigen. In principle, it should be possible to detect the expression of any transfected gene which can be assayed by these or other in situ assays. This may be particularly useful when there is no selective method to obtain stable transformants with a gene. It should be emphasized that one obtains analytical data, and that the transfected cells once fixed and stained are not available for further study. Nevertheless, DEAE dextran mediated transfection and rapid in situ analysis of transient gene expression may in many cases provide an alternative to calcium phosphate mediated stable transformation.

1184 USE OF SOMATIC CELL HYBRIDS TO STUDY DEVELOPMENTALLY REGULATED HUMAN DNA SEQUENCES, Frances J. Benham, George Banting, Peter N. Goodfellow, Imperial Cancer Research Fund, London WC2A, 3PX, England.

Cultured murine teratocarcinoma cell lines have provided an in vitro system for the study of early embryogenesis. These cell lines can be induced to undergo a series of differentiation events which recapitulate normal embryonic development. Attempts to establish analogous human teratocarcinoma cell lines in cultures have so far met with only very limited success. Thus we have used an alternative approach to obtain an in vitro system which may allow investigation of developmentally regulated changes in human DNA expression.

Using the microcell chromosome transfer technique (Fournier R and Ruddle F, PNAS 74:319-323, 1977), we have constructed somatic cell hybrids between a mouse teratocarcinoma cell line (PCC4) and various human cell lines. Hybrids which contain a single human X chromosome have been selected. These hybrid cells express human X-linked enzymes and antigens. Using retinoic acid and dibutyryl cyclic AMP to induce differentiation in these hybrid cells lines we hope to be able to influence the expression of genes on the human X chromosome. We are using these hybrid cells to try to isolate DNA sequences which are expressed during early differentiation.

1185 A PORTABLE PROKARYOTIC CONTROL REGION FOR EXPRESSION OF ANY GENE, Eric James, Connaught Research Institute, Toronto, Ontario M2N 5T8

A DNA fragment carrying the entire control region of the argF gene of Escherichia coli K12 was fused to the 8th codon of the Z gene of β-galactosidase. Chimeric molecules were opened by digestion with HindIII and resected with the exonucleases III and S1. Following cleavage with BamHI and filling of single-stranded tails using DNA polymerase I, the resultant DNA molecules were circularized by incubation at a concentration of 2 μg/ml in the presence of T4-induced polynucleotide ligase. The resulting mixture was used to transform a lac- strain to ampicillin resistance. Clones exhibiting a lac+ phenotype on XG plates were picked and purified on minimal XG plates in the presence and absence of arginine. Clones having a lac phenotype responsive to the levels of exogenous arginine were picked and characterized by the Birmboim technique. Direct nucleic acid sequence determination permitted the identification of clones having a plasmid with the argF control region directly fused to the eighth codon of the β-galactosidase at a BamHI endonuclease cleavage site. Digestion with BamHI and EcoRl permits the isolation of a DNA fragment 185 base pairs long carrying a functional arginine control region which may be fused to any desired gene sequence.

1186 TRANSFORMATION OF DICTYOSTELIUM WITH HOMOLOGOUS DNA, David I. Ratner, Scripps Clinic and Research Foundation, La Jolla, CA 92037 and Thomas E. Ward and Allan Jacobson, University of Massachusetts Medical School, Worcester, MA 01605.

A transformation system for D. discoideum should facilitate the isolation and in vivo study of genes important for slime mold differentiation. We are developing a system in which mutant slime mold amoebae unable to utilize Bacillus subtilis as a food source are converted to Bacillus "prototrophy" by treatment with wild type slime mold DNA.

The bsgA5 mutation, preventing growth of amoebae on B. subtilis, is recessive and non-leaky, maps at a single locus and reverts infrequently, all factors favorable for transformation. Mutant amoebae, after DNA treatment and brief permissive growth, are plated selectively upon lawns of B. subtilis. Large molecular weight DNA preparations from a Bsg^+ slime mold strain generate Bsg^+ colonies at an average frequency of 10^{-7}, roughly ten times that of reversion of parallel control cultures treated with calcium phosphate.

We have ligated restricted wild type slime mold DNA with DNA from the pBR322-yeast-slime mold plasmid ARS22. We are now analyzing Bsg^+ colonies obtained after treatment with recombinant DNA. In the first case examined, the Bsg^+ derivative contained two DNA fragments which hybridize to pBR322; the untreated Bsg strain contains no such fragment. Such data constitute molecular evidence for transformation and offer the possibility of recovery of the bsgA gene. Isolation of the gene should allow high frequency transformation of Dictyostelium.

1187 ROLE OF DOUBLE MINUTE CHROMOSOMES IN UNSTABLE METHOTREXATE RESISTANCE. Peter C. Brown, Stephen M. Beverley, and Robert T. Schimke, Department of Biological Sciences, Stanford University, Stanford, California 94305.
Previous studies in our laboratory have suggested that unstably amplified dihydrofolate reductase (DHFR) genes in cells selected for methotrexate (MTX) resistance are associated with small, paired extrachromosomal elements denoted double minute chromosomes (DMs) which have approximately 100-1000 kilobase pairs of DNA. We have extended these studies to new cell lines and further establish the correlation between DMs and DHFR gene amplification. Murine 3T6 cells initially resistant to 50 μM MTX and having approximately 40 DHFR gene copies per cell lose these amplified sequences and cellular resistance to MTX with prolonged growth in MTX-free medium. Coincident with these losses are the loss of DMs and DHFR sequences in fraction enriched in DMs by filtration. Additionally, the correlation between DHFR enzyme content, gene copy number and DM content was established by analysis of populations of cells prelabeled with a fluorescein derivative of MTX and sorted for fluorescence by fluorescence activated cell sorting. Finally, specific hybridization of in vitro labeled DM-enriched DNA to cloned genomic segments of the DHFR gene conclusively establish the association of DMs and amplified DHFR genes.

1188 MULTIPLE CHANGES IN THE EXPRESSION OF DIHYDROFOLATE GENES ACCOMPANY THEIR AMPLIFICATION IN ANTIFOLATE-RESISTANT CHINESE HAMSTER LUNG FIBROBLASTS, Peter W. Melera, John A. Lewis and Kathleen V. Scotto, Sloan-Kettering Institute for Cancer Research, Walker Laboratory, Rye, NY 10580
When cultured in the presence of antifolate drugs, Chinese hamster lung fibroblasts acquire resistance via the overproduction of target enzyme dihydrofolate reductase (DHFR). This overproduction is associated with an increase in the cellular amount of DHFR mRNA and with an amplification in DHFR gene number. Resistant cells overproduce either a 20K or 21K DHFR, while parental drug-sensitive cells produce the 20K enzyme. O'Farrell gel analysis has revealed that all sublines contain three isoelectric forms of DHFR, but that the distribution of these forms is correlated in overproducing cells, with the molecular weight class of enzyme synthesized, i.e., the predominant isoelectric form in sublines which overproduce the 20K enzyme is pI 6.7, while the predominant isoelectric form in sublines which overproduce the 21K enzyme is pI 6.5. The distribution of these forms in drug-sensitive parental cells is different from either of those found in the overproducing sublines. In addition, northern blot analysis has revealed the presence of 3 DHFR mRNA, i.e., 1350, 2200 and 3300 nucleotides, on the polysomes of all sublines. The distribution of these mRNAs is correlated with the molecular weight class of DHFR synthesized, i.e., the 1350 and 3300 nucleotide mRNAs predominate in sublines which synthesize the 20K and 21K DHFRs, respectively. It is apparent, therefore, that the amplification of DHFR genes in Chinese hamster lung fibroblasts is associated with the overproduction of different molecular weight classes of DHFR displaying different isoelectric patterns and with alterations in DHFR mRNA distribution.

1189 GENES CODING FOR METAL INDUCED SYNTHESIS OF RNA SEQUENCES ARE DIFFERENTIALLY AMPLIFIED AND REGULATED IN MAMMALIAN CELLS, R. A. Walters, M. D. Enger, C. E. Hildebrand and J. K. Griffith, Los Alamos Scientific Laboratory, Los Alamos, NM 87545
We have isolated cell lines which survive cadmium (Cd^{++}) concentrations 10-150 fold greater than that required to kill parental Chinese hamster cells (line CHO). Cd^{++} treatment of one of these variants, 20F4, induces the synthesis of a low complexity poly A^+ RNA class. Hybridization of cDNA (cDNAa) complementary to highly abundant RNA sequences in Cd^{++} induced 20F4 cells with 20F4 cell RNA showed that: (1) the induced abundant class has a total complexity of ∿2000 NT; (2) at least 50% of these sequences are expressed in uninduced cells; and (3) Cd^{++} induction increases the cellular concentration of these sequences ∿2000 fold above preinduction levels. Cd^{++} induction of sensitive CHO cells increases the cellular concentration of only a subset of the sequences inducible in resistant 20F4 cells and then only to a level 100 fold higher than that in uninduced cells; the remainder of the sequences cannot be induced. Thus, one may postulate a role for these sequences in Cd^{++} detoxification in resistant cells. An increased capacity for the synthesis of induction specific sequences could be by the amplification of their respective structural genes. Hybridization of cDNAa with genomic DNA from three resistant variants showed that the genes were amplified ∿10 fold in 20F4 cells, ∿3 fold in 30F9 cells, and unamplified in 2C10 cells. While sensitive CHO cells can tolerate only 0.2 μM Cd^{++}, 30F9, 20F4, and 2C10 cells are resistant to 30 μM, 20 μM, and 2 μM Cd^{++}, respectively. Thus, it would appear that gene amplification cannot be totally responsible for the observed resistance of the variant cell lines. (Supported by DOE.)

1190 LOCALIZATION OF SPECIFIC GENES WITHIN SUPERCOILED LOOP DOMAINS, Bert Vogelstein, Drew Pardoll, and Barry D. Nelkin, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Recent studies indicate that eukaryotic DNA is organized into supercoiled loop domains. These loops appear to be anchored at their bases to an insoluble nuclear skeleton or matrix. Most of the DNA in the loops can be released from the matrix by nuclease digestion; the residual DNA remaining with the nuclear matrix represents sequences at the base of the loops, and possibly other sequences which are intimately associated with the nuclear matrix for other reasons. Using a quantitative application of the Southern blotting technique, we have found this residual DNA from SV40 infected 3T3 cells to be enriched in SV40 sequences, indicating that they reside near matrix-DNA attachment points. An enrichment of 3 - 7 fold relative to total cellular DNA was found in each of three different lines of SV40 infected cells. Control experiments with alpha and beta mouse globin genes showed no such enrichment. This sequence specificity suggests that the spatial organization of DNA sequences within loops may be related to the functionality of these sequences within the cell. Further experiments to test this relationship will be presented.

1191 ENHANCED DNA MEDIATED GENE TRANSFER INTO RODENT LTK$^-$ CELLS WITH RETROVIRAL REITERATED SEQUENCES, Douglas J. Jolly, H.-Ulrick Bernard, Abby C. Esty, Inder Verma* and Theodore Friedmann, Department of Pediatrics, University of California, San Diego, La Jolla, California, 92093, and*The Salk Institute, La Jolla, California, 92037.

Mouse and hamster cells deficient in thymidine kinase (TK$^-$) have been transformed by the Herpes Simplex virus TK gene (HSV-TK) cloned into pBR322 with or without Murine Sarcoma Virus long terminal repeat sequences (LTR), or by co-transformation with the HSV TK gene and the MSV LTR sequences. Transformation with plasmids containing the LTR sequences, linked or unlinked, together with the TK gene showed a 10-20 fold increase in the number of cells which survive to HAT selection. Similar experiments with the human Alu family of interspersed reiterated sequences and with the recombinants containing the polyoma origin of replication have failed to show consistent changes in the frequency of cell transformation.

1192 INTRACELLULAR RECOMBINATION OF CO-TRANSFORMING DNA OCCURRING IN THE PROCESS OF DNA MEDIATED GENE TRANSFER, Richard A. Anderson, Teresa Krakauer, and R. Daniel Camerini-Otero, Genetics and Biochemistry Branch, NIAMDD, NIH, Bethesda, Maryland 20205

We have used restriction endonuclease digests of cellular DNA to provide evidence that co-transforming (unligated and unselected) DNA sequences become covalently linked to the selected gene sequences in phenotypically transformed cells. DNA from plasmid pBR322, from SV40, or from both, was used as the exclusive carrier DNA in an otherwise standard calcium phosphate-DNA precipitation protocol for the transfer of DNA fragments bearing the herpes simplex virus thymidine kinase gene into the mouse Ltk$^-$ cell line. The resulting frequency of transformation was similar to that observed when salmon sperm DNA was used as carrier. Individual colonies of transformants were cloned, total cellular DNA cleaved with a variety of restriction endonucleases and the resulting digests subjected to electrophoresis in agarose gels. The material was then transferred to filter paper using standard blotting techniques. Subsequent hybridization reactions showed numerous examples of bands to which both ^{32}P nick-translated viral TK gene sequences and pBR322 or SV40 sequences, respectively, annealed. The apparent size of these doubly hybridizing bands varied from 1 kbp to greater than 20 kbp. The pattern of hybridizing bands was unique for each transformed line. These results open the possibility of using intracellular recombination to form and isolate recombinant molecules that express selectable or screenable functions of interest.

1193 A BACULOVIRUS AS A VECTOR FOR TRANSDUCTION OF INVERTEBRATE CELLS, Lois K. Miller, The University of Idaho, Moscow, ID 83843

Baculoviruses possess rod-shaped nucleocapsids containing a large, circular, covalently-closed, double-stranded DNA molecule which replicates in the nucleus of an invertebrate host cell. My laboratory has been pursuing basic research on the gene organization of the model baculovirus, Autographa californica nuclear polyhedrosis virus (AcNPV). Temperature-sensitive mutants of AcNPV have been isolated and partially characterized (Lee and Miller, 1979, J. Virol. 31:240-252) and have been recently mapped by a marker rescue technique relative to a previously established physical map of AcNPV restriction endonuclease fragments (Miller and Dawes, 1979, J. Virol. 29:1044-1055). Many aspects of the nature of baculoviruses suggest that these viruses may serve as ideal transducing vectors in invertebrates and a knowledge of gene organization is essential for intelligently exploiting this possibility. Baculoviruses offer the potential for inserting large segments of passenger DNA (e.g. 20 Kb) into the virus vector without affecting the ability of the virus to package the DNA. Such large segments of passenger DNA might be accommodated by (1) variable extension of the rod-shaped nucleocapsid and/or (2) replacement of viral genes responsible for occlusion, a process which is not required for virus propagation in cell culture. Ongoing research in my laboratory includes the cloning of AcNPV DNA, in vitro translation of AcNPV mRNA and studies of enzymatic activities induced upon AcNPV infection with a view to establishing a complete functional map of AcNPV.

1194 GENE TRANSFER OF AN ENDOGENOUS MOUSE MAMMARY TUMOR PROVIRAL GENE INTO CULTURED CELLS, Bernd Groner, Nancy Hynes, Nuclear Research Center Karlsruhe, Institute for Genetics, Rob Michalides, Cancer Research Center, Amsterdam, G. Zeilmaker, University of Rotterdam

GR mice contain five copies of MMTV proviral DNA per haploid genome. A combination of techniques, construction of two congenic mouse strains and identification of MMTV specific restriction fragments in the genomes of these mice, allowed the correlation of MMTV expression and mammary tumor induction with a single proviral gene, associated with the mtv-2 locus. All five genes have been cloned into λ recombinants and shown to differ slightly in the proviral part and totally in the adjacent host DNA sequences.

One MMTV proviral gene (GR-40), which is not involved in viral expression in vivo, was cotransfected into Ltk cells with a HSV thymidine kinase gene. The transfected clones of L cells were able to transcribe and translate MMTV specific gene products indistinguishable from those made by mammary tumor cells. Dexamethasone increased the level of MMTV specific mRNA about 10 fold. A likely interpretation of our results is based on the negative control of the GR-40 MMTV gene in the animal. This negative control can be relieved after molecular amplification and reintroduction of it into a different cellular environment. The signals mediating the hormonal response are cotransfected and therefore probably located in the cloned DNA. To study the tissue specificity and the extent of expression in the animal, cloned GR-40 DNA has been injected into fertilized mouse eggs. Several dozen mice have been obtained after reinplantation of the injected eggs into pseudopregnant females. These animals are being investigated for acquisition of the injected gene and its expression.

1195 THE 500-FOLD AMPLIFICATION OF A 120 KB NUCLEOTIDE SEQUENCE IN METHOTREXATE-RESISTANT CHO CELLS. Joyce L. Hamlin, Nicholas H. Heintz, Jeffrey D. Milbrandt, W. Carlton White, and Steven M. Rothman. University of Virginia, Charlottesville, Va. 22908.

For the eventual purpose of isolating and studying a single animal cell replicon, we have developed a methotrexate-resistant Chinese hamster ovary cell line which has amplified an early-replicating DNA sequence approximately 500 times; this sequence includes the gene coding for dihydrofolate reductase (DHFR, tetrahydrofolate dehydrogenase, 5,6,7,8-folate:$NADP^+$ oxidoreductase, EC 1.5.1.3). DHFR comprises 25% of the cytoplasmic protein in this cell line, and 30% of the in vitro translatable messenger. After digestion of genomic DNA with any of several restriction enzymes, the entire amplified sequence can be visualized on agarose gels by ethidium bromide staining as a unique set of highly repetitive restriction fragments; the estimated total length of the unit repeated sequence is 120± kb. Regardless of the restriction enzyme used, a subset of these repetitive fragments hybridizes to radioactive DHFR cDNA. The cell contains approximately 1,000 copies of this 120 kb amplified sequence, which represents ca. 3% of the genome in Chinese hamster cells. The homogenously-staining regions on mitotic chromosomes in which these amplified sequences are apparently located are shown to be early-replicating, as are the highly-repeated restriction fragments themselves. These data suggest that this entire, normally unique genomic segment can be cloned and mapped with respect to origins of DNA synthesis, promoters for transcription, as well as other genetic features of interest.

1196 CONSTRUCTION AND CHARACTERIZATION OF A NOVEL RETROVIRUS TRANSFECTION VECTOR. Alexandra Joyner and Alan Bernstein, the Ontario Cancer Institute, Toronto, Ontario M4X 1K9.

Studies on the genome organization of retroviruses have indicated that these viruses are natural cloning vehicles for normal cellular genes. To determine whether genes other than those naturally acquired by retroviruses can be efficiently transfected as part of a retrovirus genome, we have subcloned the 3.4 kb Bam H-I fragment of Herpes simplex virus containing the thymidine kinase gene (TK) into a cloned infectious retrovirus genome. TK was chosen because cells with a TK^+ phenotype can be selected for in HAT medium. The retrovirus used is an integrated clone of Friend spleen focus-forming virus (SFFV), a defective murine retrovirus that produces a rapid leukemia in susceptible mice. This cloned SFFV genome, like other retroviruses, has 2 long terminal repeats (5'-LTR-SFFV-LTR-3') and 2 Bam H-I sites, 1.2 kb apart located 1.5 kb 3' to the 5' viral LTR. The 3.4 kb TK fragment was mass ligated to Bam H-I digested SFFV along with an excess of left and right arms of λGTWES (λSFFV-TK). 5×10^3 λ plaques have been screened with a TK-specific probe and of these 60 gave positive signals. Of 7 analysed by restriction endonuclease mapping and Southern blot analysis, 6 of the clones have the structure 5'-LTR-TK-LTR-5' and one has the structure 5'-LTR-TK-LTR-3', where the arrows indicate the direction of the reading frame. The TK gene in both types of clones can be expressed as measured by the ability of these clones to transform TK^- mouse L cells to a TK^+ phenotype after transfection using the calcium phosphate technique. Similar analysis of the remaining λSFFV-TK clones will be presented, as well as experiments on the rescuability of SFFV-TK from NIH/3T3 cells by superinfection of these cells with various replication-competent retroviruses. (Supported by grants from the MRC and NCI of Canada)

1197 BOVINE PAPILLOMAVIRUS DNA - A NOVEL EUKARYOTIC CLONING VECTOR, Nava Sarver,[1] Peter Gruss,[2] Ming Fan-Law,[1] George Khoury,[2] and Peter M. Howley.[1] [1]Laboratory of Pathology and [2]Laboratory of Molecular Virology, NCI, Bethesda, Maryland 20205.

A novel viral eukaryotic vector derived from the transforming region of bovine papillomavirus (BPV) was established and demonstrated to be highly effective for introducing foreign genes into animal cells. The foreign DNA is replicated as an episome, actively transcribed, and the transcripts are translated into an authentic gene product.

We have constructed a DNA hybrid molecule, BPV_{69T}-rI_1, containing the transforming region of BPV DNA and the rat preproinsulin gene I (rI_1) and used it to transform susceptible mouse cells. DNA hybridization analysis has demonstrated the presence of multiple unintegrated copies of hybrid DNA molecule with the BPV-1 DNA segment and the rI_1 gene covalently linked in selected transformed cell lines. S1-nuclease analysis revealed the presence of a correctly spliced preproinsulin transcript identical in its electrophoretic mobility to mRNA produced in rat insulinoma cells. Significant levels of a protein immunoreactive with anti-insulin serum were detected by radioimmunoassay in culture medium of transformed cells. Immunoprecipitation analysis in conjunction with competitive binding to bovine proinsulin established the identity of the protein as that of rat proinsulin.

1198 INHIBITORY EFFECT OF SPECIFIC pBR322 DNA SEQUENCES UPON SV40 REPLICATION IN SIMIAN CELLS. Monika Lusky and Mike Botchan, University of California, Berkeley, CA. 94720.

Several authors have noted that recombinant pBR322-SV40 plasmids propagated in E. coli replicate inefficiently in simian cells (see Hanahan et al., Cell 21, 1980; Benoist and Chambon, PNAS, 1980, Peden et al., Science 209, 1980); furthermore Hanahan et al. suggested that such molecules replicating in simian cells lose specific activity with respect to retransformation of E. coli cells. This phenomenon is due to pBR322 plasmid sequences, which must be Cis to the replicating viral moiety to manifest the effect; secondly, the effect is not due to poor expression of the SV40 early gene products, in the simian nucleus. In an experiment wherein multiple rounds of replication of the parental plasmids were obtained after transfection into permissive COS-7 cells (Gluzman, Cell, in press) the plasmid sequences were extracted by the method of Hirt and reintroduced into E. coli HB101 cells. Among the few isolated transformants selected most plasmids were found to have suffered deletions around the pBR322 PVUII site. These molecules must have had a strong selective advantage as this class of deletion could not be detected in the original simian cell extract. Furthermore these deleted plasmids now replicate as well as wild-type SV40 DNA in simian cells and can be returned to E. coli with specific activities equivalent to pBR322 DNA. A detailed restriction map of one such selected pBR-SV40 recombinant, taken together with the structures of other recombinant plasmids which still contain the "poison" sequences, reveals that the specific pBR322 DNA responsible for these phenomena must lie between the plasmid PVUII site and the plasmid origin of replication. We are presently testing the notion that specific eukaryotic DNA modifications in this region are responsible for the restrictions defined above.

1199 TUMOR INDUCING DNA OF AGROBACTERIUM TUMEFACIENS: STRUCTURE AND ORGANIZATION IN TRANSFORMED PLANT CELLS Patricia Zambryski, Kelly Kruger, Ann Depicker, Howard Goodman Department of Biochemistry & Biophysics, University of California San Francisco, San Francisco, California 94143

Crown gall tumors are induced in plants by infection with the soil bacterium Agrobacterium tumefaciens. A portion of the large tumor inducing (Ti) plasmid of Agrobacterium, the T-region, is transferred to plant cells during crown gall tumor induction. The transferred DNA present in transformed cells is called T-DNA. We have cloned the borders of the T-DNA from independent tumor lines of tobacco. We find two types of clones: one class bracelets the two ends of the T-DNA, and another class consists of one end of the T-DNA linked to plant DNA. These studies suggest that the T-DNA is organized as tandem repeats in the plant genome. In addition, nucleotide sequence analysis of the borders of the T-DNA suggests that specific sequences at the ends of the T-DNA may be involved in the transfer of the T-region from Agrobacterium to plant cells.

1200 STUDIES OF THE STRUCTURE AND FUNCTION OF INTERVENING SEQUENCES, Andrew R. Buchman and Paul Berg, Stanford University, Stanford, CA 94305

We have used SV40-rabbit beta-globin recombinants to determine what sequences within a gene are responsible for directing the splicing pattern and also what effects the splicing process itself has on overall gene expression. An SV40-rabbit beta-globin recombinant gene constructed with no known intervening sequences or splice junctions produces very little RNA. If the first or second intervening sequences (introns) of the globin gene are inserted into this recombinant, alone or together, there is an 80-100 fold increase in the production of RNA. Deletions within the second intron do not affect either splicing or the production of RNA, unless the deletion endpoint is very close to a splice junction. When the 3' splice junction is deleted from a recombinant containing just the second intron, RNA production is greatly reduced and the RNA appears unspliced. However, if the 5' splice junction is deleted, RNA production is again decreased, but several spliced RNA's are produced which use new 5' splice junctions within both the remaining segment of intron and the globin coding sequence. This suggests that the normal splicing pattern is simply the preferred splicing pattern and not the only one which can occur. The implications of these results in terms of possible splicing mechanisms will be presented.

1201 TI-PLASMID AS VEHICLE FOR PLANT GENETIC ENGINEERING, Robert B. Simpson, David Garfinkel, Fun Mei Yang, Milton Gordon and Eugene Nester, University of Washington, Seattle, WA 98195.

Crown gall is a plant tumor incited by a soil bacterium, A. tumefaciens. A bacterial plasmid (pTi) is required for virulence. During transformation a specific portion of the plasmid DNA (the T-DNA) is transferred to the plant cell nucleus, stably maintained, attached to plant sequences and probably integrated in the plant genome.

This system should be useful for the efficient introduction of DNA into plant cells. As a first step we are using site-directed mutagenesis to study in detail the functions of the T-DNA. Starting with E. coli cloning vehicle containing a portion of the T-DNA, we mutagenize with Tn5 and isolate such recombinant plasmids which contain the transposon within the T-DNA fragment. We then place this construct back into A. tumefaciens and force it to undergo homologous recombination. Thus, we generate Tn5-induced mutants with the insertion specifically in the T-DNA. Using similar techniques we have placed a plant gene (zein from corn) into the T-DNA. This should be efficiently transferred to the tobacco cell during tumor formation.

We would like to study the expression of such foreign DNA in a plant not a tumor. We have obtained fertile plants from a single reverted tumor cell and found that the junction fragments, but not internal fragments of T-DNA found in the tumor, remain after the cells revert. Further, this residual T-DNA is stable through meiosis suggesting that the Ti-plasmid could be useful as a vehicle for genetic engineering of plants that are propagated through seed.

1202 STRUCTURE AND EXPRESSION OF HUMAN GLOBIN GENES INTRODUCED INTO MOUSE FIBROBLASTS. Mann-Jy Chen and Arthur W. Nienhuis, National Heart, Lung and Blood Institute, Bethesda, Maryland, 20205.

A recombinant bacteriophage, λHβGl, containing both the human δ and β globin genes was introduced into mouse fibroblasts by a co-transformation with a plasmid (χ1) containing the herpes simplex thymidine kinase gene using the calcium phosphate precipitation technique λHβGl contains a 16 kilobase (kb) segment of DNA including 12 kb 5' to and 2.5 kb 3' to the β globin gene (Lawn, et al. Cell 1157, 1978). A ratio of λHβGl DNA to χ1 DNA of 3:1 was used. Of the 11 stable transformants obtained, 4 were found in which the segment of DNA containing the human δ and β globin genes was intact without apparent rearrangement as demonstrated by Southern blot analysis. RNA extracted from the transformed cells was analyzed by RNA-cDNA hybridization; no more than 200 copies of human β globin mRNA per cell was found and no human β globin was detected by radioimmunoassay. The RNA species containing human β globin sequences appeared to be 50 nucleotides shorter than authentic human β globin mRNA. To assess methylation of DNA in the segment of human DNA introduced into mouse cells, digestion with Hpa II or Msp I alone or with a second restriction enzyme was performed followed by Southern blot analysis. The sites examined near the human β globin gene in transformed cells are not methylated. Although under methylation is characteristic of normally expressed globin genes in erythroid cells, this characteristic is not sufficient to insure a high level of expression in fibroblasts.

1203 CONSTRUCTION OF SV40 RECOMBINANTS CONTAINING A FUNCTIONAL GENE FOR DIHYDROFOLATE REDUCTASE, Arthur W. Nienhuis, Valerie P. Setlow and Patricia H. Turner, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205

Metabolically normal cells into which one or more copies of a functional gene for dihydrofolate reductase (DHFR) have been introduced may be selected because of their increased resistance to methotrexate (Mtx). We wish to utilize hybrid SV40 virions to transfer the DHFH gene and therefore have assembled recombinants containing DHFR coding sequences. A late region replacement was constructed in which the mouse DHFR coding sequences derived from cDNA (Chang, et al; Nature 275:617, 1978) was inserted between the Hind III (0.947) and Bam HI sites (0.143). A virus stock was obtained by infecting monkey kidney cells with the recombinant and a tsA mutant. Northern blot analysis of RNA produced during secondary infection revealed an RNA species containing DHFR sequences of approximately 19S, present in amounts equivalent to the late 16S and 19S mRNAs produced by the helper virus. A second SV40 recombinant contained DHFR sequences in the early region. The following fragments were assembled in pBR322 by successive cloning into E. coli; SV40 origin and early promoter (0.71 to 0.645) the mouse DHFR coding sequences, the human β globin gene large intron, and the 3' exon of the sheep γ globin gene including the polyadenylation site. The entire construct replaced the early region of SV40 (0.71-0.143). A viral stock was produced by using tsB helper. During secondary infection of monkey kidney cells stable polyadenylated mRNA containing DHFR coding sequences was produced. Thus we have constructed recombinant SV40 virions into which functional DHFR genes have been inserted either into the early or late region. These are potentially useful for transferring Mtx resistance to cells.

1204 MOLECULAR CLONING OF AMPLIFIED GENE SEQUENCES CONTAINED IN DOUBLE MINUTES, Howard R. Hubbell, Carlo M. Croce and Thomas W. Dolby, the Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

Double minutes (DMs) are small, extrachromosomal fragments which naturally occur in neoplastic cells. DMs may also be artificially induced by drug treatment or viral infection. DNA was isolated from an enriched fraction of DMs obtained from a human colon carcinoid cell line COLO 320 DM. This DNA was digested to completion with PstI. The digests showed several bands not seen in a similar digest of total cell DNA. A fraction of the DM digest was ligated to the PstI site of pBR322. One of the resulting clones (446) contained an insert of 4.0 kb. PstI/Bam HI digests of the 446 insert gave two bands of 2.2 and 1.8 kb. The 1.8 kb fragment hybridized to the Alu BLUR 8 sequence of intermediate repeats. The same band also hybridized to total normal human DNA. This indicates that, under the conditions used for this experiment, the 2.2 kb band contains sequences which are unique in normal DNA. Using the 2.2 kb fragment as a probe for Southern blot analysis, PstI digested DM DNA and total parental cell DNA showed 2 bands of 4.0 and 3.2 kb. Various other tumor cells of different histological types, some of which contained DMs, and normal cell lines had a band at approximately 4.0 kb. The relative intensity of the bands indicates that this sequence is amplified in the parental line, but unique in all other lines tested. Thus, minutes arising in different histological tumor types apparently contain different sequences. Partial nucleotide sequence analysis indicates that the 2.2 kb DNA contains the poly A addition recognition site, AATAAA. Thus, it is possible that the cloned 446 sequence codes for a messenger RNA which may be amplified in the tumor either *in vitro* or *in vivo*.

1205 USE OF CAULIFLOWER MOSAIC VIRUS DNA AS A MOLECULAR VEHICLE IN PLANTS, S. H. Howell, J. T. Odell, R. W. Walden, R. K. Dudley and L. L. Walker, Biology Department C016, Calif. San Diego, La Jolla, CA 92093

Cauliflower mosaic virus (CaMV) DNA infects turnips when rubbed on the leaves of healthy plants. The CaMV genome is a relatively small (~8 kb) double-strand DNA circle, containing unusual secondary structure -- including site-specific single strand breaks and a tangled or knotted structure. Cloned CaMV DNA, lacking these unusual secondary structures, also infects plants and produces viral particles containing DNA which has reacquired single strand breaks and its normal secondary form. To infect plants, the cloned CaMV DNA must be excised from the parent recombinant plasmid but need not be recyclized. We have inserted small "linker" DNAs into cloned CaMV genome to test for sites of allowable insertion. Most insertions destroy the infectivity of the cloned DNA, although some of the cloned genomes bearing these inserts can be rescued by supplying appropriate helper functions. One insertion site has been found which does not destroy the infectivity of the cloned DNA. The nature and utility of that site for insertion of larger pieces of foreign DNA into the CaMV genome will be described.

The work is supported by grants from the USDA and NSF.

1206 ERYTHROPROLIFERATIVE ACTIVITY OF A MOLECULARLY CLONED SUBGENOMIC FRAGMENT OF SPLEEN FOCUS-FORMING VIRUS DNA, David L. Linemeyer, John G. Menke, Sandra K. Ruscetti, W. David Hankins and Edward M. Scolnick, National Cancer Institute, Bethesda, MD 20205

The Friend strain of the spleen focus-forming virus (SFFV) induces a rapid erythroid disease when injected as a pseudotype into susceptible mice. In vitro, this virus induces proliferation and differentiation of erythroid precursor cells independent of the addition of erythropoietin. In order to better understand the effect of this virus on erythroid differentiation, we molecularly cloned a 3.0 kbp proviral DNA fragment of SFFV. This fragment includes 2.0 kbp from the 3' end of the proviral genome, the long terminal repeat sequences (LTR) and 0.4 kbp from the 5' end of SFFV. The 3' area of the genome contained in this fragment encodes the previously described recombinant envelope gene of SFFV. Virus preparations released from fibroblasts after cotransfection of this fragment and cloned helper virus DNA, induce erythropoietin-independent erythroid burst formation in vitro, and induce an erythroproliferative disease in vivo. Spleens from diseased mice and fibroblast cultures transfected with the SFFV DNA fragment synthesize the env gene product, gp52. Other subgenomic viral DNA fragments as well as mutagenized viral DNA have also been examined. The usefulness of these molecularly derived viral sequences for understanding how this virus can alter normal erythroid cellular differentiation will be discussed.

1207 ISOLATION AND CHARACTERIZATION OF HUMAN DNA SEQUENCES HOMOLOGOUS TO THE SV40 ORIGIN OF REPLICATION, Susan E. Conrad and Michael R. Botchan, University of California, Berkeley, California 94720

We have screened a library of human DNA cloned into the vector Charon 4A for sequences that are homologous to the SV40 origin of replication. In a screen of 10^6 plaques, one hundred and eighty positive clones were isolated. Restriction enzyme analysis has shown that for several of the "origin-like"(SVOL)clones, the homology to SV40 does in fact span the viral origin of replication.We have tested the human SVOL sequences for homology to the Alu family of repeats found in the human genome. In many of the clones examined, the Eco R1 generated fragment that hybridizes to an SV40 origin probe does not hybridize to an Alu family probe, indicating that the sequences that we have isolated are not merely a subset of the Alu family of repeats. One SVOL sequence has been subcloned onto a plasmid vector containing the chicken tk gene. The presence of the SVOL sequence increases the frequency of tk^+colonies obtained by DNA transfection 5-10 fold. Preliminary experiments indicate the presence of free plasmid DNA in the resulting tk^+cell lines.One SVOL sequence has been subcloned, and in collaboration with R.Myers and R. Tjian has been tested for its ability to bind T antigen. Our results indicate that T antigen is able to bind to this molecule in a site-specific manner. The hybridizing region in this SVOL sequence has been sequenced, and shows structural as well as sequence homology to the SV40 origin region.We are currently attempting to map the ability to bind T antigen and to enhance the frequency of tk transformation to the region that hybridizes to the SV40 origin of replication.

1208 USE OF RECOMBINANT DNA MOLECULES TO INVESTIGATE THE NATURE OF RNA SPLICING, George Khoury and Peter Gruss, National Institutes of Health, Bethesda, Maryland 20205

For some time we have been interested in the process and potential function of RNA splicing. It appears that there are at least two categories of genes, those whose RNA's are unspliced (e.g. histones, adenovirus protein IX, herpes simplex TK, and interferon), and those that have spliced transcripts (globin, insulin, actin, collagen, growth hormone, and numerous viral genes). Certain RNAs from this latter category appear to require splicing for stabilization and/or transport to the cytoplasm. We have recently shown that a mutant of SV40 which deletes the intron from the major late transcript makes no stable mRNA - yet the introduction of an intron from a heterologous source (mouse beta globin) can rescue this defective RNA. Recombinant molecules between SV40 and rat or human insulin gave rise to stable RNA molecules which use the insulin splice site. In spite of mutations involving the insulin intron which prevent its function, we have detected the synthesis of proinsulin polypeptide in infected monkey cells. SV40 recombinant molecules with other genes not normally containing splice junctions also have given rise to their encoded proteins. The nature of the stable RNAs from these recombinants will be described.

1209 POLYOMA-YEAST LEU 2-pBR322 RECOMBINANT PLASMID AS A POSSIBLE VECTOR IN ANIMAL CELLS, Bennett Cohen and Arthur Weissbach, Roche Institute of Molecular Biology, Nutley, NJ, 07110

In developing a eukaryotic vector for recombinant DNA studies, a selection system could be based on the requirement for essential amino acids which is shown by animal cells in culture. We have chosen leucine as a model system using the yeast leu 2 gene which codes for the enzyme catalyzing the penultimate reaction in leucine biosynthesis, β-isopropylmalic acid dehydrogenase. This gene is available in the plasmid Yep 13. Expression of this yeast gene in an animal cell should permit the requirement for leucine to be replaced by β-isopropylmalic acid.

We have inserted the yeast leu 2 gene into the Sal I site of a pBR322-polyoma DNA recombinant plasmid containing an Eco R1 cut head-to-tail, tandem repeat of the polyoma genome. After transfection and selective growth in E. coli recombinant DNA plasmids (Leupy) have been obtained which contained either single or tandem head-to-tail repeats of the leu 2 gene in both possible orientations. Two of the leupy plasmids, which have a single yeast leu 2 gene in either orientation, have been used to transfect Fischer rat kidney cells. Polyoma transformed cells were selected in soft agar. These Leupy transformed rat cells are presently being tested for the presence of yeast leu 2 specific DNA and RNA. Biological testing of cells in leucine free media containing β-isopropylmalic acid is underway. In parallel experiments transfection of Ltk^- cells with the HSV thymidine kinase gene in the presence of the yeast leu 2 gene has led to isolation of an Ltk^+ cell line containing a stably incorporated yeast leu 2 gene. These cells express yeast leu 2 specific RNA and are being compared to the Leupy transformed Fischer rat cells.

1210 AGROBACTERIUM MINI-TI PLASMID CODED PROTEINS ARE SYNTHESIZED IN ESCHERICHIA COLI MINI-CELLS. M. Hagiya, S.-T. Liu and C. I. Kado, Univ. California, Davis, CA 95616

Agrobacterium tumefaciens 1D1422 harbors a Ti plasmid of 28 Mdal. This mini-Ti plasmid confers the usual oncogenic and phenotypic properties like those of the larger 120 Mdal Ti plasmid counterparts, namely oncogenicity on a number of plant hosts and opine synthesis. Mini-Ti plasmid DNA fragments, generated by restriction endonuclease Sal 1 and Pst 1, were cloned in E. coli mini-cell producing strain P67-54 using pBR325 as the vector. All cloned fragments, representing the entire pTil422 plasmid, directed the synthesis of distinct proteins in the mini-cells. The plasmid sequence directing the synthesis of each of these proteins was located on a physical map of the mini-Ti plasmid. Proteins with molecular weight of 45,000, 24,500 and 16,000 from Pst 1 fragment pTL2015 corresponded to those reported in the crown gall tumor cells.

1211 OVERPRODUCTION OF ARGININOSUCCINATE SYNTHETASE (ASS) IN HUMAN CELLS DUE TO A NOVEL MECHANISM, Arthur L. Beaudet, Hans-Georg Bock, Tsung-Sheng Su and William E. O'Brien, Baylor College of Medicine, Houston, TX 77030

The human cell line RPMI-2650 (wild-type) was used to study metabolite regulation of ASS and to isolate canavanine resistant (Can^r) variants which overproduce the enzyme. Activity of ASS in nmol/min/mg protein was as follows: wild type cells grown in arginine medium, 0.14; wild type cells grown in citrulline medium, 0.86; and Can^r1 cells grown in either medium, 25.0. Immunological studies indicated similar relative differences in amounts of enzyme antigen. A marked increase in translatable mRNA for ASS was demonstrated in Can^r cells. A sucrose gradient fraction of mRNA from Can^r cells was used to synthesize cDNA which was cloned in pBR322. A near full length cDNA clone for ASS was identified by differential filter hybridization and the identity confirmed by plasmid selected mRNA translation. Dot hybridizations revealed that the relative amounts of hybridizable mRNA correlated well with the varied levels of enzyme activity in all cells and conditions tested but that the relative amounts of hybridizable DNA were similar in wild type cells and Can^r cells. Using blot hybridization the restriction digestion pattern of genomic DNA from wild type and Can^r cells showed differences in preliminary experiments. The data indicated that (1) metabolite regulation in wild type cells and overproduction of ASS in Can^r cells are mediated by changes in steady state levels of mRNA for ASS, (2) a regulatory difference between Can^r and wild type cells allows a major change in mRNA accumulation without gene amplification, and (3) there may be a change in the DNA near the structural gene for ASS in Can^r cells.

1212 A RAT GENE, TRANSFORMED INTO MOUSE CELLS, RETAINS HORMONAL INDUCIBILITY, David T. Kurtz, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

α2u Globulin is a male rat liver protein whose synthesis is under complex hormonal control in vivo. This protein is encoded by a family of 20-25 genes which are clustered on chromosome 5. Several α2u globulin genes have been isolated from a library of the rat genome cloned in λ phage. These individual genes show identical intron-exon patterns, but are bounded by distinct flanking sequences. Two of these α2u globulin genes were introduced into mouse Ltk^- cells by cotransformation. Individual cell clones were picked and were found to have incorporated from two to ten copies of the α2u globulin gene. It has been found that α2u globulin mRNA and protein are induced in these mouse cells by administration of glucocorticoids and insulin, two hormones which induce α2u globulin in rat cells. The α2u globulin mRNA which is induced is identical in size to the mRNA found in rat cells, and the α2u globulin protein which is induced is secreted into the medium. Thus, the α2u globulin gene, resident in mouse cells, retains hormonal inducibility.

1213 AN ADENOVIRUS VECTOR SYSTEM FOR THE EXPRESSION OF FOREIGN EUCARYOTIC GENES, Carl S. Thummel, Terri Grodzicker*, and Robert Tjian, Dept. of Biochem., Univ. of Ca., Berkeley, Ca. 94720; *Cold Spring Harbor Lab, CSH, N.Y. 11724.

We have taken advantage of the high level of transcription achieved by the adenovirus major late promotor to facilitate the expression of mammalian genes inserted into the viral genome. As a prototype, we have constructed an adenovirus-SV40 hybrid virus which overproduces wild-type SV40 large T antigen in infected cells. The SV40 A gene was inserted into several different regions of the adenoviral genome and recombinants were selected by their ability to propagate in monkey cells due to the helper activity provided by T antigen. Hybrid transcripts initiated from the adenovirus late promotor contain the common tripartite leader as well as correctly spliced SV40 sequences. T antigen was purified from infected HeLa spinner cells and found to be functionally equivalent to the D2 protein and structurally indistinguishable from authentic SV40 T antigen.

The adenovirus genome can accomodate large amounts of foreign DNA without becoming too large for packaging into virions. It should, therefore, be possible to insert into these vectors a foreign eucaryotic structural gene fused to the SV40 A gene and select for recombinants by their ability to propagate in monkey cells. The powerful biological selection for hybrid viruses endowed by the expression of large T helper function should provide an indirect selection for viruses which express other foreign sequences.

1214 Cloning of the Chromosomal Rabbit Uteroglobin Gene and its Introduction into Heterologous Mammalian Cells. Savio L.C. Woo, Richard Snead, T. Chandra, and David W. Bullock, Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Uteroglobin is a pregnancy-specific protein in the rabbit uterus. The protein accummulates in the uterus lumen and peaks at day 6 of pregnancy. Subsequently the level declines to the basal pre-pregnant level at day 12. Proper concentration of the protein in the uterus lumen is imperative for implantation of the developing blastocyst which occurs at day 7. Physiological and pharmacological studies have indicated that the protein is induced by progesterone but repressed by estrogen. In order to investigate the dual mode of hormonal regulation of mammalian gene expression, we have constructed a full-length cDNA clone from partially purified uteroglobin mRNA and determined its nucleotide sequence. Analysis of steady-state RNA levels in the uterus at various days of pregnancy has shown that the induction and repression of uteroglobin synthesis is the result of accumulation and depletion of its mRNA, respectively. The chromosomal gene is 2.8 Kb in length to code for a mature mRNA of 465 nucleotides and contains 3 intervening sequences, which agrees with the size of the largest precursor RNA detected by Northern hybridization. The transcription domain of the gene as determined by S1 nuclease mapping is coincidental with the 5' and 3' termini of the chromosomal gene as revealed by direct DNA sequencing. The chromosomal uteroglobin gene was inserted into a SV40 cloning vector and introduced into monkey kidney cells. Uteroglobin RNA transcripts isolated from cells transfected with the recombinant virus were analysed by Northern hybridization and S1 nuclease mapping with respect to proper initiation and splicing.

1215 THE CLONING OF HUMAN β GLOBIN GENES IN COSMIDS AND THEIR EXPRESSION IN EUKARYOTIC CELLS. Frank G. Grosveld, Henrik M. Dahl, Torben Lund and Richard A. Flavell, National Institute for Medical Research, Mill Hill, London NW7 1AA. U.K.

A method has been developed to clone large DNA fragments ($\pm$ 40kb) in *E.coli* using cosmids containing the HSV TK gene as the vector DNA. From random human DNA libraries several overlapping clones have been isolated that span the entire β globin locus ($\pm$ 100kb) as determined by restriction enzyme analysis and Southern blotting procedures. The DNA clones were characterized for the presence of different families of repeated DNA sequences and the presence of "pseudo" globin genes. Since the cosmid vector contains the HSV TK gene these DNA clones can be directly introduced and selected for in several TK^- cell lines (e.g. L cells, Friend cells) to study their expression *in vivo*. These experiments are in progress.

1216 GENE TRANSFER USING AMPLIFIED AND/OR ALTERED DIHYDROFOLATE REDUCTASE (DHFR) GENES FROM MOUSE AND CHO CELLS TO HUMAN CELLS. J.R. Bertino, S. Srimatkandada, F.J. Carr, D.I. Scheer, B.J. Dolnick and S.K. Dube, Yale University School of Medicine, New Haven, CT 06510.

DNA mediated gene transfer has been employed for transformation of a human colon carcinoma line (HCT-8) to methotrexate resistance. Donor DNA obtained from a murine L5178Y line, elevated 300-400-fold in DHFR gene copies, was used in the calcium phosphate/DNA precipitate technique. Evidence for transfer of the mouse genes to the human cells has been obtained by restriction enzyme analysis, and the finding of 10-fold elevated DHFR activity in the human line with kinetic characteristics of the mouse DHFR enzyme. A surprising finding was a 5-fold elevation of thymidylate synthetase. Preliminary data indicates that DHFR genes from chromosomes of a CHO line with elevated DHFR levels have also been successfully transferred to a human T-cell lymphoblastic leukemia line CEM-CFR. Attempts are being made to explore the variables in gene transformation using introduction of genes into Ltk^- cells as a model system.