EUCARYOTIC GENE REGULATION

Richard Axel and Thomas Maniatis, Organizers
March 4 – March 9, 1979

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The Control of Gene Expression in Drosophila

073 REPEATED SEQUENCES AND HEAT SHOCK LOCI IN DROSOPHILA MELANOGASTER AND DROSOPHILA SIMULANS,

Matthew Meselson, Pamela Dunsmuir, Robert Freund, Robert Holmgren, Kenneth Livak, Richard Morimoto, and Miriam Schweber, Department of Biochemistry and Molecular Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138.

We have hybridized unselected cloned fragments of DNA from Drosophila melanogaster and Drosophila simulans to the chromosomes of both species separately and to those of the species hybrid. We find that sequences which hybridize only at a single nonchromocentral site in the species of origin hybridize only to the same site in the sibling species, with no significant asymmetry of labeling intensity between homologues in hybrid nuclei. There is thus no indication of loss, gain or transpostiion of such uniquely located sequences since the divergence of melanogaster and simulans. In contrast, cloned fragments which hybridize at numerous sites show patterns of hybridization in situ which differ widely in the two species. For these sequences we also find differences in the pattern of hybridization between different strains of melanogaster and even between different individuals of the same strain.

A particular multi-site sequence cloned from the heat shock locus 87C1 in melanogaster exhibits behavior like that described above.[1] This sequence, the 1.5 kb $\alpha\beta$ repeat also studied by Lis et al.[2], hybridizes at several sites in melanogaster but only in the chromocentral region in simulans. Deletion of this sequence from 87C1, where it is transcribed after heat shock, appears to have no effect on any of a number of characteristics of the heat shock response. Additional characteristics of this and other sequences associated with heat shock loci will be discussed.

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[2]Lis, J., Prestidge, L. and Hogness, D. (1978), Cell 14, 901-919.

074 GENE ISOLATION BY CHROMOSOMAL WALKING,

Welcome Bender, Pierre Spierer, and David Hogness, Biochemistry Dept., Stanford U. Med. School, Stanford, CA 94305

We have begun a chromosomal walk in the 87E region of the third chromosome of Drosophila melanogaster. There are three loci we are looking for: 1) acetylcholinesterase, genetically defined as a lethal in 87E, 2) xanthine dehydrogenase or rosy which is in the distal part of 87D, and 3) bithorax, a complex cluster of homeotic genes at 89E. A cDNA clone which by chance hybridized in situ to 87E was used as a starting probe to select several overlapping pieces of Drosophila DNA from a library of 10-20 kb random shear fragments inserted into lambda bacteriophage vectors. The new isolates were mapped, and the sequences furthest from the starting point were used to probe again for a new set of overlapping fragments. To date, we have completed about eight steps or cycles, and the overlapping fragments cover 180 kb from the 87E region. The bithorax complex is too far away to walk there directly, but several inversions exist with one breakpoint in bithorax and the other around 87E. If the inversion stock is used to construct a recombinant library, one can walk across the inversion breakpoint and "jump" into the DNA sequence at 89E. We have walked simultaneously with two libraries made with DNA from two Drosophila wild type strains, Oregon R and Canton S. The two wild type sequences are well conserved except for three large blocks of repetitive DNA, each of which is present in only one of the strains at this location in 87E.

075 SEQUENCE ORGANIZATION OF DROSOPHILA tRNA GENES, Norman Davidson, Pauline Yen, Randy R. Robinson and N. Davis Hershey. Department of Chemistry, California Institute of Technology, Pasadena, CA 91125.
The Drosophila genome has been cloned as fragments of length 15-20 kb, obtained by partial EcoRI digestion, with bacteriophage Charon 4 DNA as the vector. Recombinant bacteriophage with Dm tRNA genes have been selected and the distribution of tRNA genes mapped.

It is known that there are approximately 800 tRNA genes, and probably about 100 different species (sequences): therefore, the average repetition frequency of a tRNA gene is about 8. The fine structure mapping studies of cloned DNA in this and other laboratories, and in situ hybridization studies in this and other laboratories permit the following generalizations: a) tRNA genes are clustered at a number of specific loci on the polytene chromosomes. b) For some tRNA species, there are genes at several loci. c) At some loci, there are genes for several tRNA species and these are interspersed. d) At any one locus the genes are irregularly spaced, with no evidence for tandem repeat units.

076 ALLELIC COMPLEMENTATION AND TRANSVECTION IN D. MELANOGASTER. B. H. Judd, Department of Zoology, University of Texas at Austin, Austin, Texas, 78712.
Interactions between alleles in homologous chromosomes may offer important clues to the function and regulation of eukaryotic genes. Complementation among alleles is usually attributed to the formation of a multimeric protein where subunits encoded by two different alleles can still form a functional complex. However, the large number of loci that exhibit allelic complementation in Drosophila compels alternative explanations. The study of several complex loci suggests that those alleles that give complementation are mutant in regulatory elements. At the cut locus for example there appear to be several regulatory elements that act in cis with the structural element but which act independently of each other. In other words each regulatory element can complement other regulator units in both cis and trans. Such a pattern may be rather general among the complex loci of Drosophila. Furthermore, we should be alert to the possibility that some regulatory units within a locus may function in either cis or trans to structural sequences. If this does happen, mutations at such loci might be expected to show transvection.----E. B. Lewis (1) described transvection when he noted that the mutant phenotype of two bithorax alleles in trans configuration is enhanced by chromosome rearrangements with break points near but not in the bx locus. He suggested that such rearrangements upset homolog pairing and interfere with the interaction between bx alleles. Jack and Judd (2) have shown that the repression of the white locus by the zeste locus product occurs only if there are at least two w^+ genes paired or closely adjacent. The interpretation is that the white locus is producing a small RNA that complexes with the zeste gene product to repress w^+ activity. I suggest that fragments of RNA released during processing can be used as repressors or activators or primers. Consider also the possibility that during the maturation of mRNA, pieces transcribed from both homologs might be recombined to form some functional message even though each homolog contains a mutant allele for that locus. Allelic complementation and transvection could reflect complementation at the level of RNA processing.

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(2) Jack, J. W. and B. H. Judd. Proc. Nat'l. Acad. Sci. (Wash.) (In press)

The Control of Gene Expression in Yeast

077 ISOLATION AND CHARACTERIZATION OF YEAST GENES BY TRANSFORMATION, Gerald R. Fink, P. Farabaugh, and C. Ilgen, Department of Botany, Genetics and Development, Cornell University, Ithaca, N.Y. 14853
The his4 gene of S. cerevisiae has been isolated by transformation. A bank of 5000 E. coli clones each containing a Bam Hl insert into pBR313 was divided into pools of 100 and the heterogeneous plasmid population from each of the sub-banks was extracted. Each of these pools was used to transform a yeast his4 deletion. Only one of the 50 sub-banks was capable of transforming the his4 deletion to His$^+$. This sub-bank was fractionated further and a single plasmid pYeHIS4 was identified as the source of yeast transformants. This plasmid contains a yeast Bam insert of approximately 14 million daltons which is the HIS4$^+$ gene as well as a portion of chromosome III. This conclusion is based on several different lines of evidence.

His$^+$ transformants made with pYeHIS4 have a duplication of the his4 region and a contiguous pBR313 segment on chromosome III. Thus, in transformation the pYeHIS4 plasmid has inserted on chromosome III by homology with the resident his4 and adjacent sequences. Moreover, hybridization of pYeHIS4 with Southern blots made from restriction digests of total DNA from various deletions of his4 show that various segments of pYeHIS4 have homology with the genomic his4 region. pYeHIS4 fails to complement hisD mutants of E. coli. This result agrees with several other experiments showing that his4 fails to function in E. coli.

The regulation of the his4 region appears to be controlled by a region at the his4A end. Mutations which map outside the structural gene at this end lead to a loss of his4 function. One of these, his4-912 has bizarre genetic properties which are revealed in reversion analysis.

078 EXPRESSION OF HIS3 AND GAL GENES IN YEAST, Ronald W. Davis, Kevin Struhl, and Thomas St. John, Department of Biochemistry, Stanford University, Stanford, CA 94305.
We have studied a cloned 10 kb EcoRI fragment (Sc2601) containing the yeast his3 gene. In E. coli, expression of the wild type his3 gene results in the production of functional IGP dehydratase which resembles the yeast enzyme. We have obtained a detailed physical, genetic, and transcriptional map of Sc2601. Expression of the yeast his3 gene is compared in yeast and E. coli cells.

The his3 gene (as defined by E. coli complementation analysis) is localized to a region of less than 750 base pairs. In E. coli, transcription of the his3 gene is initiated from a promoter which maps less than 100 base pairs from the start of the structural gene. We have isolated a deletion mutant of Sc2601 which inactivates this promoter but which retains the intact his3 structural gene. In such a deletion mutant, his3 expression in E. coli occurs only when the gene is fused to a bacteriophage promoter. Deletions ending in this region also do not express his3 function when transformed back into yeast cells using a yeast vector with a yeast chromosomal replicon. We have also physically mapped the location of the lesions in cloned mutant his3 genes.

Yeast cells produce 5 discrete mRNA species which hybridize to Sc2601. Approximately 80% of this fragment is transcribed in growing yeast cells. The spacing between the different RNAs is approximately 300-500 base pairs. The his3 mRNA is about 650 base pairs in length. The 5' end of this RNA is located less than 100 base pairs from the promoter defined by expression in E. coli and in yeast. Since the location of the gene determined either by the mapping of the yeast mRNA or by E. coli and yeast complementation analysis is strikingly similar, we suggest that the genetic signals necessary for yeast and E. coli expression may share common recognition features.

cDNA probes representative of total polyA$^+$ RNA have been used to screen λ-yeast hybrids. A comparison of the labeling of individual phage with a variety of probes prepared under inducing and noninducing conditions for galactose and maltose has resulted in the isolation of DNA fragments whose sequences appear to be present in the polyA$^+$ class of total RNA only under inducing conditions. The galactose induced regions appear to be controlled by the galactose constitutive mutations GalC and gali$^-$. The RNAs encoded by this region cannot be detected in any appreciable quantity under any growth conditions tested if the strain examined is gal4$^-$. One region, contains coding sequences for three galactose controlled RNAs. Two of these RNAs are transcribed in the same direction, the third from the other strand. The strain 2894-5c (originally from Hawthorne) has been genetically characterized as a deletion of the Gal 1,7,10 gene cluster. When compared to the cloned region, this strain appears to have a deletion with an endpoint in the RNA cluster coding region.

079 CONTROL OF MATING TYPE IN YEAST, James Hicks, Amar J.S. Klar and Jeffrey N. Strathern, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724.

The interconversion of mating types in homothallic yeast represents a simple developmental event in the life cycle of that organism. Under the influence of the HO (homothallism) gene cells of one mating type (a or α) give rise to daughter cells of both mating types during mitotic division. This process is analogous to the derivation of differentiated cells from stem cells in the developing tissue of higher organisms. Changes in cell type occur in regular patterns among the progeny of homothallic cells clearly showing that the developmental potential for switching is distributed in an ordered fashion.

The mating behavior of Saccharomyces yeasts is controlled by the alleles of a single mating type locus (MAT). The efficient interconversion of mating types is the result of a reversible genetic alteration of MAT which results in the alternative expression of the MATa and MATα alleles. The molecular basis of the switching event is proposed to involve sequential transposition of DNA copies of MATa and MATα information into the MAT locus from silent (unexpressed) copies of these genes located elsewhere on the same chromosome. This proposal has been termed the "cassette model" for differentiation. In an attempt to further define the switching mechanism we have used physical and genetic methods to characterize mutations which affect the interconversion process and the expression of the MATa and MATα genes.

A major genetic prediction of the cassette model is that mutations introduced in the silent copies of MATa and MATα (the proposed sources of the genes introduced during switching) should be efficiently transposed to the MAT locus during the switching process. We have observed such transposition of mutant information for the MATa allele. This observation provides strong support for the general concept that the activation of cell type regulators can occur by site-specific transposition. In addition, we have isolated a number of mutations in the MATα allele which affect the control of cell type specific functions and make the mating type locus defective as a substrate for the interconversion process. Several of the MAT mutations affecting control of cell type have been shown to be major structural alterations of the mating type chromosome.

080 GENE AND GENOME STRUCTURE IN YEAST,*David Botstein,†Marie-Louise Bach, and †Francois Lacroute, *MIT, Cambridge, MA., USA. †Lab de Genetique Physiol., Institut de Biologie Molec. et Cell. du C.N.R.S. Strasbourg, France.

From a large population of strains of E. coli shear fragments of yeast (Saccharomyces cerevisiae) DNA attached by in vitro recombination to the plasmid vector pMB9, two hybrid plasmids were selected which relieve the pyrimidine requirement of non-reverting pyrF mutants of E. coli. An 1100 basepair DNA fragment common to the two complementing plasmids was recloned into another plasmid vector, pBR322; these new hybrids retain the ability to specify OMP-decarboxylase synthesis in E. coli. Evidence is presented that this common fragment is yeast DNA and thus apparently carries the structural information for yeast OMP decarboxylase, the product of the yeast gene ura3.

A hybrid plasmid containing the 1100 basepair fragment was used to measure levels of putative ura3 messenger RNA from yeast cultures labeled with ^{3}H-adanine. It was observed that ura3 mRNA is unstable with an apparent halflife of 10.5 min. Under different circumstances previously shown to alter the level of OMP-decarboxylase in yeast, a coordinate variation in proportion of labeled RNA complementary to the hybrid plasmid was found. These data support the hypothesis that regulation of the ura3 gene in yeast is at the level of transcription.

Recent observation on the expression of the ura3 gene in different genomic contexts will be discussed.

081 SEQUENCE ANALYSIS OF A YEAST $tRNA^{Trp}$ GENE AND ITS PRODUCT, Hyen Sam Kang, Richard C. Ogden and John Abelson, Department of Chemistry, B-017, University of California, San Diego, La Jolla, CA 92037.
It has previously been shown that the genes for yeast $tRNA^{Tyr}$ [1] and yeast $tRNA^{Phe}$ [2] contain intervening sequences. A temperature sensitive yeast mutant, ts 136 accumulates precursors to $tRNA^{Phe}$ and $tRNA^{Tyr}$ which have been shown to contain the intervening sequence.[3] We report here that a precursor to yeast $tRNA^{Trp}$ which also accumulates in ts 136 has an intervening sequence and is colinear with the $tRNA^{Trp}$ gene.

DNA from a single clone containing the yeast $tRNA^{Trp}$ gene was analyzed by restriction mapping and Southern hybridization to a pure yeast $tRNA^{Trp}$ probe. Both strands of a fragment containing the gene were sequenced according to the method of Maxam and Gilbert.[4] An intervening sequence of 34 base pairs was found adjacent to the 3' end of the anticodon.

The DNA sequence data confirms and extends fingerprint data from the $tRNA^{Trp}$ precursor isolated from in vivo labelled ts 136. The precursor tRNA contains mature 5' and 3' ends, but the oligonucleotide corresponding to a mature anticodon loop is absent. Several additional oligonucleotides are observed and have been shown to derive exclusively from the intervening sequence. In common with other tRNA precursors isolated from ts 136, pre-$tRNA^{Trp}$ contains some but not all modified nucleotides. As reported earlier,[3] pre-$tRNA^{Trp}$ can be processed in vitro using a crude yeast extract to give $tRNA^{Trp}$ containing the mature anticodon loop. By analogy with pre-$tRNA^{Tyr}$ and pre-$tRNA^{Phe}$, a secondary structure model of pre-$tRNA^{Trp}$ indicates that the anticodon can base-pair with part of the intervening sequence, but no homology to the intervening sequences in either pre-$tRNA^{Phe}$ or pre-$tRNA^{Tyr}$ exists.

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Embryogenesis and the Genetics of Development

082 TERATOCARCINOMA CELL MUTATIONS AS PROBES OF MAMMALIAN DIFFERENTIATION, Beatrice Mintz, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pa. 19111.
The malignant stem cells of mouse teratocarcinomas have been found to lose their neoplastic properties and to undergo normal differentiation if placed in a normal early-embryo (blastocyst) environment (1, 2). Under these conditions, the stem cells are developmentally totipotent: Tumor-derived cells can contribute to the formation of all somatic tissues and--if still close to euploidy--to germ cells, from which progeny are obtained. The teratocarcinoma cells thus provide a novel channel for deliberately introducing into mice specific, predetermined genetic markers. These can serve to dissect developmental processes; as probes for analyzing control of tissue-specific gene expression; and as agents for producing mouse models of human genetic diseases (3-5). The experimental possibilities rest upon the combined utilization of techniques of somatic cell genetics, molecular biology, and developmental biology. Genetic markers may be obtained by changes within the same species, or introduced from foreign species; they may be nuclear (6) or cytoplasmic (mitochondrial) (7). They are first brought into the teratocarcinoma cells in culture following mutagenesis or transfer and, after the application of appropriate selective or screening procedures, cells with the mutation of interest are placed in the in vivo setting, where gene expression may be optimally tested during differentiation.

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083 STUDIES ON EARLY DEVELOPMENT OF C. ELEGANS, David Hirsh, Scott Emmons, James Files, and Judith Kimble, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309

Caenorhabditis elegans is valuable for studying eucaryotic development because it can be manipulated easily genetically, it's cell lineages can be observed and recorded directly and it's genome is only twenty times the complexity of the E. coli genome. The cell lineages that give rise to the reproductive system have been followed and the pattern strongly suggests that whereas invertebrate development is usually considered to be mosaic, there are distinct cases of regulative development in C. elegans. More recently, the DNA of C. elegans has been shown by reassociation kinetics to have the Drosophila pattern of wide interspersion of repetitive sequences. By examining the hybridization of cloned restriction fragments to germ and somatic DNA's on Southern filters, it has been shown that no gross rearrangement of the DNA occurs during embryonic differentiation of the basic cell types. However, about 1% of the sequences differ between two interbreeding strains of C. elegans and these differences can be used as genetic markers; essentially no homology exists between DNA of sibling species of Caenorhabditis. By contrast, the structure of the ribosomal genes is extremely conserved between the two strains and very similar between the different species.

084 CELL LINEAGE IN THE DEVELOPMENT OF DROSOPHILA, Peter A. Lawrence, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH and Gines Morata, C.S.I.C., Centro de Biologia Molecular, Universidad Autonoma de Madrid, Canto Blanco, Madrid, Spain.

In Drosophila, genetic methods for marking individual cells, and their descendents, are now quite sophisticated. These methods have shown that, in early development, small groups of cells (polyclones) are set aside and each of these groups forms a precisely defined part of the adult fly (a compartment). These compartments appear to coincide with the realms of action of some homeotic mutations, suggesting that the establishment of a polyclone results from the activation of specific control genes. Garcia-Bellido (1975) has therefore proposed that the development of each polyclone is specified by a binary code word the combination of active and inactive 'selector genes'. Some of the evidence for Garcia-Bellido's theory will be examined.

Later in the development of compartments finer patterns are established, such as the rows of bristles on the legs, or the "crystalline" cell lattice in the retina. I will discuss the roles of cell lineage and compartments in the formation of these structures.

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The Organization of Specific Eucaryotic Genes

085 ORGANIZATION AND EXPRESSION OF OVALBUMIN AND RELATED CHICKEN GENES.
Pierre Chambon, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg/France

The structure of the ovalbumin gene has been established by restriction enzyme mapping of chicken chromosomal DNA and of cloned genomic fragments and by electron microscopy of hybrids between the cloned fragments and the ovalbumin mRNA (ov-mRNA). The ov-mRNA which is 1872 nucleotides in length is encoded for by a leader-coding region and 7 exons which are separated by 7 introns. The minimum length of the ovalbumin transcriptional unit is about 7500 bp. Discrete RNA molecules containing colinear transcripts of both exon and intron sequences have been isolated. The largest transcript is about 8000 nucleotides in length, which suggests that transcription of the ovalbumin gene could start at (or very close to) the leader-coding region. Biochemical and electron microscopic studies have revealed that, during the maturation of ov-mRNA, intron transcripts are removed stepwise from the primary transcript, but not necessarily in their order of transcription. DNA has been sequenced around the leader-coding region, revealing the existence of a sequence (5'-TATATAT-3') resembling those found at similar locations in other eukaryotic genes. All of the ovalbumin gene intron-exon junctions have been sequenced. In all cases comparison of the DNA and ov-mRNA sequences does not allow the unambiguous definition of the splicing (excision-ligation) points, due to the presence of short (2-4 nucleotides) directly repeated sequences at the extremities of the introns. However, the structure of all of these "boundary" sequences are closely related and it can be proposed as a general rule that the 5' and the 3' ends of all introns are defined by the dinucleotides 5'-GT-3' and 5'-AG-3', respectively.

The structure and the expression of conalbumin, ovomucoïd and other related chicken genes have also been studied and will be compared with those of the ovalbumin gene.

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086 MOLECULAR STRUCTURE OF THE NATURAL OVALBUMIN AND OVOMUCOID GENES AND THE PRECURSORS TO THEIR MESSENGER RNAs, Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

To completely define the steroid hormone-mediated expression of the ovalbumin and ovomucoid genes in the chick oviduct, it was necessary for us to understand the molecular structure and sequence organization of these genes. The natural ovalbumin gene was amplified by molecular cloning, and detailed analysis was accomplished by restriction enzyme mapping, Southern hybridization and electron microscopic mapping. We found that the structural ovalbumin gene sequences are segregated into 8 portions by 7 intervening DNA sequences of various lengths that are not represented in the mature messenger RNA. Using the cloned ovalbumin gene as a specific hybridization probe, the sequence organization of this gene in DNA from individual chickens was analyzed and genotypic alleles have been defined. We have subsequently mapped the chicken ovomucoid gene in total DNA and again found that the structural sequence is interrupted by a series of intervening sequences. These findings have been confirmed by direct analyses of cloned fragments of the natural ovomucoid gene.

Specific DNA probes prepared from structural and intervening sequences within the cloned natural ovalbumin and ovomucoid genes were used to identify the precursors of these mRNAs. Nuclear RNA from hormone-stimulated chick oviducts was electrophoresed under denaturing conditions (in the presence of methylmercury hydroxide) followed by transfer to diazobenzyloxymethyl paper and hybridization to ^{32}P-DNA probes for structural and intervening sequences. Hybridization of structural sequence probes to cytoplasmic RNA gave separate single low molecular weight bands at the expected size of mature ovalbumin and ovomucoid mRNA. However, hybridization of the same probes to nuclear RNA demonstrated that multiple species of RNA exist which are higher in molecular weight than mature ovalbumin and ovomucoid RNAs. These high molecular weight species contain RNA complementary to both structural and intervening sequences of either the ovalbumin or ovomucoid genes and thus appear to represent transcripts of these genes at various processing stages. These results are consistent with the entire ovalbumin and ovomucoid genes being transcribed into large precursor molecules, followed by excision and turnover of the intervening sequence RNA and consecutive ligation of the structural sequences to form mature ovalbumin and ovomucoid mRNAs.

087 THE ORGANIZATION AND EXPRESSION OF SEVERAL MAMMALIAN POLYPEPTIDE HORMONE GENES, Howard M. Goodman, John C. Fiddes, Peter H. Seeburg, John Shine, Joseph A. Martial and John D. Baxter, Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94143.

We have recently reported the construction of recombinant DNA plasmids containing portions of the structural genes for rat preproinsulin[1], rat growth hormone[2] and human chorionic somatomammotrophin[3]. All of these were obtained by cloning of cDNA copies of mRNA. The complete nucleotide sequence of each recombinant DNA was determined. Similar techniques have now been used to isolate a DNA fragment which codes for amino acids 24-190 of human growth hormone. It is very homologous in sequence to HCS, both in the coding region and the 3'-untranslated region, suggesting a common evolutionary origin for these two hormones.

To study the structure of these hormone genes in cellular DNA, rat and human fetal DNAs were cleaved with various restriction endonucleases and the sizes of the gene fragments examined by hydridization with cloned cDNA labeled _in vitro_ and used as radioactive probes. After agarose gel electrophoresis and _in vitro_ packaging use λgt WES. λB a 2.4 kb fragment from human DNA has been cloned and identified as containing the gene for human growth hormone. It, like many other eukaryotic structural genes, contains an intervening sequence(s).

The growth hormone (GH) gene is expressed by cultured rat pituitary (GC, GH_3) cells and is related to the genes for prolactin and chorionic somatomammotrophin. In the cells, thyroid (T_3) and glucocorticoid hormones each induce GH mRNA and the two hormones are synergistic. Further, insulin can prevent the ability of glucocorticoids to induce GH mRNA, an effect that can be overcome by T_3. Glucocorticoids and T_3 appear to affect less than 0.5% of the expressed genes. The cellular milieu can be further manipulated such that glucocorticoids deinduce GH production and chromatin capacity for polymerase binding.

In other studies on the expression of these hormone genes in bacteria, cloned cDNA to rat GH mRNA from plasmid pMB9 was ligated to the _PstI_ endonuclease restriction site in the ampicillin resistance gene to plasmid pBR322[4]. A new protein of the size expected for the fusion polypeptide between a beta-lactamase (that confers ampicillin resistance) and growth hormone was produced by the new plasmid; in addition, material from these clones, but not from other _E coli_ clones without or with pBR322 produced material that reacted to antisera to rat growth hormone. Thus, these bacteria appear to produce a protein containing amino acids of growth hormone.

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088 THE STRUCTURE AND TRANSCRIPTION OF RAT PREPROINSULIN GENES, Argiris Efstratiadis*, Stephanie Broome†, William L. Chick+, Walter Gilbert†, Lydia Villa-Komaroff‡, Peter Lomedico†, Stephen P. Naber+, Francine Perler*, and Richard Tizard†, *Department of Biological Chemistry and +Joslin Research Laboratory, Harvard Medical School, Boston, MA 02115; †Biological Laboratories, Harvard University, Cambridge, MA 02138; and ‡Department of Microbiology, University of Massachusetts, Worcester, MA 01605.

In rat there are two non-allelic genes, I and II, encoding preproinsulin, a polypeptide chain consisting of an amino terminal hydrophobic leader sequence and peptides B, C and A. Mature insulin is produced by the sequential cleavage of the leader sequence (formation of pro-insulin) and finally of the C-peptide.

By sequencing double-stranded cDNA copies of rat preproinsulin mRNA I, Ullrich et al.(1) and ourselves(2) determined the primary structure of this mRNA with the exception of the 5' noncoding region. We now have extensive sequence information for rat preproinsulin II mRNA from other cDNA clones.

By using cloned insulin DNA as probe we have studied the genomic organization of preproinsulin genes by the Southern DNA blotting technique and examined whether or not glucose (a physiological stimulus for insulin biosynthesis and release) stimulates transcription of preproinsulin sequences.

One of our double-stranded cDNA clones produces proinsulin in the form of a fused protein bearing both insulin and penicillinase antigenic determinants(2). We now have evidence that following mild treatment with trypsin the insulin synthesized by the bacteria is biologically active.

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Intercellular and Intracellular Gene Transfer

089 ANTIBODY DIVERSITY AND THE REARRANGEMENT OF ANTIBODY GENES, Philip Leder, J. G. Seidman, Edward E. Max, Barbara Norman, Marion M. Nau and Philip Hieter, National Institutes of Health, Bethesda, Maryland 20014

Studies of cloned fragments of mouse chromosomal kappa light chain genes provide direct evidence for a multiplicity of immunoglobulin variable region genes as well as for the somatic rearrangement of variable and constant region sequences. We have cloned and determined the nucleotide sequences of several immunoglobulin variable and constant region genes, both from embryonic and immunoglobulin producing cells. From a comparison of the structures of these genes, it is clear that a substantial measure of immunoglobulin diversity has arisen during evolution and is encoded in germline DNA. Furthermore, variable region genes appear to fall into many small, structurally related subgroups that share extensive homology in both their coding and flanking sequences. This homology, limited within each subgroup, suggests a recombination mechanism that could play an important role in generating additional diversity in the germline and, possibly, in somatic differentiation as well.

In addition, analysis of the cloned segments indicates that immunoglobulin kappa genes are encoded in four discrete coding segments in embryonic DNA--a leader sequence, a variable region, a joining region (J) and a constant region. Two of these, the variable and J sequences, join during somatic differentiation to form a continuous variable region that is separated from the constant region by a large intervening sequence of DNA in antibody producing cells. Thus, the J segment appears to present two faces for recombination; a DNA recombination site and a RNA splicing site. The correct joining of only one variable and J segment per cell may account for the allelic exclusion observed in antibody-producing cells.

090 GENE TRANSFER BETWEEN MAMMALIAN CELLS, Frank H. Ruddle, Department of Biology, Yale University, New Haven, Conn. 06520

Genetic information can be transferred between cells by various methods: namely, cell hybridization, microcell transfer, chromosome mediated gene transfer, and DNA mediated gene transfer. Emphasis will be placed on an evaluation of mechanisms of transformation involving the latter two systems. In both, stable and unstable transformants are obtained. Stable transformants are those which retain the selected, prototrophic marker in the absence of selection, whereas unstable transformants lose the prototrophic marker at rates of 1-10 percent of cells per generation under these conditions. Stable transformants may be derived from unstable transformants, but not vice versa. In both systems, it can be shown that the stability phenotype can be explained by the integration of the transferred genetic material (transgenome) into the host genome. Integration is not restricted to sites in the recipient genome homologous to the selected, prototrophic marker integral to the transgenome. In some instances, integration sites for a specific donor prototrophic marker have been mapped to different chromosomes. Analysis at the molecular level also provides evidence for multiple integration sites. The size of the transgenome can vary greatly, ranging from several thousand kilobases to microscopically visible chromosomal segments, representing 1-3% of the donor haploid genome (1-3x10^7 kb). In the case of chromosome mediated gene transfer, analysis of markers which are syntenic and asyntenic in regard to the selected prototrophic marker, has shown that the process of transformation can result in the disruption of original linkage relations and the formation of new ones, even between markers located on different chromosomes (congression). It will be shown how such rearrangements of linkage can be used to establish the distance relationships between genes. The nature of the transgenome in unstable transformants is still poorly understood. Experiments in progress will be described which test the hypothesis that the unstable transgenome can be considered as a kind of mammalian cell plasmid.

091 TRANSFORMATION OF MAMMALIAN CELLS WITH PROKARYOTIC AND EUKARYOTIC GENES. Richard Axel, Raymond Sweet, Angel Pellicer, Saul Silverstein, Gek Kee Sim, Barbara Wold, Michael Wigler, College of Physicians & Surgeons, Columbia University, New York, New York 10032.

Analysis of the organization of several cloned eukaryotic genes has revealed a number of surprising structural features. Transformation of appropriate eukaryotic cells with these genes may provide a system in which the relationship between gene expression and sequence organization may be examined. In our laboratory we have transferred cellular genes from complex vertebrate genomes to cultured mammalian cells. These studies, initiated with a purified viral thymidine kinase gene, were subsequently extended to the transfer of a variety of cellular genes coding for selectable biochemical markers including thymidine kinase, adenine phosphoribosyl transferase and dihydrofolate reductase. More recently, we have stably transformed mammalian cells with a variety of precisely defined prokaryotic and eukaryotic genes for which no selective criteria exist. The experimental rationale we have chosen is based upon our observation that the small selectable subpopulation of cells within a culture are competent in transformation. Transformation was therefore performed with two physically unlinked genes, only one of which was necessary for survival under selective conditions. Biochemical transformants represent the subpopulation of competent cells which integrate other unlinked genes at high frequency. In this manner, we have used the viral tk gene as a selectable marker to construct mouse cell lines that contain the tk gene along with bacteriophage ΦX 174, plasmid pBR 322, or the rabbit β globin gene sequences stably integrated into cellular DNA.

092 GENE AMPLIFICATION AND METHOTREXATE RESISTANCE IN CULTURED MAMMALIAN CELLS, Robert T. Schimke, Peter Brown, Randall Kaufman, Jack Nunberg, and David Setzer Department of Biological Sciences, Stanford, CA 94305

We have reported previously that step-wise selection of cultured murine cells for progressively increasing resistance to methotrexate (MTX) is associated with increased rates of synthesis of dihydrofolate reductase (DHFR) and a corresponding amplification of DHFR genes (1). In some cell isolates the high gene copy number is stable in the absence of continued selection pressure (MTX), whereas in other cell isolates the population loses genes upon growth in the absence of MTX.

We have analyzed the process of loss of genes by employing a fluorescein-MTX conjugate which is taken up by cells and binds quantitatively and specifically to DHFR. Cells can then be analyzed with the Fluorescence-Activated Cell Sorter for the heterogeneity of the population with respect to DHFR gene copy number (2). We conclude that in the unstable cell populations there is an unequal segregation of genes among daughter cells, and that cells with a low gene copy number have a selective growth advantage. Thus with time the population of cells will appear to have lost genes.

The DHFR mRNA contains approximately 1700 nucleotides, including at least 102 nucleotides 5' from the start codon and 900 nucleotides 3' from the termination codon. We have shown that DHFR DNA sequences derived from mRNA are expressed in E. coli (3). Restriction analysis studies of the genomic representation of the DHFR mRNA indicates the same restriction patterns in sensitive and resistant (highly amplified DHFR genes) cell lines of murine origin. It is clear that there are intervening sequences in the genomic DHFR gene, and our current estimate is that the genomic sequences containing the DHFR mRNA sequences may be as large as 20 kb.

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(3) Chang et al, Nature, 275:617, (1978).

The Control of Globin Gene Expression

093 CLONED MOUSE GLOBIN GENES,
Philip Leder, Dean H. Hamer, David Konkel, Aya Leder, Henry Miller and Yutaka Nishioka, National Institutes of Health, Bethesda, Maryland 20014

Each of the mouse α and β globin chromosomal genes that we have cloned is encoded in three discontinuous coding blocks separated by two intervening sequences of DNA. These intervening sequences occur at corresponding sites in each gene, suggesting that they have been preserved at the same locus since early vertebrate evolution and are, therefore, critical to the expression of these genes. These preserved homologies also suggest that intervening sequences will be found at these same sites in the globin genes of all vertebrate organisms. The entire nucleotide sequence of one of the genes, β-globin^{maj}, and large portions of the β-globin^{min} and α-globin have also been determined. From a comparison of these sequences we are able to identify segments of the chromosomal gene in flanking and coding sequences that have been preserved during their separate evolution and that likely play a critical role in the initiation of transcription, processing (splicing) and termination or polyadenylation of the primary transcript. Further, to provide a functional assay for the nature of these putative promoter, terminator and splicing signals, we have cloned several of the chromosomal segments in a defective SV40 vector and studied their expression in infected cells. Our initial experiments indicate that globin gene splicing and polyA addition signals are appropriately recognized if transcribed in a "sense" direction from a late SV40 promoter. Inverting the globin chromosomal segment, resulting in the transcription of the anti-sense strand, does not interfere with transcription of the segment, but eliminates the globin splicing and polyadenylation functions.

094 STUEDIES ON THE STRUCTURE AND EXPRESSION OF β GLOBIN GENES,
Charles Weissmann, Albert van Ooyen, Johan van den Berg, Anton Schamböck, Ned Mantei, Christine Gruber, Peter Curtis, Frank Grosveld and Robert Weaver, Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland.

We have cloned a rabbit chromosomal DNA segment containing a β globin gene and determined the nucleotide sequence of the gene and its neighboring regions. The coding sequences are separated into three blocks by two introns, a larger intron of about 573 base pairs following the codon for Arg^{104} and a shorter one, of 126 base pairs, following the codon for Arg^{30} (the positions cannot be defined precisely because of some sequence redundancy around the termini of the introns). The introns, particularly the large one, are strikingly rich in runs of T. A comparison with the mouse chromosomal β globin DNA cloned by Tilghman et al. (PNAS 74, 4406-4410 (1977)) showed that corresponding introns were of similar size, and located at homologous positions. The nucleotide sequences of the corresponding mouse and rabbit introns differed very much; short identical sequences were found at the junctions with the coding regions and scattered through the intron. We believe that the large and small mouse and rabbit introns are derived from common ancestral sequences, but have shown far greater evolutionary divergence than the coding regions. This would imply that there is little selective pressure for the maintenance of a particular nucleotide sequence of the introns.

Expression of the β globin gene has been studied in DMSO-induced Friend cells. We have described earlier a 15 S β globin-specific RNA (about 1500 nucleotides long) with 3' terminal poly(A) and the same caps as mature β globin mRNA. It is processed to mature mRNA with a half-life of about 2.5 min. In collaboration with P. Leder and his colleagues (Tilghman et al., PNAS 75, 1309-1313 (1978)) it was shown by R-looping that the 15 S RNA was colinear with the mouse chromosomal β globin gene. The 5' terminus of the precursor maps at the same position of the chromosomal DNA as that of the mature messenger. Double-stranded cDNA prepared from 15 S β globin RNA was cloned in pCRI. Restriction analysis showed the presence of the large and small introns.

We have isolated and characterized an uncapped RNA of about 84-87 nucleotides length (it is heterogeneous at the 3' end), from L cells and induced and non-induced Friend cells. It hybridizes to a region 1600-2700 b.p. downstream from the end of the globin gene and is identical with the RNA 16 associated with 70 S MuLV RNA described by Peters et al. (J. Virol. 21, 1031-1041 (1977)).

095 THE STRUCTURE AND EXPRESSION OF NORMAL AND ABNORMAL GLOBIN GENES,
Richard A.Flavell, Gerard C.Grosveld, Frank G.Grosveld, Ernie De Boer and Jan M.Kooter, Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam, The Netherlands

The sequence arrangement of the rabbit β-globin gene (1) has been analysed in more detail by cloning a segment of rabbit chromosomal DNA containing the rabbit β-globin gene (2; see Weissmann et al., this volume). The gene consists of three exons separated by two introns of 126 and 575 base pairs. We have characterized the β-globin gene transcripts present in rabbit bone marrow using S_1 nuclease transcription mapping (3). The largest pre-mRNA detected is a transcript of all exons plus the two introns; it is 1250-1300 nucleotides long. The 5' and 3' termini of this RNA map at the same positions as the termini of the mRNA (within the resolution of the method); there is no indication for transcription of the extragenic DNA immediately flanking the gene. Several 'intermediates' in the pathway from the pre-mRNA to the mature mRNA have been identified.

The human β-, δ- and γ-globin genes have been mapped using the genomic Southern blotting technology. The δ-globin gene is 5-7 kb to the 5' side of the β-gene (4); the Gγ-gene is 3.5 kb to the 5' side of the Aγ-gene. All genes contain the large intron within the same region of the coding sequences described previously (1). The maps of the normal genes have facilitated the analysis for deletions/insertions in and around the globin genes in the thalassaemias. Most forms of β-thalassaemia do not result from a deletion of DNA regions close to the β-globin gene. In one β°-thalassaemia, however, a 600 base pair deletion has occurred, which eliminates most of the last (i.e. 3') exon. The structure of the γ-δ-β locus in normal and thalassaemic individuals will be discussed in relation to the switch from foetal to adult globins around birth.

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096 THE STRUCTURE AND ORGANIZATION OF LINKED MAMMALIAN GLOBIN GENES,
Tom Maniatis, Eugene Butler, Edward F. Fritsch, Ross Hardison, Elizabeth Lacy, Richard Lawn, and Richard Parker, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Cloned libraries of rabbit and human DNA fragments were constructed and screened for β-globin related sequences and a number of independent, overlapping recombinants bearing closely linked globin genes were obtained. Approximately 45 kilobases of contiguous rabbit chromosomal DNA sequences containing four different β-globin related genes have been characterized. The globin genes are separated from each other by an average of 6-8 kilobases. Two of the genes hybridize efficiently to cloned rabbit adult β-globin mRNA sequences while the other two hybridize weakly to adult β-globin mRNA but strongly to rabbit embryonic globin mRNA. The latter two genes also hybridize to cloned human γ-globin mRNA sequences. Hybridization experiments using probes for the 5' and 3' regions of the gene sequences indicate that all four genes are transcribed from the same DNA strand. We are also characterizing a large segment of human DNA containing four linked β-globin related genes using gene isolation and genomic blotting procedures. Clones were obtained which carried both adult δ-and β-globin genes. Restriction endonuclease cleavage analysis, hybridization experiments and partial DNA sequencing established the identity, position, and orientation of the two genes. The two genes are separated by 5.4 kilobases of DNA and their orientation with respect to the direction of transcription is 5'-δ-β-3'. Both genes contain a large noncoding intervening sequence located between the codons for amino acids 104 and 105. A second intervening sequence was found near the 5' end of the β-globin gene. Heteroduplex formation and direct sequence analysis demonstrated regions of homology and divergence in the protein coding and noncoding regions of the two genes. The linkage arrangement between the human fetal and adult β-like globin genes is being studied using gene isolation and genomic blotting procedures. Supported by NSF-CM77-15425 & NIH-GM24716A.

097 STRUCTURE OF THE HUMAN GLOBIN GENES,
Bernard G. Forget, Cesira Cavallesco, Jon K. de Riel, Dorothy Tuan, Andrew P. Biro, John T. Wilson, Lois B. Wilson, and Sherman M. Weissman, Departments of Medicine and Human Genetics, Yale University School of Medicine, New Haven, CT. 06510 and Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA. 30902.
Recombinant DNA clones have been obtained of the human α, β and γ globin cDNAs (1) and of the human chromosomal δ and β globin genes (2). By the use of rapid DNA sequencing techniques applied to the study of these cloned DNAs we have determined the nucleotide sequence of the coding portions of the human α and γ globin genes and of portions of the genomic β globin gene. Comparison of these sequences to the previously determined sequence of human β globin mRNA (3,4), allowed us to draw parallels and contrasts between the structure of the different human globin genes. The base composition of the α globin gene has a strikingly higher GC content (64.7%) than that of the β and γ genes (51.2% and 51.6% respectively) indicating the α globin gene has diverged under different evolutionary constraints than the non-α genes and that all three globin genes have diverged significantly from the bulk of human genomic DNA which has a GC content of approximately 40%. Usage of various codons for certain amino acids is markedly biased (non random) in all three globin mRNAs, but the bias is not uniform in all cases: certain codons are used exclusively or predominantly in one mRNA and not in the others; certain codons are not used in any of the three mRNAs. Comparison of the β and γ mRNA sequences demonstrates that there is a very high rate of silent base substitutions consistent with a neutral evolutionary drift of these two related globin genes which presumably originated from a single ancestor gene by the process of gene duplication. Knowledge of the nucleotide sequence of the major globin mRNAs allowed us to construct detailed restriction endonuclease maps of the human globin genes which we have utilized in gel blotting experiments, with nick translated plasmid globin cDNA probes and probes of the cloned extragenic DNA sequences flanking the genomic δ and β globin genes, to study the extent of δ and β globin gene deletions in hereditary hemoglobinopathies such as hereditary persistence of fetal hemoglobin (HPFH) and δβ thalassemia.

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098 ORGANIZATION OF HUMAN GLOBIN GENES IN NORMAL AND THALASSEMIC CELLS,
Arthur Bank, J. Gregory Mears, Francesco Ramirez, Alexander L. Burns, and John Feldenzer, Departments of Medicine and Human Genetics, Columbia University, N.Y., N.Y. 10032.
The linked human γ-δ-β globin gene complex provides a useful system for studying eukaryotic gene regulation. Several mutants in this system permit analysis of the effect of changes in gene structure on gene function. In patients with hereditary persistence of fetal hemoglobin (HPFH)and δβ thalassemia, solution hybridization studies have shown extensive deletion of the δ and β genes. These deletions are associated with compensatory increases in γ globin gene activity and mild or no anemia. No such deletion of β-like globin gene sequences is detectable in β^{o} and β^{+} thalassemia, and γ globin gene expression is inadequate and associated with the presence of severe anemia. To study the changes in the structure and organization of the globin genes in more detail, DNA from peripheral blood cells, spleen tissue and lymphocyte and fibroblast cell lines is digested with restriction endonucleases. The resulting DNA fragments are separated by agarose gel electrophoresis, transferred to nitrocellulose filters, hybridized to ^{32}P-labeled globin cDNA and visualized by radioautography. After Eco RI digestion and hybridization to plasmid-containing ^{32}P-labeled β globin cDNA, four β-like fragments are present in normal DNA. No β-like gene fragments can be detected in HPFH DNA and a unique 4.2 kb fragment is seen in homozygous δβ thalassemia DNA indicating less deletion of β-like genes in the latter disorder. Since δβ thalassemia has less γ globin gene expression than HPFH, these results support the hypothesis that sequences in the δ gene region or between the γ and δ genes may suppress γ synthesis in δβ thalassemia. The absence of these sequences in HPFH may permit full γ globin gene expression. By contrast, in β^{+} and β^{o} thalassemia there is no evidence for extensive deletion in the γ-δ-β gene complex, again consistent with the hypothesis that sequences persist in these disorders which suppress γ globin gene expression. The possibility that small changes in the DNA sequences either flanking the δ-β gene complex or in the intervening sequences of these genes is responsible for the genetic defect in β^{+} and/or β^{o} thalassemia is currently being pursued by restriction endonuclease analysis. In hemoglobin Lepore DNA, in which restriction enzyme analysis indicates deletion of sequences between the δ and β globin genes there is also inadequate expression of the γ globin genes despite severe anemia. Thus, the region between the δ and β globin genes does not appear to regulate γ globin gene expression. The regulation of γ globin gene expression could also be associated with changes in the organization of the γ globin genes. Analyses to date using several restriction enzymes show no differences in the size or number of the γ globin gene-containing fragments in normal Lepore, HPFH, δβ, β^{+} and β^{o} thalassemia DNA. Thus, it appears that extensive deletion of γ globin genes is not responsible for the variations in γ globin gene expression in these disorders.

Mammalian Tumor Viruses

099 RNA EXPRESSION FROM THE EARLY REGIONS OF ADENOVIRUS,

Phillip A. Sharp* and Arnold J. Berk, Massachusetts Institute of Technology, Cambridge, MA 02139

The pathway for early adenovirus mRNA synthesis reflects many of the processes involved in synthesis of cellular mRNAs. Recent evidence has shown that there are at least four independent transcription units responsible for synthesis of mRNAs from the four early regions. The structure of the mRNAs from each of these regions has been defined by nuclease-gel electrophoresis procedures. In order to define nuclear precursors to the cytoplasmic mRNAs, we have pulse labeled nuclear viral RNA and followed the fate of this RNA during chases. Distinct size nuclear viral RNAs have been detected and mapped by: solution hybridization of pulse labeled RNA to restriction fragments, digestion of hybridization mix with single-strand specific nuclease, and resolution of labeled RNA/DNA hybrids by electrophoresis in an agarose.

Evidence for possible post-transcriptional regulation of mRNAs from the early regions of Ad5 will be discussed.

100 SPLICING PATTERNS AND PATHWAYS OF ADENOVIRAL RNA TRANSCRIPTS.

Louise T. Chow, Bill A. Kilpatrick, James B. Lewis, Terri Grodzicker, Joe Sambrook Richard E. Gelinas, and Thomas R. Broker. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, U.S.A.

Cytoplasmic RNAs isolated from HeLa cells at various times after infection with Ad-2, -3, or -7 or Ad2+ND1 were mapped and quantitated by electron microscopy. Nearly all the RNAs are spliced into composite transcripts derived from 2 to 7 separate segments of the genome. Each of the four early regions gives rise to multiple species with different splicing patterns. Transcripts from early regions 2 and 3 continue to be made at late times, but they originate from different promoters. Cycloheximide (CH) strongly blocks the activation of the region 2 late promoter but only partially affects the major late r-strand promoter. Both CH and araC influence the relative abundances of spliced RNA species from the early regions.

Late after Ad2 infection, a common tripartite leader of 200 nucleotides is derived from coordinates 16.6, 19.6, and 26.6 and is spliced onto a dozen different mRNAs. Sets of these transcripts form five major families that share common 3' ends. A late transcript from the l-strand has a leader derived from coordinate 16.1. This implies that late transcription diverges leftward and rightward from promoters in the interval 16.1 to 16.5.

When cytoplasmic RNAs are isolated from infected cells grown on plates rather than in suspension, presumptive processing intermediates for the common leader on the r-strand late RNAs were predominant species shortly after the onset of late transcription. These extra sequences are located between the second and third leader segments and are of various lengths. They suggest a progressive nibbling out of the intervening sequences during RNA maturation, rather than a complete deletion in a single step.

The joining of the leaders to many of the mRNAs can also be multistep processes. In addition to the common tripartite leader, one or more extra leaders can precede the main body of the mRNAs. In each case, the extra leader consists of short sequences derived from the 5' end of an upstream gene. We propose that these segments serve as carriers to transport the common leader to downstream coding sequences in the long, polycistronic precursors.

Ad3 and Ad7 leader sequences, mRNA bodies, and processing intermediates map at approximately the same respective positions found for the Ad2 late transcripts, but their tripartite leaders are not homologous to those of Ad2 and do not cross-anneal. This divergence may present a species barrier to recombination between Type B (Ad3, 7) and Type C (Ad2) strains if leader sequences must match message sequences for RNA splicing to occur.

In summary, Adenoviral RNA splicing can occur in different patterns within a single region, deletions need not occur in a single complete step, the order in which deletions occur can be variable with respect to both time and relative upstream-downstream location, and multiple pathways can be followed in the conversion of precursor to product.

101 IS THE SV40 TUMOR ANTIGEN AN ENZYME AS WELL AS A SPECIFIC DNA BINDING PROTEIN?
Robert Tjian and Alan Robbins, Department of Biochemistry, University of California, Berkeley, California 94720.

The D2 hybrid protein from adenovirus-SV40 hybrid, $Ad2^+D2$, is a phosphorylated polypeptide which is predominantly encoded by sequences from the A gene of SV40 and is related both in structure and function to the SV40 T-antigen. We reported previously that the D2 hybrid protein binds and contacts residues in the major groove of the DNA at 3 specific sites in a region encompassing the origin of viral DNA replication. Here we report that an ATPase and protein kinase activity is found associated with the purified DNA binding protein. Both of these enzymatic activities co-purify with the D2 hybrid protein through several chromatographic steps. During ion exchange chromatography 85% of the T-antigen protein elutes at 0.37 M NaCl (Form I) and is able to catalyze the hydrolysis of ATP. The remaining 10-15% of the D2 hybrid protein elutes at 0.29 M NaCl (Form II) and is able to hydrolyze ATP as well as transfer phosphorus from ATP to either the D2 hybrid protein itself or to other phosphoproteins such as phosvitin. Although both forms of the protein bind DNA equally well, the ATPase activity co-sediments with SV40 DNA more efficiently than the protein kinase activity during glycerol gradient centrifugation. The ATPase activity of Form I and the phosphorylation of the D2 hybrid protein by Form II are efficiently inhibited by addition of anti-T IgG to the reaction mixture. By contrast, phosphorylation of phosvitin by Form II is specifically blocked by anti-T IgG only when the immune complex is removed from the reaction mixture. These findings suggest that in addition to binding DNA, the SV40 A gene product may also catalyze specific enzymatic reactions.

102 SV40-GLOBIN TRANSDUCING VIRUSES, Dean H. Hamer and Philip Leder, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014.

We have constructed a series of SV40 hybrids carrying mammalian globin genes, and are using them to study regulatory sequences in both globin genes and the SV40 genome. One set of viruses contain rabbit β-globin coding sequences derived from a cloned reverse transcript (Maniatis et al., Cell 3, 163-182, 1976). With these recombinants, the expression of the foreign DNA depends critically upon the structure of the viral vector. A second type of hybrid carries a portion of the chromosomal mouse β-globin gene. These viruses have allowed us to show that the mouse genomic signals for RNA splicing and polyadenylation are active in infected monkey cells, and that as little as 18 base pairs may be sufficient to specify the 5' side of a splice junction. Finally, we have constructed recombinants containing the entire chromosomal mouse β-globin gene, and are now studying their ability to direct globin synthesis.

Gene Expression During Development

103 SEQUENCE ORGANIZATION AND GENE EXPRESSION IN SEA URCHIN DEVELOPMENT,
Eric H. Davidson, Terry L. Thomas, Amy S. Lee, Ze'ev Lev, David M. Anderson, Richard H. Scheller, Frank D. Costantini, Roy J. Britten, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

This work concerns expression of structural gene sequences and transcription of interspersed repetitive sequence elements in sea urchin embryos. The genomic recombinant DNA fragments SP88 and SP34 include single copy regions coding for polyadenylated maternal mRNA's present in the egg and in early embryo polysomes at levels typical for the rare or complex class of mRNA's. The representation of these sequences in the polysomal mRNA of various embryo stages and adult tissue has been measured, and both appear to be regulated sharply during development. However, as found previously by Wold et al. (1), transcripts of these particular sequences are present in nuclear RNA at the same level whether they are present or absent in the polysomal RNA. The expressed single copy regions of SP88 and SP34 are contiguous to interspersed repeats belonging to several repetitive sequence families. Other studies concern transcription of repeat elements. Costantini et al. (2) and Scheller et al. (3) showed that such repeats are represented in egg RNA and in nuclear RNA in a tissue-specific manner. Recent studies demonstrate that the repeat transcripts stored in egg RNA are interspersed polyadenylated molecules probably including the maternal mRNA's. This sequence organization is unlike that of mature polysomal RNA. Recombinant DNA fragments containing many members of particular repetitive sequences in their natural sequence environments are described. Individual families display different environmental sequence organization.

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104 NON-NUCLEAR STEROID REGULATION IN AMPHIBIAN OOCYTES.
L. Dennis Smith and William J. Wasserman. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

Full-grown amphibian oocytes are arrested in prophase of the first meiotic division. Meiosis is reinitiated by progesterone which is released by the surrounding follicle cells in response to gonadotropin stimulation. Meiosis can also be induced in follicle cell-free oocytes by exposure to progesterone in vitro. In response to progesterone, the oocyte nuclear membrane breaks down, the chromosomes condense and they proceed to the second metaphase at which time the oocytes can be parthenogenetically activated or fertilized. Hence, they are physiologically mature. The events of maturation also include an increase in the rate of protein synthesis (2-4 fold), but no corresponding change in the rate of RNA synthesis (1).

Enucleation studies leave no doubt that steroids exert their effect in the amphibian oocyte at an extranuclear site. Several additional lines of evidence suggest that steroids exert their initial effect at or near the oocyte surface, possibly by causing an increase in the level of intracellular free calcium (2). The observation that oocytes contain a calcium-dependent regulatory protein (CDR) suggests a possible mechanism by which steroid-induced changes in Ca^{++} activity can be mediated. In response to steroids, a factor appears in the oocyte cytoplasm, prior to nuclear membrane breakdown, which in itself can induce meiosis when microinjected into other oocytes. This maturation promoting factor (MPF) depends on protein synthesis for its appearance in the cytoplasm, but does not require protein synthesis for its action on the nucleus. MPF induces a precocious response within the recipient oocytes both from a morphological and biochemical viewpoint. Thus, it appears that MPF itself can alter the rate of protein synthesis. Recently, it has been observed that MPF is not restricted to maturing oocytes. MPF activity was detected in cleaving embryos where it cycled as a function of the cell cycle peaking during each mitosis (3). These observations suggest that MPF may be present in all dividing cells and function during the G_2-M transition of the cell cycle. Thus, it appears that the events of oocyte maturation, mediated by progesterone, represent the reentry of a G_2 "arrested" cell into a normal cell cycle, albeit meiotic rather than mitotic.

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105 THE DEVELOPMENTALLY REGULATED CHORION MULTIGENE FAMILIES, Fotis C. Kafatos, Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138.

The silkmoth chorion consists of more than 100 structural proteins, which are synthesized in succession during a two-day developmental period. These proteins are encoded by a small number of multigene families, as demonstrated by sequencing studies on chorion proteins and cloned chorion DNAs, reverse transcribed from chorion mRNA. Sequence analysis reveals pathways of evolutionary diversification within each multigene family, and indicates the existence of partial homology between the two major chorion families.
The chorion cDNA clones have been related by hybridization-affected mRNA translation to the proteins that they encode. They have also been assigned by a dot-hybridization procedure to several developmental classes.
Classical genetic analysis has revealed that at least most members of the chorion multigene families are closely linked in a single chromosome. They are tightly clustered: chromosomal DNA clones bearing as many as four chorion genes within 15 Kb have been generated. Preliminary data suggest that at least some genes are immediately adjacent to other genes expressed during the same period of choriogenesis.

Chromosome Structure and Transcription

106 THE DUAL 5S GENE SYSTEM IN XENOPUS. Donald D. Brown, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210.
The genomes of X. laevis and X. borealis contain, respectively, three and two multigene families coding for 5S RNA (1). Two of the three gene families in X. laevis and one in X. borealis encode 5S genes expressed only in oocytes; these genes are shut off in somatic cells. The "somatic" 5S RNA genes function in all cells. Repeating units of all 5 gene families have been sequenced and the spacer regions compared for sequence homologies. The two somatic 5S DNAs have GC-rich spacers; the three oocyte 5S RNAs are characterized by clusters of A's and T's. A nuclear extract prepared from germinal vesicles of Xenopus oocytes supports accurate transcription of 5S RNA from all five of these DNAs in recombinant form with a plasmid (2). Experiments are in progress to mutate and delete regions flanking the genes in order to map sequences influencing accurate initiation and termination of 5S DNA. The germinal vesicle extract is being fractionated to purify molecules involved with RNA polymerase in accurate transcription of these genes. Supported in part by NIH grant number 1 R01 GM22395.

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107 FACTORS INVOLVED IN THE TRANSCRIPTION OF PURIFIED GENES BY RNA POLYMERASE III,
R. Roeder, D. Engelke, B. Harris, S. Ng, J. Segall, B. Shastry, and P. Weil, Departments of Biological Chemistry and Genetics, Washington University, St. Louis, MO. 63110.
We have used cell-free extracts prepared from mature Xenopus oocytes and from cultured cells (human KB, murine phasmacytoma, and amphibian kidney) to study the transcription of purified DNAs containing genes known to be transcribed by RNA polymerase III. The class III RNA polymerases in these extracts accurately transcribe various class III genes including the adenovirus VA RNA genes (in adenovirus DNA), 5S RNA genes in cloned Xenopus oocyte and Drosophila 5S DNAs, and tRNA genes in cloned Xenopus and Drosophila DNAs. While the transcription signals in various class III genes are thus recognized by heterologous transcription components, there is in some cases substantial discrimination between various genes. Thus, mammalian extracts transcribe both VA RNA_I and VA RNA_{II} genes, whereas amphibian extracts apparently transcribe only the VA_I gene; and the amphibian extracts appear to transcribe primarily that Xenopus 5S RNA gene which encodes the predominant oocyte 5S RNA, whereas the mammalian extracts transcribe at least two distinct 5S RNA genes in the cloned oocyte 5S DNA. Additionally some purified genes are transcribed in extracts derived from cells which contain but do not express these genes, suggesting the loss of some transcriptional controls.
Further studies have led to the isolation, from Xenopus oocytes and from human KB cells, of subcellular fractions (devoid of detectable RNA polymerase III activity) which contain factors essential for accurate transcription of purified class III genes by purified class III RNA polymerases. Such factors have been substantially purified by various chromatographic procedures although none have yet been obtained in homogeneous form. That there may be different factors for different class III genes is suggested by the following. The highly purified fraction which directs the transcription of the adenovirus VA RNA genes supports the transcription of cloned amphibian and Drosophila tRNA genes but not the transcription of the corresponding 5S RNA genes. The activity which supports the transcription of the 5S RNA genes is recovered in a distinct fraction. That some transcriptional regulation may be mediated through the class III transcription factors is suggested by the following preliminary experiments. The Xenopus oocyte 5S RNA genes (in cloned 5S DNA) are not transcribed in extracts prepared from transcriptionally inactive unfertilized Xenopus eggs, even though these cells contain levels of RNA polymerase III comparable to those in mature oocytes; however, the addition of a partially purified factor from oocytes markedly enhances 5S gene transcription in these extracts. Studies of other aspects of transcription in these reconstituted systems and the further purification and analysis of the various transcription factors are underway.

108 GENE TRANSCRIPTION IN DNA-INJECTED OOCYTES, J.B. Gurdon and G.A. Partington,
Medical Research Council Laboratory of Molecular Biology, Hills Rd., Cambridge, UK.
The injection of purified genes into frog oocyte nuclei provides a potentially valuable technique for investigating the control of gene transcription. For this procedure to be valid, it is essential that the injected genes are transcribed correctly. It has been established that polymerase III genes are correctly and extensively transcribed. More than half of the transcripts synthesized by oocytes may be products of injected 5S or 4S genes, and almost all products made from injected 4S or 5S genes are correct transcripts. 5S genes and some 4S genes are transcribed directly into the final stable product, but some tRNA genes are transcribed into unstable precursors which are processed and modified. Polymerase I (ribosomal) genes may be transcribed entirely correctly, as judged by morphological details of nascent transcription units. Polymerase II genes are capable of yielding correct stable transcripts, but the accuracy of transcription of such genes has yet to be established. Current work in this laboratory involves the injection of random clones of Xenopus DNA. The DNA in each clone can be screened by hybridization to appropriate preparations of cDNA, with a view to determining the cell-types in which the cloned genes are normally expressed.

109 THE INTERNAL ORGANIZATION OF THE NUCLEOSOME, G. Felsenfeld, J. McGhee, B. Sollner-Webb and P. Williamson. Laboratory of Molecular Biology, NIAMDD, NIH, Bethesda, MD 20014.

We have used a variety of enzymic and chemical probes to investigate the accessibility of DNA in the nucleosome core. When the nucleosome is partially digested with any of a variety of nucleases, only a small number of discrete DNA fragments is produced. This result might suggest that only a small fraction of the phosphodiester bonds of the backbone is accessible. We show, however, that different nucleases attack different sets of sites, and that a considerable fraction of the DNA is in fact accessible to some nuclease. We next show that the N7 of guanine in the large groove of nucleosomar DNA is entirely accessible to the chemical probe, dimethyl sulfate, and that the N3 of adenine, in the small groove, also reacts similarly in the DNA of the nucleosome and in naked DNA. Finally, we have examined the template properties of 'chromatin' made by reconstituting the core histones onto phage T7 DNA. We show that under appropriate conditions; this nucleoprotein complex is indistinguishable from naked DNA as a template for E. coli RNA polymerase. These results all suggest that the histones of the nucleosome may be so designed that they can cause compaction without severely blocking the chemical and biological reactivity of the DNA.

110 THE MECHANISM OF NUCLEOSOME ASSEMBLY, Ronald A. Laskey, Barry M. Honda, Anthony D. Mills, and Richard M. Harland. M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

The assembly of nucleosomes from histones and DNA has been studied using a cell-free system from eggs of the frog Xenopus laevis. Each unfertilized egg contains a pool of stored histones (1). In addition eggs contain at least one assembly factor which promotes the ordered interaction between histones and DNA. The nucleosome assembly factor has been identified and purified (2). It is an acidic thermostable protein consisting of at least four subunits of molecular weight 29,000. A protein with similar properties has also been purified from wheat embryos. The mode of action of the assembly protein has been investi gated. A model that it acts by binding histones to organize them into nucleosome precursor complexes will be discussed. The application of this assembly system to the experimental construction of chromatin transcription templates will be considered.

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Control of Gene Expression in Drosophila

111 A HOMEOTIC GENE COMPLEX CONTROLLING ANTERIOR SEGMENTATION IN DROSOPHILA
T.C. Kaufman, R.E. Denell, B. Wakimoto and R. Lewis, Indiana University
Bloomington, Indiana 47401
Recent studies in this laboratory have demonstrated that there exists in the proximal portion of the right arm of D. melanogaster a gene complex, originally defined by the Antennapedia (Antp) mutation, which controls segmentation in the anterior portion of the organism. This locus is analogous to the bithorax (bx) complex which controls posterior segmertation. Morphological and developmental studies on several Antp complex lesions have revealed segmental transformations in the prothorax, proboscis, eye and antenna. These transformations are similar to those observed in the methathorax and abdomen under the influence of bx mutations. Utilizing two overlapping deletions for the Antp locus a series of new mutations has been recovered. Including the extant mutations we now have more than 50 lesions within the complex most of which are recessive lethals. Complementation has revealed a minimum of 7 sites. Further, based on the polar nature of some of the mutations, it appears there may be a tandem reverse repeat in a portion of the locus. The possibility of this intriguing functional arrangement is reminiscent of the bidirectional transcription units which may be present in the histone gene complex. (Supported by PHS SOS RR7031 and PHS R01 GM 24299).

112 EVIDENCE FOR MUTATIONS AFFECTING THE COORDINATE CONTROL OF THE ADJACENT GENES, Ddc AND l(2)amd, IN DROSOPHILA, J. Lawrence Marsh and Theodore R.F. Wright, Univ. of Virginia, Charlottesville, VA 22901
Two independently isolated mutants exhibit the dual phenotype of increased levels of dopa decarboxylase (DDC) activity and increased resistance to the dietary administration of α-methyl dopa (αMD) (Sherald and Wright 1974). No evidence has been found for altered DDC protein on the basis of thermolability or in vitro sensitivity to inhibitors, nor has evidence been found for activators or inhibitors in mixtures of crude extracts. An increase in Ddc gene expression is indicated by quantitative immune precipitation with anti DDC antiserum which shows an elevation in CRM identical to the increase in activity. Resistance to dietary αMD is controlled by the l(2)amd locus which is located immediately adjacent (0.001 map units) to the DDC structural gene (Ddc 2-53.9). Evidence to date indicates that altering the level of DDC activity by as much as 10-20 fold does not alter resistance to αMD. These 2 phenotypes, elevated resistance and elevated activity, are most readily explained by mutations in a region responsible for the coordinate regulation of the Ddc and l(2)amd genes or by a small duplication for the two genes. The latter is less likely since no duplication is cytologically detectable nor is the increase in Ddc activity and integral multiple of the single dose activity as is expected in duplication homo- and heterozygotes. Thus these two mutations may define a contiguous regulatory site which increases the expression of both genes coordinately. To identify precisely the nature of these mutations and to rule out the possibility of a very small partially active duplication, we are presently attempting to recover the Ddc gene from recombinant clones.

113 THE PRESENCE OF A COMPLEX CLASS OF NONADENYLATED mRNAS IN D. MELANOGASTER LARVAE, J. Lynn Zimmerman, David L. Fouts, and Jerry E. Manning, University of California, Irvine, California 92717
A technique which allows the isolation of intact, fully loaded polyribosomes from whole Drosophila melanogaster larvae (and other developmental stages) has been perfected. Chromatography of purified polyribosomal RNA over Poly-U-Sepharose or Oligo-dT-Cellulose revealed that only a small proportion of the total polysomal RNA (approximately 0.15%) contains Poly(A) sequences. This result was further substantiated by a variety of techniques which revealed that approximately 90% of the mRNA in polyribosomes at the third larval instar is nonadenylated.

Saturation hybridization experiments were performed using gap translated, single copy D. melanogaster DNA and excess amounts of polyadenylated polysomal RNA or total, EDTA released, polysomal RNA. Our measurements show that the polyadenylated mRNA from larvae represents approximately 6.6% of the single copy DNA or 6.006×10^6 nucleotide pairs. This corresponds to approximately 4000 different, translationally active mRNAs (average length 1500 nucleotides). This measurement is in excellent agreement with previously published values determined by cDNA hybridization kinetics. However, the total polysomal RNA from these larvae was shown to include approximately 22% of the single copy DNA sequence complexity or 2.002×10^7 nucleotide pairs. This corresponds to 13,300 different average size mRNAs.

These experiments indicate the presence of a very complex class of nonadenylated mRNA on larval polyribosomes. This result suggest that previous kinetic measurements may have substantially underestimated gene number in Drosophila.

114 HETEROGENEITY IN HISTONE GENE ORGANIZATION IN DROSOPHILA, Linda D. Strausbaugh and Eric S. Weinberg, Biology Dept., The Johns Hopkins University, Baltimore, MD 21218

The organization of histone DNA in Drosophila melanogaster has been analyzed using restriction enzyme digestion of genomic DNA and agarose gel electrophoresis, followed by Southern transfer and hybridization with nick-translated cloned histone DNA probe. Our results fully confirm the findings of Lifton et. al. [CSHSQB 42:1047,1978] that the major portions of histone gene repeats are organized into 4.8 kb and 5.0 kb repeat units. We have studied many strains of Drosophila melanogaster of diverse geographic origins using a number of different restriction enzymes. In the strains examined, there has been striking similarity in histone DNA organization. The restriction fragment patterns are very consistent between the strains, but differences in the ratio of 4.8 kb and 5.0 kb repeats do exist. Since individual sea urchins can show dramatic differences in histone DNA restriction fragment patterns, we were interested in investigating single chromosomes from Drosophila strains. Sub-strains isogenic for a single second chromosome were generated from the strains previously tested. Analysis of DNA from these sub-strains has shown some differences in restriction patterns of DNA of individual chromosomes which were obscured when studying the DNA of the entire population. Using a simple and quick method for restriction enzyme analysis of DNA from 1-5 flies, we have compared the organization of histone DNA in some species of the genus Drosophila to that in Drosophila melanogaster. (Supported by NSF grant SMI77-12417 and PHS grant GM06318 to LDS, and PHS grant GM22155 to ESW.)

115 GENE EXPRESSION IN THE 67B HEAT SHOCK PUFF OF DROSOPHILA MELANOGASTER, Nancy S. Petersen, Galina Moller, and Herschell K. Mitchell, Caltech, Pasadena, CA 91125

Electrophoretic varients of three heat shock proteins, 23K, 27K(1) and 27K(2) have been used to map the coding regions for these proteins by linkage to visible markers. They map to a region of the third chromosome which includes only the heat shock puff at 67B. One of these heat shock proteins, the 23K protein, is structurally different from the other two (Mirault et al, 1978). The other two proteins 27K(1) and 27K(2) and their varients have molecular weights ranging between 26.5K and 28K. The 27K(1) and 27K(2) proteins have distinct isoelectric points and tryptic digests are being done to determine their relationship to each other and to another heat shock protein the 26K protein. The three heat shock proteins we have mapped as well as the 26K protein show similar protein synthesis kinetics during recovery from heat shock suggesting that they may be coordinately regulated.

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116 GENETIC ANALYSIS OF THE DROSOPHILA HISTONE GENES, Jacqueline G. Siegel and Dan L. Lindsley. Scripps Clinic and Research Foundation and University of California at San Diego, La Jolla, California 92093.

The molecular organization of the histone genes of Drosophila has been characterized, however the minimum number of histone genes required for viability has not been defined nor has it been determined if specific histone genes are differentially expressed during development. The present work establishes a complementation map for the histone gene region in Drosophila and correlates this complementation map with the DNA sequence organization of the histone genes.To obtain a complementation map, several chromosomes have been constructed bearing either total or partial deficiencies for the histone genes. The construction of these deficienceis utilized appropriate crosses between translocations involving the Y and the left arm of the second chromosome. Several segmental aneuploids were obtained that bore either partial or total deficiencies for the histone genes. The translocation-bearing deficiencies were then irradiated to produce intact second chromosomes carrying the deficiencies present in the original segmental aneuploids. The new deficiencies were characterized by 1) crosses to stocks bearing appropriate markers in the histone gene region; 2) by inter se crossing; 3) by cytological analysis of polytene and mitotic chromosomes; 4) by in situ hybrization. The organization of the histone genes in DNA isolated from deficiency-bearing stocks was determined in collaboration with Richard Lifton at Stanford University. The DNAs, treated with restriction endonucleases, electrophoresed and transfered to nitrocellulose filters, were hybridized with a Drosophila histone gene-bearing plasmid.The number and organization of histone genes present in the deficiencies were thus determined and correlated with the genetic data.

117 TRANSLOCATABLE ELEMENTS IN DROSOPHILA,
Gerald M. Rubin, Brian Backner, William Brorein, Jr., Pamela Dunsmuir, Edward Strobel, and Elihu Young, Department of Biological Chemistry and Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115.

Elements of the dispersed repeated gene families 412, copia, and 297 display a structure similar to that of transposable genetic elements in prokaryotes (1,2). We have shown that 412, copia, and 297 elements vary in both number and location in the genomes of Drosophila tissue culture cells. Comparisons of the genomic locations of these elements between different populations of D. melanogaster, and among individuals in the same population, again reveal both quantitative and qualitative differences. These differences appear to result from translocation of 412, copia, and 297 elements. Comparisons of the structure of pairs of DNA segments, which are identical except that one DNA segment contains an inserted 412, copia, or 297 element (missing in the other), reveal certain features of the molecular mechanism(s) responsible for the observed movement of elements of these dispersed repeated gene families.

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118 TRANSCRIPTION OF DROSOPHILA MELANOGASTER RIBOSOMAL GENES,
Eric O.Long and Igor B. Dawid, National Cancer Institute, Bethesda, Md 20014

Some of the ribosomal genes in D.melanogaster are interrupted in the 28S RNA coding region by sequences called ribosomal insertions. The insertions are of two different types, not homologous to each other. This report deals with insertions of type 1, present in 50% of the ribosomal genes on the X chromosome and absent on the Y chromosome. The majority of type 1 insertions are 5kb long. It has also been shown that sequences homologous to type 1 insertions are present outside the rDNA locus. The possibility that inserted genes are transcribed into a large precursor has been investigated. Cold nuclear RNA from embryos was annealed in excess to a 5kb insertion probe. No hybrid formation was detected. The absence of a large precursor containing insertion sequences has been confirmed by transfering nuclear RNA from agarose gels to DBM-paper and hybridizing it with cloned rDNA. The largest molecule detected corresponds to the known rRNA precursor of 8kb. Even if splicing of the insertion occured extremely rapidly, the sensitivity of the techniques used would have allowed detection of transcription proceeding across the insertion, since it is known that about 5000 rRNA precursor molecules are being synthesized per embryo nucleus. When RNA transferred from agarose gels to DBM-paper was hybridized with cloned insertion fragments, several bands were detected in nuclear RNA and one 1kb band in cytoplasmic RNA. These RNA molecules are of low abundance, are complementary to only a part of the 5kb ribosomal insertion and are different in various developmental stages.

119 THE DEVELOPMENTAL EXPRESSION OF THE RUDIMENTARY LOCUS IN DROSOPHILA MELANOGASTER.
Jan Eeken, Yves Mehl, Zilmar Tosta and Bruno P. Jarry.
Centre de Biologie Moléculaire, CNRS, Marseille, France.

We have shown recently that the rudimentary locus encodes the structural regions for the first three enzymes of the pyrimidine biosynthetic pathway, namely carbamylphosphate synthetase, aspartate transcarbamylase and dihydroorotase. The enzymes have been purified under a high molecular weight complex to apparent homogeneity and an antibody raised against the purified protein into rabbits. The corresponding messenger RNA has been characterised as a 35S RNA molecule and is presently used as a probe for cloning the corresponding DNA sequence in E. coli.

As a model for the expression of those genes involved in housekeeping function in high organisms, we have followed the expression of this locus during the entire development of Drosophila, both at the messenger RNA level and at the protein level. Special emphasis has been brought towards early embryogenesis and oogenesis, where the gene function appears critical as indicated by genetical experiments with temperature-sensitive alleles of the locus.

120 A REGULATORY LOCUS IN D. MELANOGASTER, Joseph Jack and Burke Judd, The University of Texas at Austin, Austin, Texas 78712.

Gans(1953) discovered an interaction between the X-linked loci, zeste and white, of Drosophila melanogaster. We now have evidence that the zeste locus regulates the activity of w^+. Females homozygous for z and with two w^+ alleles have yellow eyes, while flies homozygous for z but with only one w^+ allele are wild type(Gans, 1953). The number and position of w^+ alleles in the genome can be varied. When z is homozygous, any w^+ that is paired or contiguous with another w^+ will be expressed only weakly as yellow eye pigmentation. However, single isolated alleles are expressed without significant decrease in activity. With z^+ or a null zeste allele, the w^+ genes are fully active. We conclude that z is a hypermorph of z^+ and that both z and z^+ probably repress w^+. Furthermore, only the proximal part of the white locus (w^{prx}) is necessary in two doses to get a zeste phenotype(Green,1959; Judd,1961). The activity of w^+ in genotypes with various doses of z, z^+, and w^+ suggests that the zeste repressor molecule is activated by w^{prx}. We hypothesize that the activator is an RNA produced by w^{prx}. We now have some new zeste alleles that behave like mutants of repressor activity and others that seem to lack the ability to bind activator.

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121 DOES POSITION-EFFECT VARIEGATION IN DROSOPHILA RESULT FROM SOMATIC GENE LOSS? Steven Henikoff, University of Washington, Seattle, WA 98195

Chromosome aberrations that place a euchromatic gene locus near heterochromatin often result in a variegated phenotype in which the gene is fully active in some cells and apparently inactive in others. Since the gene itself is not permanently altered in the germ line, the possibility exists that mutant tissue results from physical loss of the gene in some somatic cells. In order to test this possibility, polytene chromosomes were examined from heat-shocked Drosophila larvae heterozygous for a normal sequence chromosome and an aberrant homolog with a puff-site placed near heterochromatin. Occasionally, this heat-shock puff was absent on the mutant homolog in nuclei where the normal chromosome puff was fully active. ^{3}H-labeled RNA probes were used to detect puff-site sequences by hybridization in situ. In some cases, specific labeling near the euchromatin-heterochromatin junction of the aberrant homolog was apparent, even though no puff could be seen. This suggests that variegation can occur without the loss of a gene's DNA sequence.

122 ENUMERATION AND MAPPING OF THE 87A and 87C HEAT-INDUCED SEQUENCES IN DROSOPHILA MELANOGASTER.

David Ish-Horowicz, Paul Schedl*, Spyridon Artavanis-Tsakonas* and Marc-Edouard Mirault†
Imperial Cancer Research Fund, London, NW7, England; *Biozentrum der Universität, Basel, Switzerland; † Dept. de Biologie Moleculaire, Universite de Geneve, Switzerland.

The 70,000d heat shock protein of D.melanogaster (hsp 70) is encoded at 2 loci, 87A7 and 87C1 (Ish-Horowicz et al., in preparation; Schedl et al. (1978) Cell 14, 921; Livak et al. (1978), in press. We have investigated the genomic organisation of hsp 70-coding sequences and have found that the number and arrangement of coding sequences varies between different laboratory strains. Laboratory wild-type Oregon-R stocks are polymorphic in their pattern of restriction enzyme fragments that contain coding sequences. We have studied a number of strains deleted for either 87A or 87C to determine the number of coding loci at each site. A control chromosome that retains both loci has three coding sequences at 87C and 2 copies at 87A. $Df(3R)kar^{3J}$ deficiency for 87C retains 2 copies at 87A; Df(3R)229, a deficiency for 87A retains 3 copies at 87C. There are characteristic differences between the 87A and 87C coding sequences; only coding sequences from 87A are cut by Bgl I;coding sequences from 87C lack a second Sal I cleavage site found in 87A sequences. The proteins encoded at 87A and 87C are very similar. We have constructed restriction maps of both regions: 10k at 87A, 45kb at 87C including the 87C1-specific heat-induced sequences described by Lis et al. (1978) Cell 14, 901.

123 THE BIOCHEMICAL GENETICS OF CHORION SYNTHESIS IN DROSOPHILA MELANOGASTER, Mary Ellen Digan, Allan C. Spradling, Anthony P. Mahowald, Indiana University, Bloomington, In. 47401, and Gail L. Waring, Marquette University, Milwaukee, Wisconsin 53233

The chorion is synthesized by the follicular epithelium during the last stages of oogenesis in Drosophila melanogaster. Hand-isolated, in vivo labeled stage 11-14 egg chambers show sequential, stage-specific changes in synthesis of protein and poly(A)-containing RNA (1, 2). Two major bands of poly(A)-containing RNA, E3 and E4, produced by stage 12 egg chambers hybridize in situ to a single site on the X-chromosome near 7E11 (2). Two recessive female-sterile mutations map genetically to a 16-band region surrounding this site (7E10$\pm$ to 8A4 $\pm$), and cause morphological and biochemical defects in the chorion in homozygous females. Egg chambers from ocelliless homozygotes (oc/oc) underproduce E3 and E4 as well as several major chorion proteins, including c36 and c38 (3). Egg chambers from females homozygous for a new EMS-induced female-sterile mutation synthesize c38, but do not synthesize c36. Heterozygotes of these two mutations are fertile. Several cDNA clones which hybridize specifically to 7E11 have been selected from a bank of clones prepared from poly(A)-containing RNA of late stage egg chambers (Spradling, Polisky, Mahowald and Craig, ms. in preparation). Using this system, it is possible to study a specific chromosomal region of known genetic function at the DNA level. We intend to correlate particular defects in DNA to the biochemical and morphological phenotypes present in these and other newly-induced female-sterile mutations in this region.

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124 GENETIC ORGANIZATION OF THE ROSY LOCUS IN *DROSOPHILA MELANOGASTER*, Stephen H. Clark, Arthur J. Hilliker and Arthur Chovnick, The University of Connecticut, Storrs, CT 06268

Induction and analysis of XDH underproducers as putative control variants. The rosy locus (3-52.0) defines a segment of DNA associated with the biogenesis of the enzyme xanthine dehydrogenase (XDH). In addition to containing amino acid sequence coding information we assume that the locus possesses transcription and translation regulating sequences. Previous reports from this laboratory have described experiments which define the left border of the XDH structural element as well as a *cis*-acting control element immediately to the left of the structural element (See Review, Chovnick *et al.*, 1977 CSHSQB 42:1011-1021). In an effort to further define the genetic sites associated with the control of XDH biosynthesis a series of mutagenesis and selection experiments were conducted to produce an array of XDH underproducers. Several XDH "lows" were recovered and subjected to detailed biochemical and genetic analysis. The results of these studies will be discussed in light of our current understanding of the control mechanisms associated with the expression of XDH.

125 INTRONS IN THE rDNA OF THE DIPTERAN FLY CALLIPHORA ERYTHROCEPHALA, Kathy Beckingham and Ray White, Dept. Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

We have characterised the rDNA of Calliphora erythrocephala (a Dipteran fly of the same sub-order as D. melanogaster) by i) analysis of cloned fragments of the rDNA and ii) hybridisation of ^{32}P labelled rRNA to total genomic DNA cleaved with various restriction enzymes. The cloned rDNA fragments demonstrate that some rDNA cistrons contain introns positioned within the 28S rRNA coding sequence at a point indistinguishable from that of the D. melanogaster rDNA introns. Cross-hybridisation experiments show that the rDNA introns of the two species are largely unrelated in sequence although a small amount of homology between the right-ward end of the C. erythrocephala introns and the extreme right-ward end of the major class of D. melanogaster introns has been demonstrated. The hybridisation studies using genomic DNA have established the presence of two major length classes of cistron within the total rDNA, but have shown, surprisingly, that these result from two length classes of non-transcribed spacer, not from the presence/absence of an intron. Intron^{+} rDNA cistrons are probably a minor class in this species therefore. Experiments to determine more precisely what fraction of the total rDNA set they represent, and whether rDNA intron sequences exist in total genomic DNA outside the rDNA proper, are in progress.

Control of Gene Expression in Yeast

126 A GENETIC STRUCTURAL DEFECT WHICH PREVENTS GENE SWITCHING IN YEAST,
Lindley C. Blair and Ira Herskowitz, Inst. of Molecular Biology, Univ. of Oregon, Eugene, OR 97403

In haploid yeast the cell phenotype is controlled by the mating type locus (MAT), which has two wild type alleles, *a* and *α*. Haploid homothallic strains undergo frequent genetic switching between these alleles, whereas in diploid a/α strains the switching mechanism is off (for a model to account for the switching mechanism see the abstract by Kushner, Blair, and Herskowitz). A natural variant allele of the mating type locus, *MATα*-inc (inconvertible), is resistant to this frequent switching event in haploids (Takano, et al., 1973). In order to determine whether *MATα*-inc acts similarly in the presence of another mating type locus special diploids were constructed in which the switching mechanism remains on. Analysis of these diploids demonstrates that the defect in switching of *MATα*-inc is cis-acting. These results are in accordance with those of Takano and Arima (1978) and support the hypothesis that the *MATα*-inc allele contains a structural change which renders the mating type locus insensitive to switching.

127 ANALYSIS OF A YEAST *ilv 1* OPERATOR MUTATION,
Arthur P. Bollon, University of Texas Health Science Center, Dallas, Texas 75235

We have identified a cis control site specific for the *ilv 1* gene of *Saccharomyces cerevisiae*. The *ilv 1* gene codes for threonine deaminase and is repressed by L-isoleucine. We have isolated *ilv 1* regulatory mutants designated ilv 1-OP^c which are affected in a control site which affects both the basal level of expression as well as being constitutive. In strains which are *ilv 1-OP^c*, the differential rate of synthesis of threonine deaminase is 7.0 compared to 1.3 for *ilv 1-OP$^+$* strains when cells are grown in minimal medium. Under repressing conditions the differential rate for *ilv 1-OP$^+$* strains is 0.4 yet it is still 7.0 for *ilv 1-OP^c* strains. The OP^c mutation is cis dominant since the OP^c phenotype is manifest in the diploid (OP^c-ilv 1$^+$) x *OP$^+$ -ilv 1-1*) but not in the diploid (OP^c-ilv 1-1) x (*OP$^+$-ilv 1$^+$*). The *ilv 1-1* mutation used in this cis-trans analysis is a noncomplementing nonsense mutation which prevents the interference of intracistronic complementation. We found that haploids which were *OP^c-ilv 1$^-$* were resistant to thiaisoleucine (TIL) which inhibites growth of *OP$^+$-ilv 1$^+$* strains. We utilized TIL to select for recombinants since OP^c was found to be closely linked to the *ilv 1* gene. By three factor analysis in the following cross (*Arg^6-OP^c-ilv 1$^-$*) x (Arg^6-*OP$^+$-ilv 1$^+$*) we mapped the OP^c mutation outside the most proximal *ilv 1* mutation. Furthermore, the OP region may be located equidistant between the *Arg 6* and *ilv 1* genes: *Arg 6 OP ilv 1*.
New techniques are being utilized to isolate a piece of DNA containing both *Arg 6* and *ilv 1* thus permitting the correlation of DNA sequence data with various OP^c mutants.

128 REGULATION OF TRANSCRIPTION OF THE YEAST PLASMID, 2μ CIRCLE,
James R. Broach, John F. Atkins, Carolyn McGill and Louise Chow, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 11724.

We have identified two major and approximately ten minor polyA containing RNA species in *S. cerevisiae* which arise from *in vivo* transcription of the yeast plasmid, known as 2μ circle. The two major species, which are 1325 and 1275 bases in length, are transcribed from the two unique halves of the plasmid and extend into the inverted repeat sequences which separate the unique regions. The map position of the minor transcripts, which range in length from 2600 bases to 350 bases, indicate that, except for a small region of the genome in which no transcription is observed, both strands of the entire 2μ circle genome are transcribed. We have also demonstrated that RNA transcribed from 2μ circular DNA is used to program the synthesis of specific proteins in yeast: 1) Yeast RNA complementary to 2μ circle DNA can be translated *in vitro* to produce specific polypeptides of substantial size, and 2) some of the 2μ circle transcripts are found on polysomes. Finally, the pattern of transcription of 2μ circle suggests the possibility that messenger RNA species are derived by cleavage of larger transcripts and, in addition, that the intramolecular recombination of 2μ circle which occurs in yeast functions as a genetic switch to allow separate expression of two sets of genes on the 2μ circle genome.

129 GENETIC AND BIOCHEMICAL ANALYSIS OF THE HIS4ABC GENE OF YEAST,
Philip Farabaugh, Christine Ilgen, and Gerald R. Fink, Department of Botany, Genetics and Development, Cornell University, Ithaca, N.Y., 14853

We have used the technique of molecular cloning to analyze the structure of the his4 gene of *S. cerevisiae*. With Albert Hinnen, we cloned the gene as described in these abstracts (G. R. Fink, P. Farabaugh, and C. Ilgen). The plasmid (pYeHIS4) consists of a 14 million dalton BamH1 fragment from yeast inserted into the tet^r gene of pBR313 at its single BamH1 site. It complements both his4A and his4C mutations. Since the gene order is A B C, the plasmid must contain most, if not all of his4. We have identified the position of an EcoRI, SalI, and HincII restriction site in the gene by Southern hybridization of ^{32}P-labeled pYeHIS4 to restriction digests of DNA isolated from a series of his4AB deletions. A comparison of this map with the restriction map of the plasmid identifies the position of the his4 gene--approximately the middle of the insert. The Southern analysis also shows us that the insert contains no repetitive sequences longer than 50 base pairs and that the his4 gene does not appear to include intervening sequences in the first 1000 base pairs. Using these *in vivo* generated deletions and a collection of *in vitro* generated deletions, we have begun to define the physical extent of the his4 region, both its putative control region and the structural gene. We are sequencing the gene, its control region and a spectrum of point and deletion mutations; we have begun to determine the structure of the gene, the elements involved in its regulation, and the molecular basis of mutagenesis in the region.

130 "ILLEGAL" TRANSPOSITIONS OF YEAST MATING TYPE GENES,
James E. Haber and Deborah Wygal Mascioli, Brandeis University, Waltham, MA 02154

Mating type interconversion in *Saccharomyces cerevisiae* apparently involves copying a new MAT allele from one of 2 silent "library" genes and the transposition of this new allele to replace the original MAT allele. We have been trying to establish some rules that govern these MAT interconversions. 1) Can MATa switch directly to another copy of MATa or must it switch first to MATα? In order for diploids of genotype HO/ho MATα-inc/mata* hma/hma HMα/HMα to sporulate, the defective mata* must switch directly to MATa (as there are no α library alleles). The MATα-inc/mata* diploid readily switched to MATα-inc/MATa. Preliminary results with an equivalent MATa-inc/matα10 strain with only α library alleles indicate that matα10 can switch directly to MATα. 2) Can the copies of mating type at the HMa and HMα library genes be switched? When normal homothallic interconversion of MAT is prevented by the presence of the cis-acting mutations MATa-inc or MATα-inc, interconversions of mating type may occur at HMa or HMα. For example, HO HMa MATa-inc HMα strains become changed to HO hma MATa-inc HMα at a frequency of 1%. Moreover, in the course of analyzing the MATα-inc/mata* diploids described above, we found one case (out of 12 examined) in which a copy of MATa-inc had been transposed to a new location. This transposed MATα-inc allele is able to act as a library gene for the switching of MATa. The MATa allele is converted to MATα-inc which is again "stuck." The switching is only about 10% as efficient as normal. Although the MATα-inc allele is not switched to MATa, the transposed MATα-inc "library" can be used to switch MATa. Thus the mechanisms of copying and transposing the library sequence must differ from the process of excising the sequence at MAT.

131 DETECTION OF E. COLI CLONES CONTAINING SPECIFIC YEAST GENES BY IMMUNOLOGICAL SCREENING,
Ronald A. Hitzeman, A. Craig Chinault, Alan J. Kingsman, and John Carbon, University of California, Santa Barbara, CA 93106

We have developed an immunological screening procedure for the selection of antigen-producing clones from colony banks. This method involves covalent attachment of antiserum to cyanogen bromide-activated paper discs, contact of this paper with lysed colonies on agar plates, and finally detection of the bound antigen with ^{125}I-labelled antibody. Using this method several colonies of *E. coli*, containing yeast DNA inserts in plasmid ColE1, have been isolated that produce antigen to antibody made to yeast hexokinase or to yeast phosphoglycerate kinase (PGK). The characterization of the antigens produced in *E. coli* and restriction maps of the DNA sequences producing these antigens will be presented. The 10 megadalton yeast DNA insert in one clone producing PGK cross-reacting material has been shown to originate from chromosome III of yeast, using quantitative "Southern" hybridizations to DNA from yeast strains aneuploid for this chromosome. This is consistent with genetic results of Lam and Marmur (J. Bacteriol. 130: 746, 1977), who have mapped the pgk locus on the right arm of chromosome III near the centromere. In addition, stretches of DNA contiguous with this same yeast DNA sequence have been isolated by overlap hybridization screening. Since overlapping DNA segments have been obtained for the leu-2 region on the left side of the centromere, it is expected that further experiments should enable the isolation and characterization of the entire DNA sequence between pgk and leu-2, including the centromeric region.

132 REGULATORY GENE ACTIVITY IN THE GALACTOSE PATHWAY OF YEAST, James E. Hopper, James Yarger and Daniel Perlman, Rosenstiel Center, Brandeis University, Waltham, MA 02154.

Induction of galactose pathway gene expresssion in S. cerevisiae is under the control of two unlinked regulatory genes; a positive regulator, GAL4, and a negative regulatory, GAL80. To ascertain whether the GAL4 gene product is required for de novo appearance of galactose gene transcripts we have isolated yeast-pMB9 recombinant DNA molecules containing galactose genes. These recombinant molecules, which were identified by their specific capacity to hybrid-select in vitro translatable GAL1 and GAL7 specified mRNAs, were used as hybridization probes to assay for the inducible appearance of galactose gene transcripts in wild type (GAL4) cells and in cells carrying a gal4 mutant. The gal4 mutant cells produced no galactose gene transcripts detectable by this assay. We also performed two experiments designed to determine whether GAL80 controls GAL4 function at the level of GAL4 gene transcription or translation or alternatively at the level of GAL4 protein activity. One experiment involved the addition of cycloheximide 10 min prior to the addition of galactose. The appearance of GAL1 and GAL7 mRNAs as assayed by in vitro translation was normal. In the second experiment inducible gal1 cells were mated in the presence of galactose with uninducible gal4 cells containing either a dominant $GAL80^S$ encoded galactose-insensitive repressor of the normal GAL80 encoded galactose sensitive repressor. Galactokinase was expressed in the GAL80/GAL80 zygote and not in the $GAL80^S$/GAL80 zygote. We conclude that the GAL4 protein is constitutively synthesized and its activity is negatively controlled by direct interaction with the GAL80 protein. A model is presented.

133 HIGH FREQUENCY TRANSFORMATION OF YEAST BY PLASMIDS CONTAINING THE CLONED YEAST arg4 GENE. Chu-Lai Hsiao and John Carbon, Department of Biological Sciences, University of California, Santa Barbara, California 93106.

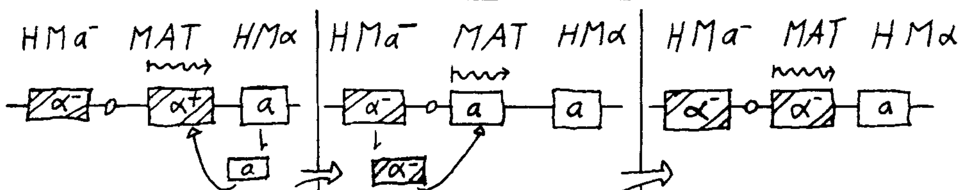
Hybrid ColE1 plasmids containing the yeast leu2 or his3 genes transform yeast mutants with low frequency (10^{-6} to 10^{-7}) and become integrated into the yeast genome (Hinnen, Hicks and Fink, PNAS 75, 1929 (1978)). In contrast, hybrid ColE1 plasmids containing cloned DNA from the yeast arg4 region (e.g., pYe(arg4)1) transform yeast arg4 mutants with a frequency of 10^{-4} (about 10^2 to 10^3 transformants per μg plasmid DNA), and replicate autonomously without integrating into the yeast genome. The yeast transformants are unstable when grown on nonselective media, but can be readily maintained and grown on minimal media lacking arginine. The existence of unintegrated replicating transforming plasmid DNA in the yeast transformants was demonstrated by Southern gel hybridization, and by transforming E. coli argH mutants with DNA preparations from yeast transformants and subsequent recovery of intact plasmid DNA from the E. coli transformants. Plasmid DNAs recovered from the yeast-E. coli "shuttle" remain essentially unchanged, as judged by DNA restriction fragment patterns. Plasmid mutations leading to increased efficiency of expression of the arg4 gene in E. coli do not appear to affect expression of the cloned arg4 gene in yeast. Appropriate derivatives of these arg4 plasmids are of potential usefulness as vectors for cloning genes in yeast, and for studying the mechanism of yeast DNA replication.

134 CHROMOSOMAL ORGANIZATION OF TRANSCRIBED YEAST DNA SEQUENCES AS INVESTIGATED BY IMPROVED METHODS OF R-LOOP MAPPING. David B. Kaback and Norman Davidson, Department of Chemistry, California Institute of Technology, Pasadena, California 91125

The organization of the genes coding for mRNA in Saccharomyces cerevisiae has been investigated by electron microscopy of R-loops. R-loops are easily recognized bubbles in the duplex DNA structure caused by RNA displacement of one DNA strand at the complementary site. These studies were made possible by new techniques which greatly improve the effectiveness of R-loop mapping. DNA was first crosslinked once per 1-5 kb using 4,5', 8-trimethylpsoralen. This permits hybridization with high molecular weight DNA at temperatures where all the DNA is effectively single stranded. As a result, R-loops can form regardless of the base compositions of the coding and flanking sequences and the double stranded molecular weight of the DNA is maintained. Total vegetative mRNA was hybridized under saturating conditions to crosslinked DNA. Under normal conditions these R-loops were unstable due to branch migration and only a small fraction of the expected R-loops were observed. We were able to stabilize R-loops by modifying the displaced DNA strand with glyoxal which prevents the displacement of the R-loops by branch migration. After this treatment 30-40% of the double stranded genome is found in R-loop structures, in close agreement with RNA complexity measurements (Hereford and Rosbash, Cell 10,403 (1977)). The spacing of the genes appears heterodisperse with a mean length of 1.5±2.0 kb on each side of a 1.0±0.8 kb R-loop. The largest observed stretch of DNA not containing R-loops was 30 kb. R-loops from abundant mRNA populations corresponding to ribosomal protein, histone and other highly expressed genes showed no obvious patterns of clustering on the genome.

135 DETERMINATION OF CELL TYPE BY MOBILE GENE CASSETTES--A CRITICAL TEST, Peter J. Kushner, Lindley C. Blair and Ira Herskowitz, University of Oregon, Eugene, OR 97403.

Cell type in haploid yeast is controlled by the mating type locus (MAT) which has two alleles, a and α. In homothallic strains the mating type locus undergoes frequent genetic switching between these alleles. According to the Cassette Model these switches (for example, from a to α) are produced by the insertion of a replica of a silent library α gene (an α cassette) into the mating type locus, a site of active gene expression. This hypothesis leads to the prediction that a strain with a mutation in, for example, the silent α gene, should switch from MAT α^+ to MAT a and then to MAT α^- (diagram below). We have isolated mutations in the genetic loci postulated to be the silent α and the silent a information (HMa and HMα, respectively) and find that the mutations are indeed "transmitted" to the mating type locus, where they have phenotypes like those of previously existing MAT α^- and MAT a^- mutations.



136 BIOCHEMICAL AND GENETIC INVESTIGATION OF THE EXPRESSION OF YEAST $tRNA^{ser}_{UCG}$ Maria Salvato, Diane Colby, Tina Etcheverry, Pyn Miake and Christine Guthrie University of California,San Francisco, Dept. Biochemistry&Biophysics,S.F., CA 94143

Certain tRNAs in Saccharomyces cerevisiae arise via precursor molecules containing intervening sequences. This lab has been investigating a serine tRNA which accumulates such a precursor in the mutant ts136. Like the tyrosine and phenylalanine tRNA precursors (Knapp et al,1978; O'Farrell et al, 1978) this precursor is also mature at both termini and contains an intervening sequence adjacent to the anticodon (Etcheverry,Colby, and Guthrie, in press). Genetic and biochemical data indicate that there is only one gene for the $tRNA^{ser}_{UCG}$, and that, unlike the genes for other yeast serine tRNAs, this is the only one containg an intervening sequence. Hence it is of great interest to investigate the expression of this particular tRNA. We have identified plasmid-bearing clones containing DNA complementary to $tRNA^{ser}_{UCG}$ for the purpose of transcribing these in a Xenopus oocyte system. Also, we have isolated mutants from serine suppressors which show simultaneous loss of suppression in several genes, indicating a possible mutation in some aspect of the processing machinery of this tRNA.

The Structure and Expression of Eucaryotic Genes

137 DEVELOPMENTALLY SPECIFIC HISTONE GENES OF THE SEA URCHIN, Michael Grunstein, Allan Lohe, Kathleen Diamond and Eva Knoppel, Molecular Biology Institute, University of California, Los Angeles, California 90024

We have recently shown that early sea urchin embryogenesis is characterized by a distinct shift in histone messenger RNA synthesis. At approximately 12 hours of embryogenesis the histone genes which were active prior to this point in time are no longer utilized and a new class of histone genes is utilized instead. We refer to these two functional subgroups as early (E) and late (L) histone genes. Our work has focused mainly on the genes coding for histone H4 messenger RNA. Differences between E and L H4 mRNAs include: a) considerable divergence in only or mostly third position degenerate bases between these two mRNAs; b) the E mRNA is 393 nucleotides long while the L mRNA is 40 nucleotides shorter; and c) this length difference manifests itself largely at the 5' untranslated end of the mRNAs.

Cloned histone genes of the sea urchin Strongylocentrotus purpuratus have been isolated. Of 100 independently isolated clones the majority are of the E-type. We have also isolated several histone gene clones which are not of the E-type. These new histone genes have altered repeat sizes, restriction enzyme patterns, and show considerable divergence from the E class histone genes. Experiments are presented regarding the stage specific nature of these genes.

138 REGULATION OF VARIANT HISTONE GENE CLUSTERS DURING EMBRYOGENESIS, Geoffrey Childs, Robert Maxson and Laurence Kedes, Stanford University, Stanford, CA. 94305.
Subtypes of the histones H1, H2A and H2B are differentially activated during sea urchin embryogenesis. Data presented will demonstrate that this regulatory event is the result of repression and activation of different sets of histone genes. We have isolated individual histone mRNA's from different embryonic stages of development on the basis of their complimentarity to cloned individual "early" histone genes. The results show that in addition to shifts in mRNA's for those proteins in which there are variant subtypes (H1, H2A and H2B), the most conserved proteins H3 and H4 also show dramatic changes in the population of their mRNA's. On the basis of size differences with corresponding purified individual early mRNA's, we can identify 1 late H1 mRNA, at least 2 late H2A mRNA's, 2 late H2B mRNA's, 3 late H3 mRNA's and 2 late H4 mRNA's. Cell-free protein synthesis reactions directed by these mRNA's mimics the protein shifts seen in vivo. Under stringent hybridization conditions, late H1 and late H2A mRNA's do not hybridize to cloned early histone genes. In addition, those purified late mRNA's which do hybridize (H2B, H3 and H4) melt 11-14°C earlier than homologous early RNA/DNA hybrids. Detailed examination of histone subtypes as well as the timing of their activation and inactivation will be discussed.

139 STAGE SPECIFIC CHANGES IN PROTEIN SYNTHESIS DURING XENOPUS OOGENESIS, Mary Lou Harsa-King and Andrea Bender, Harvey Lodish, MIT, Cambridge, Ma. 02139.
Oogenesis is a time of intense synthetic activity and product accumulation. Some of these products are essential for later development. Ovulation of mature oocytes and the concomitant dramatic increase in incorporation of exogenous amino acids into proteins in immature oocytes is triggered by hormonal stimulus. Because of the implications for subsequent development and the potentially important role played by the ovulation hormone, the regulation of gene expression during oogenesis is of special interest. The first task is to identify genes whose expression is regulated. To this end we have been investigating the changes in the pattern of protein synthesis during the six Dumont stages of Xenopus oogenesis as analyzed by two-dimensional gel electrophoresis. Several parameters are being compared: A) proteins synthesized in each of the six stages before and after hormonal (HCG) stimulation; B) proteins synthesized in stage 3,4, and 6 in vivo and in a cell-free translation system; C) newly synthesized proteins in stage 3 and 6 oocytes localized in the germinal vesicle (nucleus). Some 400 proteins can be identified on one gel and approximately 20% of them undergo stage specific qualitative or quantitative changes. There are proteins which appear to be made only during a particular stage. This is most noticeable in stage 3 oocytes in which lampbrush chromosomes are fully extended. Actin and tubulin have been tenatively identified and their synthesis appears to undergo stage specific changes as well. A few new proteins are synthesized in response to hormonal stimulation although a dramatic increase in the number of new proteins made is not observed at any stage.

140 DEVELOPMENTALLY REGULATIVE GENES IN DICTYOSTELIUM DISCOIDEUM: W. Röwekamp, Inst. für Zellforschungs-Zentrum; Heidelberg; and R.A. Firtel, Dept. of Biol., UCSD, La Jolla.
A recombinant plasmid library has been prepared from complementary DNA (cDNA) to total poly(A)$^+$ RNA from Dictyostelium cells at 6 hr. of development. By differential screening using the colony hybridization method with in vito labeled RNA isolated from vegetative (0 hr.) and 6 hr. cells, approximately 150 "developmentally regulated" colonies were identified. Three of the plasmids (pcI_{42}, pcI_{44}, and pcI_{13}) were analyzed in further detail. Hybridization of RNA pulse-labeled at various developmental stages showed the following pattern; the relative rates of synthesis of the corresponding mRNA is: 1) low or not detected at 0 hr., 2) maximal at 4-6 hrs. 3) and appreciably decreased by 10 hrs. RNA excess hybridization showed that in vegetative cells that there was approximately 0.2-2 molecules of RNA complementary to the cloned DNAs per cell, 500 at 8 hrs., and approximately 150 at 16 hrs. The repitition frequency of the nuclear DNA sequences complementary to the plasmid DNAs was determined by DNA excess hybridization kinetics and analysis of nick translated probes hybridized to Southern DNA blot filters. Two of the cloned genes (pcI_{42}, pcI_{44}) appear to be single-copy, while 1 (pcI_{13}) is reiterated approximately five times in the genome. Two recombinant plasmids, $pDdB_2$ and pWR17, which contained sequences complementary to pcI_{13} have been isolated from a genomic DNA library. Restriction analysis of these clones together with mapping of the genes in chromosomal DNA indicate that the genes are closely clustered. Moreover, although the genes have not shown any heterogeneity by restriction analysis, poly(A)$^+$ mRNA complementary to both the genomic and cDNA plasmids shows two bands on denaturing gels. The organization of the gene clustering and the regulation of the synthesis of the mRNAs and encoded proteins.

141 RNA SYNTHESIS ON CHROMATIN FROM NORMAL AND ESTROGEN-STIMULATED ROOSTER LIVER.
M. Meyers, R.F. Goldberger and K.P. Mullinix, NIH, Bethesda, Maryland 20014
Vitellogenin, the precursor of the egg yolk phoshoproteins, lipovitellin and phosvitin, is normally not synthesized by roosters or immature chicks but its synthesis can be induced in the livers of these animals by the administration of estrogen. It has been shown that the stimulation of vitellogenin synthesis by estrogen corresponds with an increase in the amount of vitellogenin mRNA in the cell. Thus, this system is an ideal one for the study of regulation of gene expression at the level of transcription.

We have studied the transcription of several genes in chromatin from estrogen stimulated and control rooster liver nuclei by a modification of procedures of Marzluff and Huang (PNAS 72, 1082-1086, 1975). No exogenous RNA polymerase was added to the transcription reactions. Under our conditions, 89% of the RNA was synthesized by RNA polymerase 2, as measured by sensitivity to 1 μg/ml α-amanitin.

RNA, labelled with ^{32}P-UMP, synthesized by liver chromatin from normal and estrogen-treated roosters was hybridized to various cloned chicken DNA sequences immobilized on nitrocellulose filters under conditions of vast DNA excess. The cloned sequences we used as probes were: albumin, a gene whose transcription *in vivo* is relatively unaffected by estrogen and vitellogenin, the transcription of which is greatly stimulated by estrogen *in vivo*. Albumin RNA synthesis by chromatin from estrogen treated animals is slightly depressed compared to normal chickens (30% reduction), whereas there is a large increase in the amount of vitellogenin RNA synthesized by chromatin from estrogen stimulated animals compared to controls.

142 SHORT REPEAT SEQUENCES AT THE 5' END OF mRNAs IN *DICTYOSTELIUM*: Alan R. Kimmel and Richard A. Firtel, B-022, Univ. Cal. San Diego, La Jolla, CA 92093
M4 is a recombinant plasmid containing *Dictyostelium* nuclear DNA. The restriction map has been determined and the 4 major fragments have been subcloned in pBR322. M4 is >90% single-copy, however, it does contain a short (300bp) sequence repeated ~100 times in the *Dictyostelium* genome. The short repeat sequence is interspersed between single-copy regions. 1-1.5% of total vegetative poly(A)$^+$mRNA hybridizes to the repeat but only 10% of the hybridization is resistant to low levels of RNase. The mRNA is heterogeneous in size and 90% of the mass is complementary to *Dictyostelium* single-copy DNA. We have also shown that one of the single-copy regions adjacent to the repeat is complementary to a low abundancy mRNA (0.01% of total mRNA). Using sandwich hybridization we have shown that unlabeled RNA that hybridizes to this single-copy region will also hybridize [^{32}P]-repeat. Exonuclease studies indicate that the repeat and single-copy region are part of a single transcription unit which produces a 1.2kb mRNA containing a short repeat at the 5' end and a single copy sequence at the 3' end which presumably codes for a specific protein. In addition RNA excess hybridization to [^{32}P] labeled separated strands of the repeat suggests that the repeat sequence is transcribed assymetrically. We have constructed a model in which this repeat sequence is adjacent to ~100 different single-copy genes and is transcribed to produce RNA molecules carrying the common repeat sequence at the 5' end. It is also suggested that initiation of transcription occurs within the repeat. Other recombinant plasmids carrying a different short, interspersed repeat which is complementary to 5-8% of poly(A)$^+$mRNA have been isolated. It is suggested that the organization of the transcripts complementary to these plasmids is the same as the M4 class.

143 CONTROL OF EXPRESSION OF EGG-WHITE PROTEIN GENES BY STEROID HORMONES, G. Schütz, A.E. Sippel, N.C. Nguyen-Huu, W. Lindenmaier, H. Land, T. Wurtz, U. Giesecke, H.J. Hauser, Max-Planck-Institut für Molekulare Genetik, 1 Berlin-Dahlem

We are studying the regulation of synthesis of the egg-white proteins ovalbumin, conalbumin, ovomucoid and lysozyme by steroid hormones. The mRNAs for these proteins have been purified by immunoadsorption of polysomes to matrix-bound antibodies. Using cDNA complementary to these mRNAs, we have shown that the steroid controlled rate of synthesis of egg-white proteins is closely correlated to the cellular concentration of the egg-white protein mRNAs. Isolated chicken oviduct nuclei were used as cell-free system to study rates of synthesis of egg-white protein mRNA sequences. Using two independent methods to distinguish newly synthesized RNA from preexisting molecules, we have shown that the cellular accumulation of egg-white protein mRNA sequences during hormone induction is primarily due to transcriptional activation of the genes.

To provide pure molecular probes for the egg-white protein genes which can be used in the analysis of these genes in genomic DNA, we have prepared recombinant plasmids containing DNA complementary to the lysozyme, ovomucoid and ovalbumin mRNA. The cloned lysozyme DNA was used to examine the organization of the lysozyme gene in chromosomal DNA. It was found that the lysozyme structural gene is not continuous in chromosomal DNA, but is interrupted by at least three intervening sequences. Fragments containing lysozyme structural gene sequences have been isolated by molecular cloning and are presently analyzed.

144 THE 3' END OF H3 HISTONE mRNA FROM STRONGYLOCENTROTUS PURPURATUS, Irmingard Sures and Laurence H. Kedes, Department of Medicine, Howard Hughes Medical Institute Laboratories, Stanford Medical School, Stanford, CA 94305.

Polysomal RNA was extracted from early blastula stages of Strongylocentrotus purpuratus embryos. H3 histone mRNA was purified from the total RNA by hybridization to DNA cellulose containing the H3 and H2a gene regions. The eluted H3 mRNA was endlabeled by ligation with RNA ligase of [5'-^{32}P]pCp to the 3'-terminal hydroxyl group (England and Uhlenbeck, Nature V.275, p.561, 1978) and further purified by gel electrophoresis. The 3'-terminal RNA sequence was determined by partial hydrolysis with T_1 and U_2 endonucleases (Donis-Keller et al., Nucleic Acids Res. V.4, p.2527, 1977) and subsequent analysis of the purine specific cleavage products on thin polyacryl sequencing gels. The resulting pattern of the cleavage products is colinear with the known DNA sequence behind the H3 protein coding region and pinpoints the end of the mRNA 57 bases downstream from the translation termination codon. This location is in the midst of a highly conserved sequence shared by other histone mRNAs from two sea urchin species.

145 TERMINATION OF TRANSCRIPTION OF THE OVALBUMIN GENE, Dennis R. Roop, Sophia Y. Tsai, Ming-Jer Tsai and Bert W. O'Malley, Baylor College of Medicine, Houston, Texas, 77030

A cloned 9.5 Kb fragment of chicken DNA which contains 2150 nucleotides of the 3' portion of the ovalbumin natural gene and 7350 nucleotides of 3' flanking sequence was digested with restriction endonucleases to prepare specific hybridization probes. These probes were used to localize the region of termination of transcription of the ovalbumin gene in vivo. This was determined by two methods: 1) hybridization of [^{3}H]DNA probes to excess oviduct nuclear RNA and 2) hybridization of [^{32}P]DNA probes to oviduct nuclear RNA which had been subjected to agarose gel electrophoresis (in the presence of methylmercury hydroxide) and transferred to diazobenzyloxymethyl paper. Both methods indicate that transcription of the ovalbumin gene is terminated within 20 nucleotides of the 3' end of the structural sequence. We have also used these probes to study the specificity of transcription in isolated oviduct nuclei. This was accomplished by the synthesis of labeled RNA in nuclei and hybridization to filters containing cloned DNA fragments. These results indicate that termination of transcription of the ovalbumin gene in vitro in nuclei is similar to that observed in vivo. This same approach is being used to determine the regions of initiation and termination of transcription of the ovomucoid natural gene.

146 THE ORGANIZATION OF MAMMALIAN PANCREATIC GENES, The Pancreas Group, Dept. of Biochem., University of California, San Francisco, CA 94143, Raymond J. MacDonald

We are analyzing the regulation of pancreatic gene expression during terminal differentiation. In order to obtain probes for several rat endocrine and exocrine genes, we have cloned cDNAs synthesized from islet and total pancreas mRNA. The cloning of rat insulin cDNA sequences has been described (Ullrich et al.(1977) Science 196, 1313). The structure of the genomic DNA sequences coding for insulin mRNA was analyzed by restriction endonuclease digestion. These studies indicated that the two rat insulin genes are not tightly linked, and that while the insulin I and II structural genes have retained extensive sequence homology, the flanking DNA sequences have diverse restriction endonuclease maps. Furthermore, we have localized the two insulin coding sequences to Hinf I fragments of 670 and 960 bp. Since approximately 350 bp are required to code for preproinsulin, introns, if present, are less than 300-600bp.

cDNA from total pancreas mRNA was also cloned. Because amylase is the only major protein common to both pancreas and parotid, hybridization of parotid cDNA should be limited to those pancreas cDNA clones which contain amylase mRNA sequences. By this criterion, 4 of 59 pancreas cDNA clones contained amylase mRNA sequences. Hybridization of the putative amylase cDNA plasmids with total pancreatic mRNA and translation of the hybridized mRNA generated a polypeptide identified as pre- secretory amylase. Analysis of the structure of the genomic amylase sequences indicated the presence of two amylase genes, presumably one expressed in the pancreas and the other in the parotid. The amylase mRNA synthesized in liver (J. Harding and W. Rutter (1978), J. Biol. Chem., in press) must therefore be coded by one of these two genes.

147 EXTINCTION OF GENE EXPRESSION BY DIFFERENTIATION., Patrick H. O'Farrell and Robert D. Ivarie, Dept. of Biochemistry, University of California, San Francisco, CA 94143.
A sensitive assay for differentiated gene products failed to detect any expression of these products in non-producing cells.
The combination of immunoprecipitation and two-dimensional gel electrophoresis permits detection of specific proteins expressed at levels as low as 1 part in 10^9 (about 100 molecules per cell) and provides excellent criteria for the identity of the detected proteins. Using this assay, we examined the expression of a liver-specific enzyme, tyrosine aminotransferase (TAT), and a product of the pituitary, growth hormone (GH), in a rat hepatoma cell line (HTC), and a rat pituitary cell line (GH 3). HTC cells make TAT at a rate of 0.1% of total protein synthesis and do not make GH at a detectable level (attained sensitivity for GH detection = 1 part in 10^9), GH 3 cells make no detectable TAT (attained sensitivity for TAT detection = 1 part in 10^6) and make GH at a rate of 1% of total protein synthesis. Thus, differentiation regulates TAT expression by at least 1,000 fold and GH expression by at least 10^7 fold. Hormones known to induce expression of these proteins in producing cells did not induce their expression to detectable levels in nonproducing cells.
Restriction mapping of the genomic GH gene sequences by B. Schachter, H.M. Goodman, and J.D. Baxter (abstract submitted to this symposium) has shown that HTC cells, though a heteroploid cell line, do contain the GH gene.
Post transcriptional events, such as RNA processing, may play a role in the restriction of expression of a protein product in nonproducing cells. To test this, we will determine whether GH-RNA is present in non-producing cells at levels above those expected from analysis of protein.

148 REGULATION OF mRNA TRANSCRIPTION IN CHICK OVIDUCT, Richard D. Palmiter & G. Stanley McKnight, Department of Biochemistry, University of Washington, Seattle WA 98195.
Relative rates of ovalbumin and conalbumin mRNA transcription were measured in isolated oviduct nuclei by allowing endogenous RNA polymerases to synthesize ^{32}P-RNA that was then hybridized to immobilized recombinant DNA containing the respective gene sequences. Administration of either estrogen or progesterone to withdrawn birds increases the rate of $mRNA_{con}$ transcription 2-3 fold to a new steady-state level within 30 min; in contrast, the rate of $mRNA_{ov}$ transcription increases >20 fold and does so gradually over 12 hr. The maximum rates of transcription achieved in 12 hr were only 10-20% of those observed after several days of hormone treatment. We have been unable to detect effects of hormones on the transciption of these genes by exogenous E. coli RNA polymerase during times when transcription by endogenous polymerases changes dramatically. The induction of $mRNA_{ov}$ and $mRNA_{con}$ in culture is quantitatively comparable to that observed in vivo. Relative rates of transcription were also measured in this system by pulse-labeling with ^{3}H-uridine. In addition, absolute rates of transcription were determined by measuring the specific activity of the UTP pool during the labeling period. The accumulation of $mRNA_{ov}$ sequences is consistent with the absolute rate of transcription measurements, indicating that this mRNA has a long $t_{1/2}$ (>20 hr) in the presence of these hormones. Comparable calculations for $mRNA_{con}$ indicate that its $t_{1/2}$ is ~8 hr in the presence of hormones. These results indicate that both estrogen and progesterone regulate the rate of transcription of these mRNAs, but several observations suggest that there may be significant effects of these hormones on mRNA stability as well.

149 "A Highly Repeated Gene Family from Dictyostelium. Stephen Poole and Richard A. Firtel, University of California, San Diego, Department of Biology, La Jolla, CA 92093. •

Plasmid pDd N1,2 contains a short 1 kb insert of Dictyostelium discoideum nuclear DNA which showed strong hybridization to vegetative poly(A)$^+$ RNA using the colony filter hybridization procedure. When a pDd N1,2 nick translated probe is hybridized to a "Northern" filter containing bound poly(A)$^+$ RNA size fractionated on a methyl-Hg agarose gel, a single RNA of 3 kb in length is observed. RNA excess hybridization of poly(A)$^+$ RNA to the pDd N1,2 probe indicates that this sequence represents 0.4-0.5% of vegetative poly(A)$^+$ RNA. DNA excess hybridization indicates that the sequence complementing to the poly(A)$^+$ RNA is repeated 100-150 fold in the genome. The 3 kb coding region therefore represents ~1% of the nuclear genomic DNA. When the pDd N1,2 probe is hybridized to Southern DNA blot filters carrying size fractionated Dictyostelium genomic DNA cleaved with Hae III or Hind III, a single, very intense band of hybridization in observed. However, when the probe is hybridized to a filter carrying genomic DNA cleaved with Eco RI, a large number of heterogeneous size bands are observed, the number of which is commensurate with the gene repetition frequency. This data suggests that the copies of the repeated are not tandemly linked and that specific Hae III and Hind III sites within the gene are conserved.

The probe was then used to screen a Dictyostelium genomic recombinant library for additional copies of the repeat. A series of plasmids carrying inserts of 5-10 kb were isolated and are being mapped. Overlapping restriction maps of these plasmids suggests that the length of the repeated sequences may be >6 kb. The coding function is being pursued.

150 CLONING AND REGULATION OF THE RAT CASEIN (C) GENES, J.M. Rosen, D.A. Richards, W.A. Guyette and R.J. Matusik, Dept. Cell Biology, Baylor College of Medicine, Houston, TX.
Prolactin (Prl) induction of C mRNA in mammary gland organ culture is being used as a model system to study the mechanism by which peptide hormones regulate specific gene expression. Within 30 to 60 min after the addition of Prl to mammary explants, a 3.5 to 4-fold increase in the rate of C mRNA transcription was observed. C specific transcripts were quantitated by pulse-labeling and cDNA-cellulose affinity chromatography. The increased rate of C mRNA synthesis was not, however, sufficient to account for the 6 to 14-fold increase in C mRNA accumulation routinely observed 24 hrs after Prl addition. Thus, Prl resulted in a time dependent increase in C mRNA half-life from 8.5 hrs in the absence of the hormone, to greater than 50 hrs in its presence. These data suggest that C mRNA accumulation is regulated at both the transcriptional and post-transcriptional levels. In order to study the possible coordinate regulation of the 3 rat Cs, the structural genes for each of the Cs and another milk protein, α-lactalbumin, have been cloned in E. coli strain X1776 using pBR322 as the cloning vector. Double-stranded DNA copies of a milk protein mRNA fraction of larger than 600 nucleotide pairs in length were selected on a neutral sucrose gradient and inserted into the Pst site of pBR322 by dG-dC tailing with terminal transferase. Several hundred transformants were then screened by colony hybridization using cDNA probes prepared against each of the purified C and α-lactalbumin mRNAs. Characterization by restriction mapping and hybrid-arrested cell-free translation has also been employed and the individual cloned probes are currently being utilized to analyze pulse-labeled RNA transcripts synthesized in response to Prl and for total DNA mapping studies.

151 THE RAT SERUM ALBUMIN GENE: ANALYSIS OF CLONED SEQUENCES. Thomas D. Sargent, José M. Sala-Trepat, R. Bruce Wallace, Antonio A. Reyes and James Bonner, Division of Biology, California Institute of Technology, Pasadena CA 91125
We have cloned the mRNA encoding rat serum albumin in the plasmid pBR322 with the E. coli strain X_{1776}. Restriction analysis of albumin cDNA clones in conjunction with "Southern blots" of restricted rat DNA have been used to elucidate gross structural features of the albumin gene.

We have also cloned the entire rat genome in the Lambda coliphage Charon 4A, screened this recombinant library with the albumin cDNA clones and have isolated approximately 32 kb of contiguous rat DNA containing all or most of the albumin mRNA complexity. Preliminary analysis of the isolated gene has partially confirmed our genome blot results and has revealed additional interruptions in the 2.2 kb albumin coding sequence, which is split into no less than five discontinuous regions and occupies at least 13 kb of genomic DNA. The gene may prove to be considerably larger than this when the exact points of transcriptional initiation and termination have been located. This work supported by USPHS grant GM 13762 and National Institute of General Medical Sciences Grant 5 T32 GM 07616.

152 SEQUENCE ORGANIZATION OF THE NATURAL CHICK OVOMUCOID GENE. Savio L.C. Woo, Joseph P. Stein, James F. Catterall, Eugene C. Lai, Anthony R. Means and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.
We have cloned recently the ovomucoid structural gene in the plasmid vector pBR322. Using the cloned ovomucoid DNA as a probe, the sequence organization of the natural chick ovomucoid gene was determined by restriction mapping and Southern hybridization. Our results indicate that the structural DNA sequences of the ovomucoid gene are not contiguous within the chick genome and are separated by multiple intervening DNA sequences. Further analysis of the sequence organization of the natural ovomucoid gene was facilitated by molecular cloning. The structural ovomucoid gene is cleaved once by EcoRI at the 3' untranslated region, and the 5' and 3' termini of the gene are contained within a 15 and a 7 kilobase EcoRI chick DNA fragment, respectively. These DNA fragments were partially purified from total EcoRI-digested chick DNA by RPC-5 column chromatography and preparative agarose gel electrophoresis. The 15 kilobase EcoRI DNA fragment, containing all of the natural ovomucoid gene except 100 base pairs of structural gene sequence at the 3'-untranslated region, was cloned using the lambda phage vector charon 4A. Restriction analysis of this cloned DNA fragment has confirmed the existence of multiple intervening sequences within this gene. The overall size of the entire ovomucoid gene is estimated to be about 8,000 base pairs in length. Only about 800 base pairs are structural gene sequences and the remainder are intervening DNA sequences. This cloned DNA fragment should be very useful for *in vitro* transcriptional studies of this eucaryotic gene.

153 SYNTHESIS OF ADENOVIRUS 2 mRNA *IN VITRO*, James L. Manley, Phillip A. Sharp and Malcolm L. Gefter, Massachusetts Institute of Technology, Cambridge, MA 02139

We are studying the synthesis of viral specific RNA in nuclei isolated from HeLa cells late after infection by Ad2. Synthesis of the major late precursor RNA is initiated *in vitro*. Specifically, the "capped" T1 undecanucleotide which is present at the 5'ends of both viral mRNA and nuclear pre-mRNA *in vivo*, and which is encoded at the site of the major late promoter, is synthesized faithfully *in vitro*. The size of the RNA is heterogeneous, although the majority of the viral specific RNA is very large, in the range of 10-25 kb. The 5'ends of the *in vitro* synthesized colinear transcripts are located predominantly at two map coordinates: 16.5, the site of the major late promoter, and 19.5, the site of the first "splice-point" observed *in vivo*. Colinear molecules with 5'ends at the sites of the other splice-points are also observed, but they are much less abundant. The transcripts are extended towards map coordinate 100, and species with 3'ends at 38.5, 49.5, 61.5 and 78.5 are detected. These sites correspond to 4 of the 5 "families" of 3'ends found on both mRNA and nuclear pre-mRNA *in vivo*. 3'ends are detected after very short *in vitro* incubations, proving that the entire precursor need not be transcribed before 3'ends are generated. The 3'ends are polyadenylated with high, although varying, effeciencies: precursors for the abundant hexon and 100 K mRNAs (78.5 & 61.5) are found exclusively in the poly A^+ fraction, while precursors for less abundant mRNAs (38.5 and 49.5) are divided between the poly A^+ and poly A^- fractions. At least the 3'ends which map at 61.5 and 78.5 are generated *in vitro* during a "chase", proving direct evidence that these molecules are generated by cleavage of a larger precursor.

154 STRUCTURE OF THE CHICKEN BETA GLOBIN GENE, Judith Strommer, UCLA, Los Angeles CA 90024; Jerry B. Dodgson, Caltech, Pasadena CA 91125; and J. Douglas Engel, Northwestern U., Evanston IL 60201

Specific DNA fragments generated from cloned chicken globin mRNA sequences have been used to orient a chicken beta globin gene cloned in bacteriophage lambda and to map its intervening sequences. Southern hybridization of nick-translated cDNA probes with gene and messenger DNA sequences electrophoresed on agarose-acrylamide gels, producing tight autoradiographic bands with fragments as short as 60 bp, together with electron microscopic visualization of R loops, has demonstrated a striking structural similarity to mammalian beta globin genes (Lawn *et al.*, Cell 15:1157 and van den Berg *et al.*, Nature 276:37). R loops formed with an embryonic beta gene linked to the adult gene indicate the same intervening sequence exists in at least two chicken beta globin genes. The presence of a short intervening sequence around amino acid 30 and a several hundred base pair intervening sequence around amino acid 104 has thus apparently been conserved in several beta globin genes through the roughly 3.5×10^8 years since the divergence of birds and mammals.

155 MAPPING DELETIONS WHICH AFFECT THE SWITCH FROM FETAL TO ADULT β-LIKE GLOBIN GENE EXPRESSION IN MAN, Edward F. Fritsch*, Richard M. Lawn*, Tom Maniatis*, Dorothy Tuan+, and Bernard Forget+, *California Institute of Technology, Pasadena, CA 91125 and +Yale University, New Haven, CN 06520

Hereditary persistence of fetal hemoglobin (HPFH) and δβ-thalassemia (δβ-thal) are two types of genetic disorders in man which are characterized by the absence of δ- and β-globin polypeptides in the adult and by the deletion of all or part of the corresponding genes. The two types of genetic abnormalities, however, differ in the relative levels of fetal γ-globin polypeptides in the adult. In HPFH, the absence of adult β-like globin chains is completely compensated by γ-globin gene expression, while in δβ-thal this compensation is only partial. These facts have led to the proposal that HPFH DNA might contain a larger deletion which removes a regulatory sequence normally reponsible for turning off fetal globin gene expression in the adult. To test this possibility we have mapped the endpoints of the deletions in each type of DNA by the procedure of genomic blotting, using γ- or β-globin cDNA plasmids or fragments of cloned genomic DNA as hybridization probes. The DNAs from two different individuals homozygous for HPFH contain deletions beginning approximately 4.0 kb 5' to the δ-globin gene and extending through both the δ- and β-globin genes. The DNA from one individual homozygous for δβ-thal contains a deletion beginning within the intervening sequence of the δ-globin gene and extending through the β-globin gene. In another example of homozygous δβ-thal, the δ- and β-globin genes, at least 4.5 kb of DNA 5' to δ-globin gene, and part or all of one of the two allelic fetal γ-globin genes are deleted. The significance of the locations of the deletions in HPFH and δβ-thal will be discussed in regards to the models for the switch in expression from fetal to adult globin genes. This work was supported by a grant from the National Institutes of Health, No. GM-24716A.

156 CHARACTERIZATION OF TANDEM AND INVERTED REPEAT SEQUENCES IN A CLOSELY LINKED CLUSTER OF RABBIT GLOBIN GENES, Che-Kun James Shen and Tom Maniatis, Division of Biology, California Institute of Technology, Pasadena, CA 91125

A number of different non-globin DNA sequences are present more than once within a 42 kilobase region of rabbit chromosomal DNA which contains four different β-like globin genes. Cross-hybridization experiments between fragments of cloned DNA from different regions of the gene cluster reveal at least 25 sequences which are repeated at least once. These sequences can be classified into at least five subgroups whose members cross-hybridize. Electron microscopic visualization of double stranded DNA regions formed by intrastrand base pairing in single strands of cloned DNA and of heteroduplexes formed between clones containing different regions of the gene cluster have been used to determine the size, location and relative orientation of the repeats. The length of the repeats varies from 100 to over 1000 base pairs. A number of different inverted repeats flank one, two, three, or all four of the linked globin genes. One set of inverted repeats is comprised of two 1.26 kilobase sequences which are located at opposite ends of the gene cluster, separated by approximately 35 kilobases of DNA. This work was supported by a grant from the National Science Foundation. No. CM 77-15425.

157 THE ORGANIZATION OF GLOBIN GENES IN THE CHICKEN CHROMOSOME. Jerry B. Dodgson, California Institute of Technology, Pasadena, CA 91125; J. Douglas Engel, Northwestern Univ., Evanston, IL 60201; and J. Strommer, U.C.L.A., Los Angeles, CA 90024

The organization of the various adult and embryonic globin genes in the chicken genome was initially studied through the use of solution and Southern hybridization techniques (Engel and Dodgson, J. Biol. Chem., in press). More recently a library of chicken DNA fragments cloned in λ Charon 4A was prepared by the EcoRI methylase, synthetic linker technique (Maniatis et al., Cell 15, 687). This library was screened for globin gene-containing clones by the Benton and Davis method (Science 196, 180). Several independent globin gene clones were obtained. Among these a clone containing the adult β-globin gene has been studied in the most detail. The chicken sequences contained in this clone also contain an embryonic β-globin gene about three kilobases (kb) from the adult gene. Both genes appear to be transcribed in the same direction with the embryonic gene 3' to the adult gene relative to the direction of transcription. No globin genes were found in the 10 kb of chicken DNA 3' to the embryonic gene; only about 1 kb of chicken DNA 5' to the adult gene is present in the cloned DNA, so other globin genes may be linked to the adult β-gene in this direction. Both genes contain 0.8 kb insertion sequences similar to those found in mammalian globin genes. Further details of the genetic organization of this clone and of other globin clones, one of which appears to contain linked α-globin genes will be presented.

158 GLOBIN GENES AND MESSENGER RNA FROM HUMANS WITH THALASSAEMIA, Nicholas J. Proudfoot, Francisco E Baralle and John Old, MRC Laboratory of Molecular Biology, Cambridge.

Work is in progress to define the precise molecular defects in various categories of the disease thalassaemia. DNA obtained from a patient with doubly heterozygous $\beta^0/\delta\beta^0$ thalassaemia has been restricted with HindIII and EcoRI and analysed by Southern transfer technology. Rabbit β-globin cDNA plasmid was used as a probe for human γ, δ and β genes. Two differences have been detected in the globin gene containing restriction patterns of normal and the thalassaemic DNAs. These are presumed to represent the β^0 and $\delta\beta^0$ globin gene alleles. DNA fractions containing these two thalassaemic globin genes have been partially purified. Experiments are being carried out to clone these two genes using EK2 lambda vectors and *in vitro* packaging procedures.

Human β-globin mRNA has been previously obtained and partially characterised from patients with homozygous β^0 thalassaemia (Old *et al.*, Cell 17, 289-198, 1978). Work is currently in progress to define the precise molecular defect of these mutant mRNAs using direct sequencing procedures.

159 STABILITY OF GLOBIN mRNA IN FRIEND ERYTHROLEUKEMIA CELLS INDUCED WITH HEMIN, Ky Lowenhaupt and Jerry B. Lingrel, Department of Biological Chemistry, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, Ohio 45267.
Hemin has been suggested to induce only early erythroid functions in Friend erythroleukemia cells and not those which occur late in the developmental program. Friend cells grown in the presence of 75 μM hemin accumulate globin mRNA. Unlike cells induced with other substances including DMSO, hemin induced cells do not cease cell division nor do they accumulate hemoglobin. The stability of globin mRNA in Friend cells after two, four, and six days of induction with hemin was investigated. Cells were pulsed with ^{3}H-uridine for two hours, the label washed away, and the cells resuspended in fresh medium without exogenous uridine. RNA was isolated from cells at various times of chase and globin mRNA was isolated from total RNA using a specific cDNA cellulose column. It was determined that globin mRNA in uninduced cells and cells induced for two, four, or six days with hemin was stable with a half-life of greater than 50 hours. This is in contrast to the specific destabilization of globin mRNA after four days of induction with DMSO. The destabilization of globin mRNA in Friend cells may, therefore, be a late function in the program of erythroid differentiation. It is probable that this program involves sequential expression of genes and the destabilization of globin mRNA requires some specific gene product. Alternatively, globin mRNA in hemin induced cells may not be translated at the same efficiency as that in DMSO induced cells. Stability of mRNA and its translation may be intimately related.

160 STRUCTURE AND SEQUENCE ANALYSIS OF TWO CLASSES OF CLONED COLLAGEN cDNAs, Forrest Fuller, John Wozney, Douglas Hanahan and Helga Boedtker, Harvard University, Department of Biochemistry and Molecular Biology, Cambridge, MA 02138
Recombinant bacterial plasmids containing chick calvaria procollagen sequences were characterized by restriction mapping, Southern gel hybridization, DNA sequence analysis and ability to positively select translatable procollagen mRNA sequences. In addition to the three pro α2 cDNA clones previously described (H. Lehrach, A. M. Frischauf, D. Hanahan, J. Wozney, F. Fuller, R. Crkvenjakov, H. Boedtker and P. Doty (1978) Proc. Natl. Acad. Sci. U.S.A., in press), three pro α1 cDNA clones have been identified. pCg1 contains an insert which is 850 base pairs long and is defined by internal Hae III fragments (110, 175 and 320 base pairs) found in double stranded procollagen cDNA and a sequence of 38+ thymine residues at one 5' end. pCg26 contains a 650 base pair long insert which is contained within the pCg1 sequence. pCg54 contains an insert of approximately 1000 base pairs and overlaps the pCg1 insert by 260 base pairs. All three inserts are colinear with mRNA as determined by hybridization protection from S1. Therefore, pCg1 and pCg54 inserts contain approximately 1600 base pairs of sequence corresponding to the 3' end of the mRNA. Sequence analysis of both α1 and α2 cloned cDNAs is being carried out in order to define them as probes to be used to study the chromosomal organization and expression of collagen genes. Supported by National Institutes of Health grant HD 01229

161 ORGANIZATION AND EXPRESSION OF IMMUNOGLOBULIN GENES, Frederick W. Alt, Vincenzo Enea, Alfred L.M. Bothwell, and David Baltimore, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
We have employed procedures similar to those used previously for the purification of dihydrfolate reductase-specific cDNA (Alt et al. J. Biol. Chem. 253: 1357-1370, 1978) to purify cDNA sequences complementary to specific immunoglobulin heavy and light chain mRNA sequences from murine myeloma lines. These probes have been used to identify cloned double-stranded cDNA sequences representing almost every type of immunoglobulin chain (α, γ_1, γ_{2a}, γ_{2b}, γ_3, several different κ and λ , and several potential μ). Currently, we are using this general approach to isolate and clone specific sequences from cell lines of the T cell lineage. These cloned sequences are being used to study the organization and expression of heavy chain genes in a number of different myeloma lines, each of which secrete a different type of heavy chain. In addition, we are also studying heavy and light chain gene expression in lines of Abelson murine leukemia virus transformed lymphoid cells, and have demonstrated the presence of specific heavy and light chain RNA sequences in a number of these lines. Various lines of evidence have suggested that some of the Abelson virus transformed lines may be counterparts of one of the earliest immunoglobulin producing cell types of the B-cell lineage. Therefore, comparison of the organization and expression of heavy and light chain genes in these lines to that found in embryonic tissue and in myeloma lines should provide a spectrum of the specific molecular events occurring over the differentiation of an immunoglobulin producing cell.

162 CHARACTERIZATION OF A MOUSE MYELOMA CLONE CONTAINING BOTH VARIABLE AND CONSTANT REGIONS OF AN IMMUNOGLOBULIN HEAVY CHAIN GENE, Mark M. Davis, Phillip W. Early, David B. Kaback, Norman Davidson, and Leroy E. Hood. California Institute of Technology, Pasadena, CA 91125.

Using purified immunoglobulin mRNA from the M603 myeloma tumor line (a phosphorylcholine binding antibody producer with alpha heavy chains and kappa light chains) and the plasmid PMB9, a cDNA clone containing a fragment of the alpha heavy chain constant region was constructed. This DNA was labeled with ^{32}P and used to screen a mouse myeloma "library" made with Eco Rl partially digested DNA from the same tumor line (M603) inserted into the bacteriophage Charon 4a. One set of genome clones thus isolated contains both the alpha constant region and a variable region very similar or identical to the phosphorylcholine binding heavy chain variable region as determined by both Southern Blots and Electron Microscopy. Furthermore, it appears that the main body of the constant region is separated from the variable region by 6800 nucleotide pairs as seen by Electron Microscopy of "R-Loops" There is also evidence for the existence of two small intervening sequences within the constant region, possibly between the domains. Supported by National Science Foundation grant No. 57668.

163 DIFFERENT NUCLEAR RNA PRECURSORS FOR COMPLETE κ LIGHT CHAIN mRNA AND κ CONSTANT REGION FRAGMENT mRNA IN MPC 11 CELLS, Edmund Choi, W. Michael Kuehl and Randolph Wall. University of California, Los Angeles, Calif. 90024 and University of Virginia, Charlottesville, Va. 22901.

Cloned MPC 11 mouse myeloma cells produce both a complete κ light chain and a light chain fragment containing the κ constant region (Rose, Kuehl and Smith; Cell 12: 453, 1977). Electrophoresis of MPC 11 mRNA in methyl mercury hydroxide gels followed by contact hybridization with cloned κ constant region DNA has confirmed that complete and fragment κ light chains are coded by 1.25 kb and .79 kb mRNAs respectively. A nonproducing variant line of MPC 11, NP.2 which synthesizes the κ constant region fragment contains only the .79 kb κ mRNA species. The κ light chain sequences in the hnRNA of MPC 11 and NP.2 cells were compared to determine whether the two κ light chain mRNAs were derived from the same nuclear RNA precursor or independently from different nuclear RNA precursors. The largest detectable nuclear RNA species containing κ light chain mRNA sequences in MPC 11 cells sediments at 28S (∿ 5.2 kb) while the largest κ specific species in NP.2 cells sediments at 21S (∿ 3.2 kb). MPC 11 and NP.2 nuclear RNAs resolved on methyl mercury hydroxide gel exhibit additional κ light chain species which appear to be processing intermediates. U.V. transcription mapping is being used to independently establish the size of transcription units coding for the nuclear RNA precursors to these two κ mRNA. These findings suggest that two different κ light chain constant region genes are simultaneously expressed in MPC 11 cells.

164 KAPPA LIGHT CHAIN GENES FROM CLONED MOPC 21 GENOMIC LIBRARIES, Michael Komaromy, Patrick Clarke and Randolph Wall, University of California, Los Angeles, Calif.90024.
We have previously determined the locations of mRNA sequences (including variable and constant regions) in the MOPC 21 κ light chain transcription unit using U.V. mapping (1). We have now constructed genomic libraries of MOPC 21 myeloma DNA to complement these studies with fine structure mapping of the cloned active κ light chain gene. Libraries were constructed by total EcoR I digestion and ligation into λgtWES, and by partial Hae III digestion, selection of 18-22 kb fragments, addition of preformed adaptors (2) and ligation into Charon 4A. An *in vitro* packaging system was used to vastly improve the cloning efficiency. The libraries were screened with nick-translated restriction fragments containing either MOPC 21 κ light chain V+C-region sequences or C-region only, generated from a cDNA clone from MOPC 21 light chain mRNA (3). A number of clones have been selected and characterized by restriction mapping, Southern mapping and R-looping. These include a clone containing V+C sequences, two clones containing only C-region sequences, and five different clones containing only V-region sequences. None of the latter V-region isolates are identical to the MOPC 21 V-region, and thus appear to represent diversity within the $V_κ$15 group (which includes MOPC 21). Nucleotide sequences from these clones will be presented. Promoter sequences are being screened for using a cloned probe from the 5' end of the mRNA.

(1) Gilmore-Hebert et al, PNAS, in press, December 1978
(2) Bahl et al, BBRC 81, 695-703 (1978)
(3) Wall et al, Nucleic Acids Research 5, 3113-3128 (1978)

165 IMMUNOGLOBULIN SYNTHESIS BY LYMPHOID CELLS TRANSFORMED IN VITRO BY ABELSON MURINE LEUKEMIA VIRUS, Edward J. Siden, Naomi Rosenberg, Dan Clark and David Baltimore, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA. 02139
The majority of cell lines derived by infection of murine bone marrow cells with Abelson murine leukemia virus synthesize a mu heavy but no detectable light chain. A minority of cell lines have been found which make no immunoglobulin, light chain alone , or transiently express a light chain as well as mu chain. Two lines have been studied in detail, one that makes mu chain and one that makes light chain. Synthesis of both polypeptides can be increased by modifying the culture conditions so as to decrease the growth rate of the cells. Although some kappa chain secretion was observed, neither secreted nor surface mu was detected. We suggest that the mu-only phenotype may be an early, normal step in the pathway of B-lymphocyte maturation.

166 STUDIES ON GENE AMPLIFICATION IN PALA-RESISTANT SYRIAN HAMSTER CELLS, Richard A. Padgett, Geoffrey M. Wahl, Gail E. Christie and George R. Stark, Department of Biochemistry, Stanford Medical School, Stanford, California 94305
PALA-resistant cell lines stably overproduce the first three enzymes of de novo pyrimidine biosynthesis by up to 120-fold. Amplification of the structural gene coding for the multifunctional protein containing the three activities appears to be the principal mechanism of overproduction. Cot analysis of DNA from various cell lines shows that the copy number of structural gene sequences is increased at least proportionately with enzyme overproduction. Genomic and cDNA clones have been prepared and the mRNA has been physically mapped relative to the DNA. Some highly drug-resistant cell lines contain both the normal 7.9 kb mRNA and an additional 10.2 kb RNA which hybridizes to the cloned cDNA.

167 STRUCTURE OF DIHYDROFOLATE REDUCTASE GENES IN METHOTREXATE-RESISTANT MOUSE LINES / EXPRESSION OF MOUSE DIHYDROFOLATE REDUCTASE IN E.COLI - JH Nunberg, RJ Kaufman, ACY Chang, HA Erlich, SN Cohen and RT Schimke, Stanford University, Stanford, Ca. 94305
Resistance to methotrexate(MTX) is associated with an increase in dihydrofolate reductase(DHFR) gene number in various mouse cell lines. We are analyzing these amplified genes to study the mechanism of amplification. A genomic 5kb BamI fragment has been cloned and mapped with respect to a DHFR cDNA clone. This 5kb fragment contains only part of the gene-the non-translated half of the cDNA sequence and part of a $>$2kb intervening sequence. Attempts are underway to obtain several independent isolates of the entire gene. Another approach is to examine the Southern hybridization of specific DHFR cDNA fragments to restricted genomic DNA. Preliminary results with total cDNA sequence as probe suggest that the unit of amplification is large($>$20 kb) and that the gene is also large and contains several intervening sequences. Experiments with specific cloned cDNA fragments should provide a clearer map.
DHFR cDNA, inserted into the PstI site of pBR322 by GC tailing, was also used to transform X2282, a thy^+ variant of X1776, and transformants selected with trimethoprim(Tp). Bacterial DHFR is 10^4-fold more sensitive to Tp than the mouse enzyme, and several 'expressors' of mouse DHFR were isolated. The best characterized, the 'strong expressors', have the cDNA inserted in the same orientation but out of phase with respect to the vector β-lactamase. The protein product has enzymatic, immunologic and also molecular weight properties of mouse DHFR. We postulate that the oligo-G tail in the vicinity of the AUG start codon is acting as a ribosome binding and initiation site resulting in 'native' mouse DHFR. Other clones which differ in structure and expression are being examined.

168 KARYOTYPIC ALTERATIONS ASSOCIATED WITH STABLE AND UNSTABLE METHOTREXATE RESISTANCE AND DIHYDROFOLATE REDUCTASE GENE AMPLIFICATION, Randal J. Kaufman, Peter C. Brown, Jack H. Nunberg, and Robert T. Schimke, Stanford University, Stanford, CA 94305
The development of high resistance to the folate analogue methotrexate (MTX) results from a stepwise selection of cells with a progressive increase in the capacity to synthesize dihydrofolate reductase (DHFR) and correspondingly high levels of DHFR mRNA and number of DHFR gene copies. Elevated DHFR gene copy numbers occur in both stable and unstable resistant cell lines. Upon growth in the presence of MTX, unstable MTX-resistant lines have become stably resistant with a 'fixation' of the DHFR genes. A fluorescein derivative of methotrexate and the fluorescence-activated cell sorter have been utilized to analyze DHFR levels in individual cells as well as to sterilely isolate living cells on the basis of DHFR gene copy number in order to develop hypotheses concerning the mechanism(s) for expansion, contraction, and fixation of DHFR gnne copies. In situ hybridization of DHFR cDNA to metaphase chromosomes has localized the amplified genes to an expanded region of chromosome #2 in a stably MTX-resistant Chinese hamster ovary line. Similar chromosome anomalies have been found in stalby MTX-resistant murine cell lines. Karyotype analysis of murine MTX-resistant lines has indicated a large number of double minute chromosomes present in unstable MTX-resistant lines but not in lines with stable MTX-resistance or in stable partial revertants. The fluorescence activated cell sorter has been used to isolate cells with high and low numbers of unstable DHFR genes and these cells have been found to contain multiple double minutes and very few double minutes, respectively. Data will be presented concerning the presence of DHFR genes in the double minutes.

169 CONTROL OF LIVER SPECIFIC FUNCTIONS IN CYTOPLASMIC-NUCLEAR HYBRIDS OF MOUSE A9 CELLS AND RAT HEPATOMA CELLS, Leah Ann Lipsich, Joseph J. Lucas and Joseph R. Kates, SUNY at Stony Brook, Stony Brook, New York 11794
Karyoplasts prepared from the mouse A9 cell line have been transplanted into cytoplasts prepared from the rat hepatoma HTC cell line to form viable whole cells. The results indicate that the mouse nucleus, when placed into rat hepatoma cytoplasm, is induced to code for the liver specific enzyme tyrosine amino transferase (TAT)-an enzyme never detected in the mouse fibroblasts. The enzyme was detected in single cells using a histochemical method and in cell extracts prepared from hybrids by the Diamondstone (1966) assay for enzyme activity and by immunoprecipitation using antibody prepared against purified TAT. That the enzyme was of mouse origin was supported by heat stability analysis, mouse liver TAT being significantly more resistant to high temperature than the corresponding rat TAT. That the cells, though expressing a liver specific function, indded contained mouse nuclei was confirmed by their resistance to azaguanine and by karyotyping. Also, as in the rat cell parent, the level of expression of TAT in the hybrids was modulated by the synthetic steroid hormone dexamethasone. The hybrids exhibited induced levels of enzyme activity which varied, from experiment to experiment, from 15-30% of that shown by control induced HTC cultures. Each hybrid culture continued to exhibit its specific level of activity constantly for 6-8 weeks after fusion, then abruptly stopped making enzyme. We feel that this system, which wxhibits both the induction and modulation of an activity not normally expressed by the nuclear parent donor cell, is particularly amenable to the study of eukaryotic gene regulation.

170 CYCLIC AMP, THE MICROFIBRILLAR SYSTEM, AND CELL REGULATION, Theodore T. Puck, Eleanor Roosevelt Institute for Cancer Research, University of Colorado Medical Center, Denver, Colorado 80262.
Increase in the level of cyclic AMP in the CMO transformed cell, causes change of morphology to that of a normal fibroblast; loss of the ability to grow in suspension although growth on solid surfaces is unchanged; disappearance of the rapidly oscillating knobs which characterize the transformed state leaving a smooth tranquilized membrane; change in activity of specific cell surface antigens; increased active transport of alpha-amino-isobutyric acid; and a change in the microtubular structure from a sparse, relatively random array to a dense pattern of microtubules parallel to each other and to the long axis of the resulting fibroblastic cell. The microtubular organization appears to be causally related to other changes since treatment with colcemid or vinblastine prevents manifestations of all but the change in growth behavior which cannot be measured in the presence of mitotic inhibitors. Addition of cyclic AMP to a hybrid of the transformed cell with a cell taken from a brain biopsy causes cessation of growth and development of neurite-like processes which unite to form a network and which contain bundles of parallel microtubules. It is postulated that, a) cyclic AMP is necessary for organization of the microtubular system within cells; b) the form which such organization takes is genetically predetermined; c) the resulting organized structure is a necessary concomitant of particular differentiation functions.

171 CLONING AND EXPRESSION OF SEA URCHIN HISTONE GENES USING SV40 DNA AS A VECTOR,
John S. Kaptein and George C. Fareed, Molecular Biology Institute, University of California, Los Angeles, California 90024.

DNA from the sea urchin (Strongylocentrotus purpuratus) histone gene cluster was digested with restriction enzymes and selected fragments were linked at one end to portions of the SV40 DNA via ligation of cohesive termini. Permissive African green monkey kidney cells (CV1-P) were coinfected with this hybrid DNA and with DNA from temperature sensitive or deletion mutants of SV40. Individual plaques appearing under restrictive conditions were expanded and screened for the presence of virus containing DNA from the sea urchin histone gene cluster, using a filter hybridization assay developed for this purpose. In each infection with the various recombinant DNAs circularization of the hybrid linear DNA molecules occurred in vivo during the transfection procedure as evidenced by various restriction endonuclease analyses. In some circularizations there was no detectable change in the size of the DNA although loss of restriction endonuclease sites at the open ends did occur. Marker rescue of SV40 genomes, and genetic rearrangements (primarily of the SV40 helper DNA) were also observed. Propagation of the hybrid genomes was achieved through lytic infections. Expression of genetic information in cytoplasmic RNA transcripts was examined for selected clones.

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172 ASSIGNMENT OF THE ADENOVIRUS-2 mRNA TRANSCRIPTS TO THEIR RESPECTIVE VIRAL POLYPEPTIDES,
Bruce M. Paterson*, Bryan E. Roberts+, and Michael B. Mathews#. *Laboratory of Biochemistry, NCI-NIH, Bethesda, MD 20014, +Dept. Biology, Brandeis University, Waltham, MA 02154, #Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Viral mRNAs produced during the early and late phases of infection have been selected by hybridization to Adenovirus-2 DNA and resolved according to molecular weight on agarose gels containing methyl mercury hydroxide. Restriction fragments of SV-40 and Adenovirus were used to calibrate the gels. By analyzing the polypeptides synthesized in a reticulocyte cell free system in response to the mRNA extracted from the gels we have been able to determine the functional size range for the majority of the mature Adenovirus-2 messenger RNAs. Our results in conjunction with previous work allow one to construct a transcription map for the virus taking into account the positions of the coding sequences within the mRNA transcripts. These results are to be presented.

173 DISCONTINUITY OF SEQUENCES CODING FOR POLY(A)mRNA IN MOUSE DNA,
Ian H. Maxwell and William E. Hahn, Department of Anatomy, University of Colorado, Denver, CO 80262.

DNA sequences in the genes coding for several specific proteins (globin, immunoglobulins, ovalbumin, ovomucoid) are known to be discontinuous. Whether sequence discontinuity is the condition of few or many structural genes is not known. To answer this question we annealed tracer amounts of a complex population of cDNA sequences (mass average size, ~1600 nucleotides), complementary to poly(A)mRNA from mouse brain, to large fragments (>15 kilobases) of genomic DNA and examined the size of the annealed cDNA after treatment with S_1 nuclease by alkaline agarose gel electrophoresis. The mass average size of the annealed cDNA recovered from S_1 nuclease-treated duplexes was about 800 nucleotides but ~15% of this cDNA was 1600 nucleotides or greater. Control experiments showed that the cDNA was not reduced in size during the incubation period required for annealing, and that S_1 nuclease did not cleave perfect duplex DNA. When the same preparation of cDNA was hybridized with poly(A)mRNA, and then treated with S_1 nuclease, only a slight shift (size decrease) in the size profile of the cDNA was observed. These results suggest that discontinuity of mRNA coding sequence is a frequent condition of structural genes. The above cDNA was also hybridized with total poly(A)hnRNA. In this case, S_1 nuclease treatment did not alter the size of most of the hybridized cDNA although a minor fraction appeared to be reduced in size. Total poly(A)hnRNA probably consists largely of processed molecules, and thus hybridization experiments with high molecular weight fractions of hnRNA are now in progress to determine whether transcription of intervening sequences is general.

174 N-6 METHYL ADENYLIC ACID IN ADENOVIRUS-2 NUCLEAR RNA IS CONSERVED IN THE FORMATION OF mRNA, S. Chen-Kiang, J. E. Nevins & J. E. Darnell, Jr., Rockefeller University, N.Y.C.

In the biogenesis of Adenovirus-2 (Ad-2) mRNAs, methylation occurs at the 5' end (cap) and internally as N^6-methyl-adenylic acid (m^6A). Accumulation kinetics of ^3H labeled Ad-2 specific m^6A and ^{14}C labeled uridine, late in Ad-2 infection, indicated a nearly complete conservation of m^6A from nucleus to cytoplasm, whereas the conservation of ^{14}C-uridine was only $\sim$20%. In accord with the accumulation kinetics, at all times the (^{14}C) U/(^3H)m^6A ratio in the cytoplasm was no greater than 40% of that of the nucleus. A mathematical model was designed to evaluate the kinetics, taking into consideration the three major parameters which affect the rate of accumulation: equilibration of S-adenosyl-methionine pool during ^3H-methionine labeling; the nuclear dwell time of RNA prior to its exit to the cytoplasm; and the stability of the cytoplasmic mRNAs. The $t_{1/2}$ for pool equilibration was determined by chromatography to be 25 min. From the best fit of data to the model, it was estimated that the nuclear dwell time was 25 $\pm$ 10 min, that the m^6A of cytoplasmic RNA turned over in 75 to 150 min, and that m^6A conservation was greater than 75%. When the group of messages that are 3' coterminal and hybridize to the Sma D restriction fragment of Ad-2 DNA (40.5-52.6% of the genome) was studied in detail, it was found that m6A was conserved within the portion of the primary transcript that was destined to become mRNA even though the joining of the 5' end to the leader sequence varies. In addition, results from 3 min labeling and DMSO-sucrose gradient centrifugation suggest that (^3H)m^6A was found mostly in the nuclear molecules larger than 5000 nucleotides (larger than any Ad-2 mRNA molecule). The conservation of m^6A and its occurence prior to processing raise the possibility that internal methylations may play a role in the formation of mRNAs.

Chromosome Structure and Transcription

175 THE NICKING-CLOSING ENZYME MEDIATES CHROMATIN ASSEMBLY IN VITRO, J. E. Germond and Douglas Brutlag, Stanford Medical Center, Stanford, CA 94305.

Studies on the assembly of nucleosomes form the four core histones (H2A, H2B, H3 and H4) and DNA can be mediated by a highly purified nicking-closing enzyme under physiological conditions of ionic strength and temperature. The histone-DNA complex assembled in vitro contains nucleosomes as judged by several criteria. When complexes are formed from relaxed circular DNA, nearly physiological numbers of superhelical turns are induced in the DNA molecule. Electron microscopy of the complexes reveals a beaded structure and a reduction of the contour length compared to the DNA. Micrococcal nuclease digestion of the histone-DNA complex results in 145 base pair DNA fragments typical of nucleosome core particles and discrete length subnucleosomal DNA fragments. The rate of chromatin assembly in vitro is consistent with the physiological rates.

176 LOCALIZATION OF THREE NONHISTONE PROTEINS IN POLYTENE NUCLEI OF DROSOPHILA MELANOGASTER, Hans Will and Ekkehard K.F. Bautz, University of Heidelberg, 69 Heidelberg, GFR

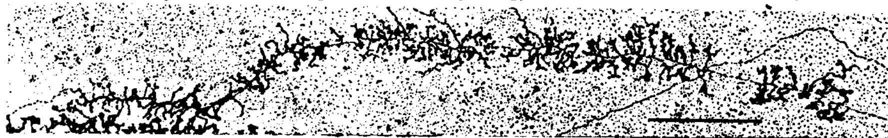
Proteins involved in the regulation of gene expression are likely to be members of a group of nonhistone proteins (NHP's). Such regulatory proteins are expected to be located at specific sites in chromosomes of eucaryotes. Using polytene chromosomes as a test system we have started to search for proteins which can be localized at defined regions in polytene nuclei. Total NHP's, obtained from nuclei of Drosophila embryos, were fractionated on a hydroxyapatite column and individual fractions or, alternatively, protein bands obtained after SDS gel electrophoresis of these fractions, were injected into rabbits. The antisera obtained were used to localize, by indirect immunofluorescence, the corresponding antigens in polytene nuclei.

One of the antisera was found to decorate primarily puffs, a second antiserum gave fluorescence almost exclusively at the chromocenter and a third antiserum proved to be specific for nucleoli. The antiserum decorating the chromocenter region was found to react more strongly with α- than with β- heterochromatin. Similar reactions were observed with other Drosophila species (D. hydei and D. virilis). The antiserum also stains metaphase chromosomes of Drosophila tissue culture cells and even of Hela cells.

The nucleolus-specific antiserum was found to react with a single polypeptide having a MW of approximately 30, 000 daltons

177 STUDIES ON THE IN VITRO ACETYLATION OF NUCLEOSOMES, Robert L. Garcea and Bruce M. Alberts, Dept. of Biochem. & Biophys., Univ. of Calif., San Francisco, Ca 94143
We have studied histone acetylation in an in vitro system. This system consists of a purified preparation of the major salt-extractable nuclear bound histone acetyl-transferase (E. Belikoff and B. Alberts, unpublished), ^{3}H-acetyl CoA, and a variety of nucleosomal substrates having different degrees of higher order packing. We find with this enzyme that only histones H2a, H2b, and (to a lesser extent) H4 are acetylated. In contrast, the pattern of acetylation in vivo, or in isolated cell nuclei in vitro, reveals preferential acetylation of histones H3 and H4 over histones H2a and H2b. Inhibition of endogenous acetylases in nuclei with NEM and subsequent readdition of the histone acetyltransferase gives predominantly H2a and H2b acetylation. Using differential NEM sensitivity, urea extraction, or increased ionic strength, we can separate the endogenous histone acetyl-transferase activity directed towards histone H3 from our acetyl-transferase activity directed towards H2a and H2b. It appears that two nuclear activities may exist: 1) the more commonly studied nuclear enzyme, which acetylates preferentially histones H2a, H2b, and H4 on nucleosomes, even though H4 and H3 are the preferred substrates when presented with equimolar amounts of free histones, 2) an activity towards histones H3 and H4, which does not purify with our acetylase and may represent a specific cofactor requirement for the major enzyme.

178 VISUALIZATION OF ACTIVE TRANSCRIPTION UNITS IN THE BALBIANI RINGS OF CHIRONOMUS TENTANS, M. M. Lamb and B. Daneholt, Karolinska Institutet, Stockholm, Sweden
Active transcription units on isolated fourth chromosomes from salivary gland nuclei of Chironomus tentans have been visualized according to the electron microscopic method developed by Miller and co-workers. These active transcription units are likely to be located in the most active sites of transcription on chromosome IV, Balbiani Ring 1 and Balbiani Ring 2, which generate 75S RNA molecules. The active transcription units most frequently observed had a mean length of 7.68 um and did not appear to be tandemly repeated. The lateral fibers on the transcription units were closely spaced with no regular pattern of gaps or evidence of processing during transcription. We have compared the active transcription units visualized by this method with structures observed in sectioned Balbiani Rings prepared for conventional electron microscopy, and with available biochemical information about the 75 S RNA generated at these sites. We consider possible models of regulation of transcription in the light of the morphology of these active transcription units.



Active transcription unit; bar indicates 1 um.

179 HISTONE GENE EXPRESSION AND CHROMATIN STRUCTURE IN CELL HYBRIDS, Nancy Hsiung and Raju Kucherlapati, Princeton University, Princeton, N.J. 08540

Partial digestion of nuclei isolated from mammalian cells with micrococcal nuclease results inDNA of discrete length. Different cell lines exhibite different repeat lengths. The H1 histone has been implicated in the determination of the variation in nucleosome repeat sizes. We have examined the expression of DNA repeat lengths and histone H1 in a series of intraspecific and interspecific somatic cell hybrids. Different mouse and human lines exhibiting distinct repeat sizes were fused. In the 24 mouse x human hybrids analyzed, the DNA was organized into repeat lengths characteristic of the mouse cells. The number and type of human chromosomes present in these hybrids had no effect on this feature.In the 5 mouse x mouse hybrids,only the repeat length of one parental line was observed.
Labeled purified histone H1 can be fractionated into 3 subfractions by gel electrophoresis. Partial peptide analysis of these proteins from mouse and human cells revealed that 1)the histone subfractions from within a species are distinct from each other and 2)in some cases,individual subfractions can be distinguished between rodent and human cells. Analysis of the mouse -human hybrids revealed the presence only of the mouse H1 histones. Because each of the human chromosomes is represented among these hybrids,we conclude that the human histone gene expression is suppressed in these hybrids.Observations that these hybrids are expressing human enzymes suggest that the H1 histone of one species can interact with the chromatin of another in a biologically active conformation.In the mouse xmouse hybrids, the histones of B82 cells are expressed. Since these hybrids show the repeat size only of the other parent line, we conclude that H1 histone is not a major factor in the determination of nucleosome repeat lengths.

180 SPECIFIC DNA FRAGMENTS ASSOCIATED WITH NONHISTONE SCAFFOLDING PROTEINS, Brian C.Bowen and U. K. Laemmli, Princeton University, Princeton, NJ 08540

The higher order structure of metaphase chromosomes may involve the repetitive attachment of specific DNA sequences to a matrix of nonhistone proteins.[1] In initial attempts to test this hypothesis, Hela chromosomes were digested with restriction endonuclease HaeIII. Upon completion of the reaction, histone proteins and 98-99% of the DNA were solubilized, and the insoluble, chromosome "scaffolds" purified by sedimentation. Digestion studies with EcoRI of the scaffold-associated DNA indicated that this DNA is highly enriched in a set of repeated fragments, suggesting a possible specific interaction with the scaffolding proteins. The two major fragments observed have sizes of 2.1 kb and 1.7 kb; they accounted for 20% of the scaffold-associated DNA. Restriction mapping of the 1.7 kb fragment shows one AluI, two Sau3A, and several (>5) HinfI sites.

By in situ hybridization, we found that the scaffold-associated DNA sequences are located on all of the Hela chromosomes, in both heterochromatic and euchromatic regions. The major EcoRI fragments, however, appear to hybridize predominantly to the centromeric regions of a subset of chromosomes. These results imply that the major fragments represent only a subset of the repeated sequences recognized by nonhistone proteins. An interpretation of the results, and the possible relation of the major fragments to previously reported human satellite DNA is discussed.

[1] Laemmli, U. K., et al (1977), Cold Spring Harbor Symp. Quant. Biol., 42, 351

181 IMMUNOFLUORESCENT LOCALIZATION OF HETEROGENOUS NUCLEAR RIBONUCLEOPROTEIN IN DROSOPHILA POLYTENE AND LAMPBRUSH CHROMOSOMES. Milan Jamrich, Dept. of Biology, Univ. of Virginia, Charlottesville, VA. 22901, Mark E. Christensen, Wallace M. Le Stourgeon, Dept. of Molecular Biology, Vanderbilt U. Nashville, Tenn. 37235

The major non-histone nuclear protein from *Physarum polycephalum* (protein B-36) is similar in certain properties to HeLa hnRNP proteins A_1, A_2, and B_1 (Christensen *et al.*, 1977, BBRC 74: 621). Antibodies raised against B-36 protein cross react with at least one of the HeLa proteins. These antibodies can be used to localize nascent hnRNA transcription or possible storage sites of the proteins or post-transcriptional RNP particles. The reaction of anti B-36 serum with polytene chromosomes produces a strong fluorescence at transcriptionally active sites. The strongest fluorescence is observed in active puffs. Antibodies also decorate the interbands, a result which is in good agreement with the localization of RNA polymerase B(II) and which supports the idea that the interbands are also involved in active transcription (Jamrich *et al.*, PNAS 74: 2079). The fluorescence obtained after staining the nuclei of *D. hydei* primary spermatocytes shows that the nucleoli contain an exceptionally high amount of antigen (the same holds for *Drosophila* tissue culture cells). Most, if not all, of the lampbrush loops show specific staining. These observations indicate that at least one of the RNP proteins has remained conserved during evolution and that some of the RNP proteins may be common for hn and pre-rRNA.

182 ABSENCE OF NUCLEOSOMES IN ACTIVE rDNA REPEATS OF *Calliphora erythrocephala* LARVAL TISSUES AND JAPANESE QUAIL MYOBLASTS. Milan Jamrich, Ellen Clark, and Oscar L. Miller, Jr., Dept. of Biology, Univ. of Virginia, Charlottesville, Va. 22901

Chromatin of *Calliphora* larval tissues and of tissue-cultured quail myoblasts was prepared for electron microscopy. Spreading conditions were varied in pH (7.5-9.0), in concentration of detergent (0-0.1% Joy, 0-0.1% Digitonin), in presence of tRNA (0-100μg/ml) and in degree of agitation. Under all conditions, rRNA genes which are not fully loaded with RNA polymerases do not exhibit nucleosomes between polymerase molecules. These chromatin segments are similar in structure to the "rho" chromatin reported for rRNA genes of *Oncopeltus fasciatus* (Foe *et al.*, Cell 9: 131). In *Calliphora*, nucleosomes are present in the regions preceding initiation sites, but nucleosome-free regions frequently are present beyond the termination points of the genes. These regions vary in length and occasionally include an entire non-transcribed spacer. The occasional presence of RNA polymerases with attached transcripts in the nucleosome-free regions beyond gene termini in *Calliphora* suggest that these regions may result from a failure of polymerase to terminate properly. In quail myoblasts, however, the spacer region of rRNA repeats appears to be free of nucleosomes. Whether or not the absence of nucleosomes in quail spacers is due to similar events remains to be determined. (USPHS Grant 5 RO1 GM2102).

183 NUCLEOSOME ASSEMBLY OCCURS ON NON-REPLICATING DNA - Ronald L. Seale, National Jewish Hospital and Department of Biochemistry, Biophysics and Genetics, University of Colorado School of Medicine, Denver, Colorado 80206.

Assembly of nucleosomes from newly synthesized histones was investigated to determine whether the sites of deposition are on newly synthesized or non-replicating DNA of HeLa cells.
1) Chromatin was labeled simultaneously with ^{14}C-BUdR and ^{3}H-lysine. Nucleosomal subunits were prepared by nuclease digestion, salt-washed to remove all proteins except the inner histones, crosslinked and banded in CsCl. The labeled proteins banded with normal density chromatin, not with the newly-synthesized, hybrid density chromatin.
2) Newly synthesized chromatin DNA bands as a light replication satellite to main band chromatin DNA in CsCl. Pulse-labeled histones were associated with the non-replicating chromatin, not with the replication satellite.
3) The kinetics of digestion of new, lysine-labeled nucleosomes from nuclei followed the parental chromatin pattern in terms of rate and size of cleavage products.

In summary, the data obtained by three different criteria indicate that nucleosome assembly takes place on non-replicating DNA.

184 THE CHROMATIN STRUCTURE OF SPECIFIC GENES, Sarah C. R. Elgin and Carl Wu, Harvard University, The Biological Laboratories, 16 Divinity Ave., Cambridge, Mass. 02138

When the chromatin of *Drosophila* is examined by digestion with DNase I or micrococcal nuclease, no general structural organization above the level of the nucleosome is revealed by the cleavage pattern. In contrast, the DNase I cleavage pattern of specific regions on the *Drosophila* chromosome shows discrete bands with sizes ranging from 2 to greater than 20 kilobase pairs. Visualization of such higher order bands was achieved by the use of the Southern blotting technique. The DNase I-generated fragments were transferred onto a nitrocellulose sheet after size fractionation by gel electrophoresis. Hybridization was then carried out with radioactively labeled cloned unique sequences of *Drosophila melanogaster* DNA. After autoradiography, distinctive patterns of higher order bands are seen. For the five chromosomal loci examined, each gives a unique and different band pattern. Restriction enzyme analyses indicate that the preferential DNase I cleavage sites in chromatin are position specific. Both these higher order bands and the nucleosome repeat pattern are disrupted to some degree at the two heat shock loci examined following gene activation. Both levels of organization are restored after heat shock. Chromatin structure of control loci (three cases) is not affected by heat shock. It is concluded that there are higher order structural domains in chromatin defined by DNase I-sensitive boundaries. Controlling packaging and disruption of DNA in nucleosomes and higher order domains may be of integral importance in the regulation of eukaryotic gene expression.

185 SPECIFIC TRANSCRIPTION OF SV40 DNA BY RNA POLYMERASE II FROM AVIAN LIVER.
W. Kastern, J. Eldridge and K.P. Mullinix, NCI, NIH, Bethesda, Maryland 20014
The mechanisms involved in the control of specific gene transcription are not known. As part of a program to develop *in vitro* systems to study specific gene transcription, we have isolated and characterized the DNA-dependent RNA polymerase II from avian liver, a tissue that is rich in this enzyme. Nuclei from rooster livers were lysed and subjected to digestion by pancreatic DNase I that we had coupled to Sepharose 4B. The RNA polymerase preparation thus obtained was completely dependent on the addition of exogenous DNA for activity. Four per cent of the activity was due to RNA polymerase I and the remaining activity was due to RNA polymerase II, with no detectable RNA polymerase III. In order to identify RNA polymerase II in complex mixtures of proteins at various stages of purification, we have used the technique of binding ^{3}H-amanitin. After the binding reaction, the protein mixture was subjected to electrophoresis on gradient polyacrylamide gels under non-denaturing conditions. We have identified two proteins that bind ^{3}H-amanitin, having molecular weights of 640,000 and 550,000, respectively. We have also utilized the ability of RNA polymerase II to bind to ^{3}H-amanitin to titrate the total amounts of this enzyme in a preparation. This determination is therefore independent of the activity of the enzyme and allows us to compare the amount of RNA polymerase II molecules present with their activity. The ability of RNA polymerase II from avian liver to initiate specific transcription has been studied. Using SV40 DNA as template, RNA polymerase II was able to initiate transcription on either the early or late gene regions, depending on the method of purification. In addition, this specific transcription was asymmetric, depending on the manner in which the polymerase had been isolated from the nuclei.

186 DYNAMICS OF NUCLEOSOME STRUCTURE STUDIED BY FLUORESCENCE, Ann E. Dieterich, Hilbert Eshaghpour, and Charles R. Cantor, Columbia University, New York, N.Y. 10027
Specifically bound fluorescent dyes have proven to be very useful probes of nucleosome structure. Nucleosomes have been reconstituted from core histones containing a fluorescent label on either histone H3 or H4. Between 10^{-4} and 0.6 M NaCl, the structure of the labelled nucleosomes has been characterized by the techniques of fluorescence polarization, collisional quenching, and singlet-singlet energy transfer. In this range of ionic strengths we observed dramatic changes in nucleosome structure, giving rise to four distinct states of the nucleosome. The kinetics of transition between some of these states has been studied, revealing in one case the presence of intermediate structures of the nucleosome. These large and complex structural changes are consistent with the variety of functions in which nucleosomes must participate in the cell.

187 COMPARISON OF SEQUENCES THAT FLANK EUKARYOTIC GENES TRANSCRIBED BY RNA POLYMERASE III, Laurence Jay Korn and Donald D. Brown, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210
Cloned and genomic *Xenopus borealis* oocyte specific 5S DNAs containing clusters of 5S rRNA genes and the adjacent AT-rich spacer DNA have been sequenced. The number of genes varies between clusters, and the distance between genes within a cluster is about 80 nucleotides. The 5S genes are accurately transcribed when added to an extract of *X. laevis* oocyte nuclei. To facilitate our search for common features in the DNA sequences immediately adjacent to the 5' and 3' ends of *Xenopus* and other eukaryotic genes transcribed by RNA polymerase III, we have developed a computer program to analyze nucleic acid sequences. Conserved sequences would be candidates for involvement in the control of transcription. The common features among the 5' flanking sequences are: 1) a purine rich region, 2) at least one direct repeat, 3) the absence of dyad symmetry, 4) transcription beginning with a purine, 5) a pyrimidine residue immediately preceding the first nucleotide of the gene, and 6) the oligonucleotides AAAAG, AGAAG and GAC, located approximately 15, 25 and 35 nucleotides respectively before the start of transcription. The ten base pair spacing between the homologous oligonucleotides is that expected for a recognition signal on one face of a DNA double helix. The 3' flanking regions contain many features in common with prokaryotic transcription termination sites. Experiments to alter the 5' flanking sequence of *Xenopus* 5S genes and study the effect of these changes on transcription are in progress.

188 ISOLATION OF A CHROMATIN FRACTION FROM *XENOPUS LAEVIS* ENRICHED IN GENES CODING FOR 5S RNA, Joel M. Gottesfeld, Scripps Clinic and Research Foundation, La Jolla, Calif.
In the frog *Xenopus laevis* the DNA sequences coding for 5S ribosomal RNA form a developmentally regulated multigene family. 5S genes are present in many thousand copies per genome and different types of 5S genes are expressed in oocytes and somatic cells. The organization of 5S genes in nucleosomes has been studied by Southern blot hybridization of labeled 5S DNA to a partial micrococcal nuclease digest of *Xenopus* chromatin (in nuclei of kidney-derived tissue culture cells). The length of 5S DNA contained in a nucleosome subunit is similar to that for the bulk of *Xenopus* chromatin (196 base pairs). The relationship of nucleosomes to the sequence of 5S DNA has been studied by the Southern blot procedure with DNA isolated from limit restriction digests of chromatin. The location of restriction enzyme cleavage sites in the sequence of oocyte-type 5S DNA and the abundance of 5S DNA in the *Xenopus* genome make this gene well suited for isolation as chromatin. Chromatin has been subjected to two cycles of restriction enzyme digestion: first with enzymes that do not cleave 5S DNA followed by a centrifugation step to remove solubilized chromatin. The remaining chromatin was then subjected to digestion with a restriction enzyme that cleaves 5S DNA once per repeating unit (HindIII). The chromatin solubilized by this enzyme was then sedimented in a sucrose gradient and the position of 5S genes in the gradient was determined. The final chromatin fractions contained less than 2% of the total DNA but most (>60%) of the 5S DNA sequences. The proteins of 5S gene chromatin have been analyzed by two-dimensional gel electrophoresis.

189 INHERITANCE OF NUCLEOSOMES DURING DEVELOPMENT. Robert M. Grainger, University of Virginia, Charlottesville, VA 22901.

The stability of nucleosomes synthesized in Drosophila embryos was determined by a density labelling approach. Radiolabelled eggs were collected from adult females fed a medium containing ^{3}H-lysine. These eggs were sterilely transferred to a completely ^{13}C-^{15}N labelled medium containing ^{14}C-lysine; thus histones synthesized postembryonically are all ^{14}C labelled and dense, while histones synthesized in the embryo are ^{3}H-labelled and light. At several times during later larval development nuclei were isolated from larvae and digested with micrococcal nuclease to yield a nearly homogeneous mononucleosome preparation. Nucleosomes were crosslinked to preserve histone octamers (which comprise the nucleosome core) using Lomant's reagent [dithiobis (succinimidyl propionate)] and samples banded in cesium formate-guanidine HCl equilibrium gradients. Control ^{14}C heavy histones and ^{3}H light histones (not crosslinked) were banded to demarcate the light and heavy density positions in the gradients. Crosslinked octamers from all developmental stages tested (through the late 3rd larval instar) segregate into a completely light ^{3}H-labelled peak and completely heavy ^{14}C-labelled peak. Thus histones in old and new nucleosomes do not mix, and embryonic nucleosomes are stable throughout this developmental period. By a DNA density labelling analysis it was possible to determine that embryonic nucleosomes were stable at least six generations. Biochemical and morphological approaches have clearly shown that the nucleosome is the structural subunit of chromatin; experiments presented here imply that it is a biologically stable structure as well.

190 SODIUM BUTYRATE INDUCES NEW GENE EXPRESSION IN FRIEND ERYTHROLEUKEMIC CELLS, Raymond Reeves and Peter Cserjesi, The University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Within twenty-four hours after treatment with millimolar concentrations of n-butyrate the histones (particularly the nucleosome core histones H3 and H4)of Friend cells have become hyperacetylated as a result of the inhibition of the histone deacetylase enzymes by the short-chain fatty acid. During this same time period, nucleic acid hybridization studies indicate that the butyrate-treated cells have also accumulated a population of about 38% new RNA transcripts synthesized from unique-sequences of mouse DNA. These butyrate-induced RNA transcripts are not detectable in control, non-fatty acid treated cells or in Friend cells treated with other inducers of overt differentiation such as dimethylsulfoxide. Furthermore, two-dimensional gel electrophoretic analysis of the treated cells also indicates that the butyrate-treated cells synthesize many new species of proteins not found in control or dimethylsulfoxide-treated cells. All of these metabolic effects of butyrate are readily reversible once butyrate is removed from the cells. The remarkable co-ordinate effect of butyate on the induction of histone acetylation, the induction of new protein synthesis and the induction of new unique-sequence gene transcripts suggests that the three phenomena may be closely linked in Friend cells and the results are not inconsistent with the idea that histone acetylation may play an important part in the initiation of new gene expression in these cells.

191 LAC REPRESSOR BINDING TO RECONSTITUTED LAC NUCLEOSOMES, Moses V. Chao, Harold G. Martinson, and Jay D. Gralla, UCLA, Los Angeles, CA 90024.

Restriction fragments of 203 and 144 base pairs containing the E.coli lac control region have been reconstituted with core histones from calf thymus to form lac DNA-containing nucleosomes. By several criteria, including DNase I digestion, sedimentation rate, and histone-histone crosslinking, reconstituted nucleosomes are similar to native nucleosomes isolated from micrococcal nuclease digestion of calf thymus nuclei. In order to map the position of the histone core on the 203 base pair lac reconstitute, Exonuclease III digestion followed by DNA sequencing was carried out. This analysis indicated that the lac operator sequence is either covered or overlapped by the histone core. Using isokinetic sucrose gradients containing known amounts of lac repressor protein, the 203 base pair lac nucleosomes were found to bind to lac repressor with high affinity and specificity. Repressor binding did not induce dissociation of the histone core. The apparent binding constant of the 203 base pair lac reconstitute is within an order of magnitude of the binding of free lac DNA with lac repressor, indicating that the presence of histones does not interfere significantly with repressor binding. The 144 base pair lac reconstitute also binds lac repressor, however, at a much lower apparent binding constant.

192 HIGHER ORDER STRUCTURE IN CHROMATIN, J. B. Rattner and B. A. Hamkalo, University of California, Irvine, CA 92717

The elucidation of a particulate chromatin subunit, the nucleosome, as a ubiquitous component of eukaryotic chromatin has led to the investigation of the manner in which these subunits are organized into higher order structures in interphase and metaphase chromatin. Minimally-disrupted higher order fibers can be visualized in chromatin derived from mouse L929 cells after gentle mechanical lysis with 0.5mm glass beads in the presence of Jokliks suspension media and preparation for electron microscopy by the Miller procedure. These uniform fibers measure 200 to 300 Å in diameter and are composed of closely-apposed arrays of nucleosomes. The higher order fibers can be seen extending from metaphase chromosomes as a series of loops 1-5 μm in length and also are observed in interphase chromatin suggesting that this fiber class is present in cells independent of the overall degree of chromatin condensation. Negatively-stained preparations show several distinct nucleosome packing patterns within the 200-300 Å fiber. The most common patterns appear as (1) bands of nucleosomes oriented diagonally relative to the fiber axis and stacked along the fiber length, (2) regions within which adjacent nucleosomes are in a staggered packing arrangement imparting a knobby or rope-like appearance to the fiber or (3) short, relatively straight segments composed of two rows of nucleosomes arranged parallel to the fiber axis. The variety of packing conformations and their variable distribution along the length of the higher order fiber suggest that, although the diameter of the fiber is uniform, it is formed by folding the basic 100 Å nucleosome-containing fiber nonuniformly. The above observations were made on inactive chromatin; higher order structures seen in active chromatin also will be discussed.

193 INITIATION OF RIBOSOMAL RNA SYNTHESIS IN VITRO BY A XENOPUS OOCYTE NUCLEAR HOMOGENATE R.A. Hipskind[1] S.L. McKnight[2] & R.H. Reeder[1] 1) Fred Hutchinson Cancer Res. Center, Seattle, WA 98104 & 2) Dept. of Embryology, Carnegie Inst. of WA., Baltimore MD 21210.

Nuclei isolated manually from mature *Xenopus* oocytes will incorporate labeled nucleoside triphosphates into RNA for at least 8 hours. The reaction product is predominantly ribosomal RNA. When incubated with γ-thionucleoside triphosphates to probe for initiation, 3-10% of the product RNA hybridizes strictly to a cloned DNA fragment containing the 5' end of the rRNA gene. With longer incubation times the bound RNA begins to hybridize to a restriction fragment from the middle of the gene. The size of this RNA on gels is consistent with its hybridization behavior. All the hybridization is to the coding strand of ribosomal DNA and is competed by cold 40S ribosomal precursor. We have also analyzed the nuclear homogenate incubated *in vitro* by electron microscopy. The micrographs show close-packed RNA polymerase molecules with attached nascent chains (initiated *in vivo*) moving further down the gene with increasing time. We calculate a chain elongation rate of 2.2 nucleotides per second for these transcripts, compared to a rate of 2-4 nucleotides per second estimated for the γ-thiotriphosphate-initiated chains. Behind these close-packed complexes are RNA polymerase-nascent chain complexes at approximately 1/4 the *in vivo* frequency. We conclude that the homogenate is reinitiating ribosomal RNA synthesis *in vitro* and that initiation occurs very near the *in vivo* initiation site. Supported by grants form the NIH to RHR and from the Helen Hay Whitney Foundation to SLM