CETUS–UCLA SYMPOSIUM

GENE REGULATION

Bert O'Malley, Organizer

March 28 – April 3, 1982

Plenary Sessions

Poster Sessions

Control of Amphibian 5S RNA Genes

0729 TRANSCRIPTION OF 5S RNA GENES: HOW THE DIFFERENTIATED STATE IS MAINTAINED, Donald D. Brown, Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, Maryland 21210.

Transcription complexes made in vitro with 5S RNA genes resemble chromatin. This has enabled us to suggest a means by which the differentiated state is maintained in eukaryotic cells. Supported in part by an NIH grant.

Organization of the Eucaryotic Genome

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0730 MOVABLE GENES OF MICE AND MEN, Philip Leder*, Philip Hieter, Gregory Hollis, Ilan Kirsch, Aya Leder, Jeffrey Ravetch and Ulrich Siebenlist, Laboratory of Molecular Genetics, NICHD, National Institutes of Health, Bethesda, Maryland 20205 and *Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115

The fact that genes are less stable than might have been expected is one of the important new lessons of molecular genetics. In the case of the immunoglobulin genes, this "instability" is part of a required developmental program that joins distant segments of DNA to form coherent and active antibody genes. In the case of other genetic systems, this instability is manifest in the germline and becomes evident only over long periods of evolutionary time. From our own studies of globin and immunoglobulin genes, we have been able to identify several different covalent alterations in chromosomal structure that involve the movements of large segments of DNA within and between chromosomes. Some of these can be reconciled by assuming they result from recombination between broadly homologous segments of DNA; others appear to require special DNA sequences and splicing enzymes, while others--a class we shall call processed genes--seem to be the result of moving a gene to a new site via an RNA intermediate. This last class is particularly interesting and we have already found two examples among the globin genes of the mouse and the immunoglobulin genes of man. In each case, the moved gene--or processed gene--bears significant evidence of RNA-type processing, including the clean loss of intervening sequences and coincident homology to the site of polyA addition. Such elements are likely to be a significant factor in genomic evolution.

0731 THE MOLECULAR DEFECT IN A CASE OF β° THALASSEMIA IS ABNORMAL SPLICING, Richard Treisman, Nicholas Proudfoot and Tom Maniatis, Harvard University, Cambridge, MA.

Genetic disorders of human β-globin gene expression are characterized either by the absence or reduced expression of normal globin polypeptide as in the case of β° and β$^+$ thalassemias, respectively. We previously reported the isolation and nucleotide sequence analysis of a β-globin gene from an individual homozygous for β°-thalassemia (1). Comparison of the nucleotide sequence of this gene with that of the normal β-globin gene (2) revealed only two differences: a G→A transition at position 1 in the second intervening sequence (IVS 2) and a G→T transversion at position 74 of IVS 2. The change at position 1 converts the highly conserved GT dinucleotide to an AT. The change at position 74 has been noted as a common sequence polymorphism in β-globin genes (S.H. Orkin, personal communication).

To determine whether the splice junction mutation prevents the normal splicing of the globin mRNA precursor, the β normal and β° thalassemia genes were introduced separately into SV40 cloning vectors by in vivo recombination using a technique developed by B. Seed (Manuscript in preparation). These β-globin gene recombinants were then transfected into COS 7 and HeLa cells (3,4). We found no difference in the amount of cytoplasmic β-globin RNA produced by the β° and normal β-globin genes. IVS 1 is correctly excised from both gene transcripts, but the normal IVS 2 splice donor site is not utilized in the β° gene transcripts. We conclude that the G→A transition completely inactivates this splice donor site. Instead, the β° gene utilizes a novel splice donor site at IVS 2 position 47 which is apparently inactive in the normal gene. The DNA sequence at this site (TGGTTAAG), is similar to previously characterized splice sites. Both the β normal and β° genes utilize the normal IVS 2 splice acceptor site. The aberrant splicing of the β° gene transcripts results in the insertion of 47 nucleotides which contains a translational stop codon. The absence of a normal splicing event at the 5' end of IVS 2 provides direct genetic evidence for the requirement of the GT sequence in mRNA splicing. The fact that a new splice donor site, which is apparently not recognized in the normal gene transcript, is utilized in the β° gene suggests that the primary sequences at splice sites are not the sole determinants of splicing competence.

(1) Shander, M., Vande Woude, S., Proudfoot, N. and Maniatis, T. (1981) J. Supramol. Struct. Cell Biochem. Suppl. 5, 229.
(2) Lawn, R.M., Efstratiadis, A., O'Connell, C., and Maniatis, T. (1980) Cell 21, 647.
(3) Mellon, P., Parker, V., Gluzman, Y. and Maniatis, T. (1981) Cell 27, 279.
(4) Banerji, J., Rusconi, S. and Schaffner, W. (1981) Cell 27, 297.

0732 HORMONAL CONTROL OF GROWTH HORMONE GENE EXPRESSION, H.M. Goodman*, M.D. Walker+, D.J. Diamond*, M.A. Conkling*, P. Kushner+,D.D. Moore*
*Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114
+Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

The rat and human growth genes have been isolated and their nucleotide sequences have been determined. The rGH gene is unique in the haploid rat genome, but there are approximately seven hGH related genes in the haploid human genome. Only two protein products, hGH and hCS, are known to be encoded by these multiple human genes. Perhaps surprisingly in the face of such potential diversity the hGH gene gives rise to two different protein products, the 22,000d. major hGH polypeptide and the smaller 20,000d. minor hGH, by two alternative splicing pathways for removal of one of the four intervening sequences present in the nuclear messenger RNA precursor.

The rGH gene has been examined in various functional states. In both the pituitary and the brain, the gene shows a lowered level of methylated cytosing residues, a state which has been correlated with active transcription in other gene systems. However, the gene is highly methylated in the GH_3 rat pituitary cell line which makes and secretes significant amounts of rGH.

The rGH gene and several thousand bases of flanking DNA have been introduced into a mouse cell line. Synthesis of rGH RNA is detectable in three different rGH containing clones, and in all of them the level of rGH RNA is increased by the addition of dexamethasone plus triiodothyronine. This indicates that at least some of the signals which cause the gene to respond to hormones are very close to the gene itself.

Nucleic acid hybridization analysis shows that the hCS gene is very homologous to the hGH gene, but the putative rCS gene is not strongly homologous to the rGH gene. Two different hypotheses for the evolutionary origin of the hCH gene have been offered to explain this paradox. According to the first, the hCS gene arose by duplication of the hGH precursor after rodents and primates diverged. Alternatively, a more ancient hCS precursor could have been changed to the observed hGH-like sequence by a recent recombination or gene conversion event involving the hGH precursor.

Viral Genomic Expression

0733 POLY(A) IN mRNA FORMATION AND STABILITY, James E. Darnell, Jr., Menachem Zeevi, Joseph Nevins, Marianne Salditt-Georgieff and Selina Chen-Kiang, Molecular Cell Biology Department, The Rockefeller University, New York, N. Y. 10027.

In the usual order of steps in mRNA processing (either virus or cell transcripts) the poly(A) segment is added before splicing. We have found however that splicing is not dependent on the physical presence of poly(A) since splicing occurs in the presence of 3' deoxyadenosine. In continuing the observations on spliced mRNA-sized molecules made in the presence of 3'dA we have now found that they enter the cytoplasm and can be found in polyribosomes. However since label does not accumulate in these molecules it appears that they turn over rapidly. The role of poly(A) may be largely (if not entirely) to increase the half-life of cytoplasmic mRNA.

Additional experiments of addition of poly(A) in the nucleus of CHO cells and in vitro poly(A) synthesis will be described as time permits.

0734 THE ANATOMY OF FUNCTION IN RETROVIRUS ONCOGENES, J.M. Bishop, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

The oncogenes of avian retroviruses provide means by which cells of different embryological lineages can be transformed to neoplastic phenotypes. Several of these genes (src, fps, yes, and ros) transform only fibroblasts and encode proteins that appear to be tyrosine protein kinases. Others (myc, erb, and myb) transform hematopoietic cells by functions as yet unidentified. All of these genes appear to have derived from "proto-oncogenes" or "cellular oncogenes" found in the genomes of both vertebrate and pre-vertebrate species. I will review: 1.) the use of molecular cloning and nucleotide sequencing to compare viral to cellular oncogenes; 2.) evidence concerning the mechanism by which oncogenes might be "transduced" from the genome of normal cells; 3.) efforts to express cellular oncogenes that have been isolated by molecular cloning and reintroduced into avian or mammalian cells in chimeric vectors; 4.) the role of subcellular localization in the function of the protein encoded by src; 5.) the role of the host cell in modulating the structure and function of the src protein; 6.) the "dose" of the src protein required to transform cells to a neoplastic phenotype; and 7.) the use of site-directed mutagenesis to map domains of function within oncogenes. At stake is the hypothesis that retrovirus oncogenes provide suitable models for "cancer genes" indigenous to normal cells.

Reference: Bishop, J.M.; Retroviruses and Cancer Genes. Adv. Cancer Res., Vol. 37, in press (1982).

0735 CHARACTERIZATION OF THE ROUS SARCOMA VIRUS TRANSFORMING GENE PRODUCT EXPRESSED IN E. COLI, R.L. Erikson and T.M. Gilmer, Department of Pathology, University of Colorado School of Medicine, Denver, CO 80262

A number of biochemical experiments suggest that the Rous sarcoma virus (RSV) src gene product is a protein kinase with the unusual capacity of phosphorylating tyrosine residues in protein substrates. Normal host cells for RSV express a variety of protein kinases, thus, in order to study $pp60^{src}$ in the absence of these enzymatic activities recombinant molecular clones were constructed that express the src gene protein in E. coli. The protein produced in E. coli was recovered in a soluble and insoluble form. The soluble form was purified extensively and shown to phosphorylate tyrosine residues in protein substrates, whereas extracts from E. coli carrying identical molecular clones lacking the src gene yielded no detectable enzyme activity. These data, taken with those previously published, lead to the conclusion that the RSV src gene encodes a protein kinase.

The RSV src gene product expressed in eukaryotic cells is a phosphoprotein and although preliminary evidence suggests p60 expressed in E. coli is not a phosphoprotein, additional studies are required. Additional studies on quantitative comparisons of the enzymatic activity of $p60^{src}$ of prokaryotic and eukaryotic origin are in progress and will be discussed, particularly with regard to the phosphorylation state of the molecule.

Gene Expression in Animal Cells

0736 STUDIES ON THE CHROMOSOMAL STRUCTURE OF DIFFERENTIATION-SPECIFIC GENES, Bert W. O'Malley, William E. Stumph, George M. Lawson, Ming-Jer Tsai, Department of Cell Biology, Baylor College of Medicine, 1200 Moursund Avenue, Houston, Texas, 77030.

Hen oviduct nuclei were subjected to pancreatic DNase I treatment under conditions known to preferentially degrade transcriptionally active genes (Weintraub and Groudine, 1976, Science 93, 848). The ovalbumin gene, its structurally related genes, X and Y, and the spacer and flanking DNA were all found to exist in a DNase I sensitive configuration. More interesting, however, the DNase I sensitive region was found to extend more than 20 Kb beyond the 5' end of the X gene and approximately an equal distance beyond the 3' end of the ovalbumin gene before it became DNase I resistant. The transition from DNase I sensitive to a resistant conformation in oviduct chromatin occurred in a gradient fashion within a 10 Kb of DNA. Thus, ovalbumin and its related genes, X and Y, exist in DNase sensitive domain which is >100 Kb in length in the oviduct tissue. In contrast, this entire domain was resistant to DNase I in spleen, liver, and erythrocyte nuclei. When the transcription of ovalbumin, X, and Y genes was eliminated by the withdrawal of hormone from estrogen-stimulated chicks, the entire domain remained in a DNase I sensitive configuration. Additionally, we have extended this concept of structural domains to other sets of avian genes. Repetitive sequences within this domain have been mapped and a particular sub-family termed CR1 has been shown to exist only at borders of this large DNase I-sensitive domain. The region of homology between these CR1 sequences extends over a region of approximately 160 base pairs. In each case, the 160 base pair region is flanked by imperfect, but homologous, short direct repeats 10-15 base pairs in length - a characteristic of transposable DNA elements. We conclude that large DNase I sensitive domains may provide the structural capability for chromosomal gene expression and appear to be a result of the differentiation process since they are cell-specific and contain potentially expressible genes of that cell type.

Gene Expression in Development

0737 REGULATION OF GENE EXPRESSION IN SEA URCHIN EMBRYOS, Eric H. Davidson, Division of Biology, California Institute of Technology, Pasadena, California 91125.

Expression of a series of cloned sea urchin embryo genes has been analyzed, including several actin genes. These genes identify a number of diverse strategies by which the prevalence of embryonic cytoplasmic transcripts is controlled. Some genes are not represented in maternal RNA and their transcripts appear suddenly during embryogenesis. Examples are provided by certain of the actin genes which have now been identified, the late histone genes, and several other cloned genes. However the majority of embryo transcripts are already represented in maternal RNA at fertilization. Many of these maternal transcripts have structural features reminiscent of nuclear RNAs rather than of the polysomal messages of the later embryo. During development the embryo replaces maternal poly(A) RNAs with newly synthesized transcripts. The point at which this occurs differs for each sequence studied. Transcript prevalences extend from a few copies of each sequence per gastrula cell to thousands or more copies per cell. Direct measurements show that the prevalence of each transcript is determined both by its specific cytoplasmic decay rate and its synthesis and flow rate into the cytoplasm during embryogenesis.

0738 A MOLECULAR ANALYSIS OF THE BITHORAX COMPLEX IN DROSOPHILA. David S. Hogness, Robert B. Saint, Michael E. Akam, Michel Goldschmidt-Clermont, and Philip Beachy, Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

The bithorax complex of Drosophila melanogaster consists of a cluster of genes that select and maintain the developmental pathways for most of the body segments of both larvae and adult flies (1). These segments include A1-A8 and T3 (i.e., all eight abdominal segments and the third thoracic segment; 1), and at least part of T2, the second thoracic segment (2). The gene cluster can be divided into left (proximal) and right (distal) halves on the basis of gene function. The left half contains the genes required for T2 and T3 development (abx, bx, pbx and ppx) and for A1 development (bxd), as well as Ubx, which is required for all three segments. This half can be viewed as a single functional unit because Ubx mutations appear to cis-inactivate the wild-type functions of its other genes (1, 2). The genes in the right half are required for the development of A2-A8; their functions are not affected by Ubx mutations (1). We have isolated cloned segments of genomic DNA that include most of the bithorax complex, and have determined the location of DNA alterations associated with a variety of mutations in the complex (3); in particular, the abx^1, bx^1, Cbx^1, Ubx^1, bxd^1 and pbx^1 apparent point mutations that were mapped in that order by recombination by E.B. Lewis (1), were shown to exhibit the same order on the molecular map, although none are true point mutations, each being associated with the insertion or deletion of small DNA segments (3). This report will concentrate on a more detailed analysis of the ~120 kb of DNA that constitutes the left half of the complex. This analysis includes the characterization of the units of transcription in that half, and the molecular mapping of a large collection of Ubx mutations. The most dramatic characteristic of the left half is the extraordinary length of the Ubx unit that is revealed both by molecular mapping of Ubx mutations and by analysis of RNA transcripts. This unit extends some 75 kb leftward (the direction of its transcription) from its right boundary near the Ubx^1 site to a position some 30 kb to the left of abx^1. The unit yields three different length classes of RNA (3.0, 4.3 and 4.7 kb) of which two contain sequences from both ends but apparently none from the intervening ~70 kb. A smaller bxd unit of transcription (~25 kb) that overlaps both bxd^1 and pbx^1 mutations yields RNAs with similar properties.

(1) E.B. (1981). In: Developmental Biology Using Purified Genes, ICN-UCLA Symp.on Mol. and Cell. Biol., Vol.XXIII (eds. D.D. Brown & C.F. Fox), Academic Press, NY, in press. (2) Morata, G.and Kerridge, S.(1981). Nature 290,778-771. (3) Hogness, D.S., Bender W.W., Akam, M.E., Saint, R.B. and Spierer, P. (1981). J. Supramol. Structure and Cell. Biochem., Supplement 5, p. 385.

Processing of RNA Precursors

0739 STRUCTURE AND FUNCTION OF RNA PROCESSING NUCLEASES, PARTICULARLY THOSE OF TRANSFER RNA BIOSYNTHESIS, Sidney Altman, Cecilia Guerrier-Takada, Madeline Baer and Robin Reed, Department of Biology, Yale University, New Haven, CT 06520.

Nucleases involved in RNA processing events are required to carry out highly specific cleavage reactions (1,2). There is little obvious relationship of known substrate recognition mechanisms to enzyme structure. A close examination will be made of the enzymes of tRNA biosynthesis (3) and, in particular, of RNase P, the enzyme which generates the 5' termini of mature tRNA sequences (4). Recent experiments have probed the points of intimate contact between the protein and RNA moieties of the enzyme with each other and with precursor tRNA substrate. In addition, the role that RNase P and other tRNA processing enzymes may play in controlling the expression of non-tRNA genes will be illustrated with specific examples from *E. coli* (1,5,6) and human mitochondria (7).

1. Altman, S., Guerrier-Takada, C., Frankfort, H.M. and Robertson, H.D., in "Nucleases" (eds. S. Linn and R. Roberts, Cold Spring Harbor, 1982) in press.
2. Perry, R.P., J. Cell. Biol. (1982) in press.
3. Altman, S., Cell 23 3-4 (1981)
4. Kole, R. and Altman, S., Biochemistry 20 1902-1906 (1981)
5. Lee, J.S., An, G., Friesen, J.D. and Fiil, N.P., Cell 25 251-258 (1981)
6. Altman, S., Model, P., Dixon, G. and Wosnick, M., Cell 26 299-304 (1981)
7. Ojala, D., Merkel, C., Gelfand, R. and Attardi, G., Cell 22 393-403 (1980)

0740 SMALL NUCLEAR RNPs AND RNA PROCESSING. Harris Busch, Ram Reddy, Dale Henning, and Paul Epstein, Baylor College of Medicine, Houston, Texas 77030

With the completion of the sequence analysis of the U-snRNA's the following have been demonstrated: 1) the U-RNA's are the only small nuclear RNA species that are "capped"; 2) the trimethyl G cap is unique to the U1 to U5 RNA species; 3) the U6 RNA has a distinct but unidentified 5'-cap different from U1 to U5 RNA; 4) all the U-snRNA species are in small particles in the nucleoplasm except for U3 RNA which is the only U-snRNA in the nucleolus; 5) the U-snRNA species are evolutionarily conserved; 6) EM pictures of hnRNP-snRNP complexes demonstrate juxtaposition of these structures but not whether their association is by RNA:RNA or protein:protein bonds; 7) preliminary analyses with crosslinking reagents show that the snRNA:hnRNA bonds exist; 8) antibodies to small nuclear RNP particles in patients with autoimmune diseases are useful markers for the disease state; 9) one autoantibody, Sm, reacts with common antigens in all U-snRNP except U3-RNP; 10) the U1-RNP autoantibody reacts specifically with only U1-snRNP; 11) the 5' terminal sequence of U1 RNA is highly complementary to the termini of the intron consensus sequence and to the 5'-termini of specific viruses; 12) models for U1 RNA structures in the U1 snRNP particles show U1 RNA is highly hydrogen bonded and is shielded by proteins from T_1 RNase; 13) preliminary immunological blocking analyses have indicated that in some systems RNA processing is blocked by antibodies to snRNP's. A nondenaturing method for preparation of the snRNP's which retains their associated enzymes for these particles is required to demonstrate their role in splicing, transport or processing of high molecular weight rRNA and mRNA precursors. The "Alu" group of snRNA's does not have a 5'-cap (4.5S RNA, 4.5I RNA and 8S RNA).

Gene Expression in Inducible Systems

0741 TRANSCRIPTIONAL REGULATION WITHIN DROSOPHILA GENE FAMILIES. Brian J. McCarthy and Janet M. Monson, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717, Jeannette Natzle and Ursula Rdest, Department of Genetics, University California, Berkeley, CA 94720. Federico Sanchez, Centro de Investigacion sobre Fijacion de Nitrogeno, UNAM, Cuernavaca, Morales, Mexico and Sara L. Tobin, Department of Genetics, University of California, Berkeley, CA 94720.

As is the case for other multi-cellular organisms, Drosophila has more than one gene for each of the structural proteins actin, tubulin and collagen. We have isolated and mapped all six actin genes, all four α- and all four β-tubulin genes and two collagen-like genes. Our interest has been in their transcriptional activity through the Drosophila life-cycle. Amounts of RNA representing individual genes present at each stage were estimated by gel blot and dot blot analysis. Several examples of these profiles of developmental expression will be discussed. Two of the actin genes, those at 79B and 88F show similar patterns during early development with peaks at 2-3 hr and 12-15 hr and increased transcription in first and second instar larvae. In pupae the 88F gene is more active than either gene at any other stage, while transcription from 79B is lower than in first and second instar larvae.

In the case of tubulin, detailed patterns of expression have been established for all eight genes. All show distinctive profiles of temporal expression and for some there are periods where expression is barely detectable. For both collagen-like genes the peak of expression occurs during the first and second larval instars, but transcripts of one can be observed earlier than those form the second. Other collagen-like genes exist but have not been explored.

We conclude that the expression of individual within gene families for structural proteins is independentally regulated.

0742 GLUCOCORTICOID REGULATION OF GENE EXPRESSION: IDENTIFICATION OF MMTV REGULATORY SEQUENCES. Gordon Ringold, Maureen Costello, Barbara Dieckmann, Deborah Dobson, J. Russell Grove, Frank Lee, and James Vannice. Department of Pharmacology, Stanford Medical School, Stanford, Ca. 94305

We have constructed gene fusions between the mouse mammary tumor virus (MMTV) LTR and a variety of selectable markers.[1] After introduction into recipient cells by DNA-mediated transformation, the selectable genes become glucocorticoid inducible. The absolute level of expression directed by the MMTV promoter is highly dependent on the cell into which it is introduced and the degree of hormone responsiveness is in large part a function of the availability of functional glucocorticoid receptors. Deletion mutants within the LTR have been constructed to delineate more precisely the region(s) required for glucocorticoid sensitivity.

In attempts to determine whether the LTR and other regions of the viral DNA are important in the hormonal responsiveness of MMTV during a natural infection we have selected mutant HTC cells that are incapable of inducing the major viral glycoprotein(gp52) in response to glucocorticoids.[2] Detailed characterization of these cells by biochemical and genetic techniques have allowed us to identify at least two complementation groups: 1) cells defective in the glucocorticoid receptor and 2) cells with cis-acting defects in the viral DNA. Analyses of the DNAs from the cells harboring defective proviruses indicate that the lesions giving rise to impaired hormonal sensitivity are not a consequence of altered DNA methylation or due to large insertions or deletions. Attempts to clone these defective proviruses are underway. Lastly, to complement our studies on the MMTV regulatory region, we are currently identifying cDNA clones corresponding to the genes for at least two rat liver proteins that are induced by glucocorticoids.

1. Lee,F., Mulligan,R., Berg,P., and Ringold,G. Glucocorticoids regulate expression of dihydrofolate reductase cDNA in mouse mammary tumor virus chimaeric plasmids. Nature 294: 228-232, 1981.

2. Grove, J.R., and Ringold,G.M., Selection of rat hepatoma cells defective in hormone-regulated production of mouse mammary tumor virus RNA. Proc. Nat. Acad. Sci. 78: 4349-4353, 1981.

0743 COMPLEX HORMONAL REGULATION OF MAMMARY GLAND MILK PROTEIN GENE EXPRESSION

Jeffrey M. Rosen, Andrew A. Hobbs, John R. Rodgers, and Li Y. Yu-Lee,
Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

The control of milk protein gene expression by both peptide and steroid hormones is a complex interactive process involving regulation at both the transcriptional and post-transcriptional levels (1). Both the rapidity and the large magnitude of induction observed in response to hormones in serum-free mammary explant cultures make this an ideal system for investigating the factors controlling specific mRNA accumulation in mammalian cells. The predominant milk proteins, the caseins, are a family of phosphoproteins, which appear to have evolved from a common ancestral gene. Considerable sequence divergence has been observed among members of this gene family in both intra- and interspecies comparisons. The three caseins are located on a single chromosome, while a fourth, abundant milk protein gene, designated pX32, is on a different chromosome (2). Analysis of the structure of genomic casein clones has revealed that the casein genes are large and complex, containing multiple intervening sequences resulting in a length of 17 Kb for the γ-, and about 25 Kb for the β-casein gene. These genes are approximately 18 and 22 times larger than their respective mRNAs. Cloned cDNAs have been used to measure the levels of these mRNAs during both normal mammary development and in cultures exposed to the hormones, prolactin and hydrocortisone (3). In organ culture both hormones were found to be essential for the maximal induction of all four mRNAs. By 24 hours after prolactin addition the levels of pX32, α-, β-, and γ-casein mRNAs had increased 4-, 8-, 35- and 250-fold, respectively. The relative proportions of the α-, β-, and γ-casein mRNAs changed, therefore, from 300:35:1 in the uninduced to 4:7.7:1 in the induced state. These changes in mRNA concentrations can be explained by differences in both the half-lives of these mRNAs, as well as their rates of transcription in the presence and absence of hormones. Recent sequence analysis of γ-casein mRNA has revealed the presence of an unusual poly(A) addition sequence, AUUAAA, 11 nucleotides 5' to the poly(A) tail. Interestingly a significantly greater proportion of γ-casein mRNA was also observed in nuclear poly(A) minus RNA. Studies are in progress to assess the significance of this observation with respect to the hormonal regulation of casein mRNA stability.

1. Guyette, et al., Cell 17:1013, 1979.
2. Gupta, et al., J. Cell Biol., in press.
3. Hobbs, et al., J. Biol. Chem., in press.

0744 SELECTIVE GENE EXPRESSION WITHIN HORMONALLY ACTIVATED MULTIGENE FAMILIES,

Jamshed R. Tata, National Institute for Medical Research, Mill Hill, London NW7 1AA.

Many hormonally regulated genes are members of small multigene families of 3-20 genes which are expressed in a highly tissue specific manner (1). These include genes coding for chicken ovalbumin, Xenopus vitellogenin, rat $\alpha_{2\mu}$-globulin and mouse α-amylase. Not all members of these multigene families are transcribed equally efficiently when a hormone activates specific genes. Thus, estrogen activates the transcription of the major ovalbumin gene in the chicken oviduct at rates that are 200 times that of the neighbouring 'X' and 'Y' ovalbumin-like genes (2). Similarly, not all $\alpha_{2\mu}$-globulin genes, which are regulated by androgen in rat liver, may be activated by the hormone (3). Particular emphasis will be placed in this talk on the selective activation by estrogen of the vitellogenin multigene family in male Xenopus liver. The hormone induces de novo the transcription of at least 4 vitellogenin genes which can be distinguished by virtue of a 20% divergence in coding sequence into 2 groups (A and B) of two genes each (4). As judged by the kinetics of hybridization, it appears that there are additional vitellogenin sequences which are not transcribed. A cell culture system was used to determine whether or not the four vitellogenin genes are co-ordinately activated at the very early stages of hormonal induction (5). We have found that the A and B groups of vitellogenin genes are not simultaneously activated and that accumulation of individual mRNAs does not proceed at identical rates at the onset of stimulation in vitro. Differential hormonal effects on conformation (as judged by DNase I sensitivity) and transcription of individual vitellogenin genes, measured simultaneously in vitro, will also be briefly discussed. Selective expression or differential rates of transcription of individual hormonally activated genes within a multigene family poses new questions concerning the regulation of their expression and the sites of interaction between the genes and hormone receptors.

1. Tata JR: J. Steroid Biochem. 15:1982, in press.
2. LeMeur M, Glanville N, Mandel JL, Gerlinger P, Palmiter R, Chambon P: Cell 23:561, 1981.
3. Kurtz DT: Nature 291:629, 1981.
4. Wahli W, Dawid IB, Wyler T, Jaggi RB, Weber R, Ryffel GU: Cell 16:535, 1979.
5. Searle PF, Tata JR: Cell 23:741, 1981.

Chromosomal Structure of Genes

0745 CHROMATIN CONFORMATION AND GENE ACTIVITY, Gary Felsenfeld, James McGhee, William Wood, Joanne Nickol, and Michael Behe, National Institutes of Health, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Laboratory of Molecular Biology, Section on Physical Chemistry, Bethesda, MD 20205

The physical properties of chromatin have been explored at the level of individual nucleosomes, extended polynucleosome filaments, and folded polynucleosome fibers. To compare the physical properties of bulk chromatin with the properties of chromatin in transcriptionally active genes, we have examined the stability of the 30 nm chromatin fiber in the neighborhood of the adult β globin gene in chicken erythrocytes. We find that the DNA in the neighborhood of this gene is indistinguishable in its degree of compaction from bulk chromatin. As the ionic strength of the solvent is decreased, the 30 nm fiber unfolds to form a 10 nm extended filament. The same salt dependence is shown by the active gene region. There appears to be no major instability, at this level of chromatin folding, associated with the potential for transcription, or with the special sensitivity to nucleases present in this region.

The sensitivity to each of 3 nucleases (DNase I, DNase II, and micrococcal nuclease) is remarkably uniform throughout the region containing the adult β globin gene, the 5' end of the neighboring embryonic ε gene, and the 2.7 Kbp of DNA separating them. A notable exception is the hypersensitive region just 5' of the adult β globin gene. We have mapped this region in detail, using a variety of nucleases, and show that it extends over about 200 base pairs of DNA between about -60 and - 260 nucleotides from the starting point of transcription. The hypersensitive region is highly enriched in the sequence CpG, the usual site of methylation. We have used the restriction enzyme Msp I to liberate a 115 bp fragment from 14 day embryo erythrocyte nuclei. About one third of the fragments are protein-free. The yield of fragment is greater than 80%; its size and accessibility suggest that the region is nucleosome-free in the nucleus. No such fragment is liberated when nuclei from 5 day embryos are incubated.

The correlation of levels of methylation with transcriptional activity in this hypersensitive region and in other active regions of the genome raises questions about the effects of methylation on DNA and chromatin structure. In order to detect such possible effects, we have examined the properties of synthetic polydeoxynucleotides with the sequence $(dm^5dC\text{-}dG)_n$. Such sequences undergo transition from the B to Z form under physiological conditions of ionic strength. We find that the binding of histones stabilizes the B form, and that Z DNA does not form a well-defined nucleosome structure. The possible significance of these results for structure <u>in vivo</u> will be discussed.

0746 CHROMATIN FINE STRUCTURE OF GENES, Abraham Worcel, Department of Biology, University of Rochester, Rochester, New York 14627.

We have previously reported that the histone gene repeat of Drosophila melanogaster is packaged into a precisely defined and characteristic structure, as follows: the non-transcribed spacers display a "normal" chromatin arrangement, with each nucleosome precisely positioned on the underlying DNA sequence; the 5' ends of all five histone genes are in an exposed configuration, highly sensitive to both micrococcal nuclease and DNase I; and the genes have an "altered" chromatin structure, as indicated by the weak and irregularly spaced nuclease cuts (1). We have now analysed the effect of salt extraction on this particular chromatin fine structure.

Treatment of nuclei from Drosophila melanogaster embryos and/or tissue culture cells with increasing concentration of salt (from 0.35M to 1.1M KCl) results in the stepwise removal of non-histone chromosomal proteins, histone H1 and finally, nucleosomal histones. Using an indirect end-label technique (1,2) we have examined the structure of the 5 Kb histone gene repeat in the extracted chromatin. The removal of the non-histone proteins with 0.35 KCl enhances a micrococcal nuclease cleavage 190 bp upstream from the 5' end of the histone H1 gene but does not otherwise effect the overall pattern of m. nuclease sites nor the DNase I hypersensitive sites at the 5' end of the genes. The quantitative extraction of histone H1 with 0.55M KCl does not cause further changes in either the m. nuclease or the DNase I cleavage pattern. Extraction with 0.75 KCl erases the preferential DNase I susceptibility at the 5' end of the five histone genes and unmasks new micrococcal nuclease sites in the long H1-H3 DNA spacer. These new sites, which correspond to m. nuclease hypersensitive sequences in the protein-free DNA, become more prominent after extraction with higher KCl concentrations. Extraction with 1.1M KCl erases the native chromatin sites and results in a m. nuclease pattern which is identical to the free DNA cleavage pattern.

The data strongly suggests that nucleosomal histones precisely positioned on the DNA sequence of the long H1-H3 non-transcribed spacer are responsible for the m. nuclease cleavage pattern observed over the same region in native chromatin. The salt concentration required to erase the DNase I hypersensitive sites (0.75 KCl) randomizes nucleosome position on DNA; thus the DNase I hypersensitivity may be due to a selective absence of nucleosomes from those 5' gene regions.

1) Samal, B., Worcel, A., Louis, C. and Schedl, P. (1981) Cell <u>23</u> 401-409.
2) Wu, C. (1980) Nature <u>286</u> 854-860.

0747 SUPERCOILED LOOPS AND GENE EXPRESSION, Bert Vogelstein, Barry D. Nelkin, Sabina I. Robinson, Don Small, The Oncology Center, Johns Hopkins University School of Medicine Baltimore, MD 21205.

The DNA in a eucaryotic nucleus is arranged into a series of supercoiled loops which are anchored at their bases to a nuclear skeleton or matrix. It can be shown that these supercoiled loops of DNA are motile, rather than stationary, structures; they are reeled through the matrix during replication, and perhaps during other nuclear processes. Using nuclease digestion, one can progressively cleave DNA from the loops, thereby isolating residual DNA that is progressively closer to the nuclear matrix anchorage sites. Using such a technique, it is shown that specific genes, such as the SV40 genes in SV40 transformed cells, are specifically located at the base of the DNA loops. The orientation within supercoiled loop domains of a variety of other genes has been assessed and correlated with cell differentiation.

Expression of Transduced Genes in Mammalian Cells

0748 EXPRESSION OF TRANSDUCED GENES IN MAMMALIAN CELLS, Paul Berg, Department of Biochemistry, Stanford University Medical Center, Stanford, CA 94305

Our laboratory has explored two approaches to achieving transduction-transformation of mammalian cells. One utilizes recombinants constructed in vitro from suitably modified cloned cDNAs or pro- and eukaryote derived genomic segments, and SV40 DNA from which segments encoding early and late viral functions have been excised. In this experimental design the recombinant viral genomes must contain the origin of SV40 DNA replication and not be larger than 5.3 kb in order to be propagated as mature virion genomes; moreover, since the recombinant genomes are defective, propagation can only occur with a helper virus to provide the missing gene product(s). A virtue of this approach is that amplification of the recombinant genomes ensures a high yield of the mRNAs and proteins expressed from the transduced genes. In my lecture I shall describe experiments by Andrew Buchman, using the SV40 lytic vector system that bear on the need for splicing in mRNA biogenesis. His experiments demonstrate a failure to accumulate stable β-globin cytoplasmic mRNA from transcripts made after infection with recombinants containing a β-globin cDNA interposed between the SV40 early region promoter and late region poly A site. Buchman has shown that this deficiency is rectified by inserting an intervening sequence at any of several locations within the recombinant's β-globin transcription unit. This system has been used to examine the splicing requirements for transcripts containing the β-globin and other protein coding segments as well as a means to correlating features of intervening sequence structure and splicing activity. The second approach exploits a new group of transducing-transforming DNA vectors for the introduction and maintenance of new genes in a variety of mammalian cells. One prototype of this class of vectors, pSV2-neo, developed by P. Southern and B. Howard, contains a DNA segment from the plasmid pBR322 to permit propagation of the recombinants in E. coli and a transcription unit comprised of the SV40 early region promoter, the dominant selectable marker, Econeo, an intervening sequence and a poly A site. Cells transformed with pSV2-neo (frequency 10^{-4}-10^{-5}) grow in media containing the inhibiting aminoglycoside G418. Unique restriction sites in pSV2-neo DNA permit the introduction of other, non-selectable genes into the same vector and thereby co-transformation of cells for these otherwise non-selectable phenotypes. In illustrating this approach I shall describe experiments, carried out by D. Canaani, which show that several clones transformed to G418 resistance with the recombinant pSV2-neogHIFN-β1 (gHIFN-β1 is the human β-1 interferon genomic DNA supplied by W. Fiers) produce an activity which protect human cells against VSV infection. Some clones produce interferon constitutively while others are induced nine-fold by exposure to poly(I)·poly(C). Clones that are inducible for the production of interferon also produce an interferon mRNA whose structure is indistinguishable from that of the induced resident counterpart gene.

Regulatory Protein-DNA Interactions

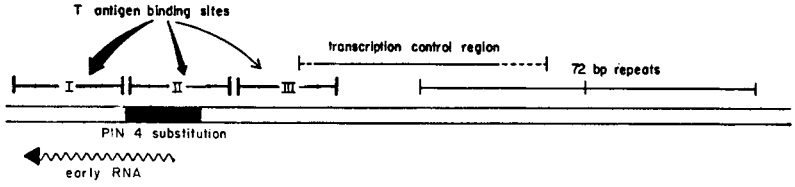
0749 λ REPRESSOR AND CRO - COMPONENTS OF AN EFFICIENT MOLECULAR SWITCH, Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

In a lysogen, most genes of phage λ are repressed; in response to a transient induction signal, they are efficiently switched on. The switch, which consists in part of a tripartite operator to which two regulatory proteins bind, depends not only upon DNA-protein interactions, but also upon effects transmitted from one DNA-bound protein to another. λ exemplifies a strategy that facilitates efficient switching between two physiological states in response to a transient signal. The repressor is both a positive and a negative regulator of gene transcription. Analysis of repressor structure by X-ray crystallography and the results of biochemical and genetic experiments suggest mechanisms for these activities.

0750 REGULATION OF SV40 TRANSCRIPTION IN VITRO. Robert Tjian, William S. Dynan, Donald C. Rio and Richard M. Myers. Biochemistry Dept., Univ. of Calif., Berkeley CA 94720

The wild type SV40 early promoter region (see figure) contains three binding sites for the SV40 early gene product, T antigen. The primary transcription control region, as defined by in vitro transcription of mutant templates, lies within an 85 bp region immediately upstream from the third T antigen binding site. The 5' ends of the early RNA lie within the middle binding site. The origin of viral DNA replication is also in this region, centered in the middle binding site. At late times in the lytic cycle, synthesis of SV40 early RNA is autoregulated. We have previously shown that this regulation operates in vitro. The presence of DNA-bound T antigen inhibits in vitro transcription by HeLa cell extracts, and this inhibition is dependent on the presence of T antigen binding site I, as well as on the spatial relationship of the binding sites and the promoter . We have recently constructed a new mutant, pIN 4, that has 31 bp of bacterial DNA substituted for 33 bp of SV40 DNA in T antigen binding site II. pIN 4 supports in vitro transcription at wild-type levels, with a novel RNA start point within the substituted region. Although a normal and functional site I is present, pIN 4 transcription is not repressed in vitro by levels of T antigen sufficient to repress wild-type transcription. This result, together with previous findings, suggests that site II is required for actual repression to occur, and that site I, which is the strongest of the three binding sites, is necessary but not sufficient. On the basis of binding studies, it appears that the primary function of site I may be to facilitate and coordinate binding to the other two sites.

In order to develop a more detailed picture of how the SV40 early promoter works, we have begun to develop a purified system for in vitro transcription. As with the adenovirus 2 major late promoter, faithful transcription of SV40 requires multiple components that do not copurify and that must be mixed in order to reconstitute selectivity. High levels of SV40-specific transcription have been obtained with a reconstituted system purified approximately ten-fold overall relative to the extract.



0751 PROGESTERONE RECEPTOR BINDING TO DNA, William T. Schrader, Department of Cell Biology, Baylor College of Medicine, Houston, Texas, 77030.

Progesterone receptor of chick oviduct has been purified extensively. One subunit of the native complex, protein A (M_r=79,000) contains a strong DNA-binding site which is exposed upon dissociation of the complex. The DNA-binding activity has been studied by velocity sedimentation, by DNA-cellulose chromatography, and by nitrocellulose filter adsorption of [^{32}P]-labeled plasmid DNA's containing various portions of the chicken ovalbumin gene, its flanking DNA, and DNA's derived from other genes not responsive to hormones. The protein has affinity for random DNA whether complexed to progesterone or not, as shown by DNA-cellulose elution studies. Nitrocellulose filter adsorption titration of linear [^{32}P] pBR322 DNA shows K_d=1 x 10^{-10}M at 50 mM KCl, 0°. Rate constants for association and dissociation are 3.4 x 10^6 M^{-1} sec^{-1} and 2.9 x 10^{-4} sec^{-1} respectively. These values are consistent with the equilibrium determinations. Titration shows no detectable DNAsequence specificity by the receptor, but rather a pronounced preference for single-stranded DNA, and for poly (dA•dT) over poly (dG•dC). Sedimentation velocity studies and S_1 nuclease digestion studies suggest that the protein may perform DNA helix destabilization. Protein A self aggregation and cooperativity of binding to double-stranded, blunt-end DNA are consistent with this interpretation. [^{32}P]DNA restriction fragments adsorbed by receptor to nitrocellulose were eluted from filters and assayed by gel electrophoresis for further studies of potential sequence specificity. Non-specific adsorption of end-labeled pBR322 (Mbo II digest) showed preferential retention of longest fragments, consistent with their increased target size. Retention studies using a restriction digest (EcoRI/Pst I) of recombinant plasmid pOV 1.7, a cloned 5'-flanking sequence of the ovalbumin gene, showed 10-fold preferential binding of receptor to the chicken DNA sequence. Competition studies were done using the homologous non-radioactive 1.7 Kb ovalbumin fragment and a 2.0 Kb 5'flanking sequence of chicken globin gene. The ovalbumin sequence was an effective competitor whereas the globin sequence was not. We conclude that progesterone receptor A protein has at least limited sequence preference for DNA of a gene regulated by the hormone; the biologic relevance of this interaction remains to be established.

0752 STRUCTURE AND EXPRESSION OF GROWTH HORMONE RELATED GENES

John Baxter, Norman Eberhardt, Mark Selby, Synthia Mellon-Nussbaum, Stephen Spindler, Michael Karin, Nancy Cooke, Nancy Lan, Arthur Guiterrez-Hartmann and Guy Cathala. Howard Hughes Medical Institute Laboratories, Univ. of Calif., San Francisco, CA 94143.

The genes for growth hormone (GH), prolactin (Prl) and placental lactogen (PL) constitute a family of related genes. cDNA and the chromosomal genes of this set have been cloned from several species. Major nucleic acid sequence homology between members of the set supports the idea that the genes were derived from a common evolutionary precursor gene. However, some genes have much more homology than expected, suggesting that genetic recombinaions occurred between members of the set. The human(h) GH and PL genes, located on chromosome 17 are closely linked. Marked nucleic acid sequence homology is found between hGH and hPL in the introns and 5'-flanking regions. This supports the recombination hypothesis and suggests that expression of hPL in placenta and hGH in pituitary is due either to more upstream structures or to only a few nucleotides. Cell-free transcription studies suggest that the small differences may be significant. The structures suggest that exons coding for specific functional domains were introduced into the genes and direct repeated structures flanking these exons could have been involved in the exon transfer. Repetitive DNA is located near and in introns of the genes, and gene transfer studies suggest that such structures may also participate in integration of the gene-containing fragment into the cellular DNA.

GH gene expression is affected by influences on: (i) gene activity; (ii) RNA processing; and (iii) mRNA stability studied in cultured rat GH cells that express rPrl and rGH. Methylation and thyroid and glucorticoid hormones affect GH gene transcription. The presence of a tRNA pseudogene in an intron of the rGH gene may result in several novel rGH gene transcripts that are not full-length. RNA processing variations can result in two mRNAs from the same gene. First, two different segments of an rGH intron can be removed yielding two GH molecules with different activities; gene transfer experiments suggest that tissue-specific factors regulate this processing. Second, two different rPrl mRNAs differ in one amino acid codon in the signal peptide region. Third, with rGH, two mRNAs differ in their polyadenylation. The larger mRNA appears in rapidly dividing cells and can be induced by thyroid hormone. The smaller mRNA is present in growth-arrested cells and appears to be translated more efficiently than the larger mRNA. This may reflect a mechanism to decrese rGH production during cell growth, possibly to conserve substrate. Finally rGH mRNA degradation appears to be regulated by glucocorticoids in GH cells and after transfer of the rGH gene to L cells; the structure dictating this appears to be inherent in the rGH gene.

Cell Free Transcription of Cloned Genes

0753 EUKARYOTIC tRNA GENE TRANSCRIPTION IS CONTROLLED BY SIGNALS WITHIN AND OUTSIDE THE MATURE CODING SEQUENCE,Dieter Söll,Donald DeFranco,Theodor Dingermann,Stephen Sharp, Department of Molecular Biophysics and Biochemistry,Yale University,New Haven,CT 06511

Drosophila tRNA genes can be faithfully transcribed *in vitro* using germinal vesicle extracts from *Xenopus laevis* (1) or in cytoplasmic extracts derived from *Drosophila* kc cells (2). An internal control region within the mature tRNA coding sequence is sufficient for transcription initiation by RNA polymerase III. An external control region located in the 5'-flanking sequence can repress transcription.

The ability of *in vitro* generated deletion clones to direct the *in vitro* synthesis of tRNA precursors was measured in the two transcription systems. Two control regions within the coding sequence were identified. The first was essential for transcription and was contained between nucleotides 8 to 25 of the mature tRNA sequence. Genes devoid of the second control region, which was contained between nucleotides 50 to 58 of the mature tRNA sequence, could be transcribed but with reduced efficiency. Thus, the promoter regions within a tRNA gene encode the tRNA sequences of the D-stem and D-loop, the invariant U at position 8, and the semi-invariant GTψC sequence (3).

Deletion analysis also has revealed an oligonucleotide sequence in the 5'-flanking region of a *Drosophila* $tRNA_2^{Lys}$ gene to be responsible for the poor transcriptional activity of this and of other tRNA genes. The oligonucleotide responsible for transcriptional repression is GGCAGTTTTTG and is located 13 nucleotides upstream from the mature-tRNA coding sequence. Since the sequence of the undecanucleotide is well conserved within the 5'-flanking region of all known *Drosophila* $tRNA_2^{Lys}$ genes we have investigated why the transcription of all these genes is not similarly repressed. Deletion or insertion of nucleotides between the mature tRNA coding region and this oligonucleotide resulted in tRNA genes with increased template activity. This observation suggests that the position of this oligonucleotide relative to some element downstream influences the extent of transcriptional repression (4).

Our studies have determined the *in vitro* transcriptional control regions for tRNA genes. The finding that tRNA gene transcription *in vitro* can be repressed by a particular 5'-flanking sequence suggests a possible mechanism for the control of tRNA gene expression *in vivo*.

(1) Schmidt,O.,Mao,J.,Silverman,S.,Hovemann,B.,Söll,D. (1978) Proc. Natl. Acad Sci. USA 75: 4819-4823

(2) Dingermann,T.,Sharp,S.,Appel,B.,DeFranco,D.,Mount,S.,Heiermann,R.,Pongs,O.,Söll,D. (1981) Nucl. Acids Res. 9:39+7-3918

(3) Sharp,S.,DeFranco,D.,Dingermann,T.,Farrell,P.,Söll,D. (1981) Proc. Natl. Acad. Sci. USA 78 in press

(4) DeFranco,D.,Sharp,S.,Söll,D. (1981) J. Biol. Chem. in press

0754 ORGANIZATION OF PROMOTERS FOR RNA POLYMERASE B (OR II) : SEQUENCE ELEMENTS CONTROLLING THE TRANSCRIPTION OF ADENOVIRUS GENES, C.Kédinger, P. Sassone-Corsi, J.L. Corden, R. Elkaim and P. Chambon, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Faculté de Médecine, Strasbourg, France.

In vitro genetic techniques were used to study the sequence requirements for initiation of specific transcription. Deletion mutants were constructed around the putative promoters of several adenovirus genes. Specific *in vitro* transcription by RNA polymerase B together with a HeLa cell extract was used as a test for promoter function.

In the case of the major late promoter, it has been shown that a conserved sequence element (the TATA-box or Goldberg-Hogness box) located about 30 base pairs upstream from the mRNA startpoint is essential for directing specific initiation of *in vitro* transcription within a narrow area. A 20 base pair sequence, containing the TATA-box, is both necessary and sufficient for specific *in vitro* transcription. Although the cap site sequences are not essential for obtaining specific discrete transcripts *in vitro*, it can be surmised that they play a role in the actual efficiency of *in vitro* transcription initiation.

Studies on the putative promoter region of the early transcription unit E3 revealed that sequences located upstream from the TATA-box are also required for efficient *in vitro* initiation.

Specific *in vitro* transcription was also obtained from the E2 transcription units. The corresponding promoters are used with similar efficiencies in the *in vitro* transcription system, although the one utilized late in the lytic cycle contains a TATA-box and the one used early in infection does not.

Taken together these results suggest that the sequence elements involved in transcription promotion are multiple and that different genes can exibit different types of control regions.

Cellular Biology of Animal Cells

0755 CONTROL OF MICROTUBULE ASSEMBLY AND DISTRIBUTION IN MAMMALIAN CELLS. B. R. Brinkley, S. L. Brenner, and W. J. Deery. Department of Cell Biology, Baylor College of Medicine, Houston, Texas. 77030

The cytoplasm of eukaryotic cells is organized around a dynamic interconnecting meshwork of microtubules, microfilaments, and intermediate filaments collectively called the cytoskeleton. The cytoskeleton functions to maintain cell shape, motility, secretion, and cell surface receptor activity. Recently, several aspects of cell metabolism including DNA sythesis, protein synthesis and gene transcription have been linked to the cytoskeleton. We have utilized tubulin antibodies to investigate several aspects of microtubule assembly and distribution in mammalian cells. Two stable arrays of microtubules exist in cells during the cell cycle; the cytoplasmic microtubule complex (CMTC) of interphase cells and the mitotic apparatus (MA) of dividing cells.[1,2] When mitosis is completed, the MA is disassembled, and its tubulin subunits reassembled into the CMTC. The initiation of tubulin assembly occurs not at random, but in association with discrete microtubule organizing centers (MTOCs). Utilizing detergent permeabilized cell models and exogenous brain tubulin we have reconsitituted the CMTC in a variety of mammalian cells in vitro.[2] Microtubules were polymerized in association with the cell's endogenous MTOC and the number, length, and distribution of reconstituted microtubules were strictly regulated in each cell. Utilizing two human antisera against MTOCs, we have shown that MTOCs undergo replication at precise intervals in the cell cycle and are equally distributed to daughter cells during mitosis.[3] Cell progeny are thereby assured of "programmed" determinants for generating specific microtubule arrays for maintaining cell shape and cytoplasmic organization. The drug taxol interferes with MTOC-associated microtubule assembly and induces "spontaneous" assembly sites throughout the cytoplasm. Other factors which influence tubulin initiation and elongation in our system include GTP, GDP, ATP, cAMP, Ca^{2+}. and calmodulin.

1. Brinkley, B. R., G. M. Fuller, and D. P. Highfield, 1975. Proc. Nat'l. Acad. Sci. 72:4981-4985.
2. Brinkley, B. R., S. M. Cox, D. A. Pepper, L. Wible, S. L. Brenner, and R. L. Pardue, 1981. J. Cell Biol. 90:554-562.
3. Brenner, S. L., D. Pepper, M. W. Berns, E. Tan, and B. R. Brinkley, 1981. J. Cell Biol. 91:95-102.

0756 CALMODULIN IS AN IMPORTANT REGULATORY MOLECULE IN CELL CYCLE PROGRESSION, Anthony R. Means, Vincent Guerriero, Lisette Lagacé, and James G. Chafouleas, Dept. Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Calmodulin (CaM) is the major intracellular Ca^{++} receptor in all non- and smooth muscle cells. This protein is a component of the mitotic apparatus as well as the microfilament bundles of interphase cells. One of the major CaM binding proteins, myosin light chain kinase, shares a similar distribution suggesting a role for this Ca^{++} receptor in regulating chromosomal movement and contractility. CaM is constitutively expressed in a variety of hormone-dependent cell types. However synthesis of this protein is tightly coupled to the G_1/S boundary of the cell cycle. Treatment of cells in exponential growth with the anticalmodulin drug W13 results in a single reversible cell cycle block in early S phase. The block is not due to an inhibition of CaM synthesis and indicates that the 2-fold elevation in the intracellular concentration of CaM that occurs in late G_1/early S may be required for DNA repair and/or synthesis. A highly significant correlation exists between the increase in CaM levels and the length of G_1. Calmodulin also seems to be important for the re-entry of plateau (G_o) cells into the cell cycle. CaM levels increase as cells enter plateau. Upon release of the cells into the growth cycle, CaM decreases 50% within the first hr and remains at this level for 4-5 hr. An increase in the intracellular concentration of this protein then occurs and it reaches the level normally observed at the G_1/S boundary. Addition of W13 at the time of treatment with fresh medium prevented entry into S phase but the changes in the intracellular concentration of CaM were not altered. When cells were treated with W13 at various times following release from plateau, a direct correlation was observed between the percentage of cells entering S phase and the time of drug addition. The labeling index increased as the interval between drug treatment and G_o release increased. However, although cells entered S phase, no progression through this period was observed when W13 was added as late as 5 hr following addition of fresh medium. These data strengthen the contention that CaM is important for the progression of cells through DNA synthesis. Experiments are in progress to determine whether any CaM-binding proteins are unique to the G_1/S boundary. In addition the full-length CaM cDNA probe is being utilized to determine whether CaM levels are regulated by transcriptional or post-transcriptional events.

Gene Expression and Gene Transfer

0757 AN EXPOSED CHROMATIN STRUCTURE AT THE 5' END OF EUKARYOTIC GENES, Carl Wu, The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Ma.02138.

Several heat-inducible genes in Drosophila exhibit a DNase I-hypersensitive chromatin structure at the 5' terminus. This exposed structure is present before (and during) induction, and is revealed by digesting gently chromatin in isolated nuclei with DNase I and mapping the sites of cleavage by a simple 'indirect end-labelling' technique that involves Southern blotting. The chromatin structure of the rat preproinsulin II gene also shows a similar DNase-sensitivity at the 5' terminus in a rat insulinoma (a beta-cell tumor) but not in kidney, liver, brain, or spleen. The region of exposure for preproinsulin II gene chromatin extends 250-300 bp upstream from the 5' end of the mRNA. The several Drosophila genes encoding the 70,000-dalton heat-shock protein show DNase I-hypersensitivity extending to about 200 bp upstream from the 5' terminus. Such exposed regions may reflect a preferential accessibility of the chromatin to regulatory molecules. Currently we are doing a fine structure analysis of the DNase I-hypersensitive structure using high resolution Southern blots and restriction endonuclease digestion of chromatin.

0758 **STUDIES OF CHROMOSOMAL GENE EXPRESSION USING SV40 RECOMBINANTS.** **Dean H. Hamer, Anthony D. Carter, Barbara K. Felber, Marie-France Jubier, Ajit Kumar, George N. Pavlakis, Carl Schmidt, MaryJane Walling, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205**

SV40, a small DNA tumor virus, can be used as a vector to transduce new genetic information into cultured animal cells. We have constructed SV40 recombinants carrying various chromosomal eukaryotic genes including those for normal and mutant mouse and human α-globins, two human growth hormones, hepatitis B surface antigen, mouse histone H4 and mouse metallothionein-I. These recombinant molecules have been introduced into various cell lines either by infection with virus particles or by direct DNA transfection. The results show that the signals for transcription initiation and termination, RNA splicing and polyadenylation, translation, and protein processing and secretion are appropriately recognized in this system. Moreover, in the case of the mouse metallothionein-I gene, it has been possible to demonstrate appropriate induction of the cloned gene by heavy metals. Thus it appears that SV40 will be useful for studying regulated as well as constitutive gene expression.

0759 HORMONAL INDUCTION OF HUMAN GROWTH HORMONE GENES IN MOUSE FIBROBLASTS, Diane M. Robins*, Peter H. Seeburg+ and Richard Axel*, *Institute of Cancer Research, Columbia University, New York, N.Y. 10032 and +Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, Calif. 94080.

The introduction of cloned genes into animal cells permits the analysis of the regulatory functions of DNA sequences in vivo. Growth hormone is a pituitary polypeptide whose synthesis is under complex hormonal control. We have asked whether information necessary for glucocorticoid regulation of expression resides in sequences about the human growth hormone (hGH) gene. Recombinant phage and plasmid clones containing inserts of chromosomal hGH DNA were introduced into tk^- mouse fibroblasts using the Herpes thymidine kinase gene as a selectable marker of transformation. Of several cotransformants examined, containing 1-10 copies of hGH DNA, half respond to glucocorticoid by increasing their level of hGH mRNA 3-5 fold. Other cotransformants appear constitutive for hGH RNA and a few make no detectable transcripts. Both constitutive and inducible RNAs are the same size as GH mRNA from human pituitary, and can be present at levels ranging from a few up to 1,000 transcripts per cell. Plasmids with only 0.5 kb of 5' sequences flanking the hGH structural gene remain responsive to glucocorticoid when transferred to L cells. This suggests that the information necessary for hormonal regulation of expression resides in the transferred DNA fragment. The construction of appropriate mutant GH genes may allow us to more precisely define these regulatory elements, and to understand why the exogenous GH genes introduced via gene transfer are transcriptionally active and inducible although their endogenous counterparts in the fibroblast remain inactive.

Gene Structure

0760 FUNCTIONAL REGIONS WITHIN THE SEMLIKI FOREST VIRUS GENOME, Hans Söderlund, Päivi Lehtovaara and Sirkka Keränen. Recombinant DNA Laboratory, University of Helsinki, SF-00290 Helsinki 29, Finland.

The genome of SFV is a single stranded 13 kb RNA molecule of positive polarity. 4 kb at the 3'end codes for 4 structural proteins, translated from a subgenomic messenger, while 8 kb at the 5'end are translated from the genome itself to yield 4 nonstructural proteins. We have compared the nucleotide sequence of the genome with that of cloned defective interfering RNAs which are generated during serial undiluted passages.

The DI RNAs consist of a population of molecules, heterogeneous both in size and sequence content. These RNAs are replicated and encapsidated only in the presence of standard virus. All DI RNAs analyzed have conserved a 106-86 bases long nucleotide stretch at the extreme 3'end. Also the 5'end of the genome is conserved, but it is rearranged and repetitive in the DI RNAs. The data indicate which regions and specific sequences are essential for replication and encapsidation of the SFV genome. Interestingly, the initiation site for the nonstructural polyprotein of the genome is conserved in the DI RNAs even if no specific translation product can be detected.

0761 CLONING OF THE HUMAN CYTOMEGALOVIRUS GENOME, Håkan Gadler and Per Stålhandske, Natl. Bact. Lab., Stockholm and Biomedical Centre, Uppsala, Sweden.

Introduction: Human cytomegalovirus (CMV) is a member of the herpesvirus group and has a propensity for establishing latent infections. In vitro latency can be established by infection of normally permissive cells in the presence of phosphonoformic acid (PFA). By nucleic acid hybridization technique we have shown an association between viral and cell DNA in these cells, a phenomenon which may be due to integration of the viral genome (Gadler & Wahren, submit. for publ.). In order to be able to closer characterize the state of the viral genome in these latently infected cells, to study the variability of the CMV genome or to study the regulation of the expression of viral sequences, pure restriction enzyme fragments of viral DNA need to be used. Since CMV has a long replication cycle in permissive cells and the amount of extracellular DNA is low, we have begun work on cloning CMV DNA fragments in a plasmid. Material and methods: Purified virion DNA, CMV strain Ad.169, was digested with HindIII. pBR322, also cleaved with HindIII was dephosphorylated and ligated with virus DNA fragments. E.coli HB10 cells were used for transformation and amp^r and tet^s clones were characterized by nick-translation of plasmid DNA and hybridization to blots of HindIII or XbaI cleaved CMV DNA. Results and discussion: 65 clones were shown to contain CMV DNA inserts and these were characterized. DNA fragments originating from both the U_L and U_S regions were cloned. As would be expected the large and the terminal fragments were not found in the clones. These will have to be cloned by other means such as cleavage with another enzyme or attachment of linkers. The clones we have obtained, will be used for studies of the CMV DNA structure, they will also be used for detection of CMV DNA sequences in clinical specimens from patients.

0762 IDENTIFICATION AND CHARACTERIZATION OF THE STRUCTURAL GENE OF GLUCAGON FROM ANGLERFISH
Ray Sanchez-Pescador, Robert Crawford, Peter Hobart and William Rutter. Dept. of Biochemistry and Biophysics, University of California, San Francisco, CA 94143.

Glucagon is a polypeptide hormone of 29 aminoacids synthesized in the A cells of the pancreatic islets. Recently we have identified an mRNA sequence from anglerfish endocrine pancreas encoding for pre-proglucagon. We have screened a genomic library of anglerfish DNA and isolated several clones hybridizing to the glucagon cDNA. We will report the nucleotide sequence of the cDNA as well as the genome DNA containing the coding sequence of the glucagon gene. The structure of the genes for the pancreatic hormones insulin, somatostatin and glucagon will be compared.

0763 DEVELOPMENT AND UTILIZATION OF A cDNA PROBE TO CALMODULIN. L. Lagace, T. Chandra, S.L.C. Woo and A.R. Means. Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Calmodulin (CaM) is a major calcium-binding protein comprised of four distinct Ca^{++}-binding domains. This multifunctional protein is ubiquitous and the amino acid sequence is highly conserved in both plant and animal kingdoms. We have constructed a full length CaM cDNA clone (pCM116) using total poly(A)$^+$ RNA from the electroplax (E. electricus). The complete DNA sequence of pCM116 reveals that this recombinant contains the entire coding region for CaM, a 3' nontranslated region of 413 nucleotides and 30 nucleotides of the 5' nontranslated region. Comparison between the amino acid sequence deduced from the nucleic acid sequence and those of bovine brain and R. reniformis CaM reveals only 3 and 5 amino acid substitutions, respectively. Comparison between the nucleic acid sequence of the Ca^{++}-binding domains shows extensive homology between all 4 domains. Highest conservation was observed between domains I and III, and domains II and IV. This suggests that the CaM gene may have evolved through two cycles of duplications of a primordial gene. The CaM cDNA was also used as a probe to study the species and tissue specificity of CaM mRNA sequences. Northern hybridization of poly A^+-RNAs obtained from eel, rat and baboon tissues with pCM116 showed multiple RNA bands. Experiments using polysomal and nuclear RNA are being performed in order to determine whether the multiple RNA species are precursor molecules.

0764 THE STRUCTURE OF THE RAT PROLACTIN GENE, Nancy E. Cooke, Arthur Gutierrez-Hartmann and John D. Baxter, UCSF, Department of Medicine, San Francisco, CA 94143.

The single rat prolactin in Prl gene is five times larger than the structurally and functionally related growth hormone (rGH) gene. These two genes evolved by duplication of a common precursor about 390 million years ago and segregated onto chromosome 6 and 17 respectively. We now report the general organization of the rPrl gene (5 exons and 4 introns constituting a total gene size of 10 kb), determine haploid gene number (1), and present extensive DNA sequence analysis including all exons, exon-intron junctions, 5' flanking region, and more than 50% of the intervening sequences. Sequence analysis and comparison to known repetitive and inserted eukaryotic elements suggests that the rPrl gene has evolved by incorporation of large segments of DNA which encode functional domains (exon 1 and exon 3) and facilitate rapid divergence of intron structure (intron D). Two non-crosshybridizing regions of dispersed repetitive DNA are located within and flanking the rPrl gene. The repetitive DNA structure within intron D is completely characterized and bears strong resemblance to the human Alu family. However, unlike the Alu repeats, the repetitive DNA structure within I_D has no RNA polymerase III transcriptional activity in vitro. This absence of transcriptional activity may correlate with I_D's lack of the polymerase III "promoter" sequences described by Fowlkes & Shenk. In support of this possibility we have recently found that another rat repetitive unit which does contain this putative RNA polymerase III promoter (e.g. I_B from rGH) is actively transcribed in vitro. While such data imply a structural & functional heterogeneity within these rat repetitive DNA elements the alternate function(s) of these elements is yet to be defined.

0765 CELL FREE TRANSCRIPTION OF THE RAT GROWTH HORMONE GENE, Arthur Gutierrez-Hartmann, Guy Cathala & John D. Baxter, UCSF Department of Medicine, San Francisco, CA 94143.

The growth hormone set of genes includes those for growth hormone (GH), chorionic somatomammotropin (CS) and prolactin (Prl). These genes are crucial for several aspects of growth and development, and are regulated by thyroid and glucocorticoid hormones. The cell free transcription as described by Manley and Gefter was used in these studies. Initial experiments revealed RNA polymerase II transcription in this system to be DNA template dependent, α-amanitin sensitive & specific for the gene cloned into pBR322. However, transcription of the rGH gene results in a complicated pattern of ~12 transcripts resistant to α-amanitin. Analysis of the rGH sequence reveals a repetitive DNA structure within intron B which seems to contain several putative RNA polymerase III promoter sequences. Hybridization studies using the in vitro labelled transcripts as probes reveal that most of these RNA's are being transcribed from repeated sequences. A comparison of the repetitive DNA within rGH-I_B to other repetitive DNA structures & RNA polymerase III transcription units shows that certain functional and structural sequences have been conserved to different degrees. Furthermore, the predicted 2^o structure of such repetitive DNA transcripts reveals a non-random tRNA-like structure. Finally, although the function of such polymerase III transcripts is not presently known, the possible interaction between polymerase II and III promoters will be discussed.

0766 THE VIMENTIN GENE IN CHICKEN: Zendra E. Zehner and Bruce Paterson, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

Both cDNA and genomic clones (18 independent isolates) coding for the intermediate filament protein, vimentin, have been isolated in chicken. Coding sequences were confirmed by mRNA selection, isoelectric focusing of selected mRNA translation products, and nucleotide sequencing. Heteroduplex analysis reveals that the various genomic clones contain overlapping DNA sequences. Southern analysis yields only those DNA fragments as predicted by the restriction map of the vimentin gene. Likewise, copy number experiments indicate that the vimentin gene is present in a single copy in the chick genome interspersed with repetitive DNA sequences present at many copies. By a Northern analysis mRNA synthesis increases during muscle development. Although the vimentin gene appears to exist as a single copy, two vimentin mRNA species (approximately 2500 and 2200 nucleotides) are detected in all the RNA's tested using either a short cDNA clone (3'-noncoding only) or a longer cDNA clone (1/2 full-length) or a gene fragment as probe. These two mRNA species are equal in abundance even in tissues where the expression of the vimentin gene is low (i.e., pre-fused muscle cultures) or in breast muscle isolated from a single chick. The existence of two vimentin mRNA species cannot be due to cross-hybridization to the other intermediate filament protein synthesized in muscle i.e., desmin. Experiements are in progress to determine how the two vimentin mRNA species arise from the transcription of the single vimentin gene.

0767 EVOLUTION AND DIVERSITY OF CRYSTALLIN GENE SEQUENCES. George Inana, Toshimichi Shinohara, Jacob V. Maizel, Jr. and Joram Piatigorsky, NEI and NICHD, National Institutes of Health, Bethesda, Maryland 20205

We have determined the nucleotide sequence of a cloned β-crystallin cDNA (pMβ Crl) derived from the mouse lens and compared its deduced amino acid sequence with the amino acid sequences of the principal β-crystallin polypeptide (βBp) and a γ-crystallin polypeptide (γII) of the bovine lens. When the amino acids were aligned, we calculated a 43% homology between the murine and bovine β-crystallin polypeptides and a 22% homology between the murine β-crystallin and bovine γ-crystallin polypeptides. As for bovine βBp and γII, there is a striking homology between the amino- and carboxy-halves of the murine β-crystallin polypeptide. The internally homologous amino acids have been preferentially conserved among the three crystallin chains. Interestingly, the nucleotides flanking the apparent initiation and termination codons for translation of the murine β-crystallin mRNA carry information for amino acids that fit within the internal homology of the polypeptide and that are homologous to βBp and γII of the bovine lens. The data show that the β- and γ-crystallins are related proteins and suggest that their genes diverged by duplications followed by the generation of new initiation and termination sites for translation as they underwent insertions, deletions and point mutations.

0768 ISOLATION AND CHARACTERIZATION OF HUMAN PREPROSOMATOSTATIN I GENE. Lu-Ping Shen, Raymond Pictet & William J. Rutter. Dept of Biochem & Biophys., Univ. of Calif., San Francisco, Ca 94143

A cDNA encoding human preprosomatostatin I was isolated from a library which was constructed from RNA of a pancreatic somatostatinoma using the anglerfish somatostatin I (1) as a probe under low hybridization stringency conditions. The nucleotide sequence of the clone predicts a complete sequence of 116 amino acids for the somatostatin precursor, which has a molecular weight of 12,727. The tetradecapeptide somatostatin is located at the COOH terminus of the protein. The sequence of somatostatin I and of 14 amino acids preceding it (termed as somatostatin-28) are identical to that of somatostatin-28 described in porcine (2), bovine and ovine species. However, the peptide moiety at the NH_2-terminus of somatostatin-28 shares very little homology with that of the 28 peptide of anglerfish somatostatin I.

The cloned cDNA has been used to isolate the human preprosomatostatin I gene. The gene contains one intervening sequence which is located within the propeptide region. Supported by NIH AM 21344.

1. Hobart, P., Crawford, R., Shen, L.P., Pictet, R., Rutter, W.J. (1980). Nature 288, 137.
2. Pradagrol, L., Jornvall, H., Mutt, V., Ribet, A. (1980). FEBS Letts. 109, 55.

0769 MOLECULAR GENETICS OF HETEROGENEOUS H2B HISTONE GENES EXPRESSED LATE IN SEA URCHIN EMBRYOGENESIS, Tim Mohun, Rob Maxson, Glen Gormezano and Larry Kedes, Howard Hughes Medical Institute and Department of Medicine, Stanford University, Palo Alto, CA.

H2B histones in the sea urchin are heterogeneous. While the presence of a single major form has been demonstrated during early embryo cleavage, by gastrula stage the synthesis of this protein has ceased and several different electrophoretic variants can be detected. This switch from early to late histone subtypes is the result of a corresponding switch in structural gene expression.

We have isolated three phage lambda recombinant clones containing late H2B sequences. Both DNA sequence analysis and S1 nuclease mapping of RNA transcripts demonstrate that these H2B genes differ strikingly from their early counterparts and from each other in protein coding sequence and the length of their untranslated 5' leaders. Putative signal sequences--including the TATA box, the transcription initiation site and a hairpin loop at the 3' terminus of the mRNA--show a remarkable degree of conservation in relation to the early H2B gene and one another. Interestingly, the 5' leader sequence of the early H2B gene appears to be a duplication of a late gene leader sequence.

Class-specific (late versus early) and gene-specific (individual versus all other H2B genes) regions of late H2B genes have been identified. Southern transfer analysis of genomic DNA demonstrates the presence of a singly copy of one of the late H2B genes and shows in addition that this locus is highly polymorphic. The relationship of these organizational features to the expression and evolution of this class of developmentally regulated genes will be discussed.

0770 THE ISOLATION AND GENOMIC ORGANIZATION OF HISTONE GENES ACTIVE LATE IN SEA URCHIN EMBRYOGENESIS, Robert Maxson, Timothy Mohun, Glen Gormezano, Geoffrey Childs and Laurence Kedes, Howard Hughes Medical Institute and Department of Medicine, Stanford University, Palo Alto, CA. 94305

The histone multigene family in the sea urchin includes two functional classes, designated "early" and "late", which are distinguished by the timing of their expression during embryogenesis. We have examined the genomic organization of late histone genes and have found them remarkably different from the early repeats. Using purified radiolabeled late histone mRNAs as hybridization probes, we have isolated four different λ recombinant clones containing late histone sequences. Positive mRNA selections demonstrate that the clones contain respectively H4 and H2B, H4, H2A and H2B, and H2B coding sequences. Cloned DNA - late histone mRNA hybrids evince high thermal stability and are thus very homologous. Mapping of the coding sequences on these clones has shown close linkage (1 kb or less) between H4 and H2B on one clone and between a different H2B and H2A on another. There are no other histone coding sequences located within 2 kb of these coding regions and within 8 kb of the H4 gene on the clone containing only that gene. Genomic Southern transfer experiments, in which coding region fragments of late H2A and H2B are used as probes, demonstrate that unlike early genes these sequences are not organized as several hundred tighly clustered, regular repeating units; rather, they are dispersed and irregularly organized and present in fewer than 20 copies. Thus two gene sets that code for protein isoforms are differentially regulatd and topologically distinct.

0771 STRUCTURE OF HUMAN TUBULIN GENES AND PSEUDOGENES, N.J. Cowan, C.D. Wilde, C.E.Crowther, and T.P. Cripe, Princeton University, Princeton, NJ 08544

Screening of two human genomic libraries with chicken α- and β-tubulin cDNA probes has resulted in the isolation of recombinant gene-containing clones that account for about two thirds of the tubulin-specific fragments observable in genomic Southern blot experiments. Structural analysis by restriction mapping, Southern blotting and electron microscopic heteroduplex formation has revealed 1) the existence of human tubulin genes with and without intervening sequences; 2) the presence of inverted repeat sequences both within the intervening sequences and in close proximity to the coding regions of both $\alpha$and β-tubulin genes; 3) the existence of a multigene family encoding α-tubulin that implies the occurrence of gene duplications involving extensive (>15kb) regions of DNA; 4)the lack of sequence conservation among intervening sequences in α-tubulin genes; 5) extensive conservation of coding sequences; and 6) the existence of restriction site polymorphism in the proximity of an α-tubulin gene. Two β-tubulin genes have been completely sequenced. One of these genes (1β) is truncated in that no recognizable sequence homology is observable 5' to aminoacid position 54 in the corresponding chicken cDNA sequence. The gene also contains a single in-phase stop codon and a short intervening sequence with correct consensus splice signals. A second gene (33β) lacks any intervening sequences, but contains four in-phase stop codons, a single base insertion and a three-base deletion. Further experiments are in progress to identify those tubulin genes that are functional, and to determine the factors that regulate their expression.

0772 Sea Urchin Late Stage H3 and H4 genes are Not Found in Tandem Repeat Units, Geoffrey Childs, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

I have analized members of the histone gene family of the sea urchin L. Pictus which are not contained in the two tandemly repeated "early" gene clusters. Three classes of clones containing histone gene sequences were obtained; 1) members of the tandem repeats which have been transposed to new genetic locations (orphons); 2) a third family of tandemly repeated "early" histone genes (pLpE) complimentary to histone mRNA from cleavage stage embryos, and; 3) members of the "late" stage gastrula specific histone genes complimentary to the unique gastrula specific histone mRNAs. The two "late" histone genes we have cloned are clustered but NOT tandemly repeated. The gene order of at least some of the "late" subtype genes is different from the conserved gene order found in all "early" sea urchin histone genes. The nucleotide sequence of a continuous 2200bp. segment of one clone encoding adjacent "late" H3 and H4 genes has been determined. Unlike any previously characterized sea urchin histone genes, these genes are transcribed off opposite strands of DNA. The late H3 and H4 proteins encoded by these genes are identical to their early counterparts, however, the choice of codons used has diverged significantly and the 5' leader region of each mRNA is shorter. Nucleotide sequence comparisons of early and late histone genes in the 5' and 3' ends of the genes reveals both homologous and diverged regions. How these sequence differences confer differential regulation of these genes remains to be determined. The fundamental difference in the organization of these differentialy regulated genes makes their evolutionary history and stability of great interest.

0773 MOLECULAR ORGANIZATION OF RAT GENES CODING FOR U1 RNA, Manuel Grez, Nikolaus Blin and Angel Alonso, German Cancer Research Center, Heidelberg, F.R.G.

Recently, small nuclear RNAs have received considerable attention. Several laboratories have proposed a role of small nuclear RNA in the splicing of intervening sequences from mRNA precursors and/or in the transport of mRNA from nucleus to cytoplasm.
From a rat liver DNA library we have isolated 7 recombinant λ-phages carrying EcoRI DNA fragments containing the gens for U1 snRNA. One of these U16 was characterized in detail. Restriction enzyme digestion and blot hybridization disclosed a complex pattern of gene clusters separated by large spacer regions. Nuclease S1 analysis revealed that most if not all cloned U1 genes deviate in their nucleotide sequence from the known U1 RNA sequence suggesting a mixed set of divergent but closely related U1 genes. From the U16 recombinant phage the EcoRI fragments were subcloned in pBR322 for further analysis. The DNA sequence of one of these (pU1-14) shows that the U1 related sequences are surrounded by a large number of sequence repeates. Furthermore, sequences related to the dispersed repetitive sequence family B1 are found 600 bp upstream from the U1 gene.

0774 LINKAGE OF A QUAIL FAST SKELETAL MUSCLE TROPONIN I GENE TO ANOTHER FAST TYPE GENE, Albert S. Baldwin, Jr., Ellen L. W. Kittler, and Charles P. Emerson, Jr., Dept. of Biology, University of Virginia, Charlottesville, Va. 22901

We are studying the structure and chromosomal linkage of the quail contractile protein genes. The interest in these genes is based on the observation that the contractile protein mRNAs accumulate coordinately when myoblasts fuse to form multinucleate myofibers. The analysis of the structure of these genes along with their flanking sequences may reveal how the genes are coordinately regulated. We report here the isolation of a troponin I gene from a genomic library of quail embryo DNA. DNA sequencing indicates that the gene codes for the fast skeletal muscle form. The gene is approximately 4 to 5 kb long and is interrupted by 3 or 4 intervening sequences. Hybridization of an overlapping lambda clone with ^{32}P-labeled cDNA synthesized from myofiber poly(A) RNA reveals that another gene is located approximately 7 kb downstream from the troponin I gene. Hybridization experiments using subcloned troponin I fragments reveal no detectable homology between the two genes. Northern analysis indicates that the linked gene is not expressed at detectable levels in myoblasts but its 2.5 kb poly(A) RNA is synthesized in cultures of differentiating myofibers as well as in adult breast (fast) muscle. DNA sequencing and Southern blot experiments with 5' end-labeled RNA indicate that the linked gene is transcribed in the same direction as the troponin I gene. Thus at least two coordinately regulated, nonhomologous genes are closely linked and transcribed in the same direction. This linkage may regulate their expression.

0775 STUDIES ON THE FUNCTIONAL ORGANIZATION OF A CLONED 135 KB NUCLEOTIDE SEQUENCE FROM CHO CELLS THAT INCLUDES THE DIHYDROFOLATE REDUCTASE GENE. Joyce L. Hamlin, Nicholas H. Heintz, Jeffrey D. Milbrandt, Jane C. Azizkhan, and Martin Montoya-Zavala, University of Virginia School of Medicine, Charlottesville, VA 22908.

In order to study basic questions of mammalian genome organization and replication, we have developed methotrexate-resistant Chinese hamster ovary cells with 1,000 copies of a 135 kb sequence that includes the gene for dihydrofolate reductase (Milbrandt et al., Proc. Natl. Acad. Sci. USA 78, 6043-6047, 1981). Because of the high copy number, it is possible to observe restriction fragments arising from this repeated sequence on ethidium bromide-stained gels of genomic digests. We have used this property to determine the location of a presumptive origin of DNA synthesis within the 20-25 restriction fragments by pulse-labelling synchronized cells at the beginning of the S period with ^{3}H-thymidine. Furthermore, since we have isolated the entire 135 kb repeating unit in overlapping recombinant cosmids, it is possible to determine which cosmid contains the origin of DNA synthesis by comparison of restriction maps of cosmids to labelled bands. By comparison to a cosmid containing the entire dihydrofolate reductase gene, we are able to determine the location of this gene relative to the nearest origin of DNA synthesis, and to ascertain when it is synthesized relative to its transcription interval in the cell cycle. These and other studies suggest to us a model for mammalian genome organization in which a single domain of DNA (possibly a loop) represents a DNA synthetic unit (replicon) as well as a transcriptional unit.

0776 MUTANTS OF BACTERIOPHAGE MU DEFECTIVE IN INVERTIBLE G SEGMENT GENES MAKE PARTICLES LACKING TAIL FIBERS, Martha M. Howe and Frank J. Grundy, Department of Bacteriology, University of Wisconsin, Madison, WI 53706.

The orientation of the 3kb invertible G segment of Mu DNA determines the host range properties of the phage. Phage with G in the (+) orientation grow on E. coli K12; those with G in the (-) orientation grow on E. coli C (van de Putte et al., Nature 286, 218, 1980). We have used serum blocking power (SBP) assays and electron microscopic observation of phage particles to study the function of gene products of the essential genes S and U encoded by the G(+) orientation of the G segment. SBP assays revealed that lysates made by induction of Mu prophages with mutations in genes S or U were greatly reduced in antiserum neutralization ability. Mutations in early genes A, B, and C, eliminating all late gene expression, were the only other mutations which caused a large reduction in antiserum neutralization ability. Since neutralizing antibodies are often directed against phage tail fibers, we examined the phage particles produced after induction of S and U mutant prophages whose G segments were fixed in the G(+) orientation by a gin mutation. Both S and U defective mutants produced phage particles lacking detectable tail fibers and containing primarily contracted tails. Addition of an amber suppressor or inversion of G to the (-) orientation restored their ability to produce normal phage particles with extended tails and up to 6 detectable tail fibers. These results demonstrate the involvement of G segment genes in synthesis and/or attachment of tail fibers to the phage particle.

0777 RECENT GENE DUPLICATIONS IN THE RAT KAPPA J-SEGMENT CLUSTER, H. W. Sheppard and G. A. Gutman, Department of Microbiology, University of California, Irvine, CA 92717

DNA segments containing the J kappa genes have been cloned from LOUVAIN rat liver, and their nucleotide sequence determined. Seven readily identifiable J kappa coding regions are evident in the rat, compared with five in the mouse; of these, six appear to be expressible (four in the mouse). The two additional J-segments in the rat appear to be the result of two sequential gene duplications occurring since the divergence of rats and mice, the most recent duplications within a multigene family described so far. The first involved a homologous but unequal crossover in a 14 base pair (bp) region spanning the 3' end of the coding region of J_1 and J_2. The second involved a crossover following unequal pairing of the two newly duplicated regions. We propose that the probability of a second duplication was greatly increased following the first as a result of the increased target for unequal pairing (370 bp of good homology vs. 27 bp in the original pairing). Intraspecific sequence comparisons of J genes shows a surprisingly high degree of conservation, both inside and outside the coding regions, when compared with other, nonimmunoglobulin, genes. This is similar to the pattern of divergence we reported previously for the kappa constant region gene. Within the coding region, the rate of accumulation of changes at silent positions is lower than that at replacement positions when comparing rat and mouse. Similarly, we find that the homology between flanking and intervening sequences of rat and mouse show the same overall degree of homology as the coding regions.

0778 SOMATIC MUTATION GENERATES DIVERSITY IN ANTIBODY GENES, Patricia J. Gearhart and Daniel F. Bogenhagen, Carnegie Institution of Washington, Baltimore, Maryland 21210 and SUNY at Stonybrook, New York 11794

Recent protein and gene sequences show that substantial diversity in immunoglobulin variable genes is generated by the hypermutation of germline genes. For example, in mice, one germline variable gene (M167) codes for seven unique light chains that bind the phosphorylcholine antigen. To determine the extent of nucleotide substitutions in immunoglobulin genes, we have cloned several rearranged M167 variable and constant genes from hybridoma cell lines. The nucleotide sequences of the genes and their flanking regions were compared to their germline counterparts. The results revealed several interesting facts about somatic mutation. (1) Mutation occurs in the region of the variable gene at a high frequency. One gene and its flanking regions have 13 base substitutions out of 806 bases sequenced (1.6%), and another gene and its flanking regions have 4 substitutions out of 734 bases (0.5%). (2) There is no specificity for any particular base to mutate, and no preference for transitions as opposed to transversions. (3) Mutation is localized to the variable gene region of DNA but not the constant gene region. No substitutions were found in the constant gene and its flanking sequences, which are 2.4 kilobases away from the variable gene. The identification of a mutational mechanism that produces extensive, localized base substitutions around the rearranged variable gene is unknown.

0779 STRUCTURE OF THE DIHYDROFOLATE REDUCTASE GENE, James W. Schilling, Stephen M. Beverley, Nancy A. Kohlmiller and Robert T. Schimke, Stanford University, Stanford, CA 94305

In excess of 200 kb of amplified DNA has been isolated from a clone library constructed from a methotrexate resistant mouse S-180 cell line which contains approximately 100 copies of the dihydrofolate reductase (DHFR) gene. The DHFR gene consists of six exons which span 31 kb of genomic DNA and five introns which range from 0.3 to 16.5 kb in length. The remaining 170 kb of DNA represents 5' and 3' flanking sequences of the DHFR gene. Heterogeneity exists among the amplified DHFR genes of the resistant S-180 cell line. One class of variant genes constitutes approximately 10% of the total amplified DHFR genes. It is identical to the normal DHFR gene to the 3' side of a site in the third intron and a novel DNA sequence occurs to the 5' side of this site. Chromosome walking studies have failed to locate the first two DHFR exons in 50 kb of DNA 5' to this site. This variant is not a cloning artifact and appears to be a partial gene which may have resulted from recombination among amplified genes or deletion of a large amount of DNA from a single amplified gene. Several classes of repetitious sequences have been found both in the introns and flanking sequences of the DHFR gene. Some flanking sequences of the DHFR gene which are fully amplified in the resistant S-180 cell line are not amplified in other independently amplified methotrexate resistant mouse cell lines. We are presently studying a resistant L5178Y cell line to determine if the sites where its amplified DNA sequence diverges from that found in resistant S-180 cells represent ends of the amplified unit.

0780 HUMAN ARGININOSUCCINATE SYNTHETASE(AS):DISPERSION OF THE AS-LIKE GENES AND REGULATION OF EXPRESSION, A.L. Beaudet, T.-S. Su, P. D'Eustachio, F.H. Ruddle, and W.E. O'Brien, Baylor College of Medicine, Houston, TX 77030, & Yale University, New Haven, CT 06510

There are between ten and twenty copies of AS-like genes in human DNA. Analysis of DNA from rodent X human somatic cell hybrids demonstrated that these genes occur on at least 8 different chromosomes including the human 9, 6, and X. Enzyme activity has been reported to map to chromosome 9, and the restriction site characteristics of the AS-like gene on that chromosome are compatible with the possibility that it is an expressed gene copy. Many of the other AS-like genes may represent dispersed pseudogenes. Location of an AS-like gene on the X chromosome is compatible with the hypothesis that transposable elements played a role in the gene dispersion. The evolutionary law that a gene which is X-linked in one mammal will be X-linked in all mammals would make chromosome rearrangement an unlikely explanation for such a finding. We suggest that dispersed pseudogenes need not follow this law. In an analysis of the expression of AS, we have examined the methylation pattern of DNA from wild type human cultured cells and from clones which overproduce AS. Analysis with MspI and HpaII demonstrated that the sites near the AS-like genes are heavily methylated. The pattern of genomic DNA fragments was complex, but there was an altered pattern of DNA methylation with a specific pattern of decreased methylation for each overproducing clone. The altered sites of methylation have not yet been related to specific AS-like gene copies because of the complexity of the genomic DNA pattern. Overproduction of AS may be accompanied by decreased methylation of DNA which might be related either to increased expression of an active gene or to activation of a gene not expressed in wild type cells.

0781 SEQUENCE AMPLIFICATION AND DIVERGENCE WITHIN THE α-FETOPROTEIN AND ALBUMIN GENES. Fern Alexander Eiferman and Shirley M. Tilghman, Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111.

The α-fetoprotein (AFP) and albumin genes arose by the duplication of a 15-exon ancestral gene. A detailed analysis of the organization and sequence of the murine AFP gene has revealed that the ancestral gene arose by successive amplification and divergence of discrete segments of DNA. The most recent of these events, thought to have occurred over 500 million years ago, involved two successive duplications of a primordial genetic domain consisting of 3 internal exons and 2 flanking exons. The 5 exons of the primordial domain itself, whose sequences can be deduced from comparisons of the six related domains within both the AFP and albumin genes, contain extensive internal nucleotide homologies which suggest that they arose from not more than two simple exons, one of which corresponds to the 5' flanking exon. The sequence of the other progenitor exon must have included a 27 base pair core, as four of the five primordial domain exons contain remnants of tandem repeats of this length. These observations have led to a model to account for the evolution of the primordial domain from two small progenitor exons by a series of duplications, followed by consolidation by deletion of intervening sequences. Thus, it is apparent that the functional protein domains in AFP and albumin were generated by the exploitation via tandem reduplication of a limited amount of genetic information.

0782 RAT SEMINAL VESICLE SECRETION (SVS) IV AND V GENES: CONSERVATION OF 3'-NON-CODING REGION AND COMPARISON OF THE 5'-FLANKING REGIONS, Stephen E. Harris, Per-Erik Mansson and Barbara Dickson, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

cDNA clones for SVS IV and SVS V have been identified and the DNA sequences have been determined for the coding and 3'-non-coding regions. Only two small conserved regions in the coding region of the genes were noted. One region (15 a.a.) has 30% homology and is very rich in glu-ser residues. Another region near the C-terminal has almost 60% homology and is very rich in basic amino acids. The rest of the coding region has diverged considerably. When the 3' end of the two sequences at the poly(A) addition site are aligned, a region of 65 nucleotides shows almost 80% homology which may indicate some biological function for this 3'-non-coding region. The rest of the 3'-non-coding region has completely diverged except a short region near the poly(A) addition site. Genomic cloned for SVS IV and V have been isolated and characterized. The SVS IV gene is 1900 bp and contains two introns. One form of the SVS IV gene has an insertion of about 200 bp in the second intron and possibly defines an allelic difference. A region 120 bp upstream from the cap site of the SVS IV gene has been identified by DNA sequencing to be able to form a cruciform structure and that region is being tested for S1 sensitivity in supercoiled plasmid and in nuclei from castrated and testosterone treated animals. Genomic clones for SVS V have been purified and are presently being mapped and sequenced.

0783 STRUCTURE AND EVOLUTION OF SMALL RNA GENES, Nikolaus Blin and Angel Alonso, German Cancer Research Center, Inst. of Exp. Pathology, 69 Heidelberg, F.R.G.

Among a number of discrete small RNA species from the cytoplasm and nucleus several had been shown to be complexed with proteins thus forming the so called ribonucleoproteins (snRNPs, scRNPs). Recently, it has been suggested that the RNPs might play a role in the metabolism of other RNA species (splicing of hnRNA, transport of mRNA from nucleus to cytoplasm).
We have focused our attention on the two most abundant snRNAs, UI and UII. After constructing cDNA clones (using rat UI and UII RNA as templates) the genomic organization of both genes was investigated. Since sequencing data revealed a substantial evolutionary conservation within particular snRNA species suggesting a conserved function we have screened a set of various genomes to examine evolutionary changes or constancy in snRNA gene organization. Rat UI cDNA shows homology to Dictyostelium genome but none to Physarum DNA. Closer related groups like mammals (rat-hamster-rat kangaroo) or amphibia (Xenopus-Triturus) display preserved sequences within each group only. Rat UI cDNA does not cross-hybridize to Xenopus DNA nor to Drosophila DNA. When studied by EcoRI digestion and blot hybridization DNA from Chinese hamster liver and DNA from a cell culture (CHV79) show a different organization of UI genes. However, rat liver DNA and DNA from Hepatoma cells exhibit an identical UI pattern of EcoRI bands.
In summary, the results indicate that, although they might help with RNP organization and RNA maturation, snUI and UII sequences are not, in general, highly conserved among distantly related species, as are e.g. the rRNA sequences. Sequences outside the RNA coding region vary even among closer related species.

0784 INTERNAL SEQUENCE ORGANIZATION OF BALBIANI RING GENES
Ulrich Wobus, Helmut Bäumlein and Fotis C. Kafatos
Zentralinstitut für Genetik, DDR-4325 Gatersleben, DDR; and
Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, USA

The Balbiani rings (BR) of the salivary gland polytene chromosomes in Chironomus contain transcribed genes which are of extraordinary size and internally repetitive. Much of each transcription unit consists of tandem repeats, usually 250 to 300 bp in length. We have investigated the substructure of these repeats by cloning and sequencing segments of BRb and BRc DNA from C. thummi (see also U. Wobus et al. 1980, Cell 22, 127-135). Sequence information is also available from BR1 of C. tentaus (A. Degalmann and C.P. Hollenberg, 1981, Chromosoma 83, 295-313). Sequence comparisons show that approximately half of each repeat unit is highly conserved, showing more than 70% identity in all three BR sequences. The remainder of the repeat unit is quite variable, and consists of short, tandem subrepeats. Two types of variable subrepeats have been encountered in the BRc sequence. This information may identify potential functional domains in the corresponding protein sequences. Furthermore, the DNA sequence organization of the BR genes suggests models for their evolution, in which tandem reduplication and deletions appear to play important roles.

0785 HISTONE GENE HETEROGENEITY IN THE SEA URCHIN, Eric S. Weinberg, Robert Donnelly, Lawrence N. Yager, and G. Christian Overton, University of Pennsylvania, Phila.PA19104

The genes coding for early embryonic histones in the sea urchin, Strongylocentrotus purpuratus are reiterated 300-400 fold per haploid genome and are transcribed until the blastula stage. Although the histone gene clusters within most single genomes are composed of similar, if not identical, repeats, gene clusters of different individual genomes have repeats of different lengths and spacer sequence. The spacer between the H4 and H2B genes is especially variable. We have examined 22 individual sea urchins and found 6 which had an H4-H2B spacer which was about 200 nucleotides longer than usual. We cloned 5 different repeats (3 with a smaller spacer and 2 with a larger spacer) and sequenced a large part of each H4-H2B spacer. The spacers consisted of two distinct regions: a highly conserved stretch of 400 nucleotides just 5' to the H2B gene and a region of variable size which differed considerably in each of the 5 repeats. The boundary between the two regions consisted of $(CTA)_n$ where n varied from 5 to 17. These differences were quite unexpected and indicate that large regions of spacer are not conserved. Heterogeneity of spacer sequence may therefore be more complex than predicted by models of unequal crossing over. Other regions of heterogeneity are under investigation including the H1-H4 spacer and the H3 gene which in one cluster occurs as a repeated pseudogene with a frameshift.

0786 TWO MULTIGENE FAMILIES ACTIVE IN COTTON EMBRYOGENESIS, Leon Dure III and Glenn A. Galau, University of Georgia, Athens, GA 30602

Genes for seed storage proteins produce abundant mRNAs at only one point in higher plant ontogeny - mid to late embryogenesis. In cotton they represent one of five different gene subsets whose expression is regulated independantly in embryogenesis. We have temporally mapped the concentration of the two principal storage protein mRNAs in embryogenic cottonseed by in vivo and in vitro protein synthesis, by northern blotting probed with cloned cDNA probes, and by the reassociation kinetics of the cloned probes to cDNA; all at different points in embryogenesis. Each of the two principal storage protein species is comprised of batteries of isoelectric isomers and constitute a multigene family containing 4-8 genes which may be clustered. The two multigene families are distantly related as shown by the immunochemical cross-reactivity of their protein products. However, extensive nucleotide homology cannot be demonstrated between the two families by northern or southern blotting. Other properties of these genes and their protein products are presented and compared with the properties of other gene sunsets active in embryogenesis.

0787 DROSOPHILA AND YEAST HAVE MULTIGENE FAMILIES RELATED TO THE MAJOR DROSOPHILA HEAT SHOCK INDUCIBLE GENE. Elizabeth A. Craig, Thomas D. Ingolia, Michael R. Slater and Lynn J. Mannseau, University of Wisconsin, Madison, WI. 53706.

Drosophila has a multigene family related to its major heat shock inducible gene (hsp 70 gene). Five distinct members of this family have been characterized. Two members, the hsp 68 and 70 genes are induced by heat shock. Three others, which are also dispersed on chromosome B are transcribed at normal growth temperatures and not induced by heat treatment. A partial DNA sequence of the protein codong regions has been determined for each gene. The predicted amino acid sequences are conserved about 75% among the five members of the multigene family.

Yeast also has a multigene family, containing between 7 and 10 members, related to the Drosophila hsp 70 gene. Preliminary results indicate that transcription of at least 1 member of the family is inducible by heat shock. The predicted amino acid sequence of two members, as deduced from partial DNA sequence, are 60-65% conserved relative to the Drosophila hsp 70.

The genes encoding the Drosophila small heat shock proteins (hsp 27, 26, 23 and 22) are partially homologous. The predicted amino acid sequences show extensive homology from amino acid 85 to 195, out of a total of about 200 amino acids. Comparison of the predicted amino acid sequences with known sequences of other proteins revealed a remarkable similarity between the region of homology and the corresponding region of mammalian alpha-crystallin. Yeast also contains DNA sequences homologous to the small heat shock genes of Drosophila

0788 Sequence Analysis of the T-DNA of the Ti plasmid of Agrobacterium tumefaciens, Wayne M. Barnes, Michael Bevan, Mary-Dell Chilton, Washington University, St. Louis, Mo 63110

Our 'kilo-sequencing' strategy has been brought to practical application. Target DNA 3 to 10 kb in size can be stably carried by our M13 vectors. Suitable targets are stretches of DNA which lack an enzyme recognition site from the following list: Pst, Xba, HindIII, BglII, EcoRI (the target region may be bordered on one side by one or two of these.) By a simple, short in vitro procedure, we create thousands of deletions which start adjacent to the commercially available dideoxy sequencing primer and extend various distances across the target DNA. Phage carrying the desired size of deletions, that is phage whose DNA as template will give rise to DNA sequence data in a desired location along the target DNA, are purified by electophoresis alive on agarose gels. Phage running in the same location on the agarose gel conveniently give rise to nucleotide sequence data from the same kilobase of target DNA.

The deletions, which carry a linker restriction site at their boundary, are available to serve other purposes besides sequencing.

We have applied this sequencing strategy to the T-DNA of the Ti plasmid T37 of Agrobacterium tumefaciens, starting from the right border, to which region the nopaline synthetase and plant-transforming genes map.

0789 VITELLOGENIN CODING SEQUENCES IN INSECTS ARE CONSERVED, Tharappel C. James, Christopher C. Maack, Ursula Bond, Shalom W. Applebaum and Jamshed R. Tata, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

Vitellogenesis among insects exhibits several unusual characteristics differing in principle or degree from avian or amphibian vitellogenesis. Besides the inherent evolutionary significance in determining whether these differences in vitellogenesis are reflected in the genes coding for vitellogenin in insects, quantitative information about the relative abundance and homology of this gene in insects may offer an insight into the hormonal regulation (juvenile hormone vs. ecdysone) of expression of this gene. Using a cloned cDNA probe to Locusta migratoria vitellogenin which was isolated in this laboratory, we have studied vitellogenin coding sequences of Carob moth, Mediterranean fruit fly, yellow meal worm, desert locust (Schistocerca gregaria) and migratory locust (Locusta migratoria). Southern blot analysis of high molecular weight DNA from these insects indicates that genomic DNA of all these different insect species has a $\sim$ 4.5 kb and a $\sim$ 3 kb EcoRl restriction fragment hybridising to vitellogenin cDNA from locust. Kinetics of homologous and heterologous hybridisation and thermal denaturation profiles of heteroduplexes formed between genomic DNA of yellow meal worm, Carob moth, Mediterranean fruit fly, or desert locust and Locusta migratoria vitellogenin cDNA probe reveal a remarkably high degree of evolutionary conservation of genes coding for insect egg-yolk proteins. The significance of these findings will be discussed with reference to their functional importance.

0790 NUCLEAR GENES ENCODING MAJOR LEAF PROTEINS, Anthony R. Cashmore, The Rockefeller University, New York, NY 10021

The major products of cytoplasmic protein synthesis in peas leaves correspond to polypeptide components of two chloroplast proteins. These polypeptides are the small subunit of ribulose-1,5-bisphosphate carboxylase and the constituent polypeptides of the light-harvesting chlorophyll a/b binding protein. Both the small subunit and the chlorophyll a/b polypeptides are encoded by nuclear genes and synthesized, on free cytoplasmic polyribosomes, as soluble precursors which function in the post-translational transport of the polypeptides from their site of synthesis into chloroplasts. Partial Eco Rl digests of pea DNA have been cloned in the lambda phage Charon 4 and cloned cDNA sequences[1], complementary to mRNAs encoding the above polypeptides, have been used as hybridization probes to isolate the corresponding nuclear DNA sequences. Two positively hybridizing phage have been isolated and characterized. One phage contains two Eco Rl inserts, one of which (8.0 kb) hybridizes to the cDNA encoding the major chlorophyll a/b binding polypeptide. A second phage contains three Eco Rl inserts (8.0, 4.7 and 3.6 kb), two of which (8.0 and 3.6 kb) hybridize to the cDNA encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. These two hybridizing fragments are not adjacent, but are separated by the 4.7 kb fragment, and they appear to represent tandemly repeated small subunit genes. Sequence studies are in progress and will be reported.

1. Broglie, R., Bellemare, G., Bartlett, S.G., Chua, N.-H., and Cashmore, A.R. (1981) Proc. Natl. Acad. Sci. USA 78, in press.

0791 CLONING OF A YEAST NUCLEAR GENE (CBP1) INVOLVED IN THE EXPRESSION OF MITOCHONDRIAL CYTOCHROME b, Carol L. Dieckmann, Columbia University, New York, N.Y. 10027

Nuclear mutants of S. cerevisiae deficient in mitochondrial respiration have been studied genetically and biochemically. A set of seven non-complementing mutations resulting in a deficiency of cytochrome b have been assigned to a single complementation group (group 60). Examination of mitochondrial RNA by the Northern blot hybridization technique has revealed that group 60 mutants produce a large number of novel apocytochrome b transcripts not detected in wild type yeast. The product of the gene affected in the mutants, therefore, appears to be required either for correct transcription or processing of apocytochrome b pre-messenger RNA. The gene has been designated CBP1. A representative mutant from complementation group 60 (N5-26) has been transformed for respiratory competency with a recombinant plasmid pool consisting of random fragments of wild type yeast nuclear DNA inserted in to a vector capable of autonomous replication in yeast and E. coli. The complementation of the N5-26 mutation has been shown for a number of independent transformants to be due to the presence of plasmid DNA. One of the plasmids (pG60/T10) was characterized and shown to have a nuclear DNA insert of 6.7 kbp. This plasmid complements the mutations of all group 60 mutants thus confirming that it contains the CBP1 gene. The gene has been subcloned and isolated in a smaller fragment of yeast nuclear DNA (2.2 kbp). DNA sequence analysis of the 2.2 kbp fragment indicates the presence of a long reading frame that can code for a basic protein with a molecular weight of 70,000. Studies are underway to purify the protein from yeast mitochondria.

0792 EVOLUTION AND STRUCTURE OF THE ACTIN GENES. Norman Davidson, Eric A. Fyrberg*, and Beverley J. Bond, California Institute of Technology, Pasadena, CA 91125 and *Johns Hopkins University, Baltimore, MD 21218.

Actin genes have been identified, cloned, and characterized in many organisms including yeast, Dictyostelium, soy beans, Drosophila, sea urchins and several vertebrates. In all cases, except yeast, they constitute an oligogene family. The following generalizations have emerged from these studies. 1) Protein coding regions of the genes and amino acid sequences of the proteins are highly conserved between organisms and between different members of the gene family for any one organism. 2) 5' and 3' untranslated regions and flanking regions are usually divergent. 3) Introns have been found in some of the genes for all organisms except Dictyostelium. Intron positions are not at all conserved between yeast, Drosophila, and the deuterostomes. There is some, but not total, conservation within the latter group. It appears that, for the actins, introns are mobile elements, whose positions have no obvious relation to functional domains of the protein. 4) For any one organism, different genes have different developmental patterns of expression. 5) The sequence data available at present do not support the view that the small number of amino acid differences between, for example, a skeletal muscle and a non-muscle, are functionally significant.

0793 CHARACTERIZATION OF THE GENOMIC CYTOCHROME P1-450 STRUCTURAL GENE IN THE MOUSE, Daniel W. Nebert, Michitoshi Nakamura, Mario Altieri, Toshihiko Ikeda, Robert H. Tukey and Masahiko Negishi, NICHHD, NIH, Bethesda, MD 20205

The Ah locus represents a group of genes controlling the induction of numerous drug-metabolizing enzyme activities by polycyclic aromatic compounds such as 3-methylcholanthrene (MC) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The major Ah regulatory gene product is a cytosolic receptor; presence of the Ah receptor appears to be essential for the induction process. Clone 46 was shown by both immunologic and genetic criteria [Negishi et al., Proc. Nat.Acad.Sci.U.S.A. 78:800-804 (1981); Tukey et al., J.Biol.Chem. 256:6969-6974 (1981)] to represent a cloned DNA sequence encoding MC-induced P1-450, a structural gene product of the Ah locus and the monooxygenase most closely associated with the metabolism of chemical carcinogens such as benzo[a]pyrene. P1-450 induction by MC or TCDD is under transcriptional and is strictly associated with an intranuclear large molecular weight precursor mRNA. With the aid of a murine plasmacytoma MOPC149 library and use of clone 46 as the probe, we have isolated and characterized clone λ3NT12. This 19-kbp fragment was found to contain mostly material 3'-ward of the genomic P1-450 gene. A fragment of λ3NT12 having 3.0 kbp was subcloned and used for isolating further hybridizable DNA segments from the murine MOPC149 library. Clone λAhP-1 (15.5 kbp) is believed to contain the 5' end of the genomic P1-450 structural gene, which extends over more than 12 kbp. P1-450 mRNA (~23 S) and another MC-inducible mRNA (~20 S) hybridize to distinct subclones of λAhP-1. These data should help elucidate the mechanism of cytochromes P-450 induction by hundreds of drugs, carcinogens, and other environmental pollutants.

0794 EVOLUTIONARY SILENCING OF THE DELTA GLOBIN GENE, Sandra L. Martin, Karen A. Vincent, and Allan C. Wilson, University of California, Berkeley, CA 94720

Delta globin is a minor, β-like polypeptide found in all higher primates except Old World monkeys (OWM). Since the gene is present in OWM, it offers a unique opportunity to study the molecular mechanisms and evolutionary consequences of gene silencing. We have cloned and sequenced the silent δ globin gene from two species, colobus and rhesus, as well as the functional β gene from colobus. In the rhesus δ, there is a single bp insertion in exon 2 which causes a termination codon to come in-phase; thus, the rhesus cannot make δ globin. Colobus does not share this insertion and has an open reading frame throughout the sequenced region. This suggests that some other event was initially responsible for the lack of δ globin in OWM. *In vitro* transcription of these genes reveals a striking difference between the two OWM δ genes and the colobus β. The β gene directs transcription of substantial quantities of specific run-off transcript, whereas, the δ genes are inefficiently and inaccurately transcribed. Examination of sequences 5' of the AUG reveals 15 substitutions and two single bp deletions by which the human δ differs from both OWM δs. None of these fall in regions previously thought to be important for regulation. δ transcripts are also not detectable *in vivo*. Taken together with the *in vitro* transcription results, it seems likely that the primary defect in OWM δ production is a drastically reduced transcriptional efficiency. A silent gene is expected to evolve much more rapidly than its functional counterpart. In the case of these OWM δ genes, an increase in point mutational evolution is observed, although it is not nearly as large as is expected. The apparent conservation of these supposedly silent genes may reflect unequal rates of sequence evolution along the hominoid and OWM lineages or an unknown function for this region.

0795 AN α-TUBULIN MUTANT IN DROSOPHILA WITH DEVELOPMENTAL ABNORMALITIES, Clarissa M. Cheney Dietmar Mischke, Mary Lou Pardue, and Allen Shearn, The Johns Hopkins University, Baltimore, Md., and Massachusetts Institute of Technology, Cambridge, Mass.

A Drosophila developmental mutant is reported here to be correlated with a defect in the α-tubulin gene located at position 85E on the third chromosome. Seven alleles of this mutant, named c43, have been isolated. As homozygotes, all alleles show alteration of larval viability and of imaginal disc size. In some alleles, some pairs of imaginal discs are reduced or absent. Four of the alleles are temperature sensitive. Recombinational mapping has assigned the mutant locus to 3-49.0, a position near four tubulin genes. Deletion mapping showed that the polytene location of the mutant locus lies between 85E1-3 and 85F1. Since an α-tubulin gene is also known to be located between these bands, the organization of the 85E α-tubulin locus was probed in these mutants. Restriction digests of DNA from larval brains and imaginal discs were hybridized with cloned 85E α-tubulin DNA. This analysis revealed an altered pattern of restriction fragments in one allele. This altered pattern indicates a deletion of the 85E α-tubulin coding region. To detect changes in the α-tubulin gene products, radiolabeled tubulins were isolated from mutant discs by vinblastine precipitation and separated by O'Farrell two-dimensional gel eletrophoresis. The allele with a deletion for the 85E α-tubulin also shows a reduced synthesis of α-tubulins. These data indicate that this α-tubulin gene is the gene identified mutationally as c43 and show that mutations at an α-tubulin locus can cause developmental abnormalities.

0796 ISOLATION OF MOUSE DNA SEGMENTS CONTAINING EPIDERMAL GROWTH FACTOR (EGF) INDUCIBLE SEQUENCES, Douglas N. Foster, Clague P. Hodgson, Harold L. Moses and Michael J. Getz, Dept. Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55905

The mechanisms by which peptide growth factors regulate specific gene expression are not known. In order to facilitate such studies, we have isolated mouse DNA segments containing sequences which are specifically inducible by epidermal growth factor (EGF). A partial EcoRI mouse genomic library was constructed in the λ vector Charon 4A and replica screened using cDNA transcribed from polyribosome-associated poly(A)$^+$ RNA isolated from quiescent cultures of AKR-2B mouse embryo cells and from such cells 6 hours following the addition of EGF to the culture medium. Twelve clones were selected, plaque purified, and utilized to estimate the levels of clone specific poly(A)$^+$ mRNA in the respective cell types. Nine of these clones were complementary to RNA sequences exhibiting a 3-7 fold increase in mass per cell following EGF stimulation. One clone (λ2B.11) exhibited an approximately 20 fold increase in complementary RNA, and two (λ2B.10 and .12) proved to contain sequences complementary to RNA species exhibiting a $\geq$80 fold increase in mass following EGF stimulation. It seems likely that λ2B.10 and .12, and possibly λ2B.11 carry sequences which are specifically induced by EGF while the remaining clones carry sequences which contribute to the general overall 4-5 fold increase in the total mass of poly(A)$^+$ mRNA which occurs in these cells following a stimulus to proliferate (Getz et al., Cell 7:255-265, 1976). Within a given cell type, poly(A)$^+$ mRNA sequences complementary to these 12 clones were found to exhibit a 40-80 fold range in relative abundance. We conclude that these clones will prove useful in studying the mechanisms underlying the regulation of specific genes by peptide growth factors.

0797 ANALYSIS OF THE CARDIAC MYOSIN HEAVY CHAIN GENES IN THE ADULT RAT: V. Mahdavi, M. Periasamy and B. Nadal-Ginard, Children's Hospital, Harvard Medical School, Boston, Ma

To study the expression and the structure of the myosin heavy chain (MHC) gene(s) in cardiac tissue and to correlate these genes with those expressed in different muscle and non-muscle tissues, we have constructed a cDNA library from rat cardiac poly(A)+ RNA. From this library, we have characterized four of them that contain the longer inserts (800-1500 bp). By hybridization and DNA sequence analysis we demonstrate that the MHC cDNA clones pCMHC21/26/34 and pCMHC5 represent the 3' most 1500 nucleotides of two different but closely related cardiac specific myosin heavy chain mRNAs. Northern blot hybridization show that a different set of cardiac MHC genes are expressed during the early and late stages of the development in the rat. The cardiac MHC cDNA clones cross-hybridize to adult skeletal and to a lesser extent to L6E9 myotube MHC mRNA but not to other tissues. The complete nucleotide sequence of these clones has been determined. By comparing these DNA sequences with those of embryonic and adult skeletal MHC cDNA clones we identified a highly conserved sequence of ∿200 nucleotides present in all the MHC clones so far analyzed. This conserved sequence is specific for striated muscle mRNAs and might be evolutionary relevant to the structure and function of the sarcomere. The cardiac MHC cDNA clones (pCMHC21/26) and pCMHC5 "encode" for two MHC proteins that share 97% homology. The major divergences are concentrated in two regions: between the 300-350 most terminal amino acids and at the very last five amino acids at the COO^- end. Furthermore, the 3' untranslated region of these two cardiac MHC mRNAs are only 55 and 100 bp long respectively and highly divergent. These gene-specific fragments are presently used to identify the corresponding MHC genes and to study their expression during development and different physiological pathological conditions.

0798 EXPRESSION AND AMPLIFICATION OF MOUSE DIHYDROFOLATE REDUCTASE MINI-GENES. Gray F. Crouse, Robert N. McEwan and Mark L. Pearson, Cancer Biology Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701.

The entire mouse dihydrofolate reductase (dhfr) gene and flanking sequences have been cloned and characterized (Crouse, G.F., Simonsen, C.C., McEwan, R.N. and Schimke, R.T., manuscript in preparation). The gene is approximately 31 kb in length, and the coding sequence is interrupted by 5 intervening sequences, ranging in length from 300 bp to 16,500 bp. In addition, there appears to be a splice in the 5' leader region of at least some of the dhfr mRNAs. dhfr minigenes have been constructed by a combination of in vitro and in vivo recombination techniques and consist of 1500 bp of 5' flanking genomic material joined, in one case, to coding sequences without any intervening sequences or, in another case, to coding sequences containing only the 300-bp intervening sequence. Both of these molecules will rescue dhfr$^-$ mutants of CHO cells, but initial transformants produce levels of DHFR substantially lower than that of the parent CHO cells. Transformants with molecules containing the 300-bp intervening sequence express higher levels of DHFR than cells with molecules which lack the intervening sequence. Transformants selected for increased levels of resistance to methotrexate appear to have amplified the mini-gene. The levels of DHFR in the methotrexate resistant lines are more than 20 times the amount found in the original parent CHO cell. Both mini-genes express high levels of RNA when cloned into plasmids containing SV40 ori and transformed into COS cells. An analysis of the RNA produced in the transformants, as well as construction of mini-genes with varying amounts of 3' and 5' flanking DNA, is now underway. Research sponsored by the National Cancer Institute under Contract No. NO1-CO-75380 with Litton Bionetics, Inc.

0799 DNA SEQUENCE AND IN VITRO TRANSCRIPTION OF PORTIONS OF THE EPSTEIN-BARR VIRUS GENOME, P. Deininger*, P. Farrell, A. Bankier and B. Barrell. MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, England. *Dept. of Biochemistry, L.S.U. Medical Center, New Orleans, La. 70112.

We have completed the DNA sequence of a 17 kilobase fragment of the B95-8 strain of Epstein-Barr virus, the Eco R1C fragment, using a modified shotgun sequencing technique. In addition, we have sequenced the region around the terminal repeat of the B95-8 strain of the virus and a portion of the same repeat from the Raji strain. These regions contain multiple large reading frames, several of which correspond well with RNA species reported for EBV transformed cells. To help characterize the transcriptional units from these regions, we have mapped the transcriptional promoters which function in a soluble in vitro system. A number of other interesting DNA features were also found, including several classes of repeated DNA sequences which had not been previously detected. The various repeats showed differing levels of divergence as well as totally different structures.

0800 SHARED SEQUENCE PRESENT IN mRNAS FOR SIX DROSOPHILA RIBOSOMAL PROTEINS. Maria Pellegrini and Steven Fabijanski, University of Southern California, Los Angeles, CA 90007

We have isolated a cloned genomic DNA segment from Drosophila melanogaster containing a ribosomal protein gene. The intact cloned DNA hybridizes to mRNAs which can be translated in vitro to give six different ribosomal proteins. Separated BamHI restriction fragments of this DNA yielded only one 2.6 kb fragment that hybridized to any translatable RNA. This RNA codes for only one of the ribosomal proteins as judged by in vitro translation. These data suggest the presence of a small sequence element within the cloned DNA that is shared by six ribosomal protein mRNAs, while the complete coding sequence for only one ribosomal protein is found within the cloned segment. However, the cloned DNA does contain four other sequences that are expressed into stable RNA species as judged by their ability to form R-loops with total poly(A) + embryo RNA. All five genes, including the ribosomal protein gene, are small, less than 1 kb. The level of R-loop formation differs among the five genes indicating that these genes give rise to differing amounts of cellular RNAs.

In summary, a Drosophila ribosomal protein gene is found to be clustered with several other small active genes and to contain a sequence element in common with several other ribosomal protein mRNAs.

0801 ORGANIZATION AND STRUCTURE OF THE α and β ACTIN GENES IN THE CHICKEN, Bruce M. Paterson, Juanita Eldridge and Zendra Zehner, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

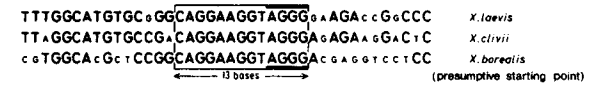
Using cDNA clones generated from RNA prepared from cultures of embryonic chick breast muscle, we have isolated the complete genomic clones for two different α genes and one β gene. This was confirmed by hybridization studies with 5' and 3' specific probes, S1 digestion patterns, and partial sequence data. Reconstruction experiments to determine gene copy number indicate there are 2 α genes and 1 β gene in the chick genome. Fragments containing the transcriptional start points for these genes have been isolated and are currently being used in promoter studies.

0802 SEQUENCE ORGANIZATION OF THE CHICKEN CALMODULIN GENE, J.P. Stein+, R.P. Munjaal+, L. Lagace, B.W. O'Malley and A.R. Means, Div. of Endocrinology, Univ. of Texas Health Science Center at Houston and Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Based on the amino acid sequence, it has been suggested that calmodulin is comprised of four calcium-binding domains and has arisen by duplications of a primordial gene. We have undertaken the cloning of the chicken calmodulin gene in order to determine if this theory is supported by the position of intervening sequences within the gene. Because the amino acid sequence of calmodulin has been highly conserved during evolution, we have used an eel cDNA probe to screen a chicken DNA library for the presence of calmodulin genomic fragments. A 10.6 Kb fragment from one of the 8 positive clones was subcloned into the Eco RI site of pBR322. Electronmicroscopic examination has failed to reveal looped heteroduplex structures between this genomic subclone and calmodulin mRNA from eel or chicken. A 1.3 Kb fragment of this genomic clone, which contains all the calmodulin sequences, was subcloned in pBR322 (pcm 1.3) and sequenced. The genomic fragment begins very near the 5' end of the calmodulin gene and extends into the 3'flanking region. 70% of the nucleotides prior to the translation termination signal are identical to the corresponding nucleotide of the eel calmodulin cDNA. When translated, 88% of the amino acid residues are identical in the two calmodulins. The DNA sequence confirms that no intervening sequences are present in the chicken calmodulin gene. Since internal sequence homologies argue that the present calmodulin gene has evolved by two gene duplication events, then either it did so without introns, or three introns were present in a primitive calmodulin gene and have been lost. A hypothesis for the evolution of the calmodulin gene is presented.

0803 SEQUENCE ORGANIZATION OF THE SPACER IN THE RIBOSOMAL GENES OF XENOPUS CLIVII AND XENOPUS BOREALIS, Marco Crippa, René Bach, Bernard Allet and Pierre-André Briand, University of Geneva, 1211 GENEVA 4, Switzerland

We have studied in X.clivii and X.borealis cloned EcoRI fragments containing the spacer located between the 28S and 18S ribosomal genes. We report for these two species the nucleotide sequences at both ends of the NTS region with special emphasis on the sequences around the transcription initiation site of the 40S rRNA precursor. In X.clivii the location of the 5' end of the precursor was mapped. In both species the sequences around the 40S origin are duplicated in the NTS. Nucleotide sequence comparison has revealed a stretch of 13 identical bases around the transcription initiation site of X.laevis and X.clivii. The same sequence is also present at the presumptive transcription initiation site of X.borealis rDNA.



Sequence homology around the 40S transcription initiation site. The heavy line corresponds to the beginning of the 40S sequence.

0804 cDNA CLONES FOR THE HEAVY CHAIN OF HLA-DR: IMMUNOPRECIPITATION OF POLYSOMES BY A MONOCLONAL ANTIBODY, Alan J. Korman, P. Knudsen, and J. L. Strominger, Harvard University, Cambridge, Ma. 02138

The mRNA coding for the heavy chain of HLA-DR antigens, the human analog of the murine Ia antigens (immune response genes) represents ~0.05% of total cell message. This mRNA has been purified 200-1000 fold by immunoprecipitation of polysomes with a monoclonal antibody directed against the isolated heavy chain of HLA-DR,and protein A-sepharose. Double stranded cDNA clones have been prepared directly from the immunopurified mRNA. In addition, cDNA prepared to the immunopurified mRNA has been used to probe cDNA libraries and Southerns. One clone, pDR·H-2 (520 bp + polyA), positively selects DR heavy chain message as assayed by cell free translation and immunoprecipitation. It contains the entire 3' untranslated region as well as coding information for 31 amino acids of the DR heavy chain, comprising the carboxy intracellular domain and part of the hydrophobic transmembrane region. Four independently hybridizing clones from a human genomic library have been isolated which share identical restriction fragments that hybridize with nick-translated pDR·H-2. Immunoprecipitation of polysomes followed by direct cloning permits the direct correlation of a single cDNA sequence with a specific protein (as defined by monoclonal antibody reactivity only). This correlation is difficult since a probe from a member of a multigene family will hybridize with all homologous sequences, making their identification laborious.

0805 ELECTRON MICROSCOPIC MAPPING OF COMPLEMENTARY SEQUENCES ON SINGLE STRANDS OF BACTERIOPHAGE LAMBDA DNA, Garret M. Ihler and Thomas D. Edlind, Texas A&M College of Medicine, College Station, Texas 77843.

Restriction fragments representing 68.3%λ to 100%λ map units were purified from the right end of bacteriophage lambda DNA containing transposon Tn903 and Tn5 insertions. These fragments were denatured to single strands and prepared for electron microscopy under partial denaturing conditions. Using the inverted repeat stems of Tn903 and Tn5 as markers, the locations of long range intrastrand base pairing were mapped. This analysis revealed two basic types of interactions. The first type consisted of largely homologous sequences arranged in opposite orientation on the DNA, illustrated by the observation of a large loop due to pairing between left (73%) and right (78%) operator and nut sequences. An additional example of this type of interaction was observed between the kil (68%) and ninR (86%) regions.

The second type of interaction reproducibly observed on lambda single-stranded DNA involved pairing within what are most likely individual transcription units, and hence reflects the structure of the corresponding mRNA. This pairing involved a set of sequences between genes cII (79%) and R (95%). As a result, rightward mRNA transcripts would be folded differently depending on the expression of specific promoters and terminators. Available lambda nucleotide sequences were analyzed for base pairing corresponding to the observed interactions. Possible roles for the observed long range base pairing in lambda development are discussed.

0806 *MOLECULAR CLONING OF POLIOVIRUS TYPE-1, Sylvie van der Werf, Helena Kopecka, Michel Dréano, and Marc Girard, Institut Pasteur, Paris, France.*

The entire poliovirus type-1 (PV1) genome was cloned into the Pst I site of pBR322 in the form of a series of overlapping inserts (van der Werf et al, 1981, P.N.A.S., 78, 5983-5987). Three groups of recombinant plasmids with inserts representing the 5', middle and 3' regions of the viral RNA, could be distinguished. Overlaps between the various inserts was ascertained by colony filter hybridization and/or by electron microscopic analysis of heteroduplex molecules.

Taking advantage of common restriction sites in the regions of overlap, a full length cDNA insert representative of the entire poliovirus genome could be reconstructed by in vitro splicing of the various plasmids.

Study of expression of the PV-1-cDNA in both prokaryotic and/or eukaryotic hosts is in progress.

0807 cDNA CLONES ENCODING THE HLA-DR HEAVY CHAIN, Claire T. Wake*, Eric O. Long*, Michel Strubin*, Nicole Gross+, Stefan Carrel+, Roberto Accolla+, and Bernard Mach*, *University of Geneva, 1205 Geneva, Switzerland, and +Ludwig Institute for Cancer Research, 1066 Epalinges, Switzerland.

cDNA clones coding for the heavy chain (M_r 34K) of HLA-DR antigen have been isolated. Monoclonal antibodies directed against HLA-DR immunoprecipitate DR antigens synthesized in frog oocytes injected with poly(A)$^+$ RNA from the human Raji cell line. Partial sequencing of the monoclonal-precipitated oocyte products gave amino acids in the positions expected from published protein sequences. Using this assay mRNA encoding the DR antigens was enriched by preparative electrophoresis and used to synthesize double-stranded cDNA which was inserted into the Pst 1 site of pBR322. Clones of transformed bacteria were screened by hybrid selection of mRNA. The hybrid-selected RNA from 100 clones was assayed by injection into oocytes and immunoprecipitation with a rabbit anti-DR serum. Two clones specifically hybridized an mRNA which directed the synthesis of a 34K protein. On two dimensional gels the 34K protein overlapped the HLA-DR heavy chain antigens labelled in vivo and immunoprecipitated with DR monoclonal antibodies. The insert from one of the HLA-DR heavy chain clones was used to screen a Raji cDNA library and overlapping clones spanning 1300 nucleotides were obtained.

0808 STRUCTURE AND ORGANIZATION OF A PROTAMINE CII GENE, J. Christopher States, Wayne Connor, Michael A. Wosnick, Lashitew Gedamu and Gordon H. Dixon, University of Calgary, Calgary, Alberta T2N 1N4 Canada

The protamines are a family of small,basic proteins that are the major structural proteins of the sperm chromatin in rainbow trout, and are coded for by a multi-gene family.The expression of this family is under developmental control as part of the terminal differentiation of the sperm. Using cloned protamine cDNA, we have isolated several genomic clones by screening an Eco RI library of trout DNA, carried in the vector Charon 4A. Analysis by restriction mapping and Southern hybridization has revealed the genes to be located in a small region in each of the clones. We have subcloned one of these regions in the plasmid pBR322 and have sequenced approximately 900 bp of this subclone, which contains the gene. This region contains a single sequence coding for the CII component of protamine, and, in addition, a 400 bp region 5' to the gene and a 250 bp region 3' to the gene. The protein coding portion contains no introns and is identical to that of pRTP59, a cDNA we have previously sequenced. However, the 3' untranslated region is not identical to that of pRTP59, indicating that this gene sequence does not correspond to this cDNA. From the data thus far, it appears that unlike the histone or globin genes, the protamine genes are not a clustered family. The other genomic clones are being analysed in a similar fashion inorder to compare several protamine genes and their surrounding sequences with a view to deducing common sequences involved in the coordinate control of expression of these non-clustered genes.

0809 ISOLATION AND NUCLEOTIDE SEQUENCE OF MOUSE HISTIDINE tRNA GENES, John D. Harding and Jang H. Han, Dept. of Biological Sciences, Columbia Univ., New York, N.Y. 10027. We have isolated several clones containing tRNA genes from a mouse genomic library. The mouse DNA insert of one of these (λMt1) was subcloned into phage M13mp7 and partially sequenced. Three M13 subclones contain identical histidine tRNA coding sequences. The unmodified base sequence of mouse his tRNA, as predicted from the DNA sequence, is (anticodon underlined): 5'-GCCGUGAUCGUAUAGGGGUUAGUACUCUGCGUUGUGGGCGCAGCAACCUCGGUUCGAAUCCGAGUCACGGCA(CCA)-3'. This putative mouse his tRNA is 82% homologous to Drosophila his tRNA; both contain an 8 residue D loop and a 4 residue variable loop. The genes do not code for the 3' CCA residues and do not contain introns. Each of the 3 M13 subclones contain a precisely conserved 3' flanking region comprising c.63 base pairs. Thereafter the sequences diverge, implying that clone λMt1 contains at least three distinct his tRNA genes. The conserved 3' region contains a typical polymerase III termination site of 6 T residues in the non coding stand. The 5' flanking sequence of one M13 subclone has been determined. It contains 2 sequence motifs present in several other eucaryotic tRNA and 5s genes; an AT rich sequence (perhaps analogous to the TATA and Pribnow boxes) and a symmetrical GT rich sequence, which are C.20 and 50bp, respectively, on the 5' side of the coding region. Comparison of the sequence of the 5' and 3' flanking sequences with the his tRNA coding region indicates that they are more homologous than is likely to have occurred at random, but less homologous than the individual copies of typical tandomly repeated genes. Therefore these his tRNA genes may have arisen as a tandomly repeated structure, some parts of which (the 5' and 3' flanking regions) have mutated extensively during evolution of the mouse.

0810 **HUMAN HLA cDNA CLONES. John Trowsdale, Janet Lee, John Jenkins and Walter F. Bodmer, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London, WC2A 3PX.**
cDNA clones were made from partially purified HLA mRNA from lymphoblastoid cell lines. One HLA-A, -B or -C clone (pHLA-A) was identified, as well as two clones (pDRH1 and 2) specific for the 34,000 M_r HLA-DR antigen glycoprotein chain. pHLA-A is about 600 nucleotides long and contains sequence information matching the C terminal 100 amino acids of the HLA-A2 antigen. pDRH1 and 2 are about 900 and 1300 nucleotides long, respectively. A nucleotide sequence in the longer clone translates into an amino acid sequence which is identical to the limited N-terminal amino acid sequence available for the HLA-DR antigen 34,000 M_r chain. In Northern blots, mRNA species hybridising to pDRH1 and 2 were expressed in B cell but not T cell or fibroblast cell cultures, whereas those for the pHLA-A clone were present in all of these cell types. Analysis of DNA from human, mouse and human-mouse somatic cell hybrid lines in Southern blots, using pDRH1 and 2 as probes, indicated that the HLA-DR heavy chain is encoded in chromosome 6. In these experiments the band patterns were simple, indicating that the sequences coding for HLA-DR heavy chain are unique in the genome. No restriction enzyme polymorphisms have yet been found. In contrast, the Southern blot patterns for the pHLA-A probe were very complex. We are isolating cosmid clones, using the cDNA clones as probes, to study the genetic structure of the HLA region.

0811 TWO DISTINCT CLASSES OF KERATIN GENES AND THEIR EVOLUTIONARY SIGNIFICANCE, Elaine Fuchs, Department of Biochemistry, The University of Chicago, Chicago, IL 60637
Mammalian keratins comprise a class of proteins (MW40-70Kd) that form 80Å cytoskeletal filaments in most epithelial cells. Although the proteins are related, different mRNAs exist for many of the keratins and it is likely that different sequences exist within the keratin family. We have constructed bacterial plasmids containing sequences specific for keratins of cultured human epidermal cells. Two separate classes were identified by positive hybrid selection: one class is specific for the mRNAs encoding the 56-58Kd keratins and the other class is specific for the 46-50Kd keratin mRNAs. Each of these classes is encoded by separate nonoverlapping multigene families (about 10 genes per family). All vertebrate DNAs from hagfish to human contain sequences similar to these two classes suggesting a coordinate evolution between the two subfamilies of keratin genes. This finding has important implications for keratin filament assembly.

Currently we are investigating the sequence-relatedness of the two keratin cDNA classes. We are sequencing one cDNA from the 56-58Kd keratin class that encompasses 80% of the mRNA sequence and one from the 46-50Kd keratin class that encompasses 90% of the mRNA sequence. In light of the lack of substantial amino acid sequence data for these proteins, the cDNA sequences will be of substantial importance in elucidating the role of these two classes of keratins in filament structure.

0812 NUCLEOTIDE SEQUENCES AROUND THE 5' AND 3' ENDS OF THE RIBOSOMAL RNA GENES IN TETRAHYMENA THERMOPHILA, Jan Engberg and Nanni Din, Department of Biochemistry B, Panum Institute, University of Copenhagen, Denmark.

The sites at which transcription of the ribosomal RNA gene in Tetrahymena thermophila is initiated and terminated were precisely localized and the surrounding nucleotides were sequenced. The DNA sequences were determined by the Maxam-Gilbert technique, and the RNA ends were localized on the gene by S1 nuclease protection of selected DNA fragments by isolated precursor or mature rRNAs, followed by a sizing of the protected fragments on sequencing gels. The purified pre-rRNA preparation contained molecules with two different and distinct 3'ends, one which is identical to the 3'end of pre-26S and mature 26S rRNA, while the other is 10-15 nucleotides longer. Immediately downstream from the 3'end of the longer pre-rRNA species is located a cluster of 6T's, and several other T clusters are found further downstream. Such stretches of t's have also been found at the end of the rRNA genes of Xenopus and yeast (but not Drosophila). No other obvious homologies have been found in the 3' spacer sequences among Tetrahymena, Xenopus, yeast and Drosophila. There is, however, sequence homology within the 3'ends of the coding regions among these organisms. The 5'end of pre-rRNA was found to map 650 nucleotides upstream from the 5'end of 17S rRNA. While the 5'sequence of Tetrahymena 17S rRNA shows a very high homology with all published 5' sequences of small mature rRNA species, no conserved sequences either in the transcribed or non-transcribed spacer have been found. We have identified several almost identical AT rich repeats preceeding the transcription initiation site. Such repeats, albeit with very different nucleotide composition, have also been found in the 5' spacer sequence of rRNA genes from other organisms.

0813 RAPID EVOLUTION OF GENES CODING FOR VARIANT SURFACE GLYCOPROTEINS (VSGs) IN TRYPANOSOMES. P.Borst, A.C.C.Frasch and J.Van den Burg, Section for Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam (The Netherlands)

The African trypanosome Trypanosoma brucei evades the immune response of its host by the sequential synthesis of different variants of a major surface protein, the VSG. Using cloned DNA complementary to each of four VSG mRNAs we have shown that each VSG is encoded in a separate gene, that VSG genes occur in families of related genes and that activation of some of these genes requires a duplication-transposition of the gene which leads to an alteration of the 3' end of the gene [1]. The evolution of VSG genes was studied by hybridization of the cDNA probes to blots of restriction digests of nuclear DNAs from related trypanosome strains. One of the genes (117) was found essentially unaltered in 11 out of 12 stocks. A second gene (118) was absent in five stocks. In the seven stocks that contained it, four forms of this 118 gene could be distinguished that differ by loss/gain of several restriction sites. A third gene (221) was only present in T. brucei 427 and in none of 11 other stocks. We conclude that a sub-set of the genes for the variant antigens evolves at a very high rate and we favour the hypothesis that this is due to local hypermutagenesis.

[1] P.Borst, A.C.C.Frasch, A.Bernards, L.H.T.Van der Ploeg, J.H.J.Hoeijmakers, A.C.Arnberg & G.A.M.Cross (1981) Cold Spring Harbor Symp.Quant.Biol. 45, 935-943.

0814 CLONING AND GENETIC ANALYSIS OF HYBRID DYSGENESIS-INDUCED ALLELES AT AN RNA POLYMERASE II LOCUS IN DROSOPHILA MELANOGASTER. Robert A. Voelker*, Lillie L. Searles†, Robert S. Jokerst†, Paul M. Bingham* and Arno L. Greenleaf†. *National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709 and †Duke University, Durham, North Carolina 27710.

The L5 locus, an allele of which ($RpII^{C4}$) confers α-amanitin resistance on RNA polymerase II of Drosophila melanogaster, maps at division 10C on the polytene X-chromosone and is lethal-mutable. Lethal alleles of this locus were generated by hybrid dysgenesis, a phenomenon in which a mobile DNA sequence known as the P-factor is mobilized and caused to insert at numerous places throughout the genome. One of these lethal alleles was shown by in situ hybridization to have a P-factor insertion in the 10C region (as well as other insertions at other sites). Probable causality between the P-factor insertion and the resulting lethality was indicated by the observation that in several independent reversions of the lethal the P-factor insertion at 10C was lost. A whole-genome charon-30 phage library of P-factor-containing mutant DNA was constructed and screened with P-factor. DNAs from the P-factor-containing phage were then hybridized in situ to wild type polytene chromosomes to identify those phages which contained unique sequences from the 10C region. One phage was identified which carried 11 kb of Drosophila DNA which included a 2 kb P-factor insert near one end. Subcloned fragments of this DNA are being used to screen other libraries to obtain genomic DNA sequences extending in both directions from the site of P-factor insertion. We plan to use these cloned sequences to identify the protein-coding and control regions of the L5 locus and to study P-factor insertion and excision.

0815 Molecular Cloning of the E. coli Genes for Translation Initiation Factor-3 and Two Aminoacyl-tRNA Synthetases. I. Schwartz, D. Elseviers*, P. Gallagher*, L. Scinto and B. Weinberg, Dept's. of Biochem. and Micro*, N.Y. Med. Coll., Valhalla, NY 10595.
In order to study the regulation of expression of E. coli genes for translational factors, the molecular cloning of the 37 minute region of the chromosome, which contains genes for IF-3, pheRS and thrRS, was undertaken. Chromosomal DNA from wild-type E. coli K-12 and plasmid pACYC 184 DNA were digested with EcoRI, mixed and recombined by DNA ligase treatment. The recombinant DNA was used to transform E. coli NP37, a mutant with a temperature sensitive lesion in the gene for the α-subunit of the pheRS which grows normally at 30°C but not at 42°C. Transformants (based on tetracycline resistance and chloramphenicol sensitivity) were screened for growth at 42°C. There were eleven such candidates from among 600 transformants. Redigestion of the isolated plasmid DNA with EcoRI indicated that there were two classes of transformants, one with an insert of 10.7kb and the other with an insert of 20.2kb. One candidate from each class was chosen for further study to determine whether there was an overproduction of the translation factors. The strain harboring the plasmid with the larger insert (pIF301) produced increased amounts of IF-3, pheRS and thrRS. In the case of IF-3 the overproduction is 8-10 fold. No detectable overproduction of any soluble protein was observed with the other transformed strain (pIF310). pIF301 DNA was used to program an in vitro transcription-translation system and directed the synthesis of IF-3, pheRS and thrRS. The restriction maps of pIF301 and pIF310 are different thus ruling out the possibility that pIF310 DNA sequences are contained within pIF301. To date a mechanism for the complementation of the temperature sensitive lesion in NP37 by pIF310 has not been established. The genes for IF-3, pheRS and thrRS are being subcloned from pIF301 DNA and these cloned DNAs will be employed to study the regulation of expression of these genes. (Supported by NIH GM24576, GM29265 and the Sinsheimer Foundation.)

0816 MAXAMIZE, A DNA SEQUENCING STRATEGY ADVISOR , Rene Bach, Peter Friedland, Doug Brutlag and Larry Kedes, Stanford University, Stanford, CA 94305
The MAXAMIZE advisory system determines from user-provided restriction maps an optimal strategy to do nucleotide sequencing by methods involving end-labeled fragments. The maps may be either simple linear restriction maps of fragments or complex circular maps including restriction sites of a vector. The whole system is interactive and is written in the GENETIC ENGLISH language provided by the GENESIS System, a molecular genetics knowledge representation and manipulation package. In addition, MAXAMIZE provides bookkeeping facilities for sequencing and offers advice on how to verify the newly obtained sequence data.

0817 HOMOLOGUES OF MITOCHONDRIAL GENES IN THE NUCLEAR GENOME OF S. PURPURATUS, Howard T. Jacobs, James W. Posakony, John T. Grula, Eric H. Davidson and Roy J. Britten, Division of Biology, California Institute of Technology, Pasadena, CA 91125.
Two non-cross-reacting clones, isolated from cDNA libraries prepared from cytoplasmic poly(A)+ RNA of S. purpuratus embryos, were found to hybridise strongly with mitochondrial DNA. Extensive genome blotting, using as tracers restriction fragments from one of these clones, showed hybridisation to bands that could not be accounted for by reaction with mtDNA. On screening a sea urchin genomic library with each of these cloned cDNAs, the identical set of overlapping clones was independently selected. These were judged to be of chromosomal origin by the following criteria : (1)various restriction fragments from them exhibited genome blot and RNA blot patterns characteristic of low and high frequency dispersed repeats, (2) heteroduplexing and cross-blotting showed only very short regions of homology with cloned sea urchin mtDNA, (3)fragments bordering the region of homology with mtDNA hybridised in genome blots to bands whose sizes were compatible with the restriction site map of the clones. Each of the two mitochondrial genes is represented only partially at the nuclear locus. Furthermore, elements homologous to one of the cDNA clones map on either side of the homologue of the other, in contrast to their organisation in mtDNA.

0818 DNA SEQUENCE ANALYSIS OF AN IMMUNOGLOBULIN GENE PROMOTER REGION, Kathryn Calame, James Berenson and Catherine Clarke, Dept. of Biol. Chem., UCLA, Los Angeles, CA 90024
Transcription of immunoglobulin genes appears to be regulated in at least two ways: 1) variable regions are transcribed only after V(D)J joining and 2) transcription in antigen-stimulated plasma cells is more efficient than in pre-B cells. In order to study both levels of transcriptional regulation, we have cloned a phosphorylcholine binding heavy chain variable gene segment, V1, from DNA representing three developmental stages--germline, an IgM producing hybridoma HPCM2 and several IgA producing myelomas. These clones are homologous by heteroduplex analyses for at least 5 kb 5' to the V1 coding region. We have mapped the transcriptional start site of the V1 gene on the HPCM2 DNA using three techniques: 1) RNA blots of poly (A+) nuclear RNA from HPCM2 cells, 2) S1 nuclease analyses of HPCM2 DNA hybridized to μ mRNA from HPCM2 cells and 3) primer extension and dideoxy sequencing of the 5' end of μ mRNA from HPCM2 cells. Our results show that transcription of V1 in HPCM2 cells initiates 60 bp 5' to the coding sequence for the leader peptide. We have determined the DNA sequence in this region and note an AT rich region at -31 bp. We are currently determining the DNA sequence of this region in both germline DNA and IgA myeloma DNA. These results will allow us to show whether sequence alterations in the region involved in transcription initiation are associated with VDJ joining or antigen stimulation and class switching. This question is particularly interesting with respect to antigen stimulation since it has recently been shown that somatic mutations in the V1 coding region are correlated with that process.

0819 NUCLEOTIDE SEQUENCE ANALYSIS OF THE RHO GENE IN E. COLI K-12, Jennifer L. Pinkham[1], Stanley Brown[2] and Terry Platt[1], [1]Yale University, New Haven, CT 06510, [2]Harvard University, Boston, MA 02115.

The rho gene has been cloned into pBR322 on a 3.6 kilobase HindIII-BglII restriction fragment. We have determined the nucleotide sequence of the rho as well as flanking regions. This analysis has revealed the following structural features. The structural gene is encoded within 1251 base pairs which allows for a polypeptide of 417 amino acid residues. The promoter for the rho gene is located in a region 240-210 base pairs upstream of the initiating AUG codon. The promoter sequence, shown below (a), has a nearly canonical -35 region spaced the optimal 17 base pairs from the Pribnow box. The Pribnow box sequence has an A at the invariant T position, but it otherwise strongly correlates to the TATAATG consensus sequence. A 226 nucleotide leader mRNA is detectable by S1 nuclease mapping. The rho terminator is located 15 nucleotides distal to a UAA stop codon and is a GC-rich region of dyad symmetry followed by a series of T's characteristic of prokaryotic terminator regions. The stem and loop is extremely stable (ΔG = -23.8 kcal/mole) and is shown below (b). The 3' end of the mRNA has been S1 mapped to within the series of uridine residues.

(a)

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      -35          -10
CTTAGTGTTGACTTAAACATACCTTATTAAGTTTGAA
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(b)

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TAAATTTGTCTTATGCCAAAAACGCCACGTGTTTACGTGGCGTTTTGCTTTTATATCT
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0820 STUDIES ON THE TERMINATION OF TRANSCRIPTION WITH MOLONEY SARCOMA VIRUS-DERIVED RECOMBINANT DNA CLONES. Dino Dina and Eric Schaeffer, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

The 600 base pair repeats (LTR) found at the ends of retroviral genomes have been implicated as sites of transcriptional control. DNA sequence data from the LTR has revealed a typical Hogness box found upstream from the 5' end of the viral RNA (capping site), and a polyadenylation signal found 59 BP downstream of the capping site. A model has been proposed to explain how readthrough of the left end LTR may be achieved, while proper termination at the right end LTR can still occur. This model is based on the potential secondary structure of nascent RNA transcripts, and involves the 70 BP region comprised between the Hogness box and the poly A site (Benz, et al. Nature 288: 665-69, 1980).

As a means of testing the above hypothesis, two recombinant clones carrying the LTR DNA have been constructed. These are P600-1 containing a single copy of the LTR, with flanking "c", and leader regions; and P600-2, a tandem LTR clone with leader and "c" regions between two 600 BP repeats.

Preliminary results have been obtained from transfections of NIH 3T3 cells with P600-1 and P600-2. Transient expression of viral sequences has been demonstrated by molecular hybridization of transcripts to specific DNA probes.

Work directed towards characterization of the transcripts synthesized in cells containing the LTR clones is in progress.

0821 ALTERATION OF A SINGLE NUCLEOTIDE AT THE SPLICE SITE ELIMINATES AN ADENOVIRUS EARLY mRNA, Craig Montell, Eric F. Fisher, Marvin H. Caruthers and Arnold J. Berk, University of California, Los Angeles, CA 90024

Early region IA of human adenoviruses encodes a function required for normal induction of early viral genes and virus induced cell transformation. The region is expressed at early times as two overlapping spliced mRNAs, 12S and 13S, encoding closely related proteins. To differentiate the functions of these proteins, a single U → G transversion was constructed which prevents splicing of the 12S mRNA. This transversion, in the second base of the 12S mRNA intron, does not alter the protein encoded by the 13S due tc degeneracy in the genetic code. Studies with this mutant demonstrated that only the 13S mRNA encodes the regulatory protein required for normal early gene expression.

0822 REGULATION OF DIHYDROFOLATE REDUCTASE mRNA PRODUCTION IN MOUSE FIBROBLASTS, Lee F. Johnson, Jin-Shyun Ruth Wu, Mark Collins and Sidney L. Hendrickson, The Ohio State University, Columbus, OH 43210

We have used a DHFR-overproducing 3T6 cell line (M50L3) to study the mechanism for regulating DHFR mRNA production. The rate of production of cytoplasmic DHFR mRNA relative to total mRNA is about 4 times lower in serum-limited or amino acid-starved cells than in exponentially growing cells. The rate increases to the growing rate several hours following growth stimulation. The technique of DNA-excess filter hybridization was used to determine the rate of labeling of DHFR hnRNA relative to total hnRNA and to monitor the efficiency of processing of DHFR hnRNA into cytoplasmic DHFR mRNA. In serum-limited, serum-stimulated, and exponentially growing cells, we found a close correlation between the rate of DHFR hnRNA synthesis and the rate of DHFR mRNA production. Furthermore, the efficiency of processing was the same in serum-limited and exponentially growing cells. In contrast, when comparing amino acid-starved with amino acid-replenished or exponentally growing cells, we found very little change in the relative rate of transcription, but a 3-4 fold difference in the efficiency of processing of DHFR hnRNA. These results suggest that DHFR mRNA production may be controlled by altering either the rate of transcription of the DHFR gene or the efficiency of processing of the mRNA precursor, depending on the physiological state of the cell.

0823 DNA POLYMORPHISM LINKED TO α1-ANTITRYPSIN DEFICIENCY AND PULMONARY EMPHYSEMA IN MAN, Savio L.C. Woo, T. Chandra, Robin Stackhouse, George Long*, Kotoku Kurachi* and Earl W. Davie*, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030, and *University of Washington, Seattle, WA 98105

Alpha-1-antitrypsin is the major plasma protease inhibitor. Deficiency of α1-antitrypsin is a genetic disorder that predisposes affected individuals to development of Chronic obstructive pulmonary emphysema. The deficiency is characterized by the presence of a mutated α1-antitrypsin gene which gives rise to a variant Z type protein instead of the normal M type protein, and is inherited by an autosomal recessive trait. The protein is a single polypeptide of 45,000 daltons in molecular weight and is synthesized in the liver. We have constructed an α1-antitrypsin cDNA clone from which the amino acid sequence of the entire protein has been deduced. The corresponding chromosomal gene was isolated from a human genomic DNA library. The human chromosomal α1-antitrypsin gene contains 3 intervening sequences and is approximately 5 Kb in length to code for a mature mRNA of about 1400 nucleotides. The entire gene resides within a 9.6 Kb Eco RI fragment, which will facilitate the cloning of the chromosomal gene from deficient individuals. Using the cloned human α1-antitrypsin gene as a specific hybridization probe, genomic DNA isolated from leukocytes of a deficient individual of the Z phenotype who is suffering from severe pulmonary emphysema was compared with that of a normal individual by restriction endonuclease mapping and southern hybridization. Polymorphic DNA patterns have been observed with certain restriction enzymes. If such polymorphic DNA patterns can be verified by analysis of a number of individuals of the 2 phenotypes, they can be used as a tool for prenatal diagnosis of recessive homozygotes.

Gene Expression

0824 PHENOTYPIC REVERSION AT THE HPRT LOCUS AS A CONSEQUENCE OF GENE AMPLIFICATION, David W. Melton, John Brennand, David H. Ledbetter, David S. Konecki, A. Craig Chinault, and C. Thomas Caskey, Baylor College of Medicine, Houston, TX 77030.

A class of HAT-resistant revertants generated by ultra-violet mutagenesis of a 6-thioguanine-resistant mouse neuroblastoma cell line (NB^-) contained a variant HPRT protein with extreme heat lability and reduced catalytic activity relative to wild-type mouse neuroblastoma (NB^+) enzyme. In vitro translation studies showed that the amount of immunologically detectable HPRT protein produced in one of these revertants (NBR4) was 25 to 50-fold elevated over the wild-type level. A cDNA bank was constructed in the plasmid vector pBR322 by reverse transcription of NBR4 mRNA and G-C "tailing." cDNA clones of the HPRT gene were identified by mRNA selection assay and used to investigate HPRT mRNA levels and gene copy number in NBR4 cells. RNA blot hybridization showed that increased HPRT mRNA levels, rather than increased translational efficiency, was the basis for HPRT protein overproduction and Southern analysis indicated that this was as a consequence of gene amplification. NBR4 cells contained a marker chromosome comprising three contiguous copies of the mouse X chromosome which was not present in NB^+ or NB^- cells. In situ hybridization studies are in progress to localize the amplified HPRT genes in the NBR4 karyotype. The sequence of the HPRT cDNA has been established and the clones, which cross-hybridize with both the human and Chinese hamster HPRT genes, are being used to investigate the nature of mutations at this locus and to probe the structure of mouse HPRT genomic sequences isolated from lambda genomic banks.

0825 UNPOLYMERIZED TUBULIN LEVELS MODULATE THE EXPRESSION OF α- AND β-TUBULIN. Don W. Cleveland,[#] Marc W. Kirschner,[+] and Margaret A. Lopata,[#] [#]The Johns Hopkins University, School of Medicine, Baltimore, MD 21205, and [+]University of California, School of Medicine, San Francisco, CA 94143.

Although numerous studies have suggested ways in which the assembly of cytoskeletal proteins can be regulated physiologically, less information has been generated on the regulation of the synthesis of these proteins. Ben Ze'ev et al. [Cell 17, 319 (1979)] recently suggested that the synthesis of tubulin in mouse 3T6 cells is affected by the state of assembly of microtubules. We have investigated the level at which this apparent modulation of tubulin synthesis takes place, using cloned cDNA probes for α- and β-tubulin mRNAs to measure the amounts of tubulin mRNAs combined with immunoprecipitation of tubulin to monitor the rate of protein synthesis. We have found that in many [but not all] cell types tubulin synthesis decreases very rapidly in response to microtubule inhibitors that increase the monomer pool. This decline in synthesis is associated with a decline in the amounts of both α- and β-tubulin mRNAs. Kinetic studies of tubulin protein synthesis and RNA levels suggest that tubulin synthesis can be shut off quickly in the cell as a consequence of 1) transcriptional modulation of the tubulin genes by the tubulin monomer pool and 2) the short half lives of the tubulin mRNAs, which appear to be only 1-2 hours. These data suggest that the cell exploits transcriptional modulation of tubulin genes combined with the instability of the tubulin mRNAs as a means to regulate precise levels of the monomer-tubulin pool.

0826 TRANSCRIPTIONAL AND TRANSLATIONAL REGULATION OF RIBOSOMAL PROTEIN SYNTHESIS IN YEAST, Jonathan R. Warner, Nancy J. Pearson, Chung Kim and Howard M. Fried, Albert Einstein College of Medicine, Bronx, N. Y. 10461.

The isolation of genes for a number of ribosomal proteins of Saccharomyces cerevisiae has enabled us to ask directly about the way in which the syntheses of these proteins is regulated. When yeast cells are shifted from 23° to 36° the synthesis of ribosomal proteins is inhibited temporarily. Pulse labeling of RNA at intervals after a temperature shift, followed by hybridization to cloned genes, revealed that transcription of ribosomal protein genes drops nearly 90% within ten minutes, but recovers to normal values by forty minutes. The transcription of non-ribosomal protein genes is relatively unaffected.

Transformation of yeast with an autonomously replicating plasmid containing a ribosomal protein gene leads to an imbalance of gene dosage for that gene. In cells carrying about five copies of a plasmid containing the ribosomal protein gene, tcm, we find that the transcription of tcm is approximately six times normal, the concentration of mRNA derived from tcm is 3 to 4 times normal, and the synthesis of the tcm gene product is only 1.5 times normal. Thus maintenance of the equimolar synthesis of yeast ribosomal proteins is due to effects at the level of translation and, perhaps secondarily, at the level of mRNA stability.

Therefore, unlike the apparent situation in E. coli, yeast appear to employ both transcription and translation for the regulation of the levels of ribosomal proteins. (Supported by NIH grant GM 25532.)

0827 MECHANISM OF RNA POLYMERASE II-SPECIFIC INITIATION OF TRANSCRIPTION IN VITRO: ATP REQUIREMENT AND UNCAPPED RUNOFF TRANSCRIPTS, D. Bunick, R. Zandomeni, S. Ackerman, and R. Weinmann, The Wistar Institute, Philadelphia, Pennsylvania 19104 The ATP analog 5'-adenylylimidodiphosphate (AMP-PNP) inhibits transcription of specific genes by the RNA polymerase II contained in crude whole HeLa cell extracts. This inhibition of transcription occurs not only with promoters that contain A as the first nucleotide of the transcript, such as the major late promoter of adenovirus 2, but also with promoters which initiate transcripts with G, such as adenovirus protein IX, or U, as in adenovirus early region IV. Other imidodiphosphate analogs can be used in vitro for elongation of transcription. In the whole cell extract, the analog AMP-PNP probably acts at the level of initiation of transcription. It can be used only for elongation by RNA polymerase II in isolated nuclei or in the whole cell extract, and by purified HeLa cell RNA polymerase II both for initiation and elongation. In contrast, RNA polymerase III in the crude system both initiates and elongates transcripts with AMP-PNP. Thus, the cleavage of the β-γ bond of ATP is an indispensable requirement for faithful and specific in vitro initiation by RNA polymerase II in the whole cell extract. In the case of the adenovirus early region IV, uncapped U-initiated transcripts were obtained in the presence of UMP-PNP, the imidodiphosphate analog of UTP. No capping can occur without cleavage of the β-γ bond of the terminal U triphosphate. The presence of the 5'-terminal imidotriphosphate at the same oligonucleotide as the cap for U-initiated precursors, established that transcription initiation and capping occur at the same site. Capping is not required for transcription by RNA polymerase II in the in vitro system, but is required for methylation of the 2' ribose of the initiating nucleotide.

0828 ANDROGENIC CONTROL OF PROSTATIC BINDING PROTEIN EXPRESSION
Malcolm G. Parker, Imperial Cancer Research Fund, P.O.Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

Prostatic binding protein contains three distinct polypeptides whose expression is stimulated by androgens. Testosterone stimulates mRNA production within one hour, but, in contrast to many steroid hormones, this appears to be due primarily to effects on turnover of nuclear RNA. Evidence to support this conclusion come from analyses of nuclear RNA by Northern blotting and measurements of RNA synthesis in isolated nuclei. The 'rate' of 10K mRNA synthesis was approximately 120 ppm in normal animals and 45 ppm in withdrawn animals; these differences are not sufficient to account for the difference in steady-state concentrations of 10K mRNA.

The organisation of the three genes, 9K, 10K and 11K, that code for prostatic binding protein have been analysed in genomic clones by Southern blotting and electron microscopy. The 9K and 11K genes are present in one copy per haploid genome whereas the 10K gene is present in 2 copies per haploid genome. The most striking feature, however, is that the 9K and 11K genes share appreciable DNA sequence homology and from cDNA sequence analysis we have found that the proteins contain a region in which 23/34 amino acids are identical. It is likely that this region is the result of duplication of an ancestral gene that has then undergone some divergence. In view of this it is likely that the 9K and 11K genes are linked but inspection of genomic clones suggest that this cannot be within 10 Kb.

0829 DEVELOPMENTAL EXPRESSION OF DROSOPHILA ACTIN GENES, S. Tobin, F. Sánchez, U. Rdest, B. McCarthy, University of California, Berkeley, CA 94720 and University of California, San Francisco, CA 94143.

Drosophila actin genes are members of a dispersed multigene family composed of six members, one at each of the cytological loci 5C, 42A, 57B, 79B, 87E, and 88F. All of these genes have been isolated by virtue of the conserved nucleotide sequences encoding actin amino acids. We are examining the transcriptional characteristics of individual actin genes during development with the use of gene-specific probes from the 3' transcribed but nontranslated regions of each gene. These probes hybridize only to the actin gene (and its transcript) from which each was derived. These gene-specific probes have been hybridized to RNAs isolated at different stages of development and dotted onto nitrocellulose. The 79B and 88F genes (both of which appear to encode muscle actin) exhibit parallel patterns of transcriptional activity until pupation, after which they differ greatly, perhaps due to differential response to hormonal signals. In contrast, the 5C gene (cytoplasmic) does not appear to be coordinated with either muscle gene. Four sequence blocs which are conserved between the 79B and 88F genes have been found at their 5' ends, though the order and placement of these blocs differ. These blocs may be remnants of the duplication events which are presumed to have led to multigene families and/or could be involved in the apparent coordinate regulation of these genes. We will also present data dealing with the putative eucaryotic control sequences for the 79B and 88F actin genes. Interestingly, the 88F gene appears to encode transcripts of two different lengths, a 1.85 kb transcript (embryos) and a 1.95 kb transcript (larvae). The 5' end of the 88F gene contains two sets of putative control sequences; the larval transcript maps to the more distal set.

0830 EXPRESSION OF NON-GLOBIN GENES DURING MOUSE RED BLOOD CELL DIFFERENTIATION,
P. R. Harrison, N. Affara, P. S. Goldfarb, Q.-S. Yang, K. Kasturi, J. O'Prey, E. Black, J. Fleming and R. Nichols, The Beatson Institute for Cancer Research, Glasgow, U.K.

Red blood cell development involves the coordination of a series of unlinked genes responsible for creating the red blood cell phenotype such as those coding for the globins, spectrin, glycophorin, carbonic anhydrase, etc. Our research concerns the structure and regulation of such non-globin genes using cloned recombinant DNA probes we have isolated which contain cDNAs transcribed from erythroblast non-globin mRNAs identified by hybridisation selection/translation experiments aided by immune precipitation with our monoclonal antibodies against red blood cell markers. Genomic DNA recombinants containing the genes coding for some of these mRNAs have also been isolated.

Our current experiments concern the stage of expression of these RNAs during the haemopoietic cell differentiation and whether they are regulated transcriptionally or post-transcriptionally.

0831 SYNTHESIS OF CAPPED ABORTIVE TRANSCRIPTS BY INTACT REOVIRIONS AND THE EFFECT OF Gp_4G AND SIMILAR CAP ANALOGS ON TRANSCRIPTION AND mRNA METHYLATION, Minoru Yamakawa, Yasuhiro Furuichi and Aaron J. Shatkin, Roche Institute of Molecular Biology, Nutley, NJ 07110

Purified reovirus converted to cores by chymotrypsin digestion synthesized capped mRNA and a molar excess of short oligonucleotides. These "initiator" oligonucleotides corresponded to the viral mRNA 5'-terminal sequence, G-C-U-A and included structures of the type ppG-C, GpppG-C, m^7GpppG-C and m^7GpppG^m-C. The same oligonucleotides were made by intact virions. However, virion nascent transcripts were not elongated, indicating that the RNA polymerase in virions is active but constrained at the initiation site. The structural changes resulting from conversion to cores released these constraints and allowed elongation, possibly by movement of genome template RNA within the particle. The results indicate that caps are present on reovirus dinucleotide transcripts, consistent with cap formation during initiation of transcription. To determine if preformed 5'-5' blocked structures can be utilized by reovirus core-associated enzymes, mRNA and oligonucleotide synthesis and methylation were assayed in the presence of Gp_4G (i.e. G^5pppp^5G) and related compounds. Methylation of mRNA was inhibited differentially (as compared to transcription) by Gp_4A and Gp_4G, Gp_5G and Gp_6G but not by Gp_2G, Gp_3G or Ap_4A. Gp_3G through Gp_6G were incorporated into initiator oligonucleotides as preformed 5'-termini. In addition, Gp_4G was utilized for mRNA synthesis, and the products were full-length transcripts. The resulting aberrant Gp_4G 5'-termini were unmethylated. The effects of altered 5'-structures of the type Gp_4G on mRNA function is being studied.

0832 Deletion of the 3' Half of the Yeast $tRNA_3^{Leu}$ Gene Does Not Abolish Promoter Function in Vitro. G. P. Tocchini-Valentini, G. Carrara, G. Di Segni, and A. Otsuka*
Laboratorio di Biologia Cellulare Consiglio Nazionale delle Richerche, Rome, Italy 00196

The promoter function of the 3' and 5' half tRNA sequences in the yeast $tRNA_3^{Leu}$ gene has been studied by in vitro transcription in Xenopus laevis germinal vesicle (GV) extracts. Truncation of the DNA template within the tRNA intervening sequence by Hpa 1 abolishes transcription. However, separation of the tRNA gene halves by insertion of a 300 bp DNA fragment at the Hpa 1 site does not affect the promoter efficiency. Further, the complete sequence of the 3' half of the tRNA is not necessary for promoter function, because removal of the 3' half of the gene by cleavage with Pvu II, within the DNA inserted at the Hpa 1 site, does not inhibit transcription.

*Present address: Department of Genetics, University of California, Berkeley, California 94720.

0833 REGULATION OF MHC GENE EXPRESSION DURING IN VITRO L_6E_9 CELL MYOGENESIS: B. Nadal-Ginard, Children's Hospital, Harvard Medical School, Boston, Ma., 02115

During differentiation of the L_6E_9 myogenic cell line myosin heavy chain mRNA (MHC) accumulates from 10 molecules per cell in log growing myoblasts up to 40,000 molecules/cell equivalent in myotubes. This accumulation is initiated in the absence of cell fusion or withdrawal from the cell cycle and it is reversible during the initail steps. Moreover, cell mutants temperature sensitive for commitment, are able to accumulate MHC mRNA in a reversible manner. In these mutant cells the induced stated is reversed upon cell division. In order to determine the molecular mechanism(s) responsible for the induction of MHC mRNA, we have determined its rate of cytoplasmic appearance, T1/2, and transcriptional rate during myogenesis using pulse labeling and chase techniques in combination with in vitro nucleic transcriptional assays and DNA-excess filter hybridization. The synthesis of the embryonic MHC gene which is expressed in L_6E_9 cells is induced at least 50 fold from undetectable levels in log growing myoblast to 3-6 mol/cell/min. in myotubes. This increased transcriptional activity, in combination with the stability of MHC mRNA (>50h), is sufficient to account for the final MHC mRNA accumulation in the cell. In L_6E_9 cells these changes in transcriptional rate are associated with changes in chromatin conformation, as detected by DNAse I digestion. The transcriptional activation of the MHC gnee is produced by changes in the cell cycle which can be dissociated from the commitment to terminal differentiation. These results suggest that biochemical differentiation and irreversible withdrawal from the cell cycle might be the result of two independent processes. The terminally differentiated phenotype can only be produced when the two programs develop in parallel.

0834 ALLELIC EXTINCTION OF THE M1 SUBUNIT OF RIBONUCLEOTIDE REDUCTASE ACCOMPANIES THE INCREASED EXPRESSION OF THE M2 SUBUNIT, David W. Martin, Jr., Ingrid W. Caras, Ruth A. Gjerset and Buddy Ullman, University of California, San Francisco, California 94143.

Ribonucleotide reductase consists of two non-identical subunits, M1 and M2. The M1 subunit mediates allosteric regulation by nucleotides and we have isolated and characterized mouse lymphoma mutants which are heterozygous for feedback resistant M1 subunits. When these mutants heterozygous for M1 alleles are selected for resistance to hydroxyurea, an inhibitor of M2 function, the resistant variants contain increased quantities of M2 activity. Concomittant with the increased M2 subunit there is a loss of the expression of one of the two M1 alleles, both of which were expressed in the hydroxyurea-sensitive parental heterozygote. From some heterozygotes hydroxyurea-resistant clones can be isolated that express either one or the other M1 allele; from other heterozygous mutants only clones that express the single wild-type allele are viable. Thus the hydroxyurea-resistant clones contain increased quantities of M2 but have become functionally hemizygous for the M1 locus.

Hydroxyurea-resistant clones selected from cells homozygous for the wild-type M1 allele also show increased M2 activity and are hemizygous for the wild-type allele. Some of the mutants heterozygous for feedback resistant M1 subunit "revert" spontaneously to wild-type ribonucleotide reductase. This event is not a true reversion but a spontaneous extinction of the expression of the mutant allele, resulting in a cell hemizygous for the wild-type allele and exhibiting a growth phenotype indistinguishable from the wild-type parent. These observations suggest that the M1 gene and the M2 gene are linked and that allelic extinction is a spontaneous event occurring in wild-type and mutant cultured T-lymphosarcoma cells.

0835 IDENTIFICATION OF DEFECTIVE IN VIVO GLOBIN mRNA SYNTHESIS, J.A. Shapiro, Y. Chiang, T. Ley, L. Killos, A.W. Nienhuis, and W.F. Anderson, NHLBI, NIH, Bethesda, MD 20205

M11X cells, formed by the fusion of mouse erythroleukemia cells (MEL) and human fibroblasts, contain the gene for human β-globin. Upon induction with HMBA, 10S or longer mRNA transcribed from the human β-globin gene is detected by Northern blot hybridization analysis. However, no human β-globin translation product is found. Although transcription occurs, improper initiation or processing of the transcript is therefore suggested. Similar data have been obtained from mouse L cells which have been microinjected with a cloned human β-globin gene. In addition, some patients with severe β-thalassemia have been shown to have a defective mRNA. The mechanisms of these defects are being investigated by S_1 mapping and sequence analysis of the mRNA. DNA probes created by cloning various segments of the human β-globin gene into the bacteriophage M13 served as templates for the synthesis of radiolabeled probes complementary to human β-globin gene transcripts. Differences between the control, M11X, and microinjected cells' human β-globin mRNA were mapped to the 5' region as determined by the length of the portion of the β-globin probes protected from S_1 nuclease by the mRNA. Synthetic DNA oligonucleotides complementary to portions of the human β-globin mRNA were used to sequence directly the mRNA using reverse transcriptase and dideoxyribonucleoside triphosphates. Studies are underway using a tridecanucleotide primer complementary to the middle of the human β-globin mRNA which allows sequencing through to the normal initiation of transcription, in order to determine where defective initiation (or processing) of the mRNA is occurring in the primary sequence.

0836 GENE DEFECTS IN THE β THALASSEMIAS, Arthur Bank, Sally E. Spence, Robert G. Pergolizzi, Maryann Donovan-Peluso, Katherine A. Kosche and Carl S. Dobkin, Columbia University, College of Physicians & Surgeons, New York, NY 10021.

The β thalassemias are a heterogeneous group of mutations in humans which affect β globin synthesis. In the β° thalassemias, no β globin is produced, while in β^+ thalassemia, some normal β globin is synthesized. In the β° thalassemias, defects have been defined which lead to either deletion of parts of the β globin structural gene, changes in the splice junction of the large intervening sequence(IVS 2)or termination codons at several positions within the β globin gene. In β^+ thalassemia, a single point mutation within the small intervening sequence(IVS 1)of the β globin gene has been reported. We describe here another β globin gene associated with the β^+ thalassemia phenotype in which there are five changes in IVS 2, in addition to a third base change at codon 2. The five changes in IVS 2 are 16, 74, 81, 666, and 705 nucleotides from the 5' end of IVS 2. The change at position 16 has been reported in both normal and thalassemia subjects using the enzyme Asu I, and is presumably a polymorphism. We believe that one or more of the other IVS 2 changes are responsible for the β^+ thalassemia phenotype for the following reasons: 1) large molecular weight β mRNA containing precursors have been found to accumulate in the bone marrow cells of this patient(Maquat et al, PNAS 77: 4287, 1980); 2) we have recently transcribed the clone containing this β globin gene in monkey kidney cells and find an abnormal splice product; and 3) computer analysis of the secondary structure of IVS 2 reveals that the changes at positions 74 and 81 together lead to a marked change in the free energy of the most stable secondary structure in IVS 2.

0837 NOVEL CONTROL OF IMMUNOGLOBULIN GENE EXPRESSION, Robert G. Hawley, Marc J. Shulman, Helios Murialdo, David Gibson and Nobumichi Hozumi, The Ontario Cancer Institute, Toronto,* Ontario M4X 1K9

In order to study immunoglobulin gene expression, a series of mutant hybridoma cell lines which produce immunoglobulin specific for the hapten trinitrophenyl (TNP) have been constructed. Two mutants which showed no or reduced synthesis of the TNP specific kappa chain have been found to have undergone rearrangement of sequences in the vicinity of the TNP specific kappa gene. Southern analysis has revealed that the nonproducer kappa gene has had the sequences coding for the hydrophobic leader and sequences upstream replaced by foreign DNA, whereas the low producer kappa gene has had an insertion of foreign DNA in the intervening sequence between the variable and constant genes. Preliminary results are consistent with what is known about transposable elements. DNA fragments containing the wild type and nonproducer genes have been cloned and compared by restriction enzyme mapping, heteroduplex analysis and DNA sequencing at the site of recombination. We have found that the recombination site is extremely A+T rich and the last 5 nucleotides at the 3' end of the foreign DNA are identical to the last 5 nucleotides found at the 3' end of the transposable element copia and the avian retrovirus spleen necrosis virus. Furthermore, there are 2 palindromes at the site of recombination and one of these has been shown to be involved in site specific recombination mediated by the tnpR gene of the transposon $\gamma\delta$. This palindrome is also present in the intervening sequence between the variable and constant genes. The cloning of the low producer gene is in progress and results concerning the characterization of these foreign DNA sequences will be presented.

0838 REGULATION OF THE MITOCHONDRIAL GENES FOR CYTOCHROME *B* AND SUBUNIT I OF CYTOCHROME OXIDASE IN YEAST, Henry R. Mahler*, Deborah K. Hanson*, Mary Rose Lamb*, C. David Zarley*; Philip S. Perlman† and Paul Q. Anziano†, *Department of Chemistry, Indiana University, Bloomington, IN 47405; †Department of Genetics, Ohio State University, Columbus, Ohio 43210

These two genes referred to as *cob* and *oxi3* are known to be split and their introns must be removed by splicing events. Some of these are dependent on polypeptides specified, at least in part, by the introns themselves. Such "maturases" participate in the splicing of I2 and I4 [Lazowska, Jacq and Slonimski, Cell 22 (1980) 333] of *cob*. The latter is of particular interest because it is the only such entity present in "short form" strains which contain only two introns and it participates directly as a positive regulator of the expression of *oxi3* [Dhawale, Hanson, Alexander, Perlman and Mahler, PNAS 78 (1981) 1778]. In this communication we demonstrate that this splicing protein is probably related to a polypeptide with an M_{app} = 27,000, which accumulates in some, but not all, mutants in I4, the base sequence of which will be described. In contrast to the I2 protein which appears to be initiated in exon sequences, the I4 protein is specified wholly within the intron itself, and we will discuss the mechanism by which this is brought about. (Research supported by NIH Research Grants GM 12228 and GM 21896.)

0839 ORGANIZATION AND EXPRESSION OF THE TUBULIN GENE FAMILY IN DROSOPHILA MELANOGASTER Jeanette E. Natzle, Brian J. McCarthy, University of California, San Francisco,CA94143

Tubulin is involved in a variety of cellular structures including the cytoskeletal network of microtubules, cilia and flagella, and the spindle apparatus. The existence of multiple gene sequences encoding tubulin in several eucaryotes raises the interesting possibility that different genes may encode tubulin proteins tailored for particular functions. Alternatively, multiple genes may exist to provide flexibility in gene regulation. We are addressing these questions in our study of the tubulin multigene family in Drosophila melanogaster. We have characterized sequences representing four different α-tubulin genes and four different β-tubulin genes. There is evidence of sequence divergence among the genes of each family based on heterogeneity in the placement of restriction endonuclease recognition sites and decreased thermal stability of inter-gene nucleic acid hybrids. The tubulin genes are dispersed throughout the genome as determined by in situ hybridization to polytene chromosomes. Genes for α-tubulin are located on chromosome 3R at 84BC, 84D, and 85E and on chromosome 3L at 67C; genes for β-tubulin are located on chromosome 3R at 85D and 97F and on chromosome 2R at 56D and 60BC. Semiquantitative analysis of RNA levels during Drosophila development using an RNA dot blotting technique shows that genes of each family are independently expressed in non-identical developmental patterns. Since more than one gene in each family is expressed during a given developmental period it is likely that the tubulin genes are regulated in either a tissue-specific or function-specific manner rather than according to developmental stage.

0840 INHIBITION OF TRANSCRIPTION BY POLIOVIRUS, Nigel Crawford, Andrew Fire, Mark Samuels, Phillip A. Sharp and David Baltimore, MIT, Cambridge, MA 02139

Poliovirus rapidly inhibits host cell RNA synthesis following infection of HeLa cells. Analysis of cell-free transcription extracts (by the procedure of Manley et al., PNAS 77, 3855-3859, 1980) prepared from mock- and poliovirus-infected cells indicates that transcription by RNA polymerase II is blocked by three hours after infection. Transcription by RNA polymerase III is also inhibited but at a slower rate, being only slightly reduced at three hours after infection. Polymerase II transcription can be restored in infected cell extracts by supplementing the extracts with a crude preparation of transcription factors (S100, see Weil et al., Cell 18, 469-484, 1979) but not by supplementing with purified polymerase II. When the S100 is fractionated on a phosphocellulose column, the restoring activity eluted between 0.35M and 1.0M KCl. These results indicate that at least one factor required for specific transcription by polymerase II is deficient in infected cell extracts. Thus, the loss of activity of one or more transcription factors may explain how poliovirus inhibits host cell RNA synthesis.

0841 THYROID HORMONES INFLUENCE THE INTRACELLULAR CONCENTRATION OF A SET OF RAT LIVER mRNA SPECIES, Howard C. Towle, Chen Liaw and Prema Narayan, Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455

The initiation of thyroid hormone action is postulated to occur through binding to a specific nuclear receptor and subsequent alterations in nuclear production of RNA. To examine the range of specific mRNA species influenced by thyroid hormone, total poly(A)-containing RNA was isolated from livers of hypothyroid, euthyroid and hyperthyroid rats. These populations were analyzed by two dimensional gel electrophoresis of translational products synthesized in the mRNA-dependent rabbit reticulocyte system. The levels of ~8% of the 250 mRNA species detected were altered with both positive and negative responses with respect to increasing plasma triiodothyronine (T_3) being observed. The earliest detectable response occurred at 1.5 hours for a mRNA species encoding a polypeptide with a Mr of 17500 and a pI of 5.0; however, other mRNA species responded with varying lag times of up to 24 hours. To study further the T_3-responsive mRNA species, specific complementary DNA probes were developed. Total hepatic mRNA of hyperthyroid rats was used to construct a cDNA library in pBR322 DNA using the dG:dC tailing method. Transformants were screened initially by differential hybridization using ^{32}P-cDNA probes to hypothyroid or hyperthyroid mRNA. Positive colonies were further examined by hybrid-selected mRNA translation and two dimensional gel electrophoresis. Eight cDNA clones were obtained in this fashion: 5 of which were higher in the hyperthyroid state than in hypothyroid and 3 which were the converse. Further studies using these hybridization probes should enable us to delineate whether T_3 is acting transcriptionally or post-transcriptionally to influence specific mRNA levels.

0842 DEVELOPMENT OF A FRACTIONATED IN VITRO TRANSCRIPTION SYSTEM FROM MOUSE ERYTHROLEUKEMIA CELLS, H. Eser Tolunay, Linda Yang, Wayne M. Kemper, Brian Safer and W. French Anderson, Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20205

Mouse erythroleukemia (MEL) cells provide a useful model system to study the regulation of globin gene expression. We have developed a cell-free transcription system for RNA polymerase II from uninduced MEL cells using a modified procedure of Manley et al. (PNAS 77, 3855, 1980) and have compared it with HeLa and KB cell extracts. Two templates were evaluated: an Eco RI-Bam HI subclone of the mouse β-globin major gene and the Sma I-F fragment of Ad2 DNA. If initiation is accurate, these templates are expected to produce 468 and 536 nucleotide long "run-off" RNAs, respectively. The MEL system directs accurate transcription of both templates. However, transcription of the mouse β-globin template appears to be about 5-fold less efficient than that of Ad2 DNA. Similar results were obtained using extracts from cells which ordinarily do not transcribe globin genes, namely, HeLa and KB cells. Using a modified procedure of Matsui et al. (JBC, 255, 11992, 1980), we have isolated three active fractions from both MEL and HeLa cell extracts which are required for accurate transcription. Comparison of equivalent fractions from MEL and HeLa cell extracts indicate that they are interchangeable. These experiments suggest that the machinery for initiation of transcription is similar in different cell types, at least to the extent that they can be assayed in these in vitro systems.

0843 SYSTEMATIC SEARCH FOR NEW MUTANTS OF GLOBIN GENE EXPRESSION, S.H. Orkin, S.C. Goff, J.P. Sexton, C. Boehm, S. Antonarakis and H.H. Kazazian, Jr., Harvard Medical School, Boston, MA 02115 and Johns Hopkins University School of Medicine, Baltimore, MD 21205
Several specific mutations in human globin genes leading to deficient globin synthesis have been characterized to date among thalassemia syndromes. These are located in coding or intervening sequences. Cloning and molecular analysis of mutant globin genes has generally been performed in essentially unselected material. We have sought to develop a strategy that permits more comprehensive and systematic study of naturally occurring mutations. Our approach combines analysis of cloned β-globin genes from thalassemic individuals and examination of DNA polymorphisms distributed throughout the β-gene cluster detected by DNA blotting. Seven polymorphic restriction sites have been used to characterize nearly 100 β-thalassemic chromosomes. Nine different patterns of cleavage (haplotypes) were observed in our panel. By cloning one or multiple β-genes from each haplotype we have identified: (1) linkage of haplotypes with specific molecular defects leading to thalassemia; (2) several new mutant β-globin genes with apparently novel putative thalassemic mutations; and (3) the existence of three distinct β-globin gene frameworks into which thalassemia mutations have been introduced. This systematic approach to the analysis of single gene mutants has yielded a candidate for the first transcriptional mutant in the human globin system: a gene with a normal DNA sequence from cap to poly(A) addition site, but a single nucleotide substitution (C→G) just upstream from the CCAAT box, a conserved region thought to lie within a promoter element. Further analysis of this gene will require application of the techniques of reversed genetics.

0844 REGULATION OF GENE EXPRESSION DURING SEA URCHIN EMBRYOGENESIS INVOLVES A CHANGE IN ABUNDANCE OF SEVERAL HUNDRED mRNA MOLECULES, Mark Boardman and Tom Humphreys, University of Hawaii, Pacific Biomedical Research Center, Honolulu, HI 96813
The mRNA sequences present in blastula and pluteus embryos of the Hawaiian sea urchin Tripneustes gratilla were studied using hybridization of poly(A)RNA to its cDNA. Analysis of the kinetics of hybridization indicate that both embryonic stages express about 12,000 rare class and 200 abundant class mRNA sequences. Most of the abundant mRNA sequences are present at the moderately abundant level of about 40 copies per cell while a few sequences are present at the very abundant level of several thousand copies per cell. Cross hybridization between the cDNA of either stage and poly(A)RNA of the other stage go to completion indicating that the same sequences are present at both stages. Since most of these sequences are rare class in both stages, we conclude that there can be little regulation of rare class sequences between blastula and pluteus stages. The cross reactions between RNA and cDNA from the two stages were further studied using purified cDNA tracers complementary to the abundant mRNA sequences. The heterologous reactions of these tracers showed that about three-fourths of the sequences that were abundant at one stage were rare at the other stage. All the regulated sequences were in the moderately abundant class of mRNA and none of the very abundant sequences, which we believe represent mitochondrial RNA, were regulated. The major component of regulation of gene activity during sea urchin embryogenesis appears to involve the repression of about 180 moderately abundant sequences to rare class levels, and the activation of about 200 sequences from rare class to moderately abundant class levels, between blastula and pluteus stages.

0845 ATTENUATION AND mRNA MODULATION IN A FEEDBACK CONTROL SYSTEM REGULATING GENE EXPRESSION IN EUKARYOTES Yosef Aloni, Nissim Hay and Hagit Skolnik-David. Department of Genetics, Weizmann Institute of Science, Rehovot 76100, Israel
We have found that SV40 RNA initiated at the major initiation site prematurely terminates in vitro 93-95 nucleotides downstream at a typical prokaryotic transcription termination structure. We have also found that the drug 5,6-dichloro-1-β-ribofuranosylbenzimidazole (DRB) augmented the premature termination process. These results suggest that the eukaryotic RNA polymerase B responds to the same signal as the prokaryotic enzyme and that an attenuation mechanism resembling that in prokaryotes regulates SV40 gene expression. Moreover, we have observed that alternative secondary structures can be predicted for the aborted RNA as well as for the initial 95 nucleotides of the major SV40 RNA: a transcription termination conformation and a readthrough conformation. Based on these results we shall present a model in which attenuation and mRNA modulation in a feedback control system regulates SV40 gene expression.
The fact that the major viral functions take place in cell nuclei via cellular machinery suggests that a similar mechanism regulates the expression of other viral and cellular genes.

0846 CELL CYCLE REGULATION OF HISTONE mRNA IN MOUSE ERYTHROLEUKEMIA CELLS, R.B.M. Alterman, S. Ganguly, D. Schulze, D. Sittman, W. Marzluff, C. Schildkraut and A. Skoultchi, Department of Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461.

Mouse erythroleukemia (MEL) cells were fractionated by centrifugal elutriation. Cell cycle position was analyzed by flow microfluorimetry and autoradiography following incubation of cells with 3H-Th. Histone mRNA was analyzed by RNA dot hybridization, Northern blotting, 3H-Ur pulse labelling, and in vitro RNA synthesis. Hybridization was carried out with either cloned sea urchin H3 and H2A genes or with a cloned mouse H3 gene probe. Northern blots showed that both the sea urchin and mouse histone gene probes hybridize specifically with 9S histone mRNAs. Autoradiograms of RNA dot hybridization quantitated by densitometry showed that the histone mRNA content of cells in mid-S phase was at least 10 times higher than in G_1 cell populations and 5 times higher than in G_2 cell populations. PolyA$^+$ and polyA$^-$ cytoplasmic RNAs were isolated from cells that had been labelled for 1 hour with 3H-Ur. Labelled histone mRNA was analyzed by hybridization to nitrocellulose filters containing cloned histone DNA. Histone mRNA was detected only in the polyA$^-$ fraction. The percent of labelled RNA which is histone mRNA was found to gradually increase as cells enter S phase, reaching a maximum value in mid-S phase at least 8 times higher than in G_1 cell populations; thereafter, the percent labelled histone mRNA decreased to 40% of the maxium value in G_2 cell populations. In contrast to a recent report (Groppi and Coffino, Cell 21:195-204, 1980) of equivalent rates of histone protein synthesis in the G_1 and S phases of certain other cultured mammalian cell lines (eg.S49 lymphoma) our results indicate that both the steady state level and production of histone mRNAs are regulated coordinately with DNA synthesis in the MEL cell cycle.

0847 CELL SPECIFIC EXPRESSION OF ACTIN GENES IN SEA URCHIN DEVELOPMENT, W.R. Crain[1], F. Bushman[1] and S.G. Ernst[2], Worcester Fndn. for Experimental Biology[1], Shrewsbury, MA, Tufts University,[2] Medford MA.

Expression of the actin gene family in the sea urchin is known to change temporally in early development, with two actin mRNA size classes (2.2 & 1.8 kb) increasing in abundance 10-20 fold between the 16-cell stage (5 h) and mesenchyme blastula (24 h). To determine if during embryogenesis cells of determined lineage differentially express these genes we have separated cell types at these stages and determined their actin mRNA content by RNA blot analysis. These separations yield micromeres and a mixture of macromeres/mesomeres from the 16-cell embryo and primary mesenchyme cells and presumptive endoderm/ectoderm from mesenchyme blastula. An actin protein-coding probe, known to react with all members of the actin gene family, shows that in 16-cell embryos both the 2.2 and 1.8 kb actin RNAs are present in both cell fractions at similar ratios indicating no differential expression of these RNAs. There is, however, differential expression of these two RNA classes in the cells of the mesenchyme blastula stage, with the 1.8 kb RNA being significantly more abundant compared to the 2.2 kb RNA in the primary mesenchyme cells than in the endoderm/ectoderm. Since the primary mesenchyme cells are the direct and only descendants of the micromeres and the endoderm/ectoderm derives from the macromere/mesomere fraction the differential expression at mesenchyme blastula must result from differential accumulation of new mRNA. In addition RNA blot analysis with probes complementary to regions located adjacent to the 3' side of actin-coding sequence from two different actin genes demonstrates that one gene codes for a 2.2 kb actin message and the other for a 1.8 kb message which is enriched in the primary mesenchyme cells.

0848 EXPRESSION OF MOUSE MUP GENES, P.M. Clissold, A.J. Clark and J.O. Bishop, Dept. of Genetics, University of Edinburgh, Edinburgh, U.K.

Mouse major urinary protein (MUP) mRNA hybridizes with a family of about 20 genes. By an analysis of genomic clones and chromosomal DNA we have detected two major sub-families (each about 10 genes) and additional variant genes which are not closely related to either of these. One of the major sub-families codes for most or all of the MUP mRNA found in male mouse liver.

The abundance of MUP mRNA is 5-fold higher in male BALB/c liver than in female liver and it cannot be detected in the male before the onset of puberty. We have examined the translation products of hybrid-selected MUP mRNA (pro-MUP) by IEF in agarose-urea gels. Male pro-MUP separated into 20 or more components, three of which were especially prominent. Only one of the three bands was prominent in female pro-MUP.

The size of the MUP mRNA precursor has been determined by Northern blots, ExoVII mapping and Southern blots of genomic clones probed with MUP mRNA. The numbers and lengths of introns and exons have been measured by electron microscopy of RNA-DNA hybrids.

0849 TRANSCRIPTION OF THE XENOPUS 5S RNA GENES, Laurence J. Korn, Mary E. Harper, Jennifer Price, Cary Queen and John B. Gurdon, Stanford University, Stanford, California 94305

We are studying the developmental control of Xenopus 5S RNA gene expression. There are two major sets of 5S RNA genes. The most abundant set codes for oocyte type 5S RNA; these genes are expressed in oocytes and are silent in somatic cells. The other set, the somatic type 5S RNA genes, is expressed in both oocytes and somatic cells. The oocyte type genes are located at the telomeres of the long arm of most or all of the 18 chromosomes. We have prepared probes for in situ hybridization to locate the somatic type 5S genes and to investigate the role of their chromosomal environment on expression. In addition, to study the selective expression of 5S RNA genes during development, we have transplanted somatic cell nuclei into Xenopus oocytes. In some cases when erythrocyte nuclei are injected into oocytes, the somatic, but not the oocyte type 5S RNA genes are expressed, i.e., the regulated state in somatic cells is maintained. Thus, 5S RNA genes as part of an intact chromosome behave differently than purified genes with respect to regulation of transcription. The surprising existence of certain frogs which have activating oocytes, i.e., express both oocyte and somatic type 5S RNA, was at first disturbing. However, when extracts from "activating" oocytes are injected along with somatic nuclei into oocytes which normally would not allow the expression of the oocyte type 5S RNA genes, these genes are now expressed. Analysis of the extract from activating oocytes may reveal the factor(s) which specifically promote transcription of the oocyte type 5S RNA genes. ¶An algorithm for finding partial homologies among a set of nucleotide sequences and other improvements have been incorporated into a commonly used computer program for the analysis of nucleic acid and protein sequence data.

0850 IDENTIFICATION OF SEQUENCES AND REGULATORY FACTORS PROMOTING THE TRANSCRIPTION OF MOUSE RIBOSOMAL GENES, Ingrid Grummt, Gert Pflugfelder and Detlef Buttgereit, Institut für Biochemie der Universität Würzburg, Röntgenring 11, D-8700 Würzburg, G.F.R.

The transcription of ribosomal RNA correlates with the proliferation rate of the cells. In order to investigate the molecular mechanisms underlying the switch-on and switch-off of rDNA expression a cell-free system for the transcription of cloned ribosomal genes both from mouse and man was established. Extracts prepared from rapidly growing cells contain the factor(s) required for the accurate initiation of RNA polymerase I on cloned rDNA. After nutritional shift-down the ability of the cell extracts to promote specific transcription is lost. The ability of active extracts to complement inactive extracts from non-growing cells has been used to isolate the proteins which regulate the transcription of rDNA. So far, an activating factor has been purified more than 1000 fold from mouse cells by ion exchange and affinity chromatography. The factor is a very labile protein which binds to DNA and seems to associate with RNA polymerase I. Mixing experiments using human and mouse rDNA and extracts from HeLa and Ehrlich ascites cells, respectively, revealed a marked species specificity of the factor(s) required for the faithful transcription of the ribosomal genes. Furthermore, specific deletion mutants were constructed in vitro and used as templates in the cell-free transcription system. The results indicate that the efficiency of specific transcription in vitro is markedly reduced if 5' flanking sequences beyond position -40 are removed.

0851 THE SPLIT INTRAGENIC PROMOTER OF TWO EUKARYOTIC tRNA GENES

Hans Hofstetter, Gabriella Galli, Armin Kressmann and Max L. Birnstiel, Institut für Molekularbiologie II der Universität Zürich, 8093 Zürich, Switzerland.

The sequences essential for promotion of transcription have been identified for a $tRNA^{met}$ and a $tRNA^{leu}$ gene of X. laevis. The cloned tRNA genes were mutated in vitro and the transcriptional activity of the mutants was then tested in frog oocytes and by homologous in vitro transcription. For both tRNA genes studied, we find two major intragenic promoter elements which are essential for transcription. These sequences closely coincide with two conserved sequence blocks present in all eukaryotic tRNA genes between nucleotides 8 to 20 and 52 to 62, suggesting that these conserved sequences are common promoter features of these genes. The sequences between the two conserved blocks play a minor role for transcription but they are indispensible for keeping the two conserved blocks at a minimal distance. The split tDNA promoter contrasts with the promoter arrangement of the 5S RNA genes, yet both gene classes are transcribed by RNA polymerase III. It permits variability in extending or shortening of the sequences lying between the two conserved blocks and is in keeping with the functional diversity of the tRNA structure.

0852 THE USE OF MONOCLONAL ANTIBODIES IN THE ANALYSIS OF A TRANSCRIPTION FACTOR FROM X. LAEVIS, Angela Krämer and Robert G. Roeder, Wash. Univ. Med. Sch., St. Louis MO 63110

Monoclonal antibodies have been raised against TFIIIa, a transcription initiation factor purified from oocytes of Xenopus laevis. This factor was previously shown to bind to the control region of 5SrRNA genes and to mediate, along with other factors, accurate in vitro transcription. Several of the antibodies obtained bind to TFIIIa and to specific polypeptide fragments obtained by digestion of the protein with CNBr, as assayed by protein blotting. One antibody studied in further detail was shown to bind the native (40,000 dalton) protein in extracts from different developmental stages of Xenopus oocytes and embryos. The antibody also bound TFIIIa in immature oocyte extracts in which the protein is complexed with 5SrRNA in 7S RNP storage particles. This result suggests that the 5SRNA-TFIIIa interaction does not interfere with binding of this particular antibody. Taking advantage of this property it was shown that the newly synthesized 5SrRNA rapidly associates with TFIIIa in vitro. Using antibody covalently attached to Sepharose it was possible to obtain a relatively pure (>90%) and transcriptionally active preparation of TFIIIa. When preincubated either with extracts from Xenopus oocytes or embryos or with purified TFIIIa (and subsequently reconstituted with other factors), these antibodies do not inhibit the transcription of 5S genes in vitro. This result suggests that the binding of the antibodies to TFIIIa does not affect the interaction of the transcription factor with the template or other proteins involved in the transcriptional process. To exploit the fact that this antibody does not interfere with other components necessary for in vitro transcription, studies are in progress to purify transcription complexes and to identify the other factors involved in accurate transcription of 5SrRNA-genes in vitro.

0853 A MOLECULAR GENETIC ANALYSIS OF RABBIT IMMUNOGLOBULIN GENE EXPRESSION, Leona Fitzmaurice, Andrea Pavirani, Frederick Jacobsen, Kenneth Bernstein, Nancy McCartney-Francis, and Rose Mage, LI, NIAID, NIH, Bethesda, MD 20205

We have constructed cDNA clones from hyperimmunized rabbit spleen mRNA and identified clones containing κ, γ, and μ sequences. We have characterized these cloned cDNAs by colony hybridization, hybridization selection of mRNA, restriction enzyme analysis, and DNA sequencing. We are using these cloned probes to study rabbit immunoglobulin gene expression at the protein, RNA, and DNA level. We have conducted cell-free translation and hybridization selection studies and have characterized κ, γ, and μ mRNA cell-free translation products ($\sim$27, 48, and 70 kilodaltons respectively) by immunological and electrophoretic methods. Using our cloned cDNA probes, we have identified κ, γ, and μ mRNAs ($\sim$1.15, 1.7, and 2.4 kilobases respectively) by "Northern" blot analysis of total mRNAs prepared from spleens of allotype-defined rabbits. We are studying the regulation of expression of these mRNAs in both total spleen and B cell populations, e.g., utilizing the highly conserved 3' untranslated region of mRNAs for the κ b allotypes to explore control of κ gene expression. Our characterized cloned cDNA probes are allowing us to define the genetic organization of immunoglobulin structural gene sequences by Southern blot analysis of rabbit liver, spleen, and sperm DNAs and to identify cloned immunoglobulin genes in genomic libraries that we have constructed from spleen and sperm DNAs prepared from rabbits of defined allotype.

0854 STIMULATION OF FETAL HEMOGLOBIN SYNTHESIS BY 5-AZACYTIDINE, Joseph DeSimone, Paul Heller, David Zwiers and Lemuel Hall, VA West Side Medical Center, Chicago, IL 60612

Methylation of the CpG dinucleotide sequences of DNA has been shown to be important in the control of gene expression, undermethylation being associated with gene expression. Since 5-azacytidine (5-azaC) is a cytosine analogue which cannot be methylated, we have chosen this agent to test the possibility that its incorporation into baboon DNA in vivo leads to γ globin gene expression in adults who normally produce only trace amounts of Hb F. The baboon has previously been shown to respond to erythropoietic stress with increased Hb F synthesis and the magnitude of the response is genetically determined ("high" or "low Hb F responders") by enumeration of the number of Hb F containing erythrocytes (F-cells) during stress. Baboons were bled daily to maintain a hematocrit of 20% and 5-azacytidine at doses of 1-8 mg/kg/day, was injected i.v. 5 days a week for periods of from 1-6 weeks. A dose of 3 mg/kg/day had the maximal effect on γ chain synthesis (40 and 80% of the non-α chain synthesis in low and high responders, respectively). Hb F levels begin to rise 5-7 days after the first injection, reach maximal levels 5-7 days later, and remain so while the animals receive the drug. After its discontinuation maximal Hb F levels are maintained for 10-12 days. The increase in γ chain synthesis is likely to be due to hypomethylation and, therefore, suggests that the CpG sequences relevant to the γ genes in the pluripotential stem cell should be normally undermethylated, and that methylation of these sequences occurs during normal red cell maturation. Relevant experiments are underway.

0855 STRUCTURE AND FUNCTION OF ENDOGENOUS RETROVIRUS PROMOTER SEQUENCES REQUIRED FOR GENE EXPRESSION, G.E. Mark and G.J. Todaro, National Cancer Institute, Frederick, MD 21701
Tissues of the Old World monkey Colobus polykomos contain multiple copies of an unexpressed endogenous virus (CPC-1). Following long term cocultivation of these tissues with a human carcinoma cell line a replication competent CPC-1 virus was isolated and subsequently found to be capable of efficient transcription (10,000 copies/cell). Sequence analysis of the CPC-1 promoter region revealed the presence of two "TATA" boxes, 29 and 62 nucleotides upstream from the cap site. Also identified were two 9-nucleotide sequences (CCAATCATA) approximately 55 nucleotides upstream from each "TATA" box. Analysis by S_1 mapping and reverse transcription of viral transcripts reveal this apparent overlapping double promoter utilizes only the TATA proximal to the cap site. Nucleotide sequence and in vitro transcription comparisons of the CPC-1 virus promoter with two CPC-1 endogenous proviral promoters indicates that as few as four base alterations (none within CCAAT or TATA) were sufficient for transcriptional inactivation. This may reflect the DNA secondary structure alterations observed in computer-generated models. A deleted thymidine kinase gene (promoter and terminator sequences removed) has been introduced into the cap site of the active CPC-1 viral promoter in a manner facilitating promoter sequence interchanging. Experiments will be discussed which compare the effects of active, inactive and mutagenized promoters on TK gene expression in vivo.

0856 SELECTIVE AND ACCURATE TRANSCRIPTION OF EUCARYOTIC CLASS III GENES IN SOLUBLE YEAST WHOLE CELL EXTRACTS. Tony Weil and Mark Klekamp, Department of Biochemistry, University of Iowa Medical School, Iowa City, Iowa 52242.

We have developed a soluble, cell-free extract derived from whole yeast cells. This extract transcribes both homologous (various tRNA and 5SrRNA) and heterologous (Xenopus laevis tRNA and Adenovirus-2 VA RNA)class III genes in vitro with varying efficiencies. Specific transcription is DNA-dependent, Actinomycin-D sensitive and mediated by endogenous (α-amanitin resistant) RNA polymerase III. We have determined optimal parameters for maximal RNA synthesis (25°, 100-150 mM NaCl, 10 mM $MgCl_2$). Specific transcription has been documented by fingerprint analyses. These extracts are also apparently able to process the in vitro tRNA gene transcripts.

Fractionation of the extract is currently under investigation as are experiments utilizing Class II (mRNA coding) genes.

0857 RAT GROWTH HORMONE PROMOTER ACTIVITIES, Patricia L. Earl, Daniel Schümperli, Martin Rosenberg, and E. Brad Thompson, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20205
The nucleotide sequence of the rat growth hormone gene promoter region was determined. To study promoter activity in vitro and in vivo, the region was subcloned into various plasmid vectors. A pBR322 derivative containing most of the coding sequence including 2 kbp of 5' flanking sequences was constructed and used in a HeLa cell in vitro transcription system. Specific rat growth hormone transcript synthesis was sensitive to α-amanitin. The sizes of "run-off" transcripts obtained using plasmid DNA cut with various restriction enzymes as the template indicated that transcription initiates approximately 30 bp downstream from a Goldberg-Hogness-like sequence. To measure promoter activity in vivo, we inserted a 600 bp fragment containing the rat growth hormone promoter into the pSVK vector (Schümperli et al., PNAS, in press). On this fusion plasmid the rat growth hormone promoter controls transcription of the E. coli gal K gene. Its product, galactokinase, is measured in mammalian cells transfected with the plasmid. Moreover, the plasmid contains, on a separate transcription unit, the E. coli xanthine-guanine phosphoribosyltransferase gene controlled by the early SV40 promoter. This second gene serves both as an internal copy number standard as well as a dominant selective marker for eukaryotic cell transfection.

0858 **REGULATION OF YEAST HISTONE SYNTHESIS,** **Lynna Hereford and Mary Ann Osley, Brandeis University, Waltham, MA 02254**

In yeast there are two copies of H2A and H2B which are organized into two genetically unlinked H2A-H2B gene pairs. In each pair the genes are divergentlly transcribed and are separated by approximately 800 bp of nonhomologous DNA. All four genes are very tightly regulated at both the transcriptional and post-transcriptional levels.

To investigate the nature of the transcriptional regulation (which results in the periodic transcription of the histone genes early in S) we have made fusions between the H2A and the H2B promotors and β galactosidase. Both promotors are functional when transformed into yeast. However, we have found that proper transcriptional regulation occurs only when sequences located at the 3' end of the H2B gene are present. In the absence of these sequences, the histone genes are expressed at low constitutive levels. We have also found that these 3' sequences contain origins of replication. We are currently in the process of determining whether these origins may be involved in transcriptional specificity.

0859 APPARENT SLIPPAGE OF RNA POLYMERASE DURING INITIATION FROM ALTERED TET PROMOTERS, C.B.Harley, J.Lawrie, M.C.Betlach, R.Crea, H.W.Boyer, and J.Hedgpeth, University of California, San Francisco, CA 94143

Prokaryotic promoters have three regions of relatively conserved DNA sequence: the point of initiation, the "Pribnow Box" and the "-35" region. However, the function of these regions is poorly understood. We have synthesized and inserted two AT-rich DNA fragments into the HindIII site of the Tet promoter of pBR322. The recombinant plasmids, pTA22 and pTA33, exhibit tetracycline resistnace to about 70% of the wild type level. With respect to the -35 region, the Tet Pribnow box is shifted downstream by 22 and 33 base pairs in pTA22 and pTA33 respectively. However, the inserts contain several sequences homologous to other known Pribnow sequences. In vitro transcripts of pBR322, pTA22 and pTA33 from HaeIII DNA fragments spanning the promoter region were studied by RNA fingerprinting and gel sequencing. Initiation within the DNA fragment from pBR322 occurs at position 45 (pppGUUUAUC...). Initiation within the recombinant fragments occurs within a stretch of A_4 in the insert. In pTA22 and pTA33 this region is at a point equidistant from the native -35 region as the wild type Tet transcript. It appears that RNA polymerase utilizes the wild type -35 and a synthetic Pribnow within the insert. However, in the case of both synthetic promoters, there is a distribution of transcripts ranging from $pppA_2U...$ to $pppA_{15}U...$, with $pppA_5U...$ being the major start. All transcripts with more than A_4 at the 5' end must be produced by an anomalous behaviour of RNA polymerase with these promoters. We hope to determine the mechanism by which these promoters produce this anomaly and in this way learn something about normal promoter activity.

0860 TRANSCRIPTION OF DROSOPHILA HEAT SHOCK AND ACTIN GENES IN HELA CELL-FREE EXTRACTS Rick Morimoto, Jean Schaffer and Matthew Meselson
Harvard Biochemical Laboratories, Cambridge, MA

The in vitro transcription of three Drosophila genes has been examined in cell-free extracts of Hela cells grown at 37 C or heat shocked at 43 C. The truncated template-runoff product assay was used to demonstrate that the genes for Drosophila actin, the 70K heat shock protein (hsp70) and hsp 83 are transcribed from sites at their 5' ends to give α- amanitin sensitive products of predicted lengths. Transcription of the hsp 83 gene and the hsp 70 gene either by itself or mixed with the actin and Ad 2 late promoter template shows that at lower heat shock template concentrations (2.5-25 ug/ml), the heat shock genes are transcribed more effectively in extracts of heat shocked cells, while the actin and Ad 2 gene show no such effect. However, at high heat shock template concentrations (100 ug/ml), transcription of hsp70 occurs in control cell extracts. This effect on template concentration appears specific since low template concentrations and increasing levels of a carrier)poly dIC;dIC) do not yield the same results. Transcription of hsp70 in heat shock extracts is observed from a template containing only 180 bp of DNA upstream from the 5' end.

0861 T ANTIGEN BINDING AND REPRESSION OF SV40 EARLY TRANSCRIPTION, Daniel G. Tenen, L. L. Haines, and T. Taylor, Sidney Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA 02115.

DNase footprint analyses of purified AD2+D2 (D2) T protein binding to SV40 origin region fragments revealed a series of four specific interactions with contiguous sequences constituting a 120 base pair block, in keeping with previous DNase protection results of others. Protection was observed to extend from a 30 base pair strong affinity site located on the early side of the replication origin (site 1) to two adjacent lower affinity sites, including the origin of replication (site 2) and an approximately 15 base pair site between site 1 and the beginning of the T/t coding sequence (site 1'). A fourth site (site 3) was noted abutting the late border of site 2. Thus, either binding to these sites is cooperative or the sequences which constitute site 1 affect the conformation of the sites 1' and 2 sequences such that they now serve as sites of more efficient D2T binding. In addition, while deletion of all of site 2 and its substitution by late viral sequences ablated processive T binding to sequences abutting site 1 on its late side, various site 2 deletions comprising up to approximately 40% of site 2 did not affect binding to a major degree. Therefore, binding to the replication origin sequence is a sequence specific event, but there may be multiple strong protein contact sites within that sequence.

In collaboration with Ulla Hansen, Philip Sharp, and David Livingston, we have performed simultaneous T footprint binding and in vitro transcription assays. Together these studies suggest a model for control of early transcription and initiation of DNA replication which will be discussed.

0862 HETEROGENEITY IN THE 5' END OF THE MULTIPLE DHFR mRNAS FROM MOUSE S-180 CELLS, Michael McGrogan, Christian Simonsen, and Robert T. Schimke, Department of Biological Sciences, Stanford University, Stanford, Ca. 94305.

The mouse dihydrofolate reductase (DHFR) gene is expressed as a family of seven overlapping mRNAs, ranging in size from 800 to 5600bases. These RNA species are colinear, all contain the DHFR coding sequence, and differ primarily in the length of their 3' untranslated region. Fine structure analysis of the 5' end of the DHFR mRNAs was performed using S1 nuclease and primer extension of labeled genomic restriction fragments. The DHFR mRNA species isolated as poly A containing cytoplasmic RNA from methotrexate resistant mouse S-180 cells were found to have at least three different colinear 5' ends, mapping at positions 115, 275, and 450 nucleotides 5'ward of the AUG in exon I of the gene. The assignment of certain 5' ends to to specific DHFR mRNAs was accomplished by hybridization of filters containing size fractionated mRNA to DNA probes derrived from the 5' flanking region. The 1600, 1000, and 800 species map with their 5' at the "115" site, where-as the 2100 and 1200 RNAs were found to have their 5' termini located in the "275" region. In addition to the multiple 5' ends on the DHFR mRNAs, one of the species, the 800 RNA, was found to have a spliced leader that originates approximately 300 bases down stream from the "115" site. The sequence of the 5' flanking region shows that the only TAATAA box is located 50-100 bp 5'ward of the 800 leader, and that there is a unique G+C rich 180 bp sequence extending 5' of the "115" site that consists of four direct 48 bp repeats each containing a CAACATA.

0863 LONG RANGE EFFECTS OF MUTATIONS ON CYTOCHROME b mRNA MATURATION IN YEAST MITOCHONDRIA, A. Halbreich,P. Pajot, C. Grandchamp and M. Foucher, Centre de Génétique Moléculaire du C.N.R.S. 91190 Gif sur Yvette, France.

The genetic locus box8 of yeast mitochondrial cytochrome b is situated in and around the third exon of this gene (1, 2). mRNA processing in one mutant of this group (G5046) was previously shown to be inhibited already in the first step of splicing which leads to the accumulation of a free covalently closed circular RNA issued from the first intron (1). In as much as this intron is situated 1.3-1.9 Kb away from box8, this suggested that a particular secondary or tertiary structure of the premRNA molecule is required in this process and is perturbed by the mutation. Recently we have analyzed mtRNAs from several other mutants of this locus. The correlation between the base alterations and the changes of mRNA maturation induced in these mutants will be discussed.

1. Halbreich et al. (1980), Cell, 19 : 321-329.
2. Lazowska et al. (1980), Cell, 22 : 333-348.

0864 CLONING AND EXPRESSION OF ESTRADIOL SENSITIVE GENES IN XENOPUS OVIDUCT, Christopher A. Maack, Tharappel C. James and Jamshed R. Tata, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

The oviduct in amphibians is the site of synthesis of the jelly coat proteins of the egg capsule. The expression of these proteins appears to be at least in part under the control of estradiol. In order to study the tissue specific regulation of gene expression by estradiol, a cDNA clone bank from estradiol stimulated Xenopus laevis oviduct polyA$^+$ RNA has been prepared. Cloned cDNAs from a number of estradiol sensitive genes have been identified, and four such clones have been analyzed in detail. The expression of three of the genes is increased by estradiol treatment and that of the fourth is reduced. Quantitation of these variations and preliminary information on the kinetics of induction have been determined. Using hybrid selected translation, the gene products of these four estradiol sensitive genes have been determined. Concurrently, monoclonal antibodies to egg jelly coat proteins have been prepared and are being used in conjunction with hybrid selected translation to determine the relationship of the cloned estradiol sensitive sequences to egg jelly coat proteins. These studies point to the usefulness of the estradiol regulation of Xenopus vitellogenin and egg jelly coat protein gene expression as an integrated model system in which to study tissue specific gene expression.

0865 FACTORS REQUIRED FOR FAITHFUL INITIATION OF TRANSCRIPTION BY RNA POLYMERASE II, H.M. Furneaux, R.A. Guggenheimer, and J. Hurwitz, Albert Einstein Col. of Med., Bx., NY 10461

We are interested in the proteins which in conjunction with RNA polymerase II catalyze the initiation of mRNA synthesis in eucaryotic cells. Accordingly, we have prepared extracts from HeLa cells which can accurately initiate transcription on a cloned DNA fragment containing the major late promoter of adenovirus. Accurate initiation have been demonstrated by the DNA-dependent incorporation of labelled GTP into a discrete (535 nucleotide) "run-off" RNA product which is sensitive to α-amanitin (1 μg/ml) and is stimulated by the addition of RNA polymerase II purified from MOPC-315 (mouse) cells. Using this "run-off" assay we have fractionated these extracts by conventional chromatographic techniques. At the present time, there appears to be at least two chromatographically distinct fractions which in addition to RNA polymerase II are absolutely required for accurate initiation. One appears to inhibit random transcription whereas the other appears to directly catalyze the formation of the 535 nucleotide "run-off" product. The purification and characterization of these fractions will be discussed.

0866 DEXAMETHASONE AND CADMIUM INDUCE DIFFERENT SETS OF PROTEINS IN LYMPHOCYTES, Edward V. Maytin and Donald A. Young, E.Henry Keutmann Laboratories, Endocrine Metabolism Unit, Departments of Medicine and of Radiation Biology & Biophysics, University of Rochester School of Medicine & Dentistry, Rochester, New York 14642.

Both steroid hormones and heavy metals probably initiate biological responses by altering gene expression. Evidence that glucocorticoids induce a set of proteins in time to account for the most rapidly-evolving metabolic effects of steroid hormones was recently demonstrated [Voris,B.P. and Young, D.A.,J. Biol. Chem. 256:11319 (1981)]. The fact that dexamethasone and cadmium each induce a common protein (metallothionein) in rodent cells *in vitro* [Mayo, K.E. and Palmiter, R.D., J. Biol. Chem. 256:2621 (1981)] also raises the possibility that the two agents might alter the expression of a larger common set of genes.

To address this possibility, we examined a large subset of cellular proteins from rat thymic lymphocytes incubated in the presence or absence of dexamethasone ($1x10^{-6}$M) or cadmium ($5x10^{-6}$M) and separated by the method of giant 2-D gel electrophoresis [Voris, B.P. and Young, D.A., Anal. Biochem. 104:478 (1980)]. We found that the two agents induce entirely separate sets of proteins. Dexamethasone induces 6 proteins, 4 of which change within the first 15-45 min after hormone addition. Cadmium induces a single protein (pI 6.5, M.W. 29,000) that does not change until 1-2 hrs. Computer scanning densitometry was used to quantitate the magnitude of induction of the different proteins. Metallothionein, located by co-migration with purified ^{109}Cd-metallothionein, is not detectably induced by either agent in these lymphocytes.

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0867 MUTANTS IN THE PROMOTER OF EUKARIOTIC tRNA GENES, Cinzia Traboni , Gennaro Ciliberto and Riccardo Cortese . E.M.B.L., Heidelberg, BRD.

The study of the transcriptional properties of deletion and insertion mutants of a $tDNA^{Pro}$ and a $tDNA^{Leu}$ from the nematode Caenorhabditis elegans has lead to the conclusion that the promoter of these genes is located within the coding sequence and is composed of at least two essential regions separated by approximately thirty nucleotides.
Newly developed methods of site-directed and localized mutagenesis, expecially optimized for small genes,have been extensively used to obtain a collection of single point mutants and single base pair deletions and insertions. The transcriptional analysis of these mutants by microinjection in the nucleus of Xenopus laevis oocytes has allowed a more precise definition of the exact sequence of eukariotic tRNA promoters.

0868 SOME HEAT SHOCK GENES ARE EXPRESSED IN NORMAL DEVELOPMENT IN DROSOPHILA, J. Lynn Zimmerman[1], William Petri[2], and Matthew Meselson[1], (1) Harvard University, Cambridge MA, 02138, and (2) Boston College, Newton, MA, 02171.
Transcription of genes for seven characteristic proteins is strongly induced in D. melanogaster by heat shock and certain other stimuli. Work in other laboratories indicates that the largest heat shock protein, hsp 83, is produced in the absence of heat shock in eggs, embryos and cultured cells. In order to study the possible activity of this and other heat shock genes in normal development, we have used cloned heat shock genes to assay directly for the presence of heat shock mRNA's in ovaries and embryos at various stages in the absence of temperature shock. We find mRNA for three of the seven major heat shock proteins, hsp 83, hsp 28, and hsp 26 in unshocked ovaries at later stages of maturation and in pre-blastoderm embryos. Hybridization in situ to sectioned ovaries indicates that the mRNA's originate in the nurse cells and are transported into the maturing oocyte. The three heat shock mRNA's disappear after blastoderm formation and that for hsp 83 reappears in late embryos. These findings suggest that hsp 83, hsp 28 and hsp 26 may perform functions required in the oocyte or early embryo while hsp 83, may additionally be required for some more general function. They further indicate that some of the heat shock genes are independently regulated, raising the possibility that some of these genes possess multiple regulatory sequences.

0869 REGULATION OF TRANSCRIPTION IN ADENOVIRUS-2, Alan R. Shaw and Edward B. Ziff, New York University Medical Center, Department of Biochemistry, New York, NY 10016
We have investigated the Ad-2 program of transcription by assaying transcription unit activity during the early stage of infection under a variety of conditions. Early during infection, Ad-2 expresses genes contained within at least 7 distinct transcription units. Early region EIa is activated first, followed by regions EIb, EIV, EII and EIII. Expression of early region EIa is believed to be required for the activation of the other early transcription units. We show that in cells which have their protein synthetic machinery blocked by anisomycin, and are therefore unable to produce EIa proteins, early regions EIa, EIb, and EIV are transcriptionally active while regions EII and EIII are repressed. We conclude that transcription of regions EII and EIII is controlled independently.
After the onset of DNA replication about 8 hours post-infection, Ad-2 transcription is dominated by the late transcription unit beginning at map unit 16.45 and extending almost to the right end of the genome at map unit 100. Each major late transcript is polyadenylated at one of five sites and processed to yield one member of five 3' co-terminal mRNA families. We have shown previously that the late transcription unit is active early in infection. The early version of the late transcript terminates, however, just to the right of map unit 40 and yields only the first 3' co-terminal mRNA family. The structure of the 3' end of the early version of the late transcript will be shown. A model, based on Ad-2 chromatin structure, of the anti-termination event associated with the late phase of transcription will be presented.

0870 ANALYSIS OF HLA AND β2-MICROGLOBULIN MESSENGER RNAs IN CELL LINES DEFICIENT FOR MEMBRANE HISTOCOMPATIBILITY ANTIGENS, Claude de Préval, Michèle Damotte, Bertrand R. Jordan and Bernard Mach, Centre d'Immunologie INSERM-CNRS de Marseille-Luminy, Case 906, 13288 Marseille cédex 9, France and Department of Microbiology, 64 Avenue de la Roseraie 1205 Genève, Switzerland.

Messenger RNAs were prepared from HLA^+ tumor lines : RAJI and P3HR-1 (B lymphomas) and from HLA^- tumor lines : DAUDI (B lymphoma, K562 (Erythroleukemic line) DUTKO-1 (DAUDI x K562 hybrid) and PUTKO-1 (DAUDI x P3H-R1 hybrid). They were compared by northern blot technique using different DNA probes : Actin cDNA, β2-microglobulin cDNA and cloned HLA genomic DNA fragments. The HLA subclones used are : the leader sequence exon, the third domain exon (α3), the transmembrane exon and the 3 cytoplasmic exons.

0871 HORMONAL REGULATION OF CALCITONIN GENE EXPRESSION, Jean-Jacques Hermod*, Fusun N. Zetinoglu†, Arnold Loera*, Ronald M. Evans†, Michael G. Rosenfeld*, *University of California, San Diego, School of Medicine, La Jolla, CA 92093; †The Salk Institute, San Diego, CA 92138

Established cell lines from rat medullary thyroid carcinomas which synthesize and secrete calcitonin and other peptides provide an ideal model system for elucidation of mechanisms of hormonal regulation of gene expression. Analysis of clonal isolates of the calcitonin gene and of several cDNA clones of mature calcitonin gene transcripts has suggested that a large primary transcript can generate a series of mature mRNAs, each encoding different component polypeptides (calcitonin and a new peptide, referred to as CGRP) as a consequence of RNA splicing events. Regulation of calcitonin gene expression was studied by quantitation of calcitonin nuclear transcripts and mature calcitonin mRNA sequences. Calcitonin, glucagon, and several other peptide hormonal agents raised calcitonin mRNA and nuclear RNA precursor concentration 3-5-fold while dexamethasone and vitamin D were without effect. Coordinate hormonal stimulation of several of the polymorphic RNA forms was consistently observed. These studies indicate that hormonal regulation of the transcription of the calcitonin gene is independent of the RNA processing regulation which determines the mature mRNA products.

0872 RECOMBINANT DNA PLASMIDS CONTAINING ABUNDANT MRNA SEQUENCES REGULATED DURING SEA URCHIN EMBRYOGENESIS, Nevis Fregien, Morton Mandel and Tom Humphreys, University of Hawaii, Honolulu, HI 96822

Cytoplasmic poly(A) containing RNA from pluteus stage embryos of the Hawaiian sea urchin (Tripneustes gratilla) was used as a template for the construction of a library of cloned cDNA sequences. Clones containing sequences complementary to mRNAs that increase in concentration between blastula and pluteus stages were assayed by in situ colony hybridizations. Initial screening reactions to the most abundant cDNAs showed no regulation in this class of RNA. A second, more sensitive, screening detected five pluteus-specific sequences from the moderately abundant class of RNA. These clones represented 4% of the total colonies tested. Quantitative dot blot hybridizations measured concentration increases of 3-40 fold for individual messages. Denaturing RNA gel blot hybridizations showed none of the pluteus-specific sequences were detectible in the RNA from 3-hour embryos, while two constitutive sequences were present throughout development. One pluteus-specific clone is complementary to an endoderm-specific mRNA and two are complementary to ectoderm-specific mRNAs. Three of the cloned cDNAs hybridize to multiple sizes of messenger RNA, with different sizes appearing predominant in different stages and tissue types. Five of the clones examined are coded by 1-3 genes in sperm DNA, and one of the cloned cDNA sequences is complementary to more than 50 restriction endonuclease generated fragments. A constitutive cloned sequence was found to be complementary to mitochondrial DNA.

0873 KERATIN GENE EXPRESSION IN MOUSE EPIDERMIS. Dennis R. Roop, Pamela Hawley-Nelson and Stuart H. Yuspa. National Cancer Institute, Bethesda, Maryland 20205.

Keratins which range in molecular weight from approximately 40Kd-70Kd are the major differentiation products of mouse epidermis. We have prepared a library of cDNA clones from total poly(A) RNA from newborn mouse epidermis. Partially purified messenger RNA was isolated for the 67Kd, 59Kd and 55 Kd keratins, which are the major keratins synthesized in this tissue, and used for the synthesis of cDNA for screening. Clones corresponding to these keratins were identified and confirmed by hybrid-selection. Although the keratins are closely related proteins, we have been successful in using short cDNA fragments which primarily consist of 3' untranslated sequence as specific probes for transcripts of these genes. By RNA blot analysis of poly(A) RNA from newborn mouse epidermis, we have identified RNA species that are approximately 2400, 2000 and 1600 nucleotides in length and are complementary to the 67Kd, 59Kd and 55Kd cDNA's respectively. We have analyzed RNA from primary cultures of newborn mouse epidermis by this same technique and find greatly reduced levels of these RNAs. However, proteins of approximately 59Kd and 55Kd are synthesized in vitro by these cells. We are currently investigating whether the changes observed in keratin gene expression in primary epidermal cultures are due to factors within the culture system or whether these changes represent a programed switch which occurs normally during development.

0874 A 3' TERMINAL FRAGMENT BEARING ONLY μ MEMBRANE EXONS CORRELATES WITH INCREASED LEVELS OF μ_S-TERMINATED C_μ GENE TRANSCRIPTS IN FUSED T-LYMPHOMA/PLASMACYTOMA CELL LINES.
David J. Kemp, Grant Morahan, Alan F. Cowman and Alan W. Harris. The Walter & Eliza Hall Institute, P.O. Royal Melbourne Hospital, Victoria 3050, Australia

Immunoglobulin μ mRNAs in B cells bear alternative μ_S and μ_M 3' termini in approximately equal amounts. After maturation to plasma cells, both the total amount of μ mRNA and the ratio of μ_S:μ_M termini are elevated about 100-fold. When B lymphoma cells are fused to plasmacytoma cells, the plasmacytoma pattern of μ mRNA is dominant. In T lymphomas transcription of the C_μ gene on non-rearranged or incompletely rearranged chromosomes results in transcripts which are spliced and bear μ_S or μ_M termini but not V_H regions.

In the present work we have examined the mechanism of control of μ_S:μ_M ratios by fusing T lymphoma cells to plasmacytoma NS1, which contains no C_μ genes. In the fused T lymphoma-plasmacytoma lines, an increase in the ratio of μ_S:μ_M terminated C_μ transcripts was observed in the absence of an increase in the abundance of C_μ transcripts. This increase in the ratio of μ_S:μ_M terminated C_μ RNAs was accompanied by the accumulation of a poly A^+ μ_M 3' termini fragment, containing only μ_M exons. We conclude that the control of 3' termination is independent of the rate of transcription and is mediated by a trans-acting factor which specifically cleaves a common precursor to the μ_S and μ_M species.

0875 IN VITRO TRANSCRIPTION OF DNA FROM THE HUMAN β-GLOBIN LOCUS, James L. Manley and Mary Colozzo, Department of Biology, Columbia University, New York, NY 10027
We have been using the human β-globin system as a model to study the steps involved in synthesis and processing of a eukaryotic mRNA in vitro. When plasmid DNA containing the human β-globin gene plus several thousand nucleotides of 5' and 3' flanking sequences is incubated in a concentrated whole-cell lysate prepared from HeLa cells, transcription initiation can be detected both at the 5' end of the β-globin gene and at a site about 4,500 nucleotides downstream from it. The latter site gives rise to an RNAP III transcript resulting from initiation within an alu-like repeat sequence, as previously described by Maniatis and coworkers. This RNA is transcribed from the same strand as is β-globin mRNA, and is quite long for an RNAP III transcript (well over 600 NT). As do several other alu-transcripts, it contains an internal oligo (A) sequence, which is sufficiently long to allow its efficient retention on oligo (dT) cellulose columns. The ionic requirements for the RNAP III initiation are quite specific, and different from any other promoter we have studied. As a result, the RNAP II and RNAP III transcripts can not be synthesized simultaneously in the same reaction mixture. Under optimal conditions, however, the RNAP III promoter is utilized with relatively high efficiency, resulting in the accumulation of 1-2 μg/ml of transcript. This amount should be sufficient to study the role of this small RNA, if any, in β-globin gene expression. Transcription initiated by RNAP II from the β-globin promoter results in the synthesis of both long, unprocessed transcripts, and also a number of smaller, related RNA species, including one the size of mature β-globin mRNA. We are currently analyzing these RNAs further by several techniques, including S1 hybridization to a cDNA probe, to determine whether any of them result from specific RNA processing.

0876 ANALYSIS OF THE 5-TERMINI OF HORMONE-INDUCIBLE MESSENGER RNAS OF HEN OVIDUCT, Robert E. Rhoads, William H. Eschenfeldt and Beth G. Cohen, and Theodore W. Munns*, University of Kentucky, Lexington, KY 40536 and *Washington University, St.Louis,MO 63110.
Previously we described a method for the analysis of 5'-termini of messenger RNA and demonstrated that eukaryotic cellular mRNA can have heterogeneous 5'-termini (Malek et al. (1981) Nucl. Acids Res. 9, 1657-1673]. This method consisted of digestion with RNase T_1, selection of capped oligonucleotides with Sepharose-bound antibodies directed against m^7Guo, labeling with [^{32}P]Cp and RNA ligase, separation of oligonucleotides by 2-dimensional gel electrophoresis, and sequencing of individual oligonucleotides by partial digestion with base-specific ribonucleases. The original analysis, performed on ovalbumin mRNA, revealed oligonucleotides which would correspond to at least three distinct sites of transcription initiation. We have extended this analysis to ovomucoid, lysozyme and conalbumin mRNAs. Ovomucoid mRNA yielded three major oligonucleotides and many minor ones. The predominant oligonucleotide corresponded to a transcriptional start at the +1 position in the sequence of Lai et. al. [(1979) Cell 18, 829-842]. Lysozyme mRNA yielded two major oligonucleotides, one of which corresponding to the -2 position in the sequence of Grez et. al. [(1981) Cell 25, 743-752]. The conalbumin mRNA pattern was by far the most complex, consisting of 20 to 30 capped oligonucleotides. The two predominant species corresponded to positions +1 and +4 in the sequence of Cochet et al. [(1979) Nature 282, 567-574]. This protocol of direct RNA sequencing avoids some of the experimental problems associated with S1 mapping and primer extension and also provides additional information on the fine structure of transcriptional initiation sites.

0877 ALTERNATE SPLICING PATTERNS PRODUCE THE γ AND γ' CHAINS OF FIBRINOGEN. G.R. Crabtree, J.A. Kant and D.M. Fowlkes, National Institutes of Health, Bethesda, Maryland 20205
Fibrinogen, coagulation factor I, is made up of three pairs of non-identical polypeptide chains: Aα, Bβ and γ. In a variety of evolutionarily divergent species the γ chain consists of two non-allelic forms, γ and γ' which by tryptic mapping differ at their carboxy termini and are present at a ratio of 10:1 in normal plasma. We have investigated the origin of the γ and γ' chains of fibrinogen by study of the mRNA's genes coding for rat fibrinogen. A cDNA library was constructed from rat liver polyadenylated RNA at the Pst 1 site of pBR322. Clones encoding the γ chains of fibrinogen were identified by nucleic acid sequence. Two homologous cDNA clones for the γ chain of fibrinogen were found. These two clones are identical from the polyadenylation site to a point 12 bp 5' to the stop codon for the γ chain. At this point there is a 530 bp insert in one mRNA which is bordered by the consensus splice sequence: 5' GT - AG 3'. The nucleotide sequence of the two clones are identical for at least 216 bp 5' to this insert. Examination of Southern blots and 21 independent genomic clones isolated by screening 8 x 10^5 plaques from two different rat genomic libraries indicates that a single 12kb region gives rise to both mRNA's and that the nucleotide sequence of the 530 bp region described above is identical to its genomic counterpart. These data indicate that the γ' chain of fibrinogen is produced by translation for 36 nucleotides into a region which acts as an intervening sequence for the 10-fold more abundant γ mRNA.

0878 BINDING SITES FOR RNA POLYMERASE II AND TRANSCRIPTION INITIATION FACTORS ON A FRAGMENT OF ADENOVIRUS 2 CONTAINING THE MAJOR LATE PROMOTER. Paul Hough, David Dignam*, Robert Roeder*, Iris Mastrangelo, Joseph Wall and James Hainfeld, Brookhaven National Laboratory, Upton, NY 11973. *School of Medicine, Washington University, St. Louis, MO 63110.
Eukaryotic RNA polymerase II is incubated with the Sma 'F' fragment of adenovirus 2 under conditions known (1,2) to give selective and accurate transcription beginning at the major late cap site, but with omission of exogenous CTP and UTP. When factors required for accurate initiation (2) are not present, polymerase II binds preferentially at the fragment ends and at a broad distribution of interior sites not obviously favoring the region of the cap site. In the presence of a complete set of initiation factors, preliminary observations show little polymerase binding at the ends (though smaller objects are seen there) and a possibly significant accumulation of large structures about 100 nucleotide pairs farther from an end than the 534 np spacing of the cap site from the end downstream (3). Binding is observed using the Brookhaven STEM (4) and a Philips 300 microscope after spreading via the N_2-discharge treatment of carbon foil (5). The method provides molecular weight and a projected M.W. distribution for bound complexes. Contour lengths for DNA show a standard deviation of about 3% over the range 300-2500 np, 5-fold smaller than Kleinschmidt (6) in the region below 500 np.

1.) Weil, Luse, Segall and Roeder (1979) Cell 18, 469-84. 2.) Matsui, Segall, Weil and Roeder (1980) J. Biol. Chem. 255, 11992-96. 3.) Gingeras et al, personal communication. 4.) Wall (1979) in: Analytical Electron Microscopy, Hren, Goldstein and Joy, Eds. (Plenum). 5.) Hough, Manley et al (1981) subm. J.Mol,Biol. 6.) Davis et al (1971) M. Enzym. 21, 413.

0879 AUXIN-REGULATED GENE EXPRESSION. Joe L. Key, John Walker, Tom Ulrich, Philip Kroner and David Baulcombe, University of Georgia, Athens, GA 30602.
The auxin class of plant hormones is involved directly in the regulation of both cell division and cell enlargement. Auxin has been known for many years to affect RNA synthesis and to require RNA and protein synthesis for its action on cellular processes. Based on DNA/RNA hybridization analyses and in vitro translation of poly(A)RNAs, auxin alters the expression of only a small number of relatively abundant genes out of some 30,000 different sequences expressed in soybean tissues. Recombinant cDNA clones made to poly(A)RNAs from different auxin-responsive tissues have been isolated. Three cloned sequences representing from 0.2 to 1% of the total poly(A)RNA of control tissue are reduced rapidly to less than 0.01% of the poly(A)RNA of auxin-treated tissue. The production of these sequences is also dramatically altered during normal growth transitions in this system. Other clones have been isolated from cDNA banks which are homologous to poly(A)RNAs, the concentration of which is increased rapidly following auxin treatment. The mechanism(s) of regulation by auxin of the production of these mRNAs is under investigation; also the relationship of the regulation of the expression of these mRNAs to auxin-regulated growth processes is being studied.

0880 CLONING OF cDNA AND QUANTITATION OF mRNA FOR AN INDUCED CYTOCHROME P-450
John B. Fagan, Julia V. Pastewka, Sang Shin Park, Fred P. Guengerich and Harry V. Gelboin, Lab. of Molec. Carcinogenesis, NCI, NIH, Bethesda, MD and Vanderbilt School of Medicine, Nashville, TN 37232

The cytochromes P-450 are a family of structurally related enzymes induced by different classes of inducers but having distinct yet overlapping specificities. The P-450s are responsible for the enzymatic activation of many compounds to mutagenic or carcinogenic forms. Not only is the gene regulation of this system of practical interest, but, as well, this system promises to be useful for studying gene families and the molecular mechanisms for the differential regulation of related eucaryotic genes. We have constructed and characterized recombinant plasmids containing DNA complementary to the mRNA for 3-methylcholanthrene (MC) induced cytochrome P-450 and have used this to isolate the corresponding gene from a library of the rat genome. By Northern hybridization, using ^{32}P-labeled P-450 cDNA as probe, we detect a single RNA species about 1900 nucleotides in length that is increased about fourfold after MC treatment.

0881 CONTROL OF DIHYDROFOLATE REDUCTASE mRNA PRODUCTION, Rodney E. Kellems and Eugene J. Leys, Baylor College of Medicine, Houston, TX 77030.

We used methotrexate-resistant mouse cells in which dihydrofolate reductase levels were approximately 500 times normal to study the effect of growth stimulation on dihydrofolate reductase gene expression. As a result of growth stimulation, the relative rate of dihydrofolate reductase protein synthesis increased threefold, reaching a maximum between 25 and 30 h after stimulation. The relative rate of dihydrofolate reductase mRNA production (i.e., the appearance of dihydrofolate reductase mRNA in the cytoplasm) increased threefold after growth stimulation and was accompanied by a corresponding increase in the relative steady-state level of dihydrofolate reductase RNA in the nucleus. However, the increase in the nuclear level of dihydrofolate reductase RNA was not accompanied by a significant increase in the relative rate of transcription of the dihydrofolate reductase genes. These data indicated that the relative rate of appearance of dihydrofolate reductase mRNA in the cytoplasm depends on the relative stability of the dihydrofolate reductase RNA sequences in the nucleus and is not dependent on the relative rate of transcription of the dihydrofolate reductase genes.

0882 IN VITRO TRANSCRIPTION OF RAT AMYLASE GENES, Anne M. Boulet, William F. Swain and William J. Rutter, Dept. of Biochemistry and Biophysics, Univ. of California, San Francisco, California 94143

Rat genomic clones containing partial or complete sequences of seven distinct α-amylase genes have been partially characterized. These genes fall into two groups based on their degree of homology with cDNAs derived from pancreatic and parotid mRNAs. Four genomic clones are closely related to pancreatic mRNA; the remaining clones show significant non-homology with both types of mRNA. The ability of RNA polymerase II to transcribe these genes in an *in vitro* system has been tested. A DNA fragment of one of the pancreas type genes is actively transcribed by RNA polymerase II. This fragment contains the first exon, the first intron and most of the second exon of the gene as well as about 130 nucleotides of 5' flanking sequence. In addition, many of the rat amylase genes are transcribed by RNA polymerase III, giving a transcript of 165 nucleotides. The coding region for one of these transcripts has been identified as a portion of one of the introns flanking the seventh exon in a pancreatic type gene. A sequence homologous to the polymerase III transcript has been found in the corresponding region of all four pancreas type genes, and related sequences are also found in other regions of these genomic clones. However, this sequence has been modified in or is absent from the non-pancreatic genes. It appears that many of these sequences are transcribed *in vitro*, but a homologous sequence located in the 5' flanking region of one of the genes is not transcribed by RNA polymerase III.

0883 DEVELOPMENTAL EXPRESSION OF THE GENES CODING FOR THE BOVINE PITUITARY HORMONES, John H. Nilson+, Jeffrey B. Virgin+, and Fritz M. Rottman*, Departments of Pharmacology+ and Microbiology*, Case Western Reserve University, Cleveland, Ohio 44106

Cloned prolactin (PRL) and growth hormone (GH) DNAs have been used in conjunction with "Northern" transfer to quantitate the levels of their respective mRNAs in fetal bovine pituitary glands. The levels of PRL mRNA, relative to total cellular RNA, increased exponentially in both males and females from 90 to 200 days of gestation (parturition occurs at 280 days). The most pronounced shift in PRL mRNA was 10-fold, and occurred over a two week interval at midgestation. In contrast, GH mRNA levels increased much more slowly during gestation, and appeared to do so in a linear fashion. Furthermore, cell-free translation of fetal RNA revealed that the relative concentration of GH mRNA exceeded that of PRL mRNA at any time during gestation. This is in contrast to the adult pituitary, where PRL mRNA levels exceed those of GH mRNA.

Recently, we have isolated several pituitary cDNA clones which cross-hybridize with cDNA prepared from mRNA of human, first-trimester placenta. Because the α-subunits of the human and placental glycoprotein hormones are identical, it is possible that cDNA from the human placenta is cross-hybridizing to clones which contain the common α-subunit of the bovine pituitary glycoprotein hormones. These pituitary clones will be further characterized by positive-selection-translation and then used to quantitate the levels of their respective mRNAs as a function of development.

0884 A MODEL FOR REGULATION OF SV40 LATE GENE EXPRESSION. Janet E. Mertz, University of Wisconsin, Madison, WI 53706. SV40 late strand synthesis is initiated at numerous sites spanning a 300 nucleotide region. The mRNAs synthesized by viable late leader region mutants differ in complex ways from those made by wild type in both their 5' termini and RNA splicing frequencies. To explain these findings, I hypothesize that the beginning of the late region contains several overlapping, yet independently functioning, promoter-regulatory regions, each responsible for controlling synthesis of one of the late virus-coded proteins via the following series of events: (1) Based upon a number of factors (e.g.s, the relative strength of the promoter, the tandem repeats region, non-histone chromosomal factors, the presence or absence of positive and/or negative regulatory elements including T antigen), each promoter-regulatory region independently determines how frequently and precisely where transcription initiates; (2) The precise primary structure of the resultant initial transcript strongly, although not necessarily absolutely, influences the choice of splice sites used in processing; (3) The precise sequence of the leader region of the resultant mRNA determines which downstream translational initiation codon is used for protein syntehsis. By regulating expression of each late gene independently, SV40 may be able to guarantee synthesis of its capsid proteins in the molar ratios needed to maximize virion production.

0885 YEAST $tRNA_3^{Leu}$ GENE TRANSCRIBED AND SPLICED IN MAMMALIAN CELL EXTRACT, David N. Standring, Patricia A. Tekamp, Alejandro Venegas & William J. Rutter, Dept of Biochemistry & Biophysics, University of California, San Francisco, Ca. 94143

A cloned yeast $tRNA_3^{Leu}$ gene containing a 33 base intervening sequence (IVS_{Leu}) is selectively transcribed by a soluble extract from HeLa cells. The 130 nucleotide $tRNA_3^{Leu}$ precursor RNA formed is colinear with the gene and contains approximately 4 leader nucleotides and up to 6 trailer nucleotides. The IVS is accurately and efficiently removed by an endogenous HeLa excision-ligase activity yielding the spliced tRNA, the free IVS, and the half-tRNA intermediates. The splicing reaction occurs without prior 5' and 3' maturation of the precursor$_{Leu}$ but, with this exception, this pattern of synthesis and subsequent maturation of the $tRNA_3^{Leu}$ precursor conforms to the scheme for tRNA biosynthesis deduced in the Xenopus oocyte system (Melton et al. (1980). Nature 284, 143-148): indeed, the two systems utilize a similar or identical $tRNA_3^{Leu}$ precursor. This result stresses the extraordinary conservation of tRNA biosynthesis in eucaryotes. We have also characterized a 3' processing enzyme (RNAse T) in yeast which precisely matures both the $tRNA_3^{Leu}$ precursor and the spliced immature product. This activity has been partially purified and shown to be an exonuclease. It appears to be different from the endonucleolytic activity previously shown to mature pre-5S RNA (Tekamp et al. (1980). J. Biol. Chem. 255, 9501-9506).

0886 RECOMBINANT RNA AS TEMPLATES FOR Qβ REPLICASE, Eleanor A. Miele, Donald R. Mills and Fred Russell Kramer, Department of Human Genetics and Development and Institute of Cancer Research, Columbia University, New York, New York 10032

T4 RNA ligase was used to join MDV-1 RNA, a small 221-nucleotide RNA template for Qβ replicase, to heterologous RNA sequences, in order to test the template activity of the composite RNAs. Heterologous sequences have been added to the 5' and 3' ends of the MDV-1 RNA sequence as well as to an internal site in MDV-1 RNA. Addition to the ends was accomplished by melting the donor RNA in the presence of spermidine, prior to ligation. The foreign sequence was inserted by site-specific cleavage of the RNA in an RNA:DNA heteroduplex, followed by ligation of the heterologous sequence to the 3'-RNA fragment and religation of the half-molecules. Since the ligations were performed with the RNA fragments hybridized to the intact DNA backbone, religation was extremely efficient. Addition to the 3' end and to the internal site of MDV-1 RNA suppressed its template activity. Addition to the 5' end of MDV-1 RNA was compatible with continued template activity, and supported read-through of the heterologous sequence in the complementary product. This read-through ability should allow rapid sequencing of heterologous RNAs using Qβ replicase with deoxyribonucleotide chain terminators. It may also be possible to use this technique for direct *in vitro* amplification of RNAs.

0887 *IN VITRO* TRANSCRIPTION OF *DROSOPHILA MELANOGASTER* tRNA GENES, George B. Spiegelman, Bhanu Rajput, Dianne DeMille and Robert C. Miller, University of British Columbia, Vancouver, B.C., V6T 1W5, Canada.

A homologous *in vitro* transcription reaction which transcribes Drosophila genes has been developed and characterized. The reaction utilizes S-100 extracts from Schneider-2 cells as a source of RNA polymerase III and cloned *Drosophila* DNA carrying tRNA genes. The extracts are highly active and carry out accurate synthesis of tRNA. Processing of the primary *RNA* product is also detectable.

Rates of RNA production as measured by formation of specific products, differ greatly depending on the DNA template introduced. Template DNA carrying val_{3B}, val_4 and ser_7 have been examined along with recombinant DNAs containing *Drosophila* sequences which differ from isolated isoacceptors by a few nucleotides ("varients"). Whether these "varient" sequences are transcribed *in vivo* is not known. A comparison of the DNA sequences in the templates has suggested nucleotides internal and external to the transcript coding region which influence transcription rate.

0888 TRANSCRIPTION FROM THE CHORION GENE REGIONS OF *DROSOPHILA MELANOGASTER*, Suki Parks and Allan C. Spradling, Carnegie Institution of Washington, Baltimore, MD 21210

The chorion of *Drosophila melanogaster* is synthesized by the ovarian follicle cells during the terminal five hours of oogenesis. It has been demonstrated taht the twenty major structural proteins are synthesized according to specific developmental programs during this time. Four chorion protein genes have been identified and localized. These genes are clustered in two areas which map to the X chromosome at 7F and the third chromosome at 66D. The chromosomal regions containing these clusters are selectively amplified in the follicle cells. Amplification begins prior to chorion protein mRNA synthesis and reaches maximal levels two hours before chorion completion.

We have found additional transcripts from these amplified regions which are expressed in choriogenic stage egg chambers. Northern analysis of the chorion gene transcripts and their neighboring transcripts has been utilized to discern the temporal program of expression. These studies demonstrate that the transcripts are expressed transiently during choriogenesis. Individual genes within each cluster show variation in patterns of accumulation suggesting that these genes may be under individual control.

The mutation *ocelliless* is a chromosomal inversion of the 7F1,2 - 8A1,2 interval which affects the differential replication of the X chromosome chorion gene region. The area lying distal to the 7F1,2 breakpoint no longer amplifies. Transcription analysis in this unamplified region demonstrates that the differential replication is not required for production of at least two transcripts.

0889 STUDY OF TRANSCRIPTION AND PROTEIN DISTRIBUTION ON XENOPUS LAMPBRUSH CHROMOSOMES. Milan Jamrich and Rahul Warrior, Yale,KBT 418, New Haven, Conn. 06511

Xenopus laevis lampbrush chromosomes were reinvestigated and found to be suitable for analysis of transcription by ^{3}H-uridine incorporation, in situ hybridization, as well as for the analysis of protein distribution by the indirect immunofluorescence technique.

Single stranded ^{3}H labelled cRNA probes of α-globin, histones and satellite sequences were prepared and hybridized to the nascent RNA transcript on these lampbrush chromosomes. The resulting labelling suggests that all of these sequences are transcribed on the lampbrush loops. Alternative explanations for this labelling are under investigation.

Most, if not all of the loops incorporate ^{3}H-uridine and so are most likely involved in transcription. Indirect immunofluorescence analysis of these chromosomes revealed that these loops carry transcripts already associated with RNP proteins. These results are in good agreement with experiments of Gall and Callan, P.N.A.S 49,544 and Okamura and Martin, J. Cell Biol. 83, 2342, as well as with our observation that RNA polymerase II is localised on the loop region of Triturus lampbrush chromosomes. A comparison of staining patterns of different antibodies on polytene chromosomes of Drosophila melanogaster and amphibian lampbrush chromosomes suggests that the bands of polytene chromosomes are functionally similar to the condensed main axis of lampbrush chromosomes, whereas the puffs and interbands are similar to the loops.

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0890 TRANSCRIPTION OF REPEATED DNA BY RNA POLYMERASE III, John J. Furth, Juel Ormsby, Chun-Yeh Su and Vera Averyhart-Fullard, University of Pennsylvania, Philadelphia, PA 19104

Transcription of calf DNA has been examined using purified calf thymus RNA polymerase III and RNA polymerase III in a HeLa cell crude extract clarified by high speed centrifugation. RNA was evaluated by polyacrylamide electrophoresis on denaturing gels and compared with RNA transcribed from DNA of a plasmid containing the Xenopus laevis oocyte 5S gene. While only a small amount of 5S RNA is transcribed from a calf DNA template by purified enzyme, 5S RNA represents a major fraction of the RNA synthesized by enzyme in crude extracts. No 5S RNA synthesis is detected with calf chromatin as template. RNA transcribed from calf DNA by purified RNA polymerase III was also evaluated by hybridization to Southern blots of DNA digested with restriction enzymes. Eco Rl digestion of calf DNA results in a 1400 bp DNA fragment. This fragment is derived from the 1.714 g/cc^{3}satellite. Purified calf RNA polymerase III transcribes RNA which hybridizes to this DNA fragment. If the purified polymerase has lost factors required for specificity, as indicated by poor transcription of the 5S ribosomal gene, transcription of the satellite DNA suggests that it contains a fortuitous promoter region for "core" RNA polymerase, or, alternatively, co-purification with the polymerase of components required for transcription of this DNA. Since calf satellite DNA has a complex structure, possibly including regions from which transcription may be initiated, transcription in vitro of satellite DNA is consistent with the possibility that portions of this DNA may be genetically active. (Supported by grant GM10390 from the NIH USPHS).

0891 CELL FREE TRANSCRIPTION OF HEPATITIS B VIRUS GENES, Leslie B. Rall, Orgad Laub and William J. Rutter,University of California, San Francisco, CA 94143

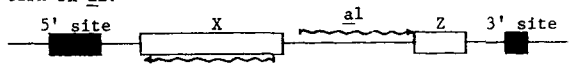
The hepatitis B virus (HBV) is a 42 nm sphere referred to as the Dane particle. It contains an outer surface antigen (HBsAg) and an inner core protein (HBcAg) that is protamine-like in structure and an endogenous DNA polymerase activity. The coding strand of the viral DNA is unit length (about 3200 bases) and nicked. The short strand overlaps the nick by about 300 bases and extends to varying degrees in the 3' direction. The virus has been cloned and sequenced, and therefore putative regions specifying the initiation of transcription have been identified. Since a reproducible in vitro system for viral replication is not available, we we have used HeLa cell-free extracts to demonstrate specific transcription from both the core and surface genes. In both cases experiments with a range of truncated gene fragments show that the site of initiation of transcription corresponds to the location predicted from the TATAA-like sequences. A second origin of HBsAg transcription is observed about 50 bases upstream from the first site. S1 mapping experiments have been carried out to precisely locate the 5' ends of the various in vitro transcripts and these results are compared with those found using poly A^{+} RNA isolated from a hepatoma cell line (Alexander cells) that produces large amounts of HBsAg.

Genomic Organization and Chromosomal Structure

0892 REGULATION OF MATING-TYPE INFORMATION IN YEAST: ACTION AT A DISTANCE, Judith Abraham, Kim A. Nasmyth, Jeffrey N. Strathern, Amar J. S. Klar, and James B. Hicks, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The genome of the yeast Saccharomyces cerevisiae contains three copies of mating-type information: an expressed copy (MAT), and two silent copies (HML and HMR). In cells carrying the homothallism (HO) gene, a copy of the information at HML or HMR can be transposed to the MAT locus, resulting in a switch in the mating type of the cell. The information at HML and HMR is normally kept silent by the action of four unlinked genes termed MAR or SIR; in mar⁻ strains, all three copies of mating-type information are expressed.

By in vitro mutagenesis of a plasmid carrying a cloned copy of HMR, we have identified two regions required for the control of HMR by MAR, one lying about 1000 bases upstream from the 5' end of the a1 gene at HMR, and one lying about 400 bases downstream from the 3' end of a1. Loss of MAR regulation by mutation of either of these two sites appears to be accompanied by a change in chromatin structure: plasmids carrying HMR have a higher supercoil density in MAR⁺ strains than in mar⁻ strains, but this topological difference is partially or completely lost in plasmids carrying mutations in the 5' or 3' sites. MAR regulation of HMR also depends on the distance between the 5' and 3' sites, in that insertions or duplications of DNA between the two sites result in partial or full expression of a1. Deletions within the X or Z regions (see figure) do not turn on a1.



0893 CHARACTERIZATION OF FUNCTIONAL GENES FOR CELL-SURFACE ANTIGENS INVOLVED IN HUMAN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), John W. Chamberlain, G.B. Price, T. Lam, S.S. Stewart and Clifford P. Stanners, Ontario Cancer Institute, Toronto, Canada M4X 1K9.

Two molecular clones, (HSAG-1 and 2), capable of transforming mouse L cells to produce a cell-surface antigen which correlates with human CLL have been isolated from a gene library of a hybrid cell (HCH-1) between a quasi-normal CHO cell (L73) and human CLL lymphocytes. This was accomplished by using a monoclonal antibody specifically reactive with HCH-1 and a fraction of CLL lymphocytes, coupled with fluorescent-cell analysis to identify small portions of the library containing functional genes for antigen expression, followed by isolation of clones containing human-specific reiterated sequences. Both clones also contain CHO-specific reiterated sequences and thus are human-CHO recombinants. The regions of HSAG-1 and HSAG-2 responsible for antigen expression have been localized by a combination of restriction enzyme and "Southern blot" hybridization analyses. Although similar, the corresponding regions are not identical, hybridizing only under non-stringent conditions. Using this antigen-producing region as a probe on HCH-1 DNA suggests a copy number for this sequence ranging from 100-1000. This agrees with the functional copy number obtained from DNA transfer experiments using the hybrid cell library. Preliminary results from "Northern" analysis of cytoplasmic mRNA suggests a high level of heterogenous mRNA molecules homologous to the HSAG-1 antigen-producing fragment in the hybrid cell compared to the CHO parent. Taken together, these results indicate that HSAG-1 and 2 are two different members of a large gene family whose expression is dramatically altered in human CLL. The results of further "Northern" and "Southern" analyses of normal and CLL RNA and DNA will be presented.

0894 HORMONAL REGULATION OF GENE EXPRESSION IN THE MURINE SUBMAXILLARY GLAND. James Scott & William J. Rutter, Dept of Biochem & Biophys, Univ of Cal., San Francisco, Ca 94143

In the mouse submaxillary gland the biosynthesis of a number of the products is under androgenic control. Androgen-regulated glandular products include: epidermal growth factor, nerve growth factor, renin, kallikrein, a number of other serine proteases, and glucose 6-phosphate dehydrogenase. In order to define the hormonally responsive regions of the genome, a cDNA library corresponding to mRNA from the adult male mouse (Swiss Webster strain) submaxillary gland has been constructed. Male specific clones representing 10% of mRNAs (1600 to 450 bases) have been selected by differential hybridization with adult male and juvenile female submaxillary gland cDNA probe. We are currently analyzing these cDNA clones.

0895 TRANSCRIPTION OF SATELLITE DNA IN THE NEWT, NOTOPHTHALMUS VIRIDESCENS, Kathleen A. Mahon and Joseph G. Gall, Yale University, New Haven, CT 06511

By in situ hybridization of cRNA probes to nascent RNA transcripts on the loops of lampbrush chromosomes, we have determined that both strands of a tandemly repeated, 222 base pair satellite DNA are transcribed in oocytes of Notophthalmus viridescens. Because this satellite flanks the histone gene clusters, we proposed that transcription in this region initiates on a histone gene promotor and proceeds uninterruptedly into the adjacent repetitive sequence (Cell, 24,639-647; 649-659, 1981).

S1 nuclease protection experiments with cloned satellite DNA now confirm that both strands of the satellite are transcribed in oocytes. In these experiments, RNA from hand isolated germinal vesicles and defolliculated, enucleated oocytes was hybridized to a labeled, single stranded satellite DNA probe. The hybrids were digested with S1 nuclease, and the protected DNA fragments analyzed on a polyacrylamide denaturing gel. In all cases the protection was insensitive to prior DNase treatment, but sensitive to RNase. Satellite transcripts were abundant in the germinal vesicle, but were not found in the cytoplasm of mature oocytes. Apparently, satellite DNA is transcribed in the germinal vesicle but the transcripts are either not transported to the ooplasm, or they are unstable there. A satellite DNA probe was also protected from S1 nuclease digestion by testis RNA. Thus expression of the satellite is not oocyte specific. We are currently analyzing RNA from other tissues for the presence of satellite transcripts. Supported by NIH Research Grant GM 12427.

0896 THE CONTROL OF VIRAL GENE EXPRESSION BY THE ADENOVIRUS E1A GENE, Nicholas C. Jones and Daniel L. Weeks, Department of Biological Sciences, Purdue University, W. Lafayette, IN 47907. Region E1a of the adenovirus genome encodes a product required both for oncogenic transformation of susceptible tissue culture cells and for the expression of the viral genes E1b, E2, E3 and E4 (Jones and Shenk, PNAS 76, 3665; Berk et al., Cell, 17, 935). In order to determine whether regulation of these genes occurred at a transcriptional or posttranscriptional level, we constructed plasmids containing chimeric genes where the upstream, promotor sequences of regions E2, E3 and E4 were linked to either the tk gene of Herpes Simplex Virus or the neomycin phosphotransferase II gene of the bacterial transposon Tn5. Expression of this latter gene confers resistance to the antibiotic G418. The expression of these chimeric genes in the presence and absence of the viral E1a gene product has been tested by 1) measuring the ability of the genes to transform tk^- cells to tk^+ or to transform G418 sensitive cells to G418 resistance, and 2) inserting the chimeric genes into a non-essential region of the Ad5 genome and studying the expression of these genes during viral infection by the S1 mapping technique.

The preliminary results demonstrate that the expression of the chimeric genes is regulated by E1a, demonstrating 1) that E1a regulation of gene expression occurs at the level of transcription and 2) that the upstream, promotor regions of the regulated genes are necessary and sufficient for this regulation. These results, together with our progress towards identifying the specific sequences involved in this regulation, will be presented.

0897 CHARACTERIZATION AND REGULATION OF THE CHICK α1(TYPE III) COLLAGEN GENE. Yoshihiko Yamada, Maria Mudryj, Silvana Obaisai, Margery Sullivan, Ira Pastan and Benoit de Crombrugghe, Laboratory of Molecular Biology, NCI, NIH, Bethesda, MD., 20205.

We have isolated the chick α1(type III) collagen gene, by stepwise screening of a chick genomic library, in a series of overlapping clones which span 50 Kb in length. The first of these clones was identified by comparing the DNA sequence of three different genomic segments with the known amino acid sequence of homologous regions in bovine type III collagen. The sequence of the segment which codes for the C terminal part of the hexical region shows features characteristic of type III collagen. The DNA of this clone hybridizes to crop RNA but not to calvaria RNA, in agreement with the presence of type III collagen in crop but not in calvaria. The size of α1(type III) collagen mRNA is somewhat larger than α1(type I) collagen mRNA. The size of two exons coding for portions of the helical segment of type III collagen agrees with our previous proposal that the ancestral collagen gene arose by amplification of a single genetic unit containing an exon of 54 bp. The amino acid sequence derived from the DNA sequence coding for the C-propeptide of chick type III collagen shows important similarities with that of the C-propeptide of chick α1 and α2(type I) collagen. The intron-exon organization of this region of the gene is similar to that of α2(type I) gene except that the last exon is larger than in the α2(type I) gene. The levels of type III collagen mRNA are severely reduced when primary cultures of 11-day chick embryos are infected with Rous sarcoma virus. This gene thus shows a similar structure and a similar regulation by $p60^{src}$ as the gene for α2(type I) collagen.

0898 DEOXYRIBONUCLEASE I SENSITIVITY OF FUNCTIONAL AND PSEUDO GOAT β-GLOBIN GENES, Paul A. Liberator and Jerry B. Lingrel, University of Cincinnati, Cincinnati, Ohio 45267
The goat serves as a good experimental model system for studies directed towards understanding the mechanism underlying the developmental expression of globin genes. Early in embryonic life, $\alpha_2\zeta_2$ is synthesized. At about day 21 following conception, this hemoglobin is replaced by HbF, $\alpha_2\gamma_2$ which, in turn, is replaced just prior to birth by HbC, $\alpha_2\beta_2^C$. During the first year of life, HbC is replaced by HbA, $\alpha_2\beta_2^A$. This laboratory has isolated and studied the sequence organization of these genes from a goat genomic recombinant library. With this information at hand, we are determining whether or not differences in structure at the chromatin level are related to differential β-globin expression. Digestion of fetal liver nuclei with DNase I demonstrated that not only the γ gene, but also the β^A- and β^C-globin genes are in a conformation which is accessible to the nuclease. Moreover, the bands corresponding to these three genes in a genomic Southern blot were digested at nearly identical rates. On the other hand, the goat pseudo-β-globin gene $\Psi\beta^X$, is resistant to digestion by this nuclease. This raises two important points. First, the pseudogene resides in a closed conformation in this tissue, a situation very much different from the three functional β-globin genes which were investigated. Second, because the pseudogene is located approximately 8kb upstream from β^C in genomic DNA, these findings roughly outline the 5' boundary of a chromosomal domain for the β^C-globin gene.

0899 REPETITIVE SEQUENCES IN THE HUMAN COLLAGEN GENES. Jeanne C. Myers, Mon-Li Chu, Francesco Ramirez, CMDNJ-Rutgers Medical School, Piscataway, N.J. 08854
Overlapping clones of the proα1(I) and proα2(I) collagen genes have been isolated from human genomic libraries. Both genes have been found to contain two repetitive sequences positioned in similar areas of the genes. Southern blotting and heteroduplex analysis show no homologies among these four sets of sequences. The first repeat is located in a large intron of the 3' end portion of the triple helical region of the proα2(I) gene. DNA sequencing of 400 base pairs show that the segment exhibits 80% homology to a member of the Alu I family. In situ hybridization studies demonstrate a specific light G banding pattern throughout human metaphase chromosomes indicating that these sequences are represented in actively transcribing areas. Northern blotting analysis using genomic fragments as probes show a slightly different localization of the second repeat in the two genes. In the proα2(I) it is located adjacent to the 3' terminus of the gene while in the proα1(I) the repeat is positioned at the end of the 3' untranslated region. Both proα2(I) repeats hybridize exclusively to an RNA species greater than 10kb far exceeding the 6kb size for mature collagen mRNA. The nature and function of these sequences interdispersed in the eucaryotic genome is unclear. However it should be emphasized the finding of these repeats in the same location in these closely related genes which furthermore are on two different chromosomes. It has been proposed that these sequences signal hotspots of recombination. Our preliminary experiments in the analysis of genomic DNA from a patient affected with a type I collagen disorder tend to support this hypothesis.

0900 REPETITIVE DNA NEAR IMMUNOGLOBULIN LIGHT CHAIN GENES, Ronald E. Wilson, Kindred A. Ritchie, Jeffrey Voss, and Ursula Storb, Univ. of Washington, Seattle, WA 98195
Extensive data derived from the cloning of eukaryotic genes has served to confirm previous liquid hybridization data showing that single copy DNA and repetitive DNA sequences are interspersed. The biological function of these repetitive DNA (rep DNA) sequences remains unclear. We examined a variety of mouse immunoglobulin (Ig) gene clones and found them to possess several regions containing rep DNA sequences. To determine whether these various rep DNA elements share homology, certain rep DNA containing restriction fragments were subcloned in pBR322, and were then used as probes in Southern blot analyses of a C-kappa, two V-kappa, and two C-lambda gene clones. Two different subclones of rep DNA sequences near a V-kappa gene shared no homology with each other or with any other rep DNA regions found on the set of Ig gene clones. One subclone of rep DNA sequences near the C-kappa gene behaved similarly. However, another C-kappa associated rep DNA element, contained in plasmid pRE103, hybridized to portions of all L-chain clones tested. While the nucleic acid sequence of pRE103 exhibits no striking homology to the major portion of the sequence of the mouse analog of the human Alu family DNA (Krayev et al. (1980) Nucl. Acid Res., 8, 1201), it does exhibit considerable homology to a 14 base pair consensus sequence derived from the Alu family sequences, sequences at or near certain viral replicon origins, and sequences of some small nuclear RNAs as described by Jelinek et al. (PNAS (1980) 77, 1398), indicating that this element could function as a replicon origin or a small nuclear RNA gene. Prelimenary data suggests that pRE103 sequences are found in cytoplasmic RNA of myelomas, but not in liver, implying possible involvement in Ig gene expression.

0901 CLEAVAGE OF INTERNUCLEOSOMAL REGIONS OF CHROMATIN BY CHEMICAL AGENTS, Iain L. Cartwright and Sarah C.R. Elgin, Washington University, St. Louis, MO. 63130.
One of the most intriguing problems in studies of chromatin structure concerns the positioning of nucleosomes with respect to given specific DNA sequences. Micrococcal nuclease has been the most commonly used probe of nucleosome positioning because of its well-known and diagnostic production of oligonucleosomal ladders from eukaryotic chromatin. However, problems of interpretation may arise because of the reported sequence-specificity of this enzyme. We have found that cleavage of chromatin of Drosophila embryo nuclei by 1,10-phenanthroline-cuprous complex produces an oligonucleosomal pattern virtually indistinguishable from that of micrococcal nuclease treated nuclei, and that chromatin-specific information can be derived from different regions of the genome, e.g. the heat-shock locus 67B. Although model studies have led to the conclusion that this agent cleaves DNA in a relatively sequence-independent manner, it is of interest that certain sequence-specified features of protein-free DNA are recognized by this chemical cleavage reagent in common with micrococcal nuclease.

0902 DELETIONS AND POINT MUTATIONS THAT PREVENT TRANSPOSITION OF YEAST MATING TYPE GENES. James Haber, Barbara Weiffenbach and David Rogers, Brandeis University, Waltham, MA 02254 and Mark Zoller, David Russell and Michael Smith, University of British Columbia, Vancouver, Canada.

Homothallic switching of the mating type (MAT) locus of Saccharomyces cerevisiae occurs by the transposition of copies of mating type sequences from one of two "silent genes", HMLα or HMRa. The initiation of MAT switching may involve the generation of a double stranded DNA cut at a specific site within the MAT locus. This site has been identified in several ways: 1) homothallic strains carrying the DNA repair mutation , rad52, suffer a lethal chromosome break at MAT when cells attempt to switch mating type. 2) Rare survivors that attempted switching in HO rad52 MATα cells have been recovered. These have proven to be variable sized deletions, all of which both prevent mating type switching and also are mutations in the MATα1 cistron. All of the deletions remove a HhaI site very close to the junction of α-specific (Yα) and common (Z1) sequences in the MAT locus. 3) Two single base pair mutations (MATα-inc and MATa-inc) that also prevent switching have been cloned and sequenced. Both changes are in the region covered by the deletions. We conclude that the sequence CGCAAC is at least part of the recognition sequence for MAT switching.

The MATa-inc mutation also prevents excision of a tandem duplication of MAT genes (MATa-URA3-pBR322-MATa-inc) created by transformation and integration of a recombinant DNA plasmid. The normal, efficient excision of the tandem duplication in HO HMLa HMRa cells results in a Ura⁻ colony. Mutations that prevent such switching remain Ura⁺. This nutritional screen has provided a powerful new method to select for new switching mutants.

0903 CHROMOSOMAL LOCALIZATION OF RAT HORMONES PROLACTIN AND GROWTH HORMONE BY HYBRIDIZATION IN SITU, Daniela S. Gerhard, Paul Szabo, Ernest S. Kawasaki* and F. Carter Bancroft, Sloan-Kettering Institute, NY 10021

Growth hormone and prolactin are two peptide hormones, having amino acid homologies, that are synthesized by the anterior pituitary and are controled by number of neuroendocrine signals. We have used ^{125}I-labeled cDNA plasmids for rat prolactin (pPRL-1) and growth hormone (pGH-1) for gene localization studies to metaphase chromosomes. The hybridization method employed in these studies relies on the formation of probe networks and has been shown to be sensitive enough to detect single copy sequences (PNAS 78, 3755, 1981). Analysis of the silver grain distribution over metaphase cells,hybridized with pPRL-1, suggested that the gene coding for prolactin is located near the telomere of an acrocentric chromosome, probably chromosome 9 (based on length and the residual G-banding observed after hybridization). Two additional chromosomes, 5 and 15, were also found to be labeled with pPRL-1 but these were also labeled in control experiments using pBR322 under the same hybridization conditions. The pattern of hybridization with pGH-1 was more complicated; besides the sites labeled by pBR322 several potential gene sites were observed and are currently being identified. The sites labeled by pGH-1 are not linked to the prolactin gene site.

*Present address: Cetus Corporation, Berkeley, CA

0904 PSEUDOGENES FOR HUMAN SMALL NUCLEAR RNA U1, U2, AND U3: EVIDENCE THAT CELLULAR RNA SPECIES CAN BE REVERSE TRANSCRIBED IN NORMAL HUMAN GERMLINE CELLS AND INTEGRATED AT NEW CHROMOSOMAL SITES, Alan M. Weiner, Laurel B. Bernstein, Scott W. Van Arsdell, and Richard A. Denison, Yale Medical School, New Haven, CT 06510

Pseudogenes for the small nuclear RNAs U1, U2, and U3 are both abundant and dispersed in the human genome [Denison, Van Arsdell, Bernstein and Weiner (1981) PNAS 78, 810], and many of these pseudogenes are exactly flanked by perfect short direct repeats of 16 to 19 base pairs [Van Arsdell, Bernstein, Denison, Weiner, Manser and Gesteland (1981) Cell 26, 11]. With one exception, in each such pseudogene the upstream direct repeat immediately precedes the 5' end of the snRNA sequence, while the downstream direct repeat immediately follows the truncated 3' end of the snRNA sequence. To explain these observations, we proposed a model in which snRNA pseudogenes are generated at some stage of human germline development by reverse transcription of the snRNA and subsequent integration of the cDNA at a staggered break. In support of this model, we have recently found that avian myeloblastosis virus (AMV) reverse transcriptase will catalyze the self-primed reverse transcription of pure human U3 snRNA *in vitro* to yield a single discrete cDNA which exactly spans the 5' fragment of U3 snRNA found in the U3.5 pseudogene. U3 snRNA can prime its own reverse transcription because the secondary structure of U3 places the 3' end of the RNA in a base-paired stem adjacent to a long single-stranded region (S. Mount, unpublished work). Self-primed reverse transcription would also explain why 3 out of 3 U3 pseudogenes examined have the same characteristic 72 base 5' fragment of the snRNA sequence flanked by different direct repeats.

0905 AMPLIFICATION OF THE SKELETAL ALPHA ACTIN GENES DURING MYOGENESIS, Robert J. Schwartz and Warren E. Zimmer, Jr., Baylor College of Medicine, Houston, Texas, 77030

The behavior of actin gene elements was studied during chicken myogenesis. Nuclear DNA samples from adult tissues as well as from embryonic and cultured myoblast stages were digested with *Eco* RI and analyzed with the Southern transfer procedure using a cloned skeletal muscle α actin cDNA probe. DNA from adult tissues and prefusion myoblasts yielded ~ seven autoradiographic bands, of which the 20Kb band was identified as containing the α actin gene. In DNA from fusing myoblasts (embryonic and in culture) two new bands were found. The 7.45Kb and the 5.4Kb. *Eco* RI bands were 8 and 85 fold greater in content than the α actin gene. Digestion of the amplified DNA with *Hha*I and *Hpa*II revealed a series of fragments which were characteristic of the chicken actin gene. Thus, the α actin gene dosage changes at least 90 fold and the amplified actin DNA sequences contains new *Eco* RI sites. The amplified DNA dissapeared from myotubes within 24 hours of its greatest concentration. The gain and loss of the extra actin genes closely paralleled the rise and fall of the α actin mRNA concentration. Thus, amplified DNA may be an intermediate in the developmental expression of α actin.

0906 CONSIDERATIONS OF THE MECHANISM OF IMMUNOGLOBULIN κ LIGHT CHAIN GENE REARRANGEMENTS. Brian G. Van Ness, Robert P. Perry and Martin Weigert. Institute for Cancer Research, Philadelphia, PA 19111.

We have carried out a systematic analysis of the fate of the DNA between V_κ and J_κ genes in cells that have rearranged κ loci. The DNA from a variety of κ producing plasmacytomas, λ producing hybridomas and κ expressing lymphocytes was digested, size fractionated and analyzed with two probes containing sequences 5' of J_κ. In 13 of 28 plasmacytomas examined the rearrangement of V_κ and J_κ appears to be accompanied by loss of DNA upstream of J_κ. However, in the rest of the plasmacytomas one or more upstream sequences are retained in a new context. In 9 of 12 λ producing hybridomas (which frequently rearrange both κ loci) one or more upstream segments were detected. These unique fragments were probably generated by a recombination event near or at the J_κ region. The extent to which the region between V and J is maintained in κ expressing lymphocytes was also measured. Most (76%) of the region upstream of J_κ is retained in the population, even though 68% of the κ loci are rearranged. We have cloned one of the upstream fragments. The sequence of this fragment contains the sequence 5' of J_1 followed by the sequence typically found 3' of V regions, suggesting it was generated as a product of a V-J fusion. That such DNA is maintained in the lymphocyte population could mean that the DNA between a recombining V and J is in the form of an episomal element. Alternatively, a more attractive possibility, which is consistent with our data, is that these elements could be retained if the V-J joining process involves unequal sister chromatid exchange.

0907 TRANSPOSABLE ELEMENTS IN HUMAN CELLS, Donald L. Robberson, Bruno Calabretta, Hugo A. Berrera-Saldana, Thymios P. Lambrou and Grady F. Saunders, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Tx. 77030. The Alu sequence family members could serve as long terminal repeats of human transposable elements in analogy with transposable elements of lower eukaryotes. In order to test this hypothesis, we have investigated segments of DNA enriched in Alu repeat sequences. A clone, λH15, with a 15 Kb human DNA insert, was found to contain at least ten Alu family repeat sequences in complex arrangement. A DNA segment within the clone, 0.8 KB in length, devoid of Alu sequences but flanked on both sides by Alu sequences in clustered arrangement was then used to investigate the general question of genetic rearrangement involving repetitive sequences. Two distinct aspects of DNA rearrangements consisting of restriction fragment length polymorphism and variation in copy number were detected by blot hybridization. Such patterns were observed among DNAs derived from normal donors, between normal and leukemic donors, and among DNAs derived from different organs of the same individual. It was also found that sequences hybridizing to the 0.8 Kb fragment occur also as extrachromosomal circular duplex DNA molecules and that they contribute substantially to the DNA rearrangements we detect. Various size classes of circular molecules containing Alu repeat sequences have been identified from different tissues. The copy number of these circular molecules range from less than one up to one hundred per cell, implying selective amplification of particular size classes of extrachromosomal DNA. The amplification observed could represent an intermediate stage preceding transposition.

0908 ISOLATION AND CHARACTERIZATION OF A NEUROTRANSMITTER GENE FAMILY FROM APLYSIA, Richard H. Sheller, James F. Jackson, Linda B. McAllister and Richard Axel, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Understanding molecular mechanisms utilized by nervous systems represents one of the ultimate challenges of modern molecular biology. Historically invertebrates have been particularly amenable to neurophysiological studies due to the large size and accessibility of their neurons. We have therefore begun to study the genes encoding peptide transmitters from the bag cell neurons of Aplysia. These neurons are responsible for governing the egg laying behavior via at least a few and probably many short neuropeptide transmitters. Using differential screening with cDNA from various tissues including the bag cells we have isolated a gene family from a CH4 genomic library encoding the 36 amino acid egg laying peptide. Hybridization selection and in vitro translation followed by immunoprecipitation reveal three different precursor peptides in the bag cells and a fourth in the atrial gland. R-looping, Northern blot analysis and nucleotide sequencing have allowed us to assign some of the various genes of the family to mRNAs and peptide precursors. Further studies on cell specific expression, processing, and development will help delineate mechanisms of synaptic transmission of neuropeptides and provide functions for the various precursors.

0909 AN ALU-LIKE MIDDLE REPETITIVE DNA SEQUENCE FAMILY IN THE CHICKEN, W.E. Stumph, P. Kristo, M.-J. Tsai, and B.W. O'Malley, Baylor College of Medicine, Houston, TX 77030 We have identified and sequenced several members of a chicken interspersed middle repetitive DNA family which we have termed CR1. Members of this family have been located in the 5' and 3' flanking DNA in the vicinity of the genes which code for ovalbumin mRNA and U1 small nuclear RNA. The two most closely related family members sequenced are homologous over a region of approximately 160 base pairs. In each case, the 160 base pair region is flanked by homologous short direct repeats 10-15 base pairs in length. Comparison of the CR1 sequence with mammalian interspersed repetitive DNA sequences (human Alu and mouse B1 families) reveals several regions of extensive homology. In addition, the short nucleotide sequence CAGCCTGG which is conserved in ubiquitous repetitive sequence families from at least four mammalian species is also conserved at a homologous position in the chicken sequences. Thus the CR1 family may possibly represent an avian counterpart of the mammalian ubiquitous repeats. We have previously shown that the ovalbumin gene and its related X and Y genes exist in oviduct tissue chromatin as a 100 kb DNase I sensitive domain which gradually becomes resistant to DNase over a transition region approximately 10 kb in length at each end of the domain. Interestingly, the CR1 sequences are preferentially located in the vicinity of these regions of transition from DNase sensitivity to DNase resistance. Although this correlation could be coincidental, it is possible that these chicken repetitive sequences could be involved in determining the boundaries of functional domains within the chicken cell chromatin.

0910 GENETIC REARRANGEMENT IN OXYTRICHA NOVA, L.A. Klobutcher, R.E. Boswell, M.T. Swanton, and D.M. Prescott, University of Colorado, Boulder, Col. 80309

In the hypotrichous ciliate Oxytricha nova all of the DNA in the transcriptionally active macronucleus is in the form of low molecular weight gene-sized DNA molecules with an average size of 2,200 base pairs. These molecules are derived in a specific manner from the more typically eukaryotic chromosomes of the micronucleus during macronuclear development. All, or nearly all, of the gene-sized macronuclear DNA molecules possess an inverted terminal sequence consisting of 5'$C_4A_4$3' repeats. We have tested the hypothesis that this terminal repeat sequence serves as a recognition signal for the excision of macronuclear genes from micronuclear chromosomes in two ways. First, a nucleic acid sequence containing 5'$C_4A_4$3' repeats has been isolated and used to probe cloned segments of micronuclear DNA that contain DNA sequences destined to be retained in the macronucleus. Second, a macronuclear DNA segment and the corresponding region of the micronuclear genome have been cloned and partially sequenced. The results of these analyses indicate that the inverted terminal repeat sequences are not present at the ends of macronuclear sequences as they exist in the micronuclear genome. Therefore, the 5'$C_4A_4$3' repeat sequence is not simply a recognition signal for gene excision, but must be added to the ends of the gne-sized DNA molecules sometime during macronuclear development. We are currently investigating the location of 5'$C_4A_4$3' repeat sequences in the micronuclear genome and when during macronuclear development the repeat sequences are added to macronuclear DNA molecules.

0911 SEQUENCE ORGANIZATION OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION GENE FAMILIES
Stephen T. Crews, Elizabeth A. Springer and Leroy E. Hood, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Understanding antibody diversity and the evolution and expression of immunoglobulin genes requires a detailed knowledge of the organization and structure of germ-line variable region gene segments. Recent approaches have utilized molecular cloning techniques to isolate genes encoding antibodies. We have focused our attention on a small family of four heavy-chain variable region gene segments, one of which contributes towards antibodies that bind the hapten, phosphorylcholine. Utilizing clones derived from libraries of BALB/c mouse sperm DNA cloned in both lambda and cosmid vectors, we have isolated and sequenced these four gene segments. This gene family exhibits features typical of a multigene family: (1) homology of gene segments (2) close linkage of genes and (3) the presence of pseudogenes. Current work is focused on the linkage of these genes and their relationship to other heavy-chain variable region gene families as well as a comparative analysis of flanking sequences.

Crews, S., et al. (1981) Cell 25, 59.

0912 REARRANGEMENTS AND DELETIONS OF DNA OCCUR IN A MURINE B CELL LYMPHOMA WHICH MAY BE UNDERGOING IMMUNOGLOBULIN HEAVY CHAIN SWITCHING. Stavnezer, J*, Marcu, K.B+, Sirlin,S*, Alhadeff, B* and Hammerling, U*, *Sloan-Kettering Institute, N.Y.C.10021, +SUNY at Stony Brook N.Y.

The B cell lymphoma, I.29, consists of a mixture of cells expressing membrane-bound IgM and IgA of identical idiotype. Most of the cells express either IgM or IgA alone, but approximately 5% of the cells express IgM and IgA simultaneously within the cytoplasm and on the cell membrane. IgM+ cells, purified from the lymphoma and then passaged in mice or cultured in vitro, convert to IgA+ cells. These properties suggest that cells of the I.29 lymphoma may undergo immunoglobulin heavy chain switching. We have performed Southern blotting experiments on genomic DNAs isolated from I.29 cells in the process of progressing from IgM to IgA synthesis and on a number of cell lines derived from the lymphoma. The results are consistent with the deletion model for H chain switching, as the IgM+ cells contain rearranged μ genes, and α genes in the germline configuration on both the expressed and non-expressed H chain chromosomes, whereas the IgA+ cells have deleted both μ genes and contain one rearranged and one germline α gene. Although μ genes appear to be deleted from both chromosomes simultaneously as the cells switch from expression of IgM to IgA, the sites of DNA recombination differ on the two chromosomes. On the expressed chromosome, Sμ sequences are recombined with Sα sequences, whereas on the non-expressed chromosome, Sμ sequences are recombined with sequences located 5' (probably $S_{\gamma 3}$ sequences) to the γ_3 gene. These results suggest that the switch recombinase acts on both chromosomes, and that the switch recombinase is not class-specific.

0913 DNA REARRANGEMENTS IN MPC 11 IMMUNOGLOBULIN HEAVY CHAIN CLASS SWITCH VARIANTS, L.A. Eckhardt‡ S.A. Tilley‡ R.B. Lang† K.B. Marcu† and B.K. Birshtein‡ Albert Einstein Coll. of Medicine‡ Bronx NY 10461, State U. of New York† Stony Brook NY 11794

Comparisons of germline and myeloma immunoglobulin genes indicate that gene rearrangement plays a major role in the developmental regulation of immunoglobulin heavy chain gene expression. The discovery that myeloma cells can undergo a heavy chain class switch raises the possibility that additional information concerning class switch mechanisms will come from a further examination of myelomas as a model system. In the IgG2b-producing MPC 11 myeloma, IgG2a-producing cells arise at a high frequency. In some cases, switch variants producing normal-sized (55,000 MW) γ2a heavy chains have arisen spontaneously from a mutagen-induced "intermediate" (ICR 9.7.1) that produces an unusually large (75,000 MW) heavy chain. Other switch variants have been isolated directly from the parent cell line.

The expressed and unexpressed γ2b genes of MPC 11 can be distinguished in restriction endonuclease digests of total genomic DNA so that DNA rearrangements detected in MPC 11 variants can be directly associated with one or the other of these two genes. We describe here DNA rearrangements occurring on the expressed heavy chain chromosome of several MPC 11 γ2a "switch" variants as well as on the expressed chromosome of the ICR 9.7.1 intermediate. Our data indicate that all of these variants express the parental V_H region gene, supporting previous protein studies. We provide mapping data for the expressed gene of both ICR 9.7.1 and one of the IgG2a-producing variant cell lines (ICR 9.9.2.1) derived from it and discuss the advantages of an in vitro switching system for examining the dynamics of the immunoglobulin heavy chain class switch.

0914 THE EVOLUTION OF THE DEVELOPMENTALLY REGULATED GLOBIN GENES OF THE GOAT, Jerry B. Lingrel, Eric A. Schon, Michael L. Cleary, Joel R. Haynes, Steven G. Shapiro, Tim Townes and Sue Wernke, Departments of Microbiology and Biochemisty, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

We have recently cloned the embryonic (ε^{I}, ε^{II}, ε^{III}); fetal (γ); pre-adult (β^{C}) and adult(β^{A}) globin genes of the goat as well as two pseudo globin genes and two α globin genes and have determined their complete nucleotide sequence. Several interesting features of this gene set have emerged. The two pseudo genes, $\psi\beta^{X}$ and $\psi\beta^{Z}$, contain many of the same alterations which make these genes defective indicating that they have arisen from the duplication of an already defective gene. The nucleotide sequence of the γ, β^{C} and β^{A} globin genes are very homologous suggesting that they have arisen relatively recently in evolution and comparative sequence analysis of the $\psi\beta^{X}$ and $\psi\beta^{Z}$ genes and the β^{A} and β^{C} globin genes indicates that both these gene pairs diverged at the same time in evolution. This suggests that ε^{II}-$\psi\beta^{X}$-β^{C} and the ε^{III}-$\psi\beta^{Z}$-β^{A} linkage groups which we have established must have arisen by a block duplication. The divergence analysis also demonstrates that the goat γ gene evolved much later than the human γ globin gene. This means that a gene which is expressed only during fetal life has evolved more than once during evolution. Another interesting feature of this gene set is the presence of an insertion in the large intron of the γ, β^{C} and β^{A} globin genes. The insertions contain many of the hallmarks of a transposable element including direct and inverted repeats. The element appears in one position in the β^{A} and β^{C} globin genes but in another location in the γ gene.

0915 DNA SEQUENCES ASSOCIATED WITH NUCLEAR MATRIX, CHROMOSOMAL SCAFFOLD AND SYNAPTONEMAL COMPLEX, M. Tien Kuo, The Univ. of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

The nuclear matrix (NM), chromosomal scaffold (CS) and synaptonemal complex (SC) have been thought to play an important role in supporting the chromatin domain structure in interphase nuclei, metaphase chromosomes and pachytene spermatocytes, respectively. They are thought to be related since they are all resistant to treatment with high molar salt and nuclease digestion. The object of this research is to determine whether any specific DNA sequences are associated with these structural entities. NM, CS and SC were prepared from chicken MSB-1 cell nuclei, metaphase chromosomes and rat pachytene nuclei (fractionated from testicular cells by centrifugal elutriation), respectively, by sequential treatments with high molar salts, nuclease digestion (either micrococcal nuclease or restriction endonuclease) and ultracentrifugal sedimentation or column chromatography. Sequence complexity and enrichment of specific gene sequences in the DNA isolated from the prepared NM, CS, and SC were analyzed by liquid hybridization or by Southern blotting hybridization techniques. We have found that the transcribing gene is enriched in the DNA associated with NM but not with the CS. Neither enrichment nor depletion of nontranscribing gene sequences was found to be associated with NM or CS. The enrichment of the transcribing gene could have been due to a co-purification of transcriptional complexes during the purification of NM. However, sequence complexity analyses of DNA isolated from SC revealed a significant depletion (about 50% less) in repetitive sequences. These results suggest a possible transient interaction between DNA and proteins in these structural entities.

0916 CHARACTERIZATION OF ANTINUCLEAR ANTIGEN (SS-B/La)-ANTIBODY COMPLEX THAT CONTAINS ADENOVIRUS INDUCED RNAs, Lewis Pizer, Eng M. Tan, Jau-Shyong Deng, Richard Stenberg, University of Colorado School of Medicine

We have employed sera from patients with antoimmune diseases to study the interaction of the nuclear antigen SS-B/La with its associated RNAs. Antibodies against this antigen produce complexes containing four major populations of RNAs, designated SS-B, 1,2,3 and 4. Analysis of adenovirus infected cells reveals an additional population of RNAs that arise late in infection which we refer to as adenovirus induced RNAs (AdI-RNA). One of these RNAs is the virus associated RNA of adenovirus (VA-RNA). The others hybridize to the DNA containing the gene for VA-RNA and may be derived from VA-RNA. Based on studies it appears that the interaction of the SS-B/La antigen with these RNAs involves a single component which is or contains a protein of 49,000 daltons. Polacrylamide gel electrophoresis of the antigen-antibody complex produced with extracts of cells grown in radioactive phosphate demonstrated that the 49,000 dalton protein is phosphorylated and invitro labeling of SS-B/La antigen with ^{32}P-ATP confirms this observation.

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Statement of Other Interests - Our laboratory has been concerned with the regulation of transcription in Herpes virus infected cells. We have special interest in the effect of infection on the expression of cellular genes and the expression of viral genes inserted into bacterial plasmids. While a poster session would be presented on the material described in the above abstract we will participate in sessions dealing with these other topics.

0917 IDENTIFICATION OF A SINGLE STRANDED DNA BINDING PROTEIN FROM RAT LIVER WITH HIGH MOBILITY GROUP PROTEIN 1, Catherine Bonne, Michel Duguet, Pierre Sautière and Anne-Marie de Recondo, Institut de Recherches Scientifiques sur le Cancer B.P. N° 8 - 94802 VILLEJUIF CEDEX.

The rat liver single-stranded DNA binding protein (1) (2) isolated by differential DNA cellulose affinity chromatography from the cytosol of either normal liver (S_{2c}) or regenerating liver (HD25) was compared to the high mobility group proteins HMG 1 and HMG 2 isolated from rat liver chromatin by the technique of Goodwin et al. (3). Analysis of their amino acid composition, electrophoretic mobility and tryptic peptide map reveals the identity of the SSB with HMG 1 protein, implicating that this non histone chromatin protein bears additional properties previously demonstrated for the rat liver SSB :
In particular, it is able to both destabilize a double helix of DNA and to stimulate homologous DNA polymerases only when rat liver cells enter a phase of DNA synthesis, possibly after a specific modification.

(1) Duguet, M. and De Recondo, A.M. (1978), J. Biol. Chem., 253, 1660-1666.
(2) Bonne, C., Duguet, M. and De Recondo, A.M. (1980), Nucleic Acids Res., 8, 4955-4968.
(3) Goodwin, G.H., Sanders, C. and Johns, E.W., (1973), Eur. J. Biochem., 38, 14-19.

0918 AN ATP-DEPENDENT DNA TOPOISOMERASE FROM RAT LIVER, Michel Duguet, Catherine Lavenot and Anne-Marie de Recondo. Institut de Recherches Scientifiques sur le Cancer, Villejuif, France.

Type II DNA topoisomerases are known to catalyse multiple changes in the topology of DNA in vitro. Their in vivo functions are only partly elucidated : a number of data indicate that these enzymes are very likely involved in the initiation and presumably in the elongation and termination steps of the eplication of both the bacterial chromosome and of the eukaryotic chromatin. Moreover, the catenation/decatenation reaction performed by topo II may be one of the major activities of these enzymes in the cell by controlling the condensation.decondensation of chromosomal DNA.
We have isolated and partially purified an ATP-dependent DNA topoisomerase from rat liver nuclei.The purification included lysis, polyethylene glycol precipitation, phosphocellulose, hydroxyapatite, and DNA cellulose chromatographies. The enzyme is able to catenate closed double-stranded DNA circles in an Mg^{++}, ATP, and histone H_1 dependent reaction at low ionic strength (43 mM KCl). The products of this reaction are catenated dimers, trimers, tetramers, pentamers... and networks. The enzyme also performs the decatenation of the huge networks of kinetoplastic DNA from Trypanosoma (up to 20000 catenated molecules) in 1.48 kBP mini circles. The reaction is Mg^{++} and ATP dependent and takes place in the absence of H_1 and at moderate ionic strength (85 mM KCl). The possible in vivo roles of this enzyme are under investigation. With this in mind, preliminary experiments indicate that the amount of the enzyme is increased in regenerating liver nuclei compared to normal liver (the amount of topo I is not significantly changed).

0919 UNUSUAL CHROMATIN STRUCTURE OF TRANSCRIBED AND NONTRANSCRIBED SEGMENTS OF MOUSE RIBOSOMAL RNA GENES, William T. Garrard, Alan H. Davis, and Tim L. Reudelhuber, Division of Molecular Biology, Department of Biochemistry, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

We have examined the chromatin structure of mouse ribosomal RNA genes in specific regions of the 44 kb repeat unit. Recombinant plasmids that harbored mouse ribosomal sequences encompassing the start of transcription, the upstream nontranscribed spacer, or the transcription unit were each used as hybridization probes. The pattern of DNA fragments generated by micrococcal nuclease cleavage within ribosomal repeats of cultured mouse cell nuclei was analyzed by blot-hybridization experiments. Chromatin fractionation following nuclease treatment led to a striking enrichment of the vast majority of ribosomal sequences in an insoluble fraction, suggesting that these sequences may be intimately associated with the nucleolar matrix. Ribosomal sequences probed by each of the above recombinant plasmids in this insoluble fraction lacked the canonical nucleosomal repeat that was exhibited by bulk DNA. Rather, a series of sharp bands above a diffuse background were generated by nuclease digestion. In contrast, minor fractions of the same ribosomal sequences which were rendered soluble upon chromatin fractionation possessed nucleosomal repeats. These results suggest that "active" and "inactive" ribosomal chromatin can be separated based on solubility properties after micrococal nuclease cleavage, and that the "active" fraction, including the upstream nontranscribed spacer region, lacks a canonical nucleosomal configuration.(Supported by Grant GM-22201 from NIH and Grant I-823 from The Robert A. Welch Foundation.)

0920 ULTRASTRUCTURE OF YEAST CHROMATIN, B.A. Hamkalo[1], J.B. Rattner[2], J.R. Davie[3], and C.A. Saunders[3], [1]Univ. of CA., Irvine, CA. 92717, [2]Univ. of Calgary, Alberta, Canada, [3]Oregon State Univ., Corvallis, OR. 97330

The ultrastructural orgainzation of chromatin released from spheroplasts or isolated nuclei of Saccharomyces cerevisiae was investigated using the Miller spreading technique. Up to 95% of the dispersed chromatin exhibited a beads-on-a-string morphology typical of the nucleohistone fiber of all eukaryotes although internucleosomal linkers are quite variable in length. A small portion of the material exhibited a smooth contour but is not naked DNA, judged by diameter and staining characteristics. In an attempt to eliminate the possiblity that structural differences observed are artifactually generated by a trans-acting substance, we co-spread yeast and mouse cells. Mouse chromatin maintained its typical appearance of regularly-spaced nucleosomes with no nucleosome-free chromatin observed while yeast chromatin appeared as described. The smooth fibers may represent a subset of nucleosomes which unfold during preparation, or they may represent regions which are unfolded in vivo but which maintain histone-DNA contacts. Nascent transcription complexes were readily visualized along yeast chromatin when material was prepared in the presence of Actinomycin D. Transcripts are associated with both smooth and beaded chromatin, but all smooth chromatin does not possess nascent RNPs. Among the transcription units observed along smooth fibers are putative ribosomal genes. If samples are lysed mechanically in 5 mM Mg^{++}, fibers 200-300 Å in diameter are observed. They are made up of units separated by chromatin fibers devoid of nucleosomes. Thus, although yeast does not possess a typical histone H1, it is apparently capable of forming higher order chromatin structures which bear some resemblance to those of higher eukaryotes.

0921 SMALL NUCLEAR RIBONUCLEOPROTEIN COMPLEXES FROM DROSOPHILA MELANOGASTER
Su-yun Chung, Roger Cone and John Wooley, Biochemical Sciences Department, Princeton University, Princeton, New Jersey 08544

Small nuclear RNA species or snRNAs are ubiquitous among higher eukaryotes and are associated with proteins, forming small nuclear ribonucleoprotein complexes or snRNPs. The most abundant class of snRNPs contain members of the U-family of snRNAs which may participate in RNA splicing and other genetic processes. To exploit the unique features of Drosophila for studying genetic processes, we have isolated and partially characterized snRNPs from nuclei of the D. melanogaster Kc_0 cell line. Velocity sedimentation partially fractions snRNPs present in conventional nuclear extracts; analysis of fractions so obtained suggests in general that one snRNP contains one snRNA. At least eleven species of small RNAs are present in Drosophila nuclei; among them we have identified potential homologues to mammalian U1, U2, U3 and U4 based on sequence homology (ranging between 70 to 75%). Furthermore, anti-SM autoimmune sera recognize nuclear antigens in Drosophila and precipitate a set of snRNAs (as RNPs) which correspond strikingly in gel mobility to the U-family of mammalian snRNAs (which were themselves originally shown to be precipitated by anti-SM sera by Lerner and Steitz, PNAS 76, 5495-5499, 1979). Immunoprecipitation experiments indicate the protein components are also conserved: notably anti-SM recognizes a 26,000 dalton polypeptide (on transfers from SDS gels) in both HeLa and Drosophila snRNP extracts. (Research supported by NIH GM26332.)

0922 EVIDENCE FOR EVOLUTIONARY CONSERVATION OF AN EUKARYOTIC HELIX-DESTABILIZING PROTEIN AND ITS INVOLVEMENT IN TRANSCRIPTION RATHER THAN REPLICATION, Gordhan L. Patel and Peter E. Thompson, Department of Zoology, University of Georgia, Athens, GA 30602

A nucleic acid helix-destabilizing protein (HDP) purified from rat liver in our laboratory mimics the behaviour in vitro of the gene 32 protein (a bacteriophage HDP). We have elicited monospecific antisera against this eukaryotic HDP and have exploited immunological approaches to explore its functions in vivo. We report here the following observations. (1) Heterogeneous extracts of proteins from mammalian, insect and fungal sources, when separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto diazobenzyloxymethyl paper for reaction with antiserum, exhibited an immunoreactive band in all cases similar in size to the subunit (30,000 daltons) of the purified rat liver HDP; gene 32 protein kindly provided by Dr. Bruce Alberts did not cross react with the antiserum. (2) Indirect immunofluorescence staining of salivary gland polytene chromosomes from Drosophila melanogaster larvae revealed a non-uniform distribution of HDP, with a dramatic concentration of immunoreactivity in normal developmental puffs and in heat-shock puffs (e.g., 87A and 87BC) known to be active in transcription. In many respects, the chromosomal distribution of HDP resembled that reported by others for RNA polymerase. (3) Similar immunocytochemical analyses of Sciara coprophila polytene chromosomes, while confirming the above observations, showed absence of immunoreactive HDP in the unique replication puffs, which incorporate thymidine in this system. Thus, this eukaryotic HDP is conserved through evolution and, unlike the gene 32 protein known to be involved in replication, apprears to be involved in some aspects of gene transcription. (Supported by NIH Grant CA 31138)

0923 A GENETIC TEST FOR THE ASSOCIATION OF HISTONE H2A AND H2B SUBTYPES IN YEAST, Michael Grunstein, David Kolodrubetz, Mary Rykowski and Joonho Choe, University of California, Los Angeles, California 90024

There are two histone H2A subtypes (H2A1, H2A2) and two histone H2B subtypes (H2B1, H2B2) in yeast. Using in vitro mutagenesis followed by gene replacement, we have asked the following questions:

1) Can the yeast cell cycle be completed in the absence of any one of these histone subtypes?
2) Can either of the histone H2A subtypes function exclusively with either of the H2B subtypes on the chromosome?

We have concluded that 1) yeast can survive and its cell cycle can be completed in the absence of either H2A1 or H2A2 but not both H2A1 and H2A2. These results are similar to those already presented for histone H2B1 and H2B2 (Cell (1981) 24: 477-487). 2) By crossing $H2A^-$ subtype mutations into $H2B^-$ subtype mutant backgrounds and analyzing the histones of the progeny we have been able to show that the yeast cell cycle can be completed when any of the H2A and any of the H2B subtypes are associated with each other.

0924 GENETIC DETERMINANTS FOR THE NUCLEASE-SENSITIVE SITE IN SV40 CHROMATIN, Walter A. Scott[1], Robert D. Gerard[1] and Mary Woodworth-Gutai[2] 1) Univ. of Miami School of Medicine, Dept. of Biochemistry, Miami, FL 33101 2) Roswell Park Memorial Institute, Dept. of Cell and Tumor Biology, Buffalo, NY 14263

Endonuclease digestion of SV40 chromatin extracted from the nuclei of infected cells has revealed that a short segment of the viral genome adjacent to the origin of replication is hypersensitive to nuclease cleavage. Chromatin from a partially duplicated, nondefective variant of SV40 [in(Or)-1411; Shenk, Cell 13:791, 1978] displays two such hypersensitive sites -- one for each copy of the duplicated segment. This allows quantitative evaluation of the effects of mutations in one copy of the duplicated segment by comparison with nuclease-sensitivity in the unaltered segment. We have constructed a series of deletion mutants and determined their effects on viral chromatin. Deletions which encompass the predominant sites for nuclease cleavage essentially abolish nuclease-sensitivity. Short deletion mutants affecting the 72 bp segment (a sequence known to play a unique role in in vivo transcription) have substantial effects on the nuclease-sensitivity of nearby chromatin. Our results suggest that sequences in the neighborhood of the 72 bp segment act at some distance along the viral genome to influence nucleoprotein structure and that they modulate the degree of nuclease-sensitivity rather than provide primary determinants for the cleavage sites. (Supported by the NIH.)

0925 DOES METHYLATION UNDERLIE X CHROMOSOME INACTIVATION? Stanley F. Wolf and Barbara R. Migeon, The Johns Hopkins University, Baltimore, Maryland 21205

Numerous suggestions have been made that DNA methylation is responsible for regulation of developmental processes. Among these differentiation events is that resulting in the inactivation of all X chromosomes but one in somatic cells of mammals. The tissue specific association of undermethylation with gene activity and abundant methylation in the early embryo has led to speculation that the inactive X chromosome might be highly methylated. Recently, it has been suggested, based on studies of interspecies cell hybrids, that 5-azacytidine can induce local derepression of the inactive X chromosome presumably because the cytidine analog has blocked methylation at relevant sites on the inactive X. Using cloned DNA fragments unique to the X chromosome, we have investigated the stability of X DNA methylation and its relation to X chromosome activity in normal human placentas, skin fibroblast cultures, and derivative clones. Approximately 28 KB of X chromosome DNA was assayed by Southern blot analysis using the cloned probes and restriction enzymes Hpa II and Msp I. Not only were no consistent differences observed with respect to the number of X chromosomes or transcriptional activity, but the pattern of X DNA methylation in these normal cells varied after replication. The effects of hypomethylation induced by 5-azacytidine on expression of inactive X genes was also determined in clonal populations of normal human fibroblasts carrying heterozygous X linked markers. No derepression was detected. These findings along with species differences in stability of methylation suggest that methylation is not the sole determinant of X chromosome activity.

0926 METHYLATION STATUS OF THE α2(I) COLLAGEN GENE DOES NOT ALTER ITS CHROMATIN STRUCTURE AS DETERMINED BY DNase I SENSITIVITY. Catherine McKeon, Ira Pastan and Benoit de Crombrugghe, Laboratory of Molecular Biology, NCI, NIH, Bethesda, Md., 20205.

The methylation patterns of three discrete regions of the α2(I) collagen gene were studied in DNA isolated from 5 tissue: Chick embryo fibroblasts (CEF) in which type I collagen accounts for 1-2% of proteins systhesized, Rous sarcoma virus-transformed CEF (RSV-CEF) in which collagen synthesis decreases 10-fold, brain in which only glial cells synthesize small amounts of collagen, erythrocytes and sperm which synthesize no detectable collagen. We find that the 5' region of this gene is not methylated in all cell types studied. The central and 3' region of the gene are methylated to a similar extent in all cell types studied. The level of expression of the α2(I) collagen gene is, therefore, independent of the methylation of its DNA. In chromatin from brain and sperm, the unmethylated and methylated regions of the α2(I) collagen gene show equal sensitivity to DNase I. Hence, there is no correlation among the different segments of the gene between their methylation status and their chromatin sensitivity to DNase I. In CEF chromatin, the 5' region of the gene contains a site hypersensitive to DNase I which is near the start site of transcription. This site is not found in brain chromatin. We conclude that the absence of methylation at the 5' end of the α2(I) collagen gene and the chromatin modification detected by DNase I hypersensitivity correspond to two different and independent mechanisms. Therefore, the demethylation of the DNA does not control the alteration of chromatin structure which accompanies gene activation.

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0927 LEFT-HANDED Z-DNA: ITS RECOGNITION BY ANTIBODIES AND PROTEINS, Alfred Nordheim,* Eileen M. Lafer,+ Achim Müller,* B. David Stollar,+ Mary Lou Pardue* and Alexander Rich*

*Department of Biology, Massachusetts Institute of Technology, Cambridge, Mass. 02139 USA
+Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Mass. 02111

To test for the natural occurrence and biological function of Z-DNA we have stabilized this left-handed DNA helix in low-salt solution and used it as immunogen for the production of specific anti-Z-DNA antibodies. These antibodies were used in indirect immunufluorescence studies of the Drosophila polytene chromosomes. A specific binding of the anti-Z-DNA antibodies to interband regions of the polytene chromatin was found. Further characterization of the anti-Z-DNA antibody binding to decondensed chromatin during puff formation will be reported.

Finally, left-handed Z-DNA is used in affinity chromatography experiments and the recent progress in the isolation of Z-binding proteins shall be discussed.

0928 CHROMOSOMAL LOCALIZATION OF XENOPUS SOMATIC-TYPE 5S DNA, Mary E. Harper and Laurence Jay Korn, Agouron Institute, La Jolla, California 92037 and Stanford University, Stanford, California 94305

Xenopus and other amphibian genomes contain two major classes of 5S RNA genes, the oocyte-type and somatic-type. The more abundant class of genes (20,000 copies/haploid genome) codes for oocyte-type 5S RNA and is transcribed only during oogenesis. In contrast, the somatic-type 5S RNA genes (400 copies/haploid genome) are expressed in somatic cells as well as in oocytes. Whereas the two types of coding regions exhibit 95% homology, their spacer regions do not cross-hybridize. Previous in situ hybridization of ^{3}H-labeled 5S RNA to Xenopus chromosome preparations resulted in labeling of the telomeric regions of the long arms of many if not all chromosomes. These experiments most likely localized only the oocyte-type 5S genes due to their higher abundancy. Because chromosome location may play a part in the differential expression of these genes, we cloned a 459 bp spacer region from Xenopus laevis somatic-type 5S DNA in order to localize the somatic-type genes. This cloned DNA, which hybridizes to a single band on Xenopus genomic DNA blots, was ^{3}H-labeled by nick translation and hybridized in situ to Xenopus metaphase chromosome preparations according to a more sensitive, recently developed technique. Preliminary results show relatively heavy labeling of the distal end of a small number of submetacentric chromosomes, usually one to two per mitosis. In contrast, hybridization of a genomic clone containing the 5S RNA coding region resulted in uniform labeling of the telomeres of the long arms of most chromosomes.

0929 THE EFFECT OF THE B-Z TRANSITION IN POLY(dG-m^{5}dC)·POLY(dG-m^{5}dC) ON THE BINDING OF HISTONES, Joanne Nickol, Michael Behe and Gary Felsenfeld, NIADDK, National Institutes of Health, Bethesda, Maryland 20205

We have studied the properties of complexes between histones and the methylated synthetic polydeoxynucleotide, poly(dG-m^{5}dC)·poly(dG-m^{5}dC). This polymer undergoes the transition from B DNA to left-handed Z DNA under moderate ionic strength conditions. When the polymer is in the Z form it will bind histones, but nucleosomes are not detected. When the polymer in the B form is combined with equimolar quantities of the four core histones and digested with micrococcal nuclease, particles are formed that behave in all respects like normal nucleosome cores. If these core particles are now placed in solvents that would result in conversion of the protein-free polymer to the Z form, no transition is observed. The formation of a nucleosome core particle thus stabilizes the B form, while the presence of the Z form prevents nucleosome formation. The results suggest that if Z DNA is present in eukaryotic nuclei, it will serve to disrupt the normal chromatin structure.

0930 CHROMATIN STRUCTURE OF DOUBLE ORIGIN SV40

Chae, C-B*, Mathis, D.J, Jongstra, J., Oudet, P., Benoist, C. and Chambon, P. Laboratoire de Génétique Moléculaire des Eucaryotes du C.N.R.S., Faculté de Médecine, Strasbourg, France, and *University of North Carolina, Chapel Hill, NC, USA.

The SV40 replication origin region (Hind III - Hha I; map coordinate 5171 - 343 bp) and the various DNA fragments derived from this region were inserted into a site situated near the transcription termination site (Hpa I, 2666 bp) in a deletion mutant of SV40 (dl 2122) which lacks 234 bp in the T antigen intron. These viable recombinant SV40s containing an additional replication origin region were used to infect CV-1 cells for studies on the DNA sequences involved in the assembly of nucleosome-free and nuclease-sensitive SV40 chromatin structure. During the late infectious cycle (40-42 hrs) there are three major DNase I sensitive regions in the original and the second replication origin regions: two are centered in the two respective "72 bp" repeats and the third on the "TATA" box. The nuclease sensitivity in the second 72 bp box requires the DNA sequence upstream (toward the late region) from the "72 bp" and the DNA sequence within the "72 bp" itself. The sensitivity in the "TATA" box requires the sequence downstream from the "TATA" box. The assembly of nucleosome-gap structure requires the DNA sequences involved in all three of the nuclease sensitive regions.

0931 PRECISTE DETERMINATION OF THE TISSUE SPECIFIC METHYLATION PATTERNS OF THE RAT INSULIN II GENE, Richard L. Cate and Walter Gilbert, Harvard University, Cambridge, Ma. 02138

We have measured the level of methylation of cytosine at six restriction sites within or 5' to the rat insulin II gene in various tissues and cell lines. In an insulin producing tumor and an insulin producing cell line derived from that tumor, a cytosine residue 490 nucleotides upstream from the cap site is undermethylated. In all other tissues examined, this site is 100% methylated, including a cell line derived from the rat tumor, that expresses somatostatin but not insulin. However a site within the coding region of the gene was found to be undermethylated not only in the insulin producing tissues but in kidney and liver as well. Thus only at certain sites does the level of methylation correlate with insulin gene activity. The tissue specific methylation patterns are very reproducible and allow a defining of subpopulations of DNA molecules, each population possibly reflecting different cell types or groups of many similar cell types within that tissue. Consistant with this premise, the individual methylation patterns of the insulin gene in T and B cell DNA were shown to be subclasses of the total spleen DNA methylation pattern.

0932 SEVERAL NUCLEAR "RIBONUCLEOPROTEIN PARTICLES" CAN BE DISTINGUISHED IN DROSOPHILA MELANOGASTER CELLS BY THE USE OF MONOCLONAL ANTIBODIES, Werner Risau, Peter Symmons, Harald Saumweber and Rudolf Burberg, Max-Planck-Institut für Virusforschung, D7400 Tübingen, FRG

Of a collection of monoclonal antibodies prepared against nuclear proteins of Drosophila melanogaster, some show a selective staining of puffs and prominent interbands (Saumweber et al., Chromosoma 80, 253-275, 1980). We used four antibodies showing this staining pattern to get a further insight into the function(s) of the antigen that each detects. The antigens could be enriched in a nuclear extract which also contained a large proportion of rapidly labelled RNA. This extract was further separated on sucrose gradients. Analysis of the gradients by radioimmunoassay and protein blotting revealed at least three different RNase-sensitive particulate structures. These structures are different in size, in the relative proportion of antigen they contain, in their content of rapidly labelled RNA compared to unlabelled RNA and a number of other parameters. Evidence from indirect immunofluorescent studies will be presented showing that the protein antigens are also differentially distributed among active sites on the chromosome. The possible role of the protein antigens in hnRNA packaging and processing will be discussed.

0933 TRIIODOTHYRONINE AND DEXAMETHASONE RECEPTOR PROTEINS ARE HIGHLY ENRICHED IN MICROCOCCAL NUCLEASE HYPERSENSITIVE DOMAINS CONTAINING GROWTH HORMONE GENE SEQUENCES, Beatriz Levy-Wilson, Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717.

Chromatin from rat pituitary tumor (GH_3) cells was fractionated into transcriptionally active and inactive domains. When the various chromatin fractions were assayed for their content of growth hormone gene sequences, it was found that these sequences were highly enriched in those chromatin fractions most sensitive to micrococcal nuclease (S_1 and MN_1). Furthermore, fraction S_1, showed the highest enrichment in growth hormone genes and was impoverished in histones H_3 and H_4. The distribution of specifically bound thyroid and glucocorticoid receptors in chromatin fractions enriched and depleted in growth hormone gene sequences was also examined. Both thyroid and glucocorticoid receptors are highly enriched in the transcriptionally active chromatin domains encompassing growth hormone genes. Within active chromatin, both types of receptors exist in more than one molecular form.

0934 INTERACTIONS AMONG DNASE I HYPERSENSITIVE SITES 5' TO A *DROSOPHILA* GLUE PROTEIN GENE, Steven K. Beckendorf, Antony W. Shermoen and William McGinnis, University of California, Berkeley, CA 94720

The chromatin structure adjacent to the *Drosophila* glue protein gene *Sgs-4* changes drastically when the gene is active. In nuclei from embryos in which *Sgs-4* is inactive, there are three DNase I hypersensitive sites 3' to the gene but none near its 5' end. In the nuclei of late third instar salivary glands, *Sgs-4* is actively transcribed and a complex of five DNase I hypersensitive sites appears in the first 500 bp 5' to the gene. The two sites closest to the gene are located at +30±50 and -70±50 and may correspond to promoter sequences. The three more distal sites, -330, -405, and -480, are affected by small deletion mutations which greatly reduce or eliminate gene expression. Thus the hypersensitive sites, or DNA sequences within 50 bp of them, seem to be required for normal gene expression. The *Sgs-4* null mutant BER 1 removes sequences corresponding to two hypersensitive sites, -405 and ≈480 (M. Muskavitch and D. Hogness, personal communication). When chromatin from the salivary glands of this mutant is examined, none of the 5' hypersensitive sites is found even though sequences corresponding to three of them remain. This result suggests that hierarchical interactions among the regions 5' to *Sgs-4* are required for its full expression.

0935 MONOCLONAL ANTIBODIES TO NUCLEAR MATRIX PROTEINS, Donald D. Newmeyer and Betsy M. Ohlsson-Wilhelm, U. Rochester, Rochester, NY and Ronald Berezney and Linda A. Buchholz, S. U. N. Y., Buffalo, NY

Involvement of the nuclear matrix in DNA replication, RNA maturation and viral processes has been suggested by several investigators. However, which of the many molecular species present in the nuclear matrix actually participate in these functions is not known. We have been interested in identifying individual proteins that play structural or functional roles in the nucleus and, in particular, in the nuclear matrix. To that end, we have generated a set of monoclonal antibodies that recognize proteins in the cell nucleus of mammals and amphibians. Certain of these antibodies recognize nuclear matrix proteins. In particular, one antibody (J9) binds to a set of four rat liver matrix polypeptides of molecular weight 60 to 74K; these have been localized by in situ immunofluorescence microscopy in the nuclear periphery, the nuclear interior and inside nucleoli. A second monoclonal antibody (J16) is specific for a 72K matrix polypeptide (comigrating with one of the J9 antigens) residing exclusively in the nuclear periphery. These findings are consistent with a distinction in protein composition between the inner nuclear matrix and the peripheral lamina. Other monoclonal antibodies have been shown to recognize a) a 200K protein at the nuclear periphery and b) a set of proteins (between 60K and 200K) present in the mitotic spindle and discrete intranuclear bodies which are separate from nucleoli.

0936 STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE HUMAN METALLOTHIONEIN GENE FAMILY, Michael Karin, Metabolic Research Unit, Department of Medicine, UCSF, San Francisco,Ca. 94143.

allothioneins (MT) are low molecular weight, cysteine rich, heavy metal binding proteins. make possible the molecular analysis of the human MT genes, a cDNA "clone bank" was pre- ed from cadmium induced Hela cells. The cDNA bank was screened using 32P-cDNA probes made m control and induced Hela cells RNA. Colonies that hybridized preferentially with the in- ed cDNA probe were picked for further analysis by "Southern blotting" of plasmid DNA and ridization with mouse MT cDNA. One of the positive plasmids was subjected to nucleotide uence analysis and was found to contain an almost full length cDNA insert of human MT-II. ng the cDNA clone as a probe, a human genomic library was screened, and 53 positive bac- iophage λ clones were isolated. The exact number of absolutely different MT gene clones is clear yet but seems to be around 10. 2 genes that correspond to the MT-II cDNA were sub- ted to further analysis. One of them contains 2 intervening sequences and the other is sing them completely. I am currently investigating, using gene transfer techniques whether two genes are expressable in mouse cells, and whether or not their expression is regulated heavy metals or glucocorticoids.

0937 CHROMATIN STRUCTURE OF THE TRP-1 ARS-1 PLASMID IN YEAST, Lawrence W. Bergman, National Institutes of Health, Bethesda, MD 20205

I have examined the chromatin structure of the 1453 bp circular plasmid containing the yeast trp-1 gene and a chromosomal replicator present extrachromosomally in yeast cells. The plasmid was partially purified by passage of a cytoplasmic lysate over a Sepharose 2B column such that in the pooled trp-1 containing column fractions the trp-1 plasmid DNA is the only detectable DNA species present. Sedimentation analysis indicates that the native trp-1 plasmid sediments approximately 2.5 times faster than protein-free trp-1 DNA. Digestion with either Staphylococcal nuclease or DNase II reveals that the trp-1 minichromosome is fully saturated with 9 nucleosomes with a nucleosome repeat length of 161 bp. These results indicate that the trp-1 plasmid is assembled in a condensed chromosome-like structure very similar in structure to yeast nuclear chromatin. Furthermore, it appears that the replicator portion of the molecule is more accessible to staph nuclease digestion than the trp-1 gene portion of the molecule. Fine structure analysis indicates that there are DNase I hypersensitive regions near the 5'-end of the trp-1 gene and in the replicator region of the molecule, suggesting that these areas may be more accessible for interaction with other protein molecules. Finally, it appears that each of 9 nucleosome are located in a precise position (ie. phased) on the minichromosome. It is not known whether the precise arrangement is related to the replication, transcription or DNA sequence of the molecule.

0938 ROLE OF NUCLEAR MATRIX IN THE LYTIC INFECTION OF SIMIAN VIRUS 40, Clint Jones and Robert T. Su, University of Kansas, Lawrence, Kansas 66045

The role of nuclear matrix in eucaryotic chromosome replication was investigated using simian virus 40(SV40) as a model system. The nuclear matrix of SV40 infected cells consists of peripheral layer of nuclear pore complexes, connecting lamina, residual nucleolar and fibrillar structure as seen under electron microscope. The amount of SV40 DNA and viral tumor(T) antigen on matrix coincide with the replication cycle of virus. Matrix T antigen is highly phosphorylated and organized into complexes different from the cytoplasmic T antigen. At least seven phosphorylated proteins other than the known 56K protein are co-immunoprecipitated by anti-T sera. Matrix T antigen is not readily phosphorylated *in vitro* in the presence of γ-32P-ATP. Enhanced α DNA polymerase activity is also detected on matrix from infected cells compared to normal cells. Matrix-bound polymerase is capable of performing limited DNA synthesis on either endogeneous or exogeneous templates. Complexes containing DNA polymerase activity and T antigen appear to cosediment on sucrose gradient. The results support the model that nuclear matrix might be the site for DNA replication.

0939 SELECTIVE ARRANGEMENT OF NUCLEOSOMES WITHIN THE GENOME, James Barsoum, Louis Levinger and Alexander Varshavsky, Department of Biology, Massachusetts Institute of Technology Cambridge, Ma. 02139

We have used a new approach, two-dimensional hybridization mapping of nucleosomes (Levinger et al., J. Mol. Biol. 146:287-304, 1981) to compare the structures of mononucleosomes from different regions of the Drosophila and mouse genome. In murine methotrexate-resistant cells (L5178Y-R), the highly amplified, transcriptionally active gene for dihydrofolate reductase was not found to be enriched in HMG14 and 17, unlike the transcribed globin genes in chicken erythroid cells. Virtually no ubiquitin-H2A(uH2A) semihistone was found in mononucleosomes isolated from L5178Y-R cells, suggesting the presence of active uH2A isopeptidase. In cultured non-shocked Drosophila melanogaster cells, approximately one in two nucleosomes of the transcribed *copia* and heat shock 70 genes contains uH2A. In striking contrast, fewer than one in twenty-five nucleosomes of the tandemly repeated, non-transcribed 1.688 satellite DNA contain uH2A, suggesting that most if not all of the nucleosomal uH2A is located in transcribed or potentially transcribed genes. Mononucleosomes containing the 1.688 satellite DNA, in addition to being deubiquitinated, contain a specific 50-kd protein, D1.

0940 CHARACTERIZATION OF G-BAND DNA FROM MAMMALIAN CHROMOSOMES. Gerald Holmquist, Baylor College of Medicine Dept. of Medicine. Houston, Tx. 77030

G-band DNA from Chinese hamster V79-8 cells was separated from R-band (interband) DNA and the two fractions were characterized. Chromosomes from cells labeled with BrdUrd in the 1st or last half of S-phase show reciprocal BrdUrd quenched fluorescent patterns which are 98% coincident with trypsin G-bands; the non-coincident bands are usually the small regions of heterochromatin which also C-band. In well synchronized cultures, d(DNA)/dt is bimodal splitting S-phase into two halfs; the R-bands complete or are completing replication before the G-bands initiate it. Thus, R-band and G-band DNA from synchronized cultures can be alternately labeled with isotopes or BrdUrd substituted and density fractionated. On CsCl gradients, alternate labels show identically shaped distributions displaced by a density corresponding to 4% AT content difference; G-band DNA is the AT richer. Alternate labels in chromatin show similar digestion kinetics with DNase I and nick translated G or R-band probes are driven to hybridization similarly by total poly A-RNA; both G and R-band DNAs are thus equally transcribed. Both DNAs digest similarly with Msp I and Hpa II; no gross methylation differences were seen. Both fractions contain the same % snapback for fragments $>$2kb. Thus, the mammalian euchromatic genome is divided into about 2,000 longitudinally differentiated bands half of which replicate early and are cytologically hypersensitive to trypsinization. The basis for this genome partitioning remains unclear.

0941 COMPARISON OF THE T15 GENE FAMILY AMONG DIFFERENT MOUSE STRAINS, Johanna A. Griffin, Roger M. Perlmutter, and Leroy Hood, California Institute of Technology, Pasadena, CA 91125

Most native antibodies elicited in response to phosphocholine are encoded by a single germline gene segment, T15, in BALB/c mice. This gene segment is included in a family of four immunoglobulin heavy chain variable region (V_H) gene segments that are greater than 85% homologous to each other. Strain-specific variation in both the number and arrangement of members of the T15 family was revealed by Southern blot analysis of genomic DNA from multiple mouse strains hybridized to a T15 probe. A B10.P mouse strain sperm DNA-λL47 phage recombinant library was screened with the same probe used to identify the T15 gene family in BALB/c mice. There are striking similarities between the analogous V_H gene segments in the BALB/c and B10.P mouse strains even though these are among the most distantly related of the inbred lines. The possible evolutionary significance of these findings will be discussed. (Supported in part by NIH grants AI 06261, AI 18088, and AI 16913.)

0942 PURIFICATION OF CENTROMERIC HETEROCHROMATIN FROM MOUSE CELLS, Lorraine Lica*, Barbara Hamkalo*, Joanna Olmsted+, and John Cox+, *UCI, Irvine, CA 92717 and +Rochester University, Rochester, NY 14627

Hybridization of mouse satellite DNA in situ is restricted to centromeres of virtually all mouse chromosomes. This highly repeated DNA fortuitously is resistant to digestion by the restriction endonucleases Eco RI and Alu I. We have exploited these properties to purify centromeric heterochromatin from metaphase cells. When L929 cells in metaphase are digested with either Eco RI or Alu I, chromatin containing about 85% of the DNA is released. Sedimentation of these digests through preformed Percoll™ gradients yields two DNA-containing bands. The densest band contains about 15-20% of the DNA which is high molecular weight. Buoyant density sedimentation of DNA obtained by sarkosyl dissociation of the material in the dense band shows that it is at least 4-fold enriched in satellite sequences. When the material in this band is incubated with anti-centromere serum from a human CREST scleroderma patient and then with rhodamine-conjugated anti-human IgG, fluorescence appears in the form of paired dots. This is the same pattern of fluorescence observed when whole metaphase chromosomes are stained in this manner. Since phase contrast microscopy shows the absence of chromosome arms in the digested preparations, the fluorescence staining pattern and the enrichment of satellite DNA sequences together support the conclusion that the structures in the dense band are indeed centromeres.

0943 NON-RANDOM ASSOCIATION OF REPEATED DNA SEQUENCES WITH THE NUCLEAR MATRIX, Don Small, Barry Nelkin, and Bert Vogelstein, Johns Hopkins University School of Medicine, Oncology Center, Baltimore, Maryland 21205

The DNA in a eukaryotic nucleus is arranged into a series of supercoiled loops which are anchored at their bases to the nuclear matrix. We have analyzed the DNA sequences that are closest to the matrix attachment points for their relative content of specific repeated sequences. In mouse cells, there was a substantial enrichment of satellite sequences in the matrix DNA fraction. This enrichment was found in both fibroblastic and erythroleukemic cells of the mouse. In contrast, mouse nuclear matrix DNA had proportionately less of a major interspersed repeat (the Eco RI repeat) than did total nuclear DNA. Tandemly repeated sequences were not always associated with the nuclear matrix, however; for example, the alpha component of African green monkey cell DNA formed proportionately less of the matrix DNA than it did of total nuclear DNA. The human Alu family repeat, an interspersed repeat homologous to the origins of replication of several mammalian viruses, was substantially enriched in human nuclear matrix DNA preparations, as judged by restriction endonuclease and hybridization analyses. The results are discussed in terms of a non-random arrangement of genes with respect to nuclear DNA loops.

0944 POTENTIAL Z- DNA FORMING SEQUENCES ARE HIGHLY DISPERSED IN THE HUMAN GENOME , Hiroshi Hamada and Takeo Kakunaga, Lab. of Molecular Carcinogenesis, NCI, NIH, Bethesda,MD 20205

Some synthetic DNA polymers have a novel conformation(Z-DNA) with left-handed twist. However, their existence in naturally occuring DNA or their biological implication is not known. We have been studying the structure and differential regulation of human actin gene family by molecular cloning.
In the course of characterizing a number of cloned genomic actin genes, we have found a potential Z-DNA forming sequence, 25times repeats of TG/CA dinucleotide , in a intron of a human cardiac muscle actin gene. This is the first demonstration of potential Z-DNA forming sequence in a natural DNA. Furthermore, by hybridization using ^{32}P-labled poly(TG).poly(CA) as probe, we detected about 10^5 copies of this sequence throughout human genome. Attempt are underway to know its biological functions

0945 THE ORGANIZATION AND EXPRESSION OF THE GLOBIN GENES OF XENOPUS LAEVIS R.K. Patient*, R.M. Kay+, D. Banville+ and J.G. Williams+. *King's College, Department of Biophysics, London WC2B 5RL, U.K. and + ICRF, Mill Hill, London, NW7 1AD, U.K.

In X.laevis, the major adult α- and β-globin genes (α' and β') reside on the same chromosome separated by 8kb. Two of the minor adult globin genes (α^2 and β^2) are also linked and separated by the same distance. There is no evidence that the major and minor loci are linked and X. tropicalis, a diploid relative of the tetraploid X. laevis, contains only one α-β locus. These results suggest that there has been gene duplication by tetraploidization and that the major and minor loci reside on separate chromosomes. We have since discovered a major tadpole α-globin gene (αT') 5 kb upstream of α' and a minor tadpole α-globin gene (αT^2) 11 kb upstream of α^2. This result suggests that an insertion/deletion event between αT and α occurred at some time after the appearance of the major and minor loci. We have also isolated genomic and cDNA clones for the major tadpole β-globin, as well as other minor adult and tadpole globins. The locations of these genes relative to the two characterized loci are currently under investigation. The complete nucleotide sequences of α' and β' and their flanking regions have been determined. The start points of transcription have been identified and the conserved ATA, CCAAT, AATAAA and GT-AG sequences have been found in the same positions as in other genes transcribed by RNA polymerase II. By analysis of nuclear RNA from erythroid cells, full length precursors, and splicing intermediates in their processing, have been detected. Repetitive sequences and RNA polymerase III transcripts have been mapped 5' and 3' of α' and 3' of β'. Nucleotide sequences from the major tadpole α- and β-globin genes (αT' and βT') will also be presented. We are currently studying the expression of these various genes by microinjection into oocytes and fertilized eggs.

0946 EVOLUTIONARY CONSERVATION OF TWO PROTEINS ASSOCIATED WITH U1 SMALL NUCLEAR RNA AND THEIR BEHAVIOR DURING HEAT SHOCK IN DROSOPHILA. Eric Wieben and Thoru Pederson, Worcester Fdn. for Exptl. Biology, Cell Biology Group, Shrewsbury, MA 01545.

We have identified two proteins (14,000 and 26,000 mol. wt.) in nuclear extracts of Drosophila Kc cells which are specifically precipitated by a human autoimmune antibody selective for U1 small nuclear RNA. These proteins comigrate on SDS-polyacrylamide gels with the B and D proteins previously identified in mammalian U1 small nuclear RNP. Electrophoresis of RNA from the antibody-precipitated Drosophila RNP reveals a single major RNA species that comigrates with HeLa cell U1 RNA. Analysis of the Drosophila U1 RNP on sucrose gradients reveals that the two antibody-precipitable proteins co-sediment at 9-18 S, which is similar to the sedimentation of U1 RNP from mammalian cells. Further experiments revealed that the antigenic integrity of Drosophila U1 RNP is not disrupted by heat shock. Moreover, the rate at which the 14,000 dalton polypeptide is synthesized and assembled into an antigenically active RNP is not reduced after heat shock. These findings are consistent with the hypothesis that U1 RNP performs an indispensable function in the cell nucleus, perhaps in mRNA splicing. The evolutionary conservation of the 14,000 and 26,000 mol. wt. proteins in U1 RNP from insect and mammalian cells, and the apparent absence of any additional proteins in the Drosophila U1 RNP, strongly suggests that these two proteins are functional components of these RNPs.

0947 DEVELOPMENTAL EXPRESSION OF A TRANSCRIBED REPEAT SEQUENCE AND ASSOCIATED SINGLE-COPY GENES IN DICTYOSTELIUM. Alan R. Kimmel, LNE/NIADDK, NIH Bethesda, MD 20205

M4 is a short, repeat sequence interspersed with single-copy regions and transcribed on the 5'-ends of a set of mRNAs from Dictyostelium (Kimmel and Firtel (1979) Cell 16: 787; Kimmel and Firtel (1980) N.A. Res. 8: 5599). 1-1.5% of total vegetative poly(A) + mRNA hybridizes to the repeat. The mRNA is heterogeneous in size and >90% of the mass is complementary to single-copy DNA. The data suggest that most of the single-copy genes associated with the M4 repeat are expressed at a low abundancy level during vegetative growth. We have isolated different recombinant M4 genomic fragments and associated single-copy regions. The DNA sequences of a number of these have been compared to determine their common region. In addition, we have generated a series of deletions with Bal-31 exonuclease and used these to confirm that the M4 repeat lies only within the predicted sequence. A set of cDNA clones which contain the M4 repeat has also been isolated; the M4 repeat lies at the 5'-end of these cDNAs. During Dictyostelium development, the expression of the M4 repeat increases > 5-fold relative to vegetative growth. Additionally, using single-copy probes to specific M4 mRNAs we have shown that these individual mRNAs exhibit a relative increase in their developmental expresion which is similar to that of the total M4 mRNA population. There may be a co-ordinate increase in expression of the M4 gene set during development. We are interested in the possible function of this interpersed repetitive element in the regulation of developmental gene expression.

0948 EXPRESSION OF THE MATERNAL AND EMBRYONIC GENOMES DURING EMBRYOGENESIS OF SEA URCHINS Bruce P. Brandhorst, Frank Tufaro, and André Bédard. McGill Univ., Montreal, P.Q. Can.

We have investigated the expression of the paternal genome in sea urchin interspecies hybrids in an attempt to define the timing and extent of expression of the embryonic genome. We have used reciprocal crosses between S. purpuratus and S. droebachiensis as well as a cross between S. purpuratus eggs and L. pictus sperm. Based on two-dimensional electrophoretic analyses no proteins specific to the paternal species were detectibly synthesized at the 4 cell stage. The synthesis of a few paternal proteins was detected at hatching, but most distinctly paternal proteins were never detected even in mature plutei. Hybridization of radioactive cDNA transcribed from polysome-enriched poly(A)-RNA to homologous sperm DNA or hybrid embryo DNA indicated that DNA coding for mRNA normally translated in embryos of the paternal species is fully retained in hybrid gastrulae. Hybridization of these probes to excess poly(A)-RNA of hybrid embryos indicated a substantial underrepresentation of paternal transcripts, particularly those which are normally prevalent. These results indicate that either the expression of the paternal genome is largely but not uniformly restricted in hybrid embryos, or the mRNA coding for many of the proteins synthesized as late as pluteus stage is stored maternal RNA. Comparison of the products of cell-free translation of purified RNA and protein synthesis in vivo indicate that there is a close correlation between the presence of a translatable mRNA and its utilization in vivo. Thus if maternal mRNA codes for proteins whose synthesis begins during embryonic development it must be stored in a form untranslatable in cell-free systems. Cloned cDNA's are being used to sort out the various possible explanations for these observations.

0949 YEAST ARS SEQUENCES ARE ORIGINS OF IN VITRO REPLICATION. J.L. Campbell, S.E. Celniker, C.-L. Kuo, California Institute of Technology, Pasadena, CA 91125

In vitro replication systems have been instrumental in elucidating mechanisms of regulation of plasmid and chromosomal DNA replication in E. coli. In order to study the control of replication in eukaryotes, we have developed an enzyme system for studying plasmid replication in Saccharomyces cerevisiae (baker's yeast). In this system both the endogenous yeast plasmid 2 μm circle, or ScpI, and E. coli plasmids containing yeast chromosomal sequences that mediate autonomous replication of such DNAs in yeast (ARS sequences) serve efficiently as templates: twenty-five percent of input DNA is replicated. The most compelling evidence that the in vitro system uses the same replication machinery as is used in vivo is the fact that extracts of strain cdc8, a known yeast DNA replication mutant, are defective in in vitro synthesis, but can be complemented by Cdc8 protein purified in our laboratory by an independent assay.

We have shown that the ARS1 locus is an origin of directional replication by determining the order of labeling of restriction fragments during in vitro replication. Furthermore, electron microscopic analysis of replication intermediates has allowed precise mapping of the initiation site within ARS1. The relative efficiencies of deletion mutants of ARS1 plasmids as templates in vitro has defined neighboring sequences that influence origin activation. Similar results have been obtained with ScpI and ARS3. Thus, the control of DNA replication can now be studied biochemically and genetically in this in vitro system.

Information Transfer and Regulatory Molecules

0950 ALTERNATIVE RNA PROCESSING EVENTS IN CALCITONIN GENE EXPRESSION, Susan G. Amara*, Vivian Jonas*, Ronald M. Evans†, and Michael G. Rosenfeld*, *University of California, San Diego, School of Medicine, La Jolla, CA 92093; †The Salk Institute, San Diego, CA 92138

The neuroendocrine gene for calcitonin encodes an mRNA precursor which is alternatively spliced in a tissue-specific fashion to generate multiple mRNAs. The sequence of cDNA clones of two of the resultant mRNAs and the map and partial sequence of a genomic clone of the calcitonin gene are consistent with a model in which functional domains encoding component polypeptides are alternatively spliced during processing of the primary RNA transcript. Two mature RNA species analyzed share an identical 5' coding domain but differentially contain one of two alternative 3' coding exons. As a result these mRNAs encode protein products having identical N-termini, but which can generate different peptides from their C-termini during proteolytic processing. The physiological and functional significance of these "peptide switching" events is suggested by the observation that the mRNA encoding the protein precursor of calcitonin represents the major species in C-cells of the thyroid, while a second alternatively spliced mRNA appears to be preferentially produced in the hypothalamus. The consequence of "peptide switching" is that one neuroendocrine gene can be expressed as one product, the hormone calcitonin, in the thyroid and a second product, a new putative hormone referred to as CGRP, in the hypothalamus.

0951 ALTERNATIVE SPLICING IN NORMAL AND MUTANT HUMAN α-GLOBIN GENES, Barbara K. Felber, Stuart H. Orkin* and Dean H. Hamer, National Institutes of Health, Bethesda, MD 20205 and *Childrens Hospital, Boston, MA 02115

Sequence analysis of the α_2-globin gene from an α-thalessemia patient revealed a pentanucleotide deletion in the first intervening sequence immediately 3' to the first structural sequence. To test the functional consequences of this mutation we inserted the thalassemic gene, together with its normal counterpart as a control, into a pBR322-SV40 vector and transfected the recombinant molecules into cultured monkey cells. Both genes are transcribed, at similar levels, into approximately full-length poly(A)-containing RNA. The RNA from the thalessemic gene is spliced from a donor-like sequence in the middle of the first structural sequence to the normal 3' acceptor site. No RNA spliced at the normal site was present and there was no accumulation of RNA retaining the first intervening sequence. In contrast, most of the RNA from the normal gene is spliced at the usual donor site, but significant amounts of RNA spliced at the novel donor site were detected. Alternatively spliced RNA was also present in the bone marrow and peripheral blood from the α-thalessemia patient and, in lower quantities, from β-thalassemia and normal individuals. These results show that the deletion of the first donor site is sufficient to account for the thalassemic phenotype and that alternative RNA splicing may be a general feature of normal as well as mutant globin genes.

0952 ALTERNATIVE SPLICING OF PRECURSOR β GLOBIN mRNA IN β^+-THALASSEMIA. Bernard G. Forget, Edward J. Benz Jr., Prabhat K. Ghosh, Yasuyuki Fukumaki, and Sherman M. Weissman. Yale University School of Medicine, New Haven, Connecticut 06510.

Nucleotide sequence analysis of a cloned β^+-thalassemic (β^+-thal) globin gene has suggested a molecular mechanism for deficiency of normal β globin mRNA in this disorder: a base substitution within the first intervening sequence (IVS-1) of the gene creates a potential new 3'-splice site for processing of the pre-β mRNA. We have now directly demonstrated the presence of abnormal (alternative) β mRNA splicing in β^+-thal in two ways: 1) by analysis of reticulocyte β mRNA of a number of different β^+-thal patients using S_1 nuclease and primer extension techniques; 2) by analysis of the β mRNA synthesized by monkey kidney cells infected with an SV40 recombinant virus containing the cloned β^+-thal gene. Analysis of reticulocyte RNA from over 12 unrelated individuals with β^+-thal revealed that, in the majority of the cases, an abnormally long β mRNA species of the expected size (19 additional nucleotides) could be detected in addition to normal sized β mRNA. The RNA synthesized in monkey kidney cells infected with the SV40 recombinant virus also contained the abnormally long β globin mRNA species in addition to normal sized β mRNA. However, in the monkey kidney cells the abnormal β mRNA species was the predominant species whereas in reticulocyte RNA, it was a minor species compared to the normal β globin mRNA. These results are interpreted as demonstrating that the alternative splice site generated in IVS-1 by the base substitution in β^+-thal is preferentially used over the normal 3'-splice site but the abnormally spliced β mRNA, because it is present in such low amounts in reticulocyte RNA, is presumably unstable and rapidly degraded.

0953 Unstable Beta-Globin mRNA in mRNA-Deficient β^{o} Thalassemia. Alan J. Kinniburgh, Lynne E. Maquat, Eliezer A. Rachmilewitz*, and Jeffrey Ross. McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706. *Department of Hematology Hadassah University Hospital, Mount-Scopus, Jerusalem, Israel.

The molecular defect in four Kurdish Jews with homozygous, mRNA-deficient β^{o} thalassemia was investigated. Electrophoretic profiles of pulse-labeled α- and β-globin RNAs are similar to those of non-thalassemics; therefore, at least one of the thalassemic β-globin alleles is transcribed. During a 30 min actinomycin D chase, most of the α- and β-globin mRNA precursors and processing intermediates are converted to mRNA-sized RNA. Thalassemic and non-thalassemic β-globin RNAs are indistinguishable, as determined by S_1-nuclease mapping and RNA blotting. Non-thalassemic β-globin mRNA is stable during a 30 min actinomycin chase, but 30-75% of the thalassemic mRNA-sized molecules is degraded during that period. We conclude that the absence of β-globin mRNA in this disease results from rapid turnover of β-globin mRNA-sized molecules.

0954 TRANSCRIBED AND NON-TRANSCRIBED REGIONS OF THE RIBOSOMAL RNA GENE OF TETRAHYMENA EXHIBIT DIFFERENT CHROMATIN STRUCTURES, Ted E. Palen, Daniel E. Gottschling, and Thomas R. Cech, University of Colorado, Boulder, CO 80309

Nuclei isolated from log phase or starved Tetrahymena thermophila were incubated to various extents with either micrococcal nuclease, DNase I, or DNase II. The DNA was purified and electrophesed on agarose gels. Southern blots of the gels were then hybridized with labeled probes derived from either the gene, central spacer, or terminal spacer region of the rDNA. Both the gene and central spacer regions had micrococcal nuclease digestion patterns indicative of a nucleosomal structure. The repeat distance was 175 base pairs for the gene and 195 base pairs for the central spacer, the latter equal to that of the bulk chromatin. The terminal spacer revealed a subnucleosomal pattern with a repeat of about 100 base pairs. A number of sites near the 5' end of the gene were found to be hypersensitive to DNase I and DNase II when DNA was digested in nuclei, but not when deproteinized DNA was digested. Major sites occurred at or near the origin of replication and the initiation point of transcription. Similar patterns were obtained with the DNA from both log phase and starved nuclei. However, the nucleosomal patterns and the Dnase I and Dnase II hypersensitive sites appeared at low levels of nuclease digestion (1-5% TCA solubility) with the DNA from log phase nuclei and at medium levels of digestion (10-25% TCA solubility) with DNA from starved nuclei.

0955 EXPRESSION OF HUMAN β-INTERFERON LINKED TO HSV-1 TK PROMOTER SEQUENCES IN MOUSE CELLS AND XENOPUS OOCYTES. G.R. Reyes, E.R. Gavis, B. Raj, G.S. Hayward and P.Pitha, Johns Hopkins School of Medicine and Cancer Center, Baltimore, Maryland 21205

The human fibroblast β-interferon (HFβ) structural gene has been subcloned from a cDNA-pBR322 plasmid (pHFβcDNA) downstream to the herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) promoter region. A BglII linker modified 560 bp HFβ fragment was inserted into the single BglII site of HSV-1 TK located 55 bp before the authentic viral TK AUG start, and 77 bp after the TATTAA box. The regulatory sequences necessary for accurate initiation (-37 to -109) were maintained (McKnight et al., 1981). The hybrid HFβ gene was assayed by oocyte injection for authentic IFN production. Only the sense orientation of the HFβ insert expressed active IFN, in contrast to both the reverse orientation, and the original pHFβcDNA. By virtue of a selectable HSV-2 TK gene linked to the hybrid HFβ, Ltk$^-$ mouse cells were transfected to the TK$^+$ phenotype. The cell lines generated did not demonstrate constitutive IFN production but all were inducible with polyrI:rC. A single TK$^+$ cell line receiving the hybrid gene in the sense orientation was also inducible upon infection with HSV-1. The kinetics of HSV-1 induction mimicked that seen with poly rI:rC induction of IFN in human cells and of TK synthesis in infected mouse cells. However, IFN synthesis after poly rI:rC induction in the mouse cells continued unabated for longer than 72 hr. The specificity of the IFN produced in oocytes and Ltk$^+$ mouse cells was confirmed to be human β-IFN by differential indicator cell assays and by neutralization with antisera.

0956 PARTIAL PURIFICATION OF THE NUCLEAR ACCEPTOR SITES WHICH BIND THE AVIAN OVIDUCT PROGESTERONE RECEPTOR, Bruce A. Littlefield, Hiroo Toyoda and Thomas C. Spelsberg, Department of Cell Biology, Mayo Clinic and Graduate School of Medicine, Rochester, MN 55905. The nuclear binding site (acceptor site) for the avian oviduct progesterone receptor (PR) contains specific chromatin proteins called acceptor proteins (Thrall and Spelsberg, 1980, Biochemistry 19, 4130). These proteins are tightly bound to DNA and thus the majority of chromosomal proteins can be removed from chromatin without losing the acceptor sites. Previous studies have shown that PR binding to these acceptor sites in vitro displays the same requirements and properties as nuclear PR binding in vivo. In the present studies, partially deproteinized hen oviduct chromatin (nucleoacidic protein or NAP) containing the acceptor sites was digested with DNase I and bound with PR. Acceptor sites remained after 80 to 90% acid solubilization of the DNA; DNA length was reduced from 20,000 to 100 base pairs (bp) average. These remaining DNA segments were nuclease resistant. Preparation of slightly larger fragments (150 to 200 bp) obtained with only 40 to 60% acid solubilization followed by complete deproteinization allowed nick translation and sequence complexity analysis of the DNA. This analysis revealed that the protected sequences were repetitive; no unique sequences were observed. Presently, DNase I digested NAP fragments are being fractionated by isoelectric focusing and hydroxylapatite chromatography, yielding further enrichment of the nucleoprotein containing the acceptor sites for PR. The DNA in these complexes will be analyzed and sequenced. (Supported by HD 9140-B and the Mayo Foundation.)

0957 THE EXPRESSION IN ESCHERICHIA COLI OF CLONED HUMAN SERUM ALBUMIN cDNA, Barbara Wallner Philipp and Walter Gilbert, Harvard University, Cambridge, Ma 02138

Recombinant plasmids containing human fetal liver cDNA were screened for human serum albumin (HSA) sequences using cloned mouse albumin cDNA as hybridization probe. These plasmids had been constructed by insertion of double stranded cDNA copies of fetal liver poly(A)RNA in the Pst I site of the expression vector pKT218. Eighteen positive clones were isolated and the HSA cDNA inserts confirmed by DNA sequencing and comparison to the known amino acid sequence of HSA. Two of these clones were ligated at a common restriction site, producing a 2100 base pair clone pcHSA11. The DNA sequence of this plasmid shows that it contains the coding sequence for prepro-HSA including 250 base pairs of noncoding sequences at the 3' terminal.

Expression of HSA in pcHSA11 transformed E. coli was determined by radioimmuno assays and by comparison of the synthesized protein with mature HSA on SDS polyacrylamide gels. Transport of bacterial HSA antigen into the E. coli periplasm was shown by standard radioimmuno assays of the respective fractions.

0958 ORGANIZATION AND EXPRESSION OF A FAMILY OF CYTOCHROME c GENES IN RAT, Richard C. Scarpulla and Ray Wu, Cornell University, Ithaca, NY 14853

We isolated and sequenced a gene encoding the entire rat cytochrome c polypeptide (J. Biol. Chem. 256, 6480-6486 (1981)). A family of approximately 25 different sequences representing intact or nearly intact gene copies in the rat genome hybridize to this gene. These cytochrome c-specific sequences are divided into 3 groups based upon homology with the gene of known structure. A subgroup of about 6 members have sequences common to a 73 nucleotide portion of the 5' non-coding DNA immediately preceding the AUG initiation codon. Four of these are also included in the highest homology group of 7 members whose coding sequences differ by less than 2% mismatched bases. The 3 remaining genes in this class each have base substitutions which would result in the replacement of amino acid residues that are invariant in all known eucaryotic cytochromes c. One of these also has an internal termination codon and is most likely a pseudogene.

Three cytochrome c mRNAs of sizes 1.4, 1.1 and 0.7 kb are identified in adult liver, kidney, testis and heart. Thermal dissociation experiments show that the coding sequences of all 3 mRNAs are indistinguishable from that of the rat gene we originally sequenced. They also have regions complementary to the same portion of the 5' non-coding region as the 4 high homology DNA sequences. The major cytochrome c transcripts in these adult tissues, therefore, appear to arise from a small group of closely related genes that have extensive homology at the 5' non-coding region.

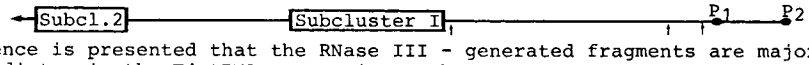
0959 THE EFFECTS OF SPECIFIC RHIZOBIUM MUTATIONS ON HOST PLANT GENE EXPRESSION DURING SYMBIOTIC NITROGEN FIXATION, Wynne Szeto, J. Lynn Zimmerman and Fred Ausubel, The Biological Laboratories, Harvard University, Cambridge, MA 02138

The formation of nitrogen (N_2) fixing nodules in the roots of the legume alfalfa (Medicago sativa) is a symbiotic process which results from specific interactions with the invading bacteria Rhizobium meliloti. In order to obtain probes to examine the molecular and biochemical mechanisms underlying the reciprocal signalling which activates the developmental process leading to symbiotic N_2 fixation, a series of R. meliloti mutants carrying mutations in the nitrogenase structural gene region were generated by site-specific transposon Tn5 mutagenesis (G. Ruvkun, Ph.D. Thesis, Harvard University). We have used these mutants to induce ineffective (Fix^-) nodules in alfalfa and have examined the proteins synthesized in these Fix^- nodules. Our results show that there are both quantitative and qualitative differences between the proteins of the Fix^- nodules and those of nodules induced by wild type Rhizobium. Furthermore, the transcriptional properties of leghemoglobin - the most abundant nodule specific plant gene product made during symbiosis - in the Fix^- nodules were also analyzed. The results of these analyses, and their relevance to the developmental program leading to effective nitrogen fixation, will be discussed.

0960 ENDONUCLEASES INVOLVED IN PROCESSING OF TRANSCRIPTS OF BACTERIOPHAGE T4 tRNA GENE CLUSTER, Alexander Goldfarb[X], Tamar Barkay[+] and Violet Daniel[+]

[X]Max-Planck-Institut für Biochemie, 8033 Martinsried b. München, FRG and [+]Biochemistry Department, Weizmann Institute of Science, Rehovot, Israel

The tRNA gene cluster of bacteriophage T4 consists of ten closely linked genes arranged in two subclusters separated by a spacer of about 0.6 kb. The two promoters serving these genes are situated at distances of about 1 kb and 1.35 kb upstream from the first tRNA gene and a terminator shortly after the last gene of the cluster. Isolated in vitro transcripts specified by these signals were used for the study of the tRNA processing. Purified RNase III cleaves the transcripts at three primary sites situated at the leader sequence between the promoters and the first tRNA gene as shown by arrows on the scheme:



Evidence is presented that the RNase III - generated fragments are major intermediates in the T4 tRNA processing pathway.

A new endonuclease was purified from E. coli extracts by using the in vitro transcripts as assay substrates. This enzyme attacks the fragment generated by RNase III and cleaves it into monomeric and dimeric tRNA precursors which are known to be the substrates for RNase P.

0961 TRANSFERENCE OF GLUCOCORTICOID REGULATION OF RAT GROWTH HORMONE EXPRESSION TO CULTURED MOUSE CELLS, Marcia Barinaga, Johannes Doehmer, Wylie Vale, Michael G. Rosenfeld, Inder Verma, and Ronald M. Evans, Salk Institute, San Diego, CA 92138

It has been proposed that glucocorticoid hormones exert their regulatory effect on gene expression at the nuclear level, through a direct interaction with the chromatin. Our lab has shown that, in growth hormone producing GH cells, dexamethasone regulates the growth hormone gene at the transcriptional level. The sites of glucocorticoid-DNA association might be determined by the primary structure of the DNA, or by chromosomal structure. To test the hypothesis that specific DNA sequences may be responsible for the interaction, we have taken a genetic approach, and reintroduced the rat growth hormone gene into mammalian cells.

In our first of several approaches, we used a vector derived from Maloney Murine Sarcoma Virus (MSV). The MSV was cloned in pBR322, and the entire growth hormone gene, with several kilobases of flanking DNA was inserted into the MSV DNA, in an orientation with growth hormone transcription proceeding in the opposite direction from MSV transcription. One of the clonal isolates obtained, showing MSV transformation, was characterized in detail. By northern analysis, this cell line was shown to be producing a growth hormone reactive mRNA, and was secreting authentic growth hormone into the medium. The growth hormone produced was immunologically and biologically active. To test the postulate that the dexamethasone regulatory element had been transferred with the gene, the cells were given dexamethasone, and a 2.5-fold induction of growth hormone levels was observed.

0962 PROCESSING OF RNA IN E. COLI AND BACTERIOPHAGE T4, David Apirion, Bheem Bhat, Michael Gurevitz, Swatantra Jain, Andras Miczak, Monoj Roy and Ned Watson, Washington University, St. Louis, MO. 63110

Analysis of RNAs accumulated in mutants defective in RNA processing showed that four processing endoribonucleases, RNase III, E, F and P participate in the maturation of rRNA and tRNA in E. coli. Two of these enzymes, RNase III and E also have profound effects on mRNA metabolism. RNase E is responsible for the formation of p5 rRNA. It introduces two successive staggered cleavages in a stem formed by nucleotides which flank the 5S rRNA sequences. In all seven rRNA genes of E. coli, the stems in which RNase E introduces its cleavages are identical. Moreover, the sequence between the 23S and the 5S is also conserved in all the 7 rRNA genes, and at least some of these sequences are involved in the RNase E reaction.

The synthesis of T4 tRNAs is carried out by the same host enzymes. RNase III introduces a cleavage 6 nucleotides upstream from the first tRNA in the tRNA cluster. This cut is followed by cleavages at or near the 3' ends of the tRNAs, probably by RNase F, and RNase P introduces the last cleavages which form the 5' ends of the tRNAs. The mutant RNase E enzyme causes inhibition of RNase F cleavages, and brings about the accumulation of new precursor RNAs. One of these precursors, p2Sp1, which contains species 1 (a tRNA like molecule; this RNA is the last species in the T4 tRNA cluster) and the sequence 3' to it (about 75 nucleotides) was used to identify a new endonucleolytic activity which we call RNase F. The enzyme cleaves between a C and an A, producing a 3' phosphate and a 5' hydroxyl groups. The cleavage is in a putative stem and loop structure, separating the stem from the loop. The specificity of this enzyme could be, the introduction of a cleavage near the 3' ends of tRNA molecule in RNA precursors.

0963 ALU AND OTHER SnRNAs, Ramachandra Reddy, Dale Henning, Wen-Yu Li, Paul Epstein, Eng Tan, and Harris Busch, Baylor College of Medicine, Houston, Texas 77030

7-8S SnRNAs of rat and human cells have been characterized (Reddy et al, JBC 256, 8452-8457, 1981). Four RNAs, 7-1, 7-2, 8S and 8-2, were found to be nucleolar and 7-3 RNA was a nucleoplasmic localization. The 7S RNA was found to be partly nuclear and partly cytoplasmic; its nucleotide sequence for potential binding to abundant and dispersed Alu family sequences were studied. The 5'-end 80 nucleotides and a portion near the 3'-end of 7S RNA was found to be homologous to human Alu family sequences, mouse B1 sequences and La 4.5S RNA. The sequence homology between 5'-end of 7S RNA and a portion of human Alu DNA sequence is shown below. The asterisks indicate nucleotide identity.

```
7S RNA pppGCCGGGC.. .GGUG.CGCA CGCCUGUAGU CCCAGCUACU CGGGAGGCUG AGACAGGCAG AUCGCUUGAG
          ** ***     **** * ** ******** * *****    *  *******   ** ******* *** * ****
Human Alu GCUGGG.AG  GGUGGCUCA CGCCUGUAAU CCCAGAAUUU UGGGAGGCCA AGGCAGGCAG AUCACCUGAG
sequence
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Recently, small RNPs precipitated by antibodies from patients with Scleroderma were characterized. Two nucleolar RNPs containing RNAs 7-2 and 8-2 were precipitated by "anti-To" serum. Since the isolated RNAs were not precipitable by these antibodies, nucleolar 7-2 and 8-2 RNAs are probably complexed with protein(s), like other snRNAs. In addition, antibodies from five different patients precipitated nucleolar U3 RNP complexed with preribosomal RNPs.

0964 EVIDENCE FOR AN SV40 LATE TRANSCRIPTIONAL CONTROL FACTOR: MIXED INFECTION OF A LATE LEADER DELETION MUTANT AND WILD TYPE EXHIBIT A TRANS EFFECT ON LATE TRANSCRIPTION. James C. Alwine, University of Pennsylvania, Philadelphia, Pa., 19104

Mixed infections involving equal multiplicities of wild type Simian Virus 40 (SV40) and viable deletion mutant d1861, in comparison to individual infections, result in decreased cytoplasmic levels of wild type derived late mRNA as well as very low to undetectable levels of mutant derived late mRNA. The d1861 deletion removes 16-25 base pairs from the late leader region. This deletion was shown to be the direct cause of the mixed infection effect; replacement of the deletion with wild type sequences restored normal levels of late mRNAs in mixed infections. Other viral functions, e.g. early gene expression and replication, were found to be uneffected by the d1861 deletion. Further examination of the mixed infection effect showed that the levels of unspliced, nuclear precursors of late mRNA, derived from each genome, were decreased or undetectable in accord with the cytoplasmic results. Thus the effect appears to be occuring at the transcriptional level. Overall the data demonstrated a trans acting effect on late transcription, which is detectable due to the presence of the d1861 mutant in the mixed infection. This finding is indicative of a diffusable factor which exerts a control on SV40 late gene expression at the transcriptional level. Based on the data a positive control model can be postulated. 11/11/81.

0965 STUDIES ON THE HUMAN COLLAGEN α1(I) GENE AND PROCOLLAGEN mRNA. K.S.E. Cheah, E. Weiss, R.A. Flavell and E. Solomon, Imperial Cancer Research Fund & National Institute for Medical Research, London, U.K.

The differentiated state of connective tissues is characterised by the synthesis of specific collagen molecules of which 5 distinct species are known, encoded for by at least 9 genes. Regulation of the expression of collagen genes is basic to vertebrate development and the characterisation of these genes is a prerequisite to the study of controls governing the synthesis of collagen and its precursor, procollagen. We have isolated a 38kb genomic clone (cosHcol.1) from a human cosmid DNA library which has been identified as the collagen α1(I) gene as follows: 1.5kb of cosHcol.1 was sequenced and contained 5 exons covering 99 amino acids collagen α1(I). The cell-free translation of procollagen α1(I) mRNA was specifically arrested by hybridisation of cosHcol.1 to human fibroblast mRNA. Also, fragments of cosHcol.1 selected pro-α1(I) mRNA from human mRNA. The extent of the α1(I) gene contained in cosHcol.1 was investigated. All EcoR1 fragments of cosHcol.1, except 1.5kb at the extreme 5' end hybrid-selected pro-α1(I) mRNA and hybridised to mRNA on a Northern blot. At the 3' end, sequences for the carboxy terminus of pro-α1(I) are situated close to the vector. The data suggests that cosHcol.1 may contain the entire human collagen α1(I) gene. CosHcol.1 hybridises to 2 distinct mRNA species 5.7 and 6.9kb. It is possible that there are 2 mRNA's of different size for human pro-α1(I) mRNA, alternatively one may be a precursor of the other. Experiments are described comparing the hybridisation of cosHcol.1 to various types of procollagen mRNA from different species and its use in the study of procollagen mRNA synthesis on viral transformation of human fibroblasts.

0966 LOCALIZATION OF SEQUENCES RESPONSIBLE FOR HEAT SHOCK INDUCED TRANSCRIPTION, V.G. Corces, A. Pellicer*, R. Axel* and M. Meselson, Dept. Biochem. and Mol. Biol., Harvard Univ., Cambridge, MA 02138 and *College of Physicians and Surgeons, Columbia Univ., New York, NY 10032.

Heat shock and certain other stimuli coordinately induce in D. melanogaster vigorous transcription of genes coding for seven characteristic proteins. We have cloned these genes and have found regions of homology near the origin of transcription. In order to determine if these sequences play a role in controlling the heat shock response, we introduced a Drosophila heat shock gene into mouse Ltk$^-$ cells by cotransformation with the herpes tk gene. The Drosophila segment consists of the 2.25 kb transcribed region for the heat shock protein hsp 70 along with 1.1 kb of flanking 5' sequences and 0.2 kb of 3' sequences. Prior to temperature elevation no Drosophila RNA is detected. After heat shock approximately 800 copies of Drosophila mRNA accumulate per transformed mouse cell. Most of the transcripts have 5' and 3' termini indistinguishable from those induced in Drosophila embryos. These results indicate that regulatory sequences involved in the induction of the heat shock response are present in the transforming DNA. To localize them we constructed a hybrid gene containing 1.3 kb of the 5' sequence of the Drosophila hsp 70 gene including the transcribed region up to the first ATG spliced to the coding region of a human growth hormone gene (including the ATG initiation codon). The hybrid gene is expressed in mouse fibroblasts only after temperature elevation, indicating that sequences in the 1.3 kb of 5' Drosophila DNA are sufficient to place the growth hormone gene under heat shock control.

0967 AMPLIFICATION AND EXPRESSION OF DNA INTRODUCED INTO MAMMALIAN CELLS, Randal J. Kaufman and Phillip A. Sharp, Massachusetts Institute of Technology, Cambridge, MA 02139

A dihydrofolate reductase (DHFR) mini-gene has been constructed in pBR322 with a DHFR cDNA and the adenovirus major late promoter. DNA mediated transfer of this gene, in the absence of carrier DNA, transforms Chinese hamster ovary DHFR$^-$ cells to the DHFR$^+$ phenotype. Transformants contain one to several copies of the transfected DNA integrated into the host genome. Clones subjected to growth in increasing concentrations of methotrexate eventually give rise to lines containing greater than 1000 copies of the transforming DNA present on expanded chromosomes. A large poly (A)$^+$ RNA species encoding DHFR comes from initiation of transcription at the adenovirus major late promoter. The adenovirus leader sequence is spliced to a 3' splice site junction cloned into the mini-gene and the RNA is not polyadenylated at sequences in the DHFR cDNA, but rather uses polyadenylation signals downstream from the DHFR cDNA.

Since DHFR synthesis is regulated with cell growth (i.e. it's synthesis is repressed as cells are arrested in stationary phase of growth), clones with amplified mini-genes were tested for growth phase regulation. Most were found not to be regulated. However, one clone does show growth dependent DHFR synthesis. Since all lines tested utilized the same promoter and have identical 5' ends of their mRNA, the regulation must result from differences in the 3' end of the RNA or in differences in the location of the transforming DNA in the genome. In addition, adenovirus infection specifically represses the synthesis of DHFR from the mini-genes. Data will be presented concerning these regulatory events.

0968 RNA POLYMERASES AND THE SWITCH FROM EARLY TO LATE TRANSCRIPTION DURING INFECTION OF INSECT CELLS BY A NUCLEAR POLYHEDROSIS VIRUS, Robert F. Weaver, L. Yolanda Fuchs, Patricia Buller and Qin Jun-chuan. University of Kansas, Lawrence, KS 66045.
During the first eight hours after infection of fall armyworm (Spodoptera frugiperda) cells with autographa californica nuclear polyhedrosis virus, the viral RNA synthesis is completely inhibited by α-amanitin, a specific inhibitor of host RNA polymerase II. However, by 24 hr post-infection the viral RNA synthesis is totally resistant to α-amanitin, and therefore is apparently directed by another enzyme. In these studies, viral RNA synthesis is assayed by labeling RNA in infected nuclei, then extracting the RNA and hybridizing it to viral DNA-cellulose. The nature of the polymerase involved in late viral RNA synthesis is not known, but our studies suggest that it is a viral-coded or viral-modified enzyme. Specifically, we find that late viral RNA synthesis is stimulated about 50% by α-amanitin, while none of the host polymerases show this behavior. Furthermore, levels of 7(N-dibenzylamino)-α-amanitin that completely inhibit the host RNA polymerases in vitro and in intact nuclei only inhibit viral RNA synthesis in infected nuclei by 75%. Thus, the switch from early to late gene expression in this eukaryotic virus seems to involve a switch in RNA polymerase. Late viral RNA is predominantly (75%) poly(A)$^-$, as assayed by oligo (dT) cellulose chromatography. The distinction between poly(A)$^+$ and poly(A)$^-$ late viral RNA was reinforced by electrophoresing these two classes of RNA from infected cells, "Northern" blotting and hybridizing to (^{32}P) viral DNA. The virus-specific bands in the poly(A)$^+$ and poly (A)$^-$ fractions are distinct sub-sets. Finally, both poly(A)$^-$ viral RNA's are capped with typical cap 1 structures (m^7GpppX^mpYp), and are thus probably mRNA's.

0969 EXPRESSION OF NORMAL AND THALASSEMIC GLOBIN GENES, T. Ley, K. Humphries, G. Pepe, M. Goldsmith and A.W. Nienhuis, NHLBI, Bethesda, MD 20205
Several patients with β-thalassemia have been shown to have increased levels of β globin mRNA precursor, thus implying the presence of mutations which affect RNA processing. We have examined splice junctions which are derived from mRNA processing by determining the lengths of portions of several probes protected from S_1 nuclease by total bone marrow mRNA obtained from a number of Greek and Italian thalassemic patients. The single stranded probes were prepared from M13-β globin gene recombinants which served as templates for synthesis of uniformly labeled probes complementary to portions of β globin gene transcripts. We found that 6/14 patients examined had an abnormal RNA species implying the presence of a mutation which creates an alternative splice site 22 bp upstream from the 3' end of IVS1 (Spritz et al., PNAS 78:2455, 1981). In addition, 7/13 patients examined had increased precursor RNA from which IVS1 sequences had not been removed. Cloned human α, δ, and β globin genes were replicated in monkey kidney ("COS") cells by using a plasmid expression vector containing the SV40 origin of replication but only a portion of one of the 72 bp direct DNA repeats found near the SV40 origin of replication. Although the α globin gene produced high levels of correctly processed mRNA in this vector, the β globin gene required activation by an additional SV40 segment which contains two complete 72 bp direct DNA repeats of SV40. This element activates the β globin promoter yielding high levels of correctly processed mRNA. δ globin gene expression was approximately 20-fold less than β with this modified expression vector, reflecting the relative level of expression of these two genes in normal bone marrow cells. Cloned thalassemic genes from patients with and without identified processing defects are being studied in COS cells.

0970 BOVINE PARVOVIRUS CONTAINS OVERLAPPING TRANSCRIPTION UNITS, Parris R. Burd, Robert C. Bates, Muriel Lederman, and Ernest R. Stout, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061
Bovine parvovirus (BPV) contains a linear, single-stranded DNA of approximately 5.5 kb. This DNA codes for three capsid proteins of 80, 70 and 57 kd which exceeds the coding capacity of the genome. A transcription map of the genome was prepared to assess the extent of transcription overlap. Total cellular RNA was isolated from BPV-infected cells at 16-24 hours p.i. by centrifugation through guanidine thiocyanate-CsCl gradients. This RNA was electrophoresed on 2.2M formaldehyde-agarose gels, transferred to nitrocellulose by the method of Thomas and probed with restriction fragments end-labeled by T4 DNA polymerase-mediated exchange. Hybrids treated with S1 nuclease and RNAs selected by hybridization chromatography on BPV DNA coupled to DBM-cellulose were analyzed by blot hybridization. Sizes determined for S1 nuclease-treated hybrids were 5.25, 2.6, 2.3, 1.6, 0.85 and 0.52 kb while for untreated RNAs sizes were 5.25, 2.85, 2.4 and 1.8 kb. Restriction fragment blot hybridization indicated that restriction fragments *Bgl* II (A) (map position 1.00-0.51) and *Bgl* II B (0.51-0.20) hybridized to RNA species 2.8, 2.6, 2.4, 2.3 and 1.6. In addition *Bgl* II C (0.20-0.00) hybridized weakly to RNA species 2.8, 2.6, 2.4 and 2.3. Similarly, hybridization with *Hinc* II C (0.23-0.105) and *Hinc* II D (0.11-0.00) show that this hybridizable sequence is limited to map position 0.15-0.05 suggesting a leader sequence at the 5' end of the 2.6 and 2.3 kb RNAs. These overlapping RNAs direct an in vitro translation system to synthesize immunoprecipitable viral proteins of the expected sizes.

0971 CHARACTERIZATION OF YEAST MITOCHONDRIAL mRNAs, Barbara E. Thalenfeld, Columbia University, New York, N.Y. 10027

The precursor transcripts and mRNAs coding for subunits 2 and 3 of cytochrome oxidase, subunit 9 of the ATPase and apocytochrome b have been studied by Northern blot hybridization analysis and S1 mapping. With the exception of apocytochrome b, these mitochondrial proteins are encoded by co-linear genes. The apocytochrome b gene, however, contains two intervening sequences. Maturation of the pre-messenger RNAs occurs by discrete cleavages at the 5' and 3' ends in the precursors. In the case of the apocytochrome b transcripts, there are additional cleavage and ligation events leading to the excision of the intervening sequences. The four mRNAs studied have 5' and 3' non-coding sequences of varying lengths. The mRNA for subunit 2 of cytochrome oxidase has a 54 nucleotide long 5' leader whereas the three other mRNAs have leaders exceeding lengths of 500 nucleotides. There are no apparent sequence homologies at the 5' termini of the mRNAs suggesting that processing involves features in the secondary structures of the RNA. The 3' termini, however, reveal a common 7 nucleotide sequence (5'-ATTCTTA-3') that could function as a recognition site for a 3' specific endonuclease. In several instances the protein coding sequences are co-transcribed with tRNA genes. The processing of the tRNAs may therefore provide still another means for mRNA maturation. The four mRNAs have been found to contain sequences upstream of their AUG initiation codons that can base pair with a sequence in the 3' tail of the 15s rRNA. Although the yeast mitochondrial 15s rRNA sequence differs from the Shine-Dalgarno sequence of the E. coli rRNA, its function may be analogous.

0972 VARIANT HUMAN BREAST CANCER CELLS THAT SYNTHESIZE LARGE AMOUNTS OF PROGESTERONE RECEPTORS DESPITE ESTROGEN AND ANTIESTROGEN RESISTANCE. K.B. Horwitz, University of Colorado Health Sciences Center, Denver, Colorado 80262.

In all estrogen targets studied to date, progesterone receptors (PgR) are synthesized under control of estradiol, acting through estrogen receptors (ER). We have developed and studied a variant of T47D human breast cancer cells which synthesize extraordinary amounts (300,000 sites per cell) of PgR in the absence of estradiol, and in the presence of high concentrations (1 μM) of the antiestrogen nafoxidine. The cells are estrogen and antiestrogen resistant as shown by growth studies and PgR synthesis. The ER are anomalous: there are no unfilled ER in cytoplasm or nuclei; only filled nuclear receptors are present which appear to be in a persistently activated, or "processed" state. We have studied the binding, translocation, nuclear turnover and replenishment of PgR in detail following progesterone treatment: cytoplasmic depletion is progestin specific; nuclear translocation is rapid (1-2 min) and stoichiometric: nuclear turnover is extensive (∿ 80% total cell PgR are lost in 30-60 min) but chloroquine (100 mM) inhibitable; replenishment is protein synthesis dependent but estrogen independent. We have purified and characterized the human breast tumor PgR by standard techniques and by photoaffinity labeling: receptors consist of two dissimilar subunits whose MW is 108K and 79K; only one subunit binds DNA-cellulose. Despite their resistance to estradiol and antiestrogen, PgR are not constitutively synthesized; BudR (20 μg/ml) and butyrate (10 mM) can selectively inhibit PgR production. In sum, these variant cells have anomalous ER, their PgR independent yet they retain some characteristics of inducible proteins.

0973 ASSOCIATION OF LIPOSOME INTRODUCED snRNAs WITH LARGE POLY A-CONTAINING NUCLEAR RNA IN DROSOPHILA, Robert H. Gross and Martin S. Cetron, Department of Biological Sciences, Dartmouth College, Hanover, NH 03755

Small nuclear RNAs (snRNAs) have been implicated in the splicing of heterogeneous nuclear RNA (hnRNA) to messenger RNA (mRNA). The proposed mechanism involves the transient base-pairing of part of an snRNA molecule to a specific region(s) of the hnRNA. In order to study this interaction in vivo, we have developed a protocol for efficiently packaging Drosophila snRNAs into liposomes (15-20% efficiency) and delivering them to Drosophila tissue culture cells (30% fusion). By packaging radiolabeled snRNAs and delivering them into unlabeled cells through liposome fusion, we have constructed cells in which the only label resides in the snRNAs. It is therefore possible to unambiguously follow the fate of these RNAs. Once introduced into the cells, the snRNAs are relatively stable (compared to whole cytoplasmic RNA) and are transported into the nucleus leading to a 25-fold higher concentration in the nucleus than in the cytoplasm. This implies that there is a signal(s) residing on the snRNA for its stability and translocation. Once in the nucleus, 40-50% of these snRNAs (which are poly A$^-$) can be bound to an oligo (dT)-cellulose column. This binding can be disrupted by prior heating of the sample. Further analysis of the oligo (dT)-binding snRNAs shows them to sediment very rapidly in sucrose velocity sedimentation gradients, again through a heat labile interaction. We conclude that a significant portion of the nuclear snRNAs are associating with large poly A-containing hnRNAs through a hydrogen-bonding type of interaction.

0974 RNA SPLICING AND HIGH LEVEL EXPRESSION OF HUMAN GENES IN A RETROVIRUS VECTOR, Joe Sorge and Stephen H. Hughes, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The v-src gene of avian sarcoma virus DNA was deleted to create a helper-independent, non-transforming vector for stable DNA integration and high level expression of selected genes. A cDNA copy of the alpha human chorionic gonadotropin (HCG) gene was inserted and the recombinant DNA was transfected into normal chicken fibroblasts. When infection had spread throughout the entire culture, virion and total cellular RNA were analyzed. Cells produced a level of RNA, containing the complete HCG sequence, equivalent to that found in term human placenta (0.5% poly A+). Recombinant virus passaged to uninfected cells produced full infection with no deletion or rearrangement of the HCG sequence for at least two months. Helper virus was never needed and infected cells retained their normal morphology.

When a genomic version of the HCG gene was inserted into the vector, resulting viral RNA was shown to contain populations of either perfectly spliced or unspliced copies of the insert in a ratio of 2:1 respectively. Although more data is needed, the preliminary interpretation is that viral RNA destined for packaging into virions is protected from splicing, whereas viral RNA destined for protein synthesis is spliced normally.

0975 DELETION MUTANTS OF THE MOUSE MAMMARY TUMOR VIRUS LTR WHICH DO NOT AFFECT THE CONTROL BY GLUCOCORTICOID HORMONE, Helmut Ponta, Nancy Hynes, Bernd Groner, Ursula Rahmsdorf and Peter Herrlich, Institut für Genetik und Toxikologie, Kernforschungszentrum Karlsruhe, Postfach 3640, D-7500 Karlsruhe 1, Federal Republic of Germany

Two kinds of molecular clones were modified by reverse genetics: i) a 13 kb fragment of mouse DNA containing one of the proviral mmtv genes. ii) a chimeric gene fusing the right half of an exogenous mmtv gene including the right LTR to the herpes thymidine kinase (tk) gene. Upon transfection into mouse cells, the expression of the proviral gene and the herpes tk gene respectively are induced by dexamethasone. With the chimeric gene, dexamethasone induces a fusion RNA. In addition, the LTR mediates enhanced hormone-independent transcription from the normal tk promoter (remote effect). Induction is 8-20 fold depending on clone picked. Deletions were constructed ranging into the left or right LTR respectively and removing increasing parts of the LTR. The largest deletions removed also the TATA box or the whole LTR. All deletion mutants were analysed by transfection and scored for their ability to promote transcription, for the remote effect and for the hormone inducibility of direct LTR dependent transcription. At the date of this abstract, deletions ranging up to 500 nucleotide 5' of the TATA box had been through the procedure. These deletion did not alter the hormone response.

0976 EXPRESSION OF RED CELL-SPECIFIC GENES IN COS-1 CELLS, James Douglas Engel and Sheryl Niemiec, Northwestern University, Evanston, IL 60201. We have cloned and sequenced (partially or completely) all of the chromosomal globin genes of the chicken, as well as a number of other red cell-specific genes. These have been introduced into COS-1 cells using a variety of plasmid vectors containing both SVori and one or more red cell-specific genes. The pattern of expression of unmodified and modified genes introduced in *cis* or in *trans* will be discussed.

0977 REVERSION BY HYPERMETHYLATION OF A DNA MEDIATED THYMIDINE KINASE TRANSFORMANT, Stephen C. Hardies, David E. Axelrod, Marshall H. Edgell, and Clyde A. Hutchison III, University of North Carolina, Chapel Hill, NC 27514

After DNA mediated transfer with the HSV thymidine kinase (TK) gene, we have isolated a cell line which is unusually prone to revert to TK^- by hypermethylation. Methylation of the TK sequences was monitored by the loss of an Eco RI site which is flanked by the dinucleotide CG. Some of the revertants also exhibited methylated Hpa II sites in the TK genes. This line was named HM for "hypermethylater". Clones of HM TK^- revertants, containing a full complement of 200 methylated TK genes, reverted back to TK^+ at very low frequency (10^{-6}). The TK^+ survivors did not de-methylate their TK genes, but rather amplified them 10 fold. Therefore, the hypermethylation is a genetically stable shutdown which is compensated for rather than reversed in rare back revertants. All transformants investigated showed a gradual increase in methylation even while under TK^+ selection. However, only HM showed hypermethylation (approaching 100% of the RI sites blocked) of TK^- revertants. Hypermethylation was a reproducible result in HM revertants after selection with either BUdR or acycloguanosine. Consequently, we have genetically defined two different mechanisms for placing methyl groups at new sites in the genome. The hypermethylation in HM is a concerted effect over a region of at least 10^6 base pairs. The sequences governing the potential for hypermethylation of TK genes in HM are presumably not in the TK genes themselves, since these were present in transformants which would not undergo this process.

0978 ANALYSIS OF THE RABBIT β-GLOBIN PROMOTER BY DNA RESTRUCTURING AND SITE-DIRECTED MUTAGENESIS. Peter Dierks, Albert van Ooyen, Carl Dobkin, Jakob Reiser, Hans Weber and Charles Weissmann, Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland.
Promoter sequences which influence the transcription of the rabbit β-globin gene were identified by analyzing the transient expression of modified β-globin genes in mouse 3T6 cells. The region containing the ATA box sequence (-31)CATAAAA(-25) constitutes the major determinant in directing the site of initiation of transcription. The ATA box is also required for efficient transcription. Mutants in which the ATA box was partially or completely removed expressed β-globin RNA at 1%-12% of the wild type level. In addition, a segment containing the CCAAT box sequence (-77)GGCCAATCT(-69) and a less well defined region located between positions -82 and -109 are also required for efficient transcription. Deletion of either region resulted in an 8- to 12-fold reduction in transcription. The importance of the ATA box and CCAAT box canonical sequences was verified by analysis of point mutants. Single or double point mutations at positions -26 to -28 reduced transcription 2 to 5-fold, and transition mutations at positions -73 to -75 reduced the level of transcription 4 to 8-fold. Transition mutations at several other locations had little or no effect.

0979 POLYOMA MUTANT INFECTION OF TERATOCARCINOMA STEM CELLS, Frank K. Fujimura and Elwood Linney, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

Although wild-type polyoma virus can adsorb to and penetrate mouse embryonal carcinoma (EC) cells, the stem cells of teratocarcinomas, the infection process appears to be blocked at a step before viral early protein synthesis. Differentiation of EC cells can lead to cell types that are permissive for polyoma infection, suggesting that cellular factors regulating polyoma gene expression may be altered during cell differentiation. We have isolated and characterized polyoma mutants that productively infect the EC cell line F9. These mutant DNAs are characterized by a point mutation near and to the late side of the origin of DNA replication. One mutant, PyF441, has only the point mutation while another, PyF101, has a perfect tandem duplication of DNA sequences encompassing the point mutation. These mutants do not rescue replication of wild-type polyoma DNA during a mixed infection of F9 EC cells. Mixed infection of 3T6 cells, which are permissive for both wild-type and mutant polyoma, leads to efficient replication of both viral genomes. A double mutant having the PyF101 tandem duplication and a temperature-sensitive lesion in large T antigen is temperature-sensitive for growth in F9 EC cells. The double mutant can be rescued at the restrictive temperature by coinfection of F9 EC cells with PyF441. (Supported by funds from the NSF, the NCI, the American Cancer Society, and the Leukemia Society of America).

0980 INSECT IMMUNITY - A MODEL SYSTEM FOR SELECTIVE GENE EXPRESSION, Hans G Boman, University of Stockholm, S-106 91 STOCKHOLM, Sweden.
The immune response of diapausing pupae of the Cecropia moth (Hyalophora cecropia) is an excellent model system for the study of selective gene expression, because of the following facts: 1) During the diapause the metabolism of the pupae is reduced to only a few percent of the level of a developing animal. Such dormant insects can respond to an infection by turning on only the genes forthe immune defence. 2) The immune response involves de novo synthesis of RNA and about 15 proteins. Some of these proteins have potent antibacterial activities which are easy to assay. Depending on their mobilities in SDS-PAGE the main protein fractions were designated P1-P9.The two major immune proteins are P4 and P5.

The P9 proteins, called cecropins, constitute only a few per cent of the newly synthesized proteins but they are responsible for most of the antibacterial activity towards Gram negative bacteria. We have purified 7 members of this family (designated A-G) and studied the structure of A-F. The most abundant cecropins are A, B and D and the minor components are belived to be metabolites of the major forms. Cecropin A and B contain 37 amino acid residues, they differ in only 12 residues and both have a blocked C-terminal. Cecropin D has been purified from Cecropia and from the Chinese oak silk moth, Antheraea pernyi. Both D forms are very similar and resemble cecropin A but they start with residue 2 of the A form. Present data indicate that cecropins A, B and D may have arisen through a serie of gene duplications. A method has been worked out for the isolation of mRNA from immunized Cecropia pupae. Cell free synthesis of proteins with this mRNA mixture indicates that only 10-12 polypeptide chains are synthesized. The mRNA preparation was used for the synthesis of cDNA. The product was inserted into pBR322 and clones have been identified which have fragments corresponding to immune proteins P4 and P5.

0981 METAL REGULATION OF HERPES THYMIDINE KINASE IN A FUSION GENE CONTAINING PLASMID, pMK, IN MOUSE L-CELLS, MOUSE OOCYTES AND ADULT MICE. R. Warren and R.D. Palmiter, Howard Hughes Medical Institute, Seattle, WA 98195; R. Brinster, University of Pennsylavia, Philadelphia, PA 19103.

A plasmid containing the regulatory sequences of mouse metallothionein-I gene fused to the structural gene sequences of herpes thymidine kinase (TK) has been inserted into various mouse cells and the regulation of herpes TK by factors known to induce metallothionein, i.e., heavy metals and glucocorticoids, has been investigated. In mouse L-cells transformed with the plasmid pMK, TK activity increases as a result of Cd exposure but not glucocorticoids. Similarly after injection of pMK into mouse oocytes only Cd induces TK activity. When a modified fusion plasmid containing 227 base pairs 5' to the site of gene fusion is analyzed, Cd still induces TK activity. TK activity and induction is particularly sensitive to the removal of palindromic sequences located within the 227 base pair region.

The fusion gene containing plasmid has also been injected into fertilized mouse eggs in order to assess the somatic expression of herpes TK in mice. In several mice containing pMK, herpes TK activity was induced by Cd. Primary cell cultures derived from these mice are being analyzed.

0982 TRANSCRIPTION OF THE CHICKEN LYSOZYME GENE AFTER TRANSFECTION IN EUKARYOTIC CELLS, Rainer Renkawitz, Patrick D. Matthias, Manuel Grez and Günther Schütz, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, F.R.G.

The chicken lysozyme gene codes for one of the four major egg white proteins, which are expressed under the control of steroid hormones. The lysozyme gene is about 4 kb long and interupted by three introns. To study the transcription and the regulation of this gene we are using four approaches: a) in vivo transcription, b) in vitro transcription, c) transfection in HeLa cells and other non-avian cells, d) microinjection in chicken oviduct cells.

a) In vivo transcription of chicken lysozyme gene starts at three different positions on the genomic DNA (Grez et al., Cell 25, 743 (1981)).
b) Using an HeLa cell extract for in vitro transcription, the same start sites are used. But in addition minor starts at new sites have been detected.
c) After CaPO4-transfection of the lysozyme gene into HeLa cells, transiently made RNA is isolated. Detectable RNA transcription is only found, if SV40 "enhancer" sequences are located upstream of the lysozyme promoter. The initiation starts correlate exactly with the three starts seen in vivo.
d) For microinjection of the lysozyme gene into chicken oviduct cells, a primary cell culture was established. To distinguish the expression of the injected DNA from the endogenous gene, we replaced the body of the cloned lysozyme gene with the body of the SV40 T antigen gene. The expression was detected by immunofluorescence against T and found to be dependent on the lysozyme upstream sequences, but does not depend on SV40 "enhancer" sequences.

Currently we are studying the regulation of the transcription by hormones.

0983 SYNTHESIS AND RELEASE OF FOREIGN GENE PRODUCTS FROM BACILLUS SUBTILIS USING A "SECRETION VECTOR". Ralf Pettersson,Ilkka Palva,Kenneth Lundström,Matti Sarvas and Leevi Kääriäinen. Recombinant DNA Laboratory, University of Helsinki, Finland.

To promote the expression of foreign genes in *Bacillus subtilis* and to facilitate the secretion of the gene products out of the cell, we have constructed a set of plasmids using the promoter and signal sequence regions of the α-amylase gene from *B. amyloliquefaciens*. The α-amylase gene was isolated by molecular cloning from the chromosomal DNA in the plasmid pUB110 using *B. subtilis* as a host. The N-terminal region and the region upstreams from the coding region were sequenced and the promoter region and the sequence coding for a 31 amino acid-long signal peptide were identified (Palva *et al.*, Gene 15:43:1981). By restriction enzyme treatment followed by digestion with Ba131 nuclease and ligation of restriction endonuclease linkers to the ends, a series of plasmids containing the whole signal peptide sequence and no or a few nucleotides coding for amino acids of the structural gene was constructed.

The gene for β-lactamase from pBR322 was isolated, its signal sequence removed and the modified gene containing HindIII linkers was ligated to different plasmids. Expression of β-lactamase was obtained with several different constructions. In one case the enzymatic activity was cell-associated, whereas in other cases virtually all the enzymatic activity was recovered from the culture medium. We also studied the expression of the gene coding for the envelope glycoprotein E1 of Semliki Forest virus (Garoff *et al.*, Nature 288:236:1981). The gene,from which the C-terminal hydrophobic region had been removed, was ligated to different secretion plasmids. The results of these experiments will be presented and discussed.

0984 STUDIES ON POLIOVIRUS PRODUCED IN CELLS TRANSFECTED WITH CLONED VIRAL cDNA. Vincent R. Racaniello and David Baltimore, M.I.T., Cambridge, MA 02139

Poliovirus is a positive-strand animal virus containimg a genome of one RNA molecule. Recently we have begun to study this RNA virus in new ways using recombinant DNA techniques. It was previously shown that a cloned, full-length DNA copy of the poliovirus genome is infectious in mammalian cells. The RNA and proteins of wild-type poliovirus (used to construct the cDNA clone) and six plaque-purified viruses released from cells transfected with cloned DNA were compared. The sequences of the first 5' 35 nucleotides of the viral RNAs were determined by primer extension. The sequences so obtained showed that each of the viruses produced by DNA transfection contained RNA with a 5' end identical to that of wild-type virus. Preliminary results of two-dimensional RNAse T_1 oligonucleotide fingerprinting indicated that the genomes of viruses obtained by DNA transfection were very similar to that of wild-type virus. Polypeptides induced in infected cells by transfection-derived poliovirus were indistinguishable in number or in migration, on SDS-polyacrylamide gels, from polypeptides induced by wild-type virus. Experiments are currently in progress to determine whether viruses obtained from transfected cells (1) contain VPg at the 5' end of their RNA, and (2) contain a 3'-terminal nucleotide sequence identical to that of wild-type virus. Our studies to date suggest that poliovirus produced from cloned DNA is identical in many ways to the wild-type virus from which the clone was derived. The infectious cDNA clone will therefore be extremely useful for performing specifically directed genetic manipulations with poliovirus.

0985 TRANSFER AND EXPRESSION OF HUMAN GROWTH HORMONE GENES. George N. Pavlakis and Dean H. Hamer, National Cancer Institute, NIH, Bethesda, MD 20205

We have constructed simian virus 40 recombinants carrying two different human growth hormone (hGH) genes. Cultured monkey cells infected with these recombinants synthesize, process and secrete hGH. The product of one gene is indistinguishable from pituitary hGH by several criteria. The product of the second gene differs from the pituitary hormone in its physicochemical and immunological behavior but binds efficiently to hGH receptors. This indicates that the second gene encodes a protein closely related to hGH but with as yet unidentified function. We have also constructed a series of mutants missing some or all of the 5' flanking and intervening sequences of the normal gene. One such hGH "mini-gene" was fused to the promoter of a cadmium-inducible mouse metallothionein gene and introduced into various cell lines by acute transfection with simian virus 40 vectors or by transformation with a bovine papilloma virus vector (in collaboration with P. Howley). The expression of the hybrid gene is regulated by cadmium in both systems. Some of the stable transformants produce more than 10^8 molecules/cell/8 hours of hGH under optimal induction conditions.

0986 DIFFERENT FORMS AND FUNCTIONS OF SV40 LARGE T ANTIGEN, Carol Prives, Lori Covey-Nichols, and Arno Scheller, Department of Biological Sciences, Columbia University,N.Y.,N.Y.10027

The affinities of SV40 large T antigen for unique viral DNA sequences were examined by binding Bst N-1 digested SV40 DNA in extracts of virus infected or transformed cells, and then immunoprecipitating the T antigen-DNA fragment complex with anti T serum. The 311 bp G fragment which spars the viral origin of replication (ori) was quantitatively bound to the T antigen. In addition, the binding of a small quantity of the 552 bp D fragment which contains a putative attenuator region was also detected. Various monoclonal antibodies were tested for their ability to immunoprecipitate specific DNA binding activity. Two monoclonal antibodies isolated by Gurney and colleagues (J.Virol. 34 752 (1980)) provided insight into the T antigen function. Mcl 7 which recognizes only a very small fraction (5%) of the total T antigen molecules from infected or transformed cells, immunoprecipitated the vast majority of their ori binding activity. Therefore only a small subpopulation of SV40 T antigen is active in origin-specific DNA binding. Mcl 122 which recognizes a 53000 dalton host protein which forms a complex with T antigen, immunoprecipitated 50% of the ori binding activity in SV40 transformed cells. This demonstrated that T antigen can bind to the viral origin when associated with the host antigen. There studies, and others which will be discussed, suggest that T antigen is a complex, multifunctional protein comprising several subpopulations with different activities.

0987 Construction of vectors from the genome of MoMuLV for the expression of nonselectable genes in mammalian cells; Eli Gilboa, J. S. Park, S. Hwang, R. Kucherlapati, K. Noonan, H. Freeman and M. Kolbe.

We have functionally identified and physically isolated viral DNA fragments required for the expression of the viral gag/pol and envelope coding sequences. Using several procedures, we have generated DNA fragments from the genome of MoMuLV, enzymatically joined to DNA fragments carrying the coding sequences of Herpes TK, mouse DHFR and bacterial neo^r genes; transfected the hybrid DNA into mouse cells and followed their expression.

Using this information we have constructed a set of versatile vectors for the expression of nonselectable genes (including cDNA copies of mRNAs) by effectively replacing the coding sequences of one of the viral genes with a selectable marker like Herpes TK and replacing the second gene with other nonselectable cDNA fragments.

In vitro reconstructed genes are used to study the mechanism of mammalian gene expression. The use of selectable vectors enables the isolation and analysis of cells carrying a defective gene construct.

0988 IN VIVO ACTIVITY OF THE CLONED MOUSE β-GLOBIN^{maj} GENE PROMOTER, P.E. Berg,[+] D. Schumperli,[*] M. Rosenberg,[*] E. Diacumakos,[§] and W.F. Anderson,[+]
[+]Laboratory of Molecular Hematology, NHLBI, [*]Laboratory of Biochemistry, NCI, NIH, Bethesda, MD 20205 and [§]Rockefeller University, New York 10021
The aim of these experiments is to define the promoter sequences required for expression of the mouse β-globin^{maj} gene and further to determine if there are sequences 5' to the β-globin gene which are necessary for induction of β-globin in MEL cells. An expression vector has previously been developed from pBR322 (Schumperli, Howard and Rosenberg, PNAS, in press) which contains as separate removable segments: a) a promoter region, b) a coding sequence (gal K from E. coli), c) a signal for RNA splicing derived from the SV40 t splice region, and d) a poly (A) addition signal from the SV40 early region. Our approach was to clone the mouse β-globin^{maj} promoter into the promoter region of this vector. This promoter was obtained by Hinc II digestion of the 7 kb fragment containing the mouse β-globin^{maj} gene, yielding a 500 bp fragment whose 3' end lies between the capping site and ATG initiation codon. This construction allows the unique opportunity to assay the function of the mouse β-globin^{maj} promoter directly by galactokinase levels in mouse MEL cells which already have a normal β-globin^{maj} gene. In addition, this vector contains a functional gpt gene which permits primary selection as well as a standard against which galactokinase activity can be compared. The levels of galactokinase and xanthine-guanine phosphoribosyl transferase are being assayed in CV-1 cells (transformed using the calcium phosphate method) and in MEL cells (transformed by physical microinjection) both before and after induction.

0989 INTACT BPV-HUMAN DNA RECOMBINANT PLASMIDS THAT PROPAGATE AS EPISOMES IN MOUSE AND BACTERIAL CELLS, D. DiMaio, R. Treisman, T. Maniatis, Harvard Univ., Cambridge, Mass.

We have been using a subgenomic fragment of bovine papillomavirus (BPV) DNA as a vector to introduce cloned eucaryotic genes into mammalian cells in culture. This vector is unique in its ability to propagate as an episome in transformed cells, but its use has been hampered by a several hundred-fold reduction of transformation efficiency when the BPV fragment is inserted into the bacterial plasmid pBR322. Thus, the cloned BPV DNA must be separated from the pBR322 DNA before it can be used to transform mammalian cells. We have found that BPV plasmids containing a 7.6 kb human β globin gene DNA fragment stably transform mouse cells about 500-fold more efficiently than does the analogous construct without human DNA. Blotting experiments on transformed cell DNA show that the recombinant DNA is maintained as an episome with no detectable rearrangements and with a copy number of about 25 per cell. Plasmids indistinguishable from the input DNA can be recovered by transformation of bacteria with DNA isolated from transformed mouse cells. Analysis of transformed mouse cell RNA indicates that the human β globin gene is transcribed at a high level and that the 5' ends of these transcripts are identical to those of authentic human β globin mRNA. Thus, the β globin gene in the BPV recombinant appears to be transcribed from its own promoter. In addition, both intervening sequences of the human mRNA precursor are accurately excised. The ability to shuttle cellular genes between bacterial and mammalian cells may provide a rapid means of analysing and recovering individual mutants of a cloned gene from a pool of mutagenized molecules. These vectors may also allow the isolation of selectable genes from mammalian cells.

0990 EXPRESSION OF HETERLOGOUS GENES IN XENOPUS EGGS, L.D. Etkin, M. Roberts and B. Pearman Dept. of Zool., Univ. of TN., Knoxville, TN. and S. Bektesh Dept. of Genetics, Univ. of Washington, Seattle, WA. We have analyzed the fate and expression of genes coding for Drosophila alcohol dehydrogenase (ADH) and sea urchin histones following their microinjection into fertilized eggs of Xenopus laevis. Southern blot analysis of microinjected DNA reveals that the genes persist until at least the feeding tadpole stage and that they probably replicate during early cleavage. Some of the DNA co-migrates with high molecular weight Xenopus cellular DNA, but the majority of the DNA molecules appear to remain extra-chromosomal. Microinjected DNA persists as the relaxed form (form II or form I^r) during early cleavage, but is converted to the supercoiled (form I) configuration at the late blastula-early gastrula stage of development. This difference in configuration is also observed in DNA incubated in vitro in extracts of embryos from either the early cleavage stages or the blastula stage. Aberrant transcripts are detected from both genes during early cleavage stages, but transcripts which co-migrate with authentic ADH mRNA or sea urchin histone mRNA appear at the late blastula-early gastrula stage of development. We also detect the presence of sea urchin histone proteins at the late blastula-early gastrula stage. These results demonstrate that microinjected heterologous genes survive, replicate, and are transcribed during development of the Xenopus embryo. The proper expression of these genes may be dependent upon the interaction of the DNA with molecular components in the embryo and the assumption of a specific physical configuration of the DNA molecules. Supported by N.S.F.

0991 EHANCEMENT OF TK TRANSFORMATION BY SEQUENCES OF BOVINE PAPILLOMA VIRUS DNA. Monika Lusky, Leslie Berg and Mike Botchan, University of California, BerkeleyCA94720

We have chosen to use Bovine Papilloma Virus (BPV) as a model system for studying the effect of autonomous replication on transformation in mammalian cells. BPV DNA is maintained as an autonomous replicon in the plasmid state in multiple copies in transformed rodent cells (Law et al.PNAS 81). Recombinant plasmids carrying the Herpes simplex virus TK gene (HSVTK) with and without the BPV genome were transfected into rodent TK^- cells. The transformation efficiency to TK^+ was increased up to 100 fold with BPV-TK plasmids compared to that of TK plasmids alone. In addition the enhancement increased as the stringency of the selective conditions increased. Surprisingly, Southern blot analysis of total cellular DNA of the TK^+ cell lines did not show any detectable free plasmid DNA. Thus the enhancement of TK transformation by BPV appears unrelated to autonomous replication. Preliminary experiments using the SV40 system indicate that the BPV genome contains a regulatory element which activates gene expression. The SV40 72 bp repeated sequence upstream from the SV40 early region has been shown to play an essential role for early gene expression. SV40 recombinant plasmids lacking the 72 bp repeat do not express T-antigen at any detectable level and do not replicate in monkey CVI cells.Subcloning of the BPV genome both 5' and 3' to the SV40 A-gene and in either oreintation restored both functions.The sequences responsible for this effect have been mapped to within a 2.9 Kb fragment of BPV DNA. We are attempting to map this regulatory function more precisely. Furthermore we are testing in independent experiments wether the same sequences in the BPV genome are responsible for both effects: enhancement of TK transformation and substitution of the SV40 72 bp region.

0992 TRANSCRIPTION OF THE HEPATITIS B SURFACE ANTIGEN GENE IN MOUSE CELLS TRANSFORMED WITH CLONED VIRAL DNA,Marie F. Dubois,Christine Pourcel, Anne Louise and Pierre Tiollais, Institut Pasteur, Paris, France

Mouse L cells transformed with recombinant plasmids carrying hepatitis B virus (HBV) DNA fragments were used to study the transcription of the viral surface antigen gene (gene S). A HBV specific poly(A)$^+$ RNA of 2.3 kb was mapped on the HBV genome. This RNA hybridized with approximately 75% of the genome and excluded the region of the HBV core antigen gene (gene C). The 2.3 kb species was present only in the HBsAg producing cell lines. A HBV specific 2.3 kb RNA was also detected in the HBsAg producing PLC/PRF/5 human hepatoma cell line. Study of the gene S expression in the transformed mouse L cells allowed us to localize the regions of initiation and termination of gene S transcription. These results strongly suggest that the 2.3 kb RNA molecule is the messenger RNA of the major polypeptide of the envelope, which carries the viral surface antigen determinants.

0993 ACTIVATORS, CONTROLLING ELEMENTS REQUIRED FOR EFFICIENT TRANSCRIPTION
Peter Gruss, Michael Kessel, Lou Laimins, and George Khoury. National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205
We have been interested in the basic mechanisms which control expression of eucaryotic genes. Using SV40 as a model system, we initially defined the 72 bp repeats as a cis-essential element required for the activation of SV40 genes. In extending these studies, we subsequently demonstrated that nucleotide sequences which differ significantly from the SV40 repeats can, nevertheless, harbor a similar functional activity. Specifically a 167 bp fragment from the Moloney murine sarcoma virus (MSV) LTR which contains another 72 bp direct repeat of mouse origin was capable of functionally replacing the SV40 repeats. This recombinant molecule, containing the MSV repeats, expressed SV40 gene functions efficiently and generated viable virus. Using the SV40 repeat-minus mutant, or derivatives thereof as a recipient, present studies are directed at defining activator-like sequences in eukaryotic DNA. In particular, we are interested in determining whether such sequences might play a role in development or tissue tropism.

0994 TRANSFORMATION AND EXPRESSION OF HUMAN GLOBIN SEQUENCES IN FRIEND CELLS, Moses V. Chao*, Pamela Mellon+, Barbara Wold*, Tom Maniatis+, and Richard Axel*, *Columbia University, New York, N.Y. 10032 and +Harvard University, Cambridge, Mass. 02138.
We have investigated the expression of cloned human hemoglobin genes which have been introduced into murine erythroleukemic (Friend) cells by DNA mediated gene transfer. The α_1 and β globin gene as well as a mouse 5' β-human 3' β hybrid gene have been introduced separately by cotransformation into Friend cells deficient in either tk or aprt activity. Stable transformants were isolated and shown to contain from 1-100 copies of the exogenous globin gene. Upon induction of the α_1 and β containing Friend cell transformants with DMSO or HMBA, we have not as of yet observed any human mRNA induction, even though the endogenous mouse β globin gene is induced. A constitutive level of synthesis of human globin mRNA is observed in these lines. Induction of the mouse-human hybrid β globin gene in Friend cells indicates that the fusion 3'-human mRNA is induced to levels of 2000 copies of RNA/cell, a value closely approximating the endogenous mouse globin gene. These data imply that the information required for the inductive process resides within approximately 1.3 Kb of 5'-flanking mouse DNA. We are presently using in vitro truncations of this region to define more precisely those DNA sequences which are involved in the induction phenomenon.

0995 EXPRESSION OF THE CLONED CHICKEN OVALBUMIN GENE IN A HUMAN ESTROGEN RESPONSIVE CELL-LINE. Eugene C. Lai and Mary E. Riser, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030
To study the regulation of expression of the chicken ovalbumin gene by steroid hormones, the entire ovalbumin gene and flanking sequences have been cloned together with the bacterial gene for Xanthine-Guanine Phosphoribosyltransferase (XGPRT) in plasmid pBR322. This recombinant plasmid was linearized and used to transform an estrogen-responsive breast carcinoma cell-line (MCF-7) which possesses estrogen receptors. Transformants were selected by their ability to grow in a medium containing mycophenolic acid and xanthine. The entire ovalbumin gene was integrated into high molecular weight DNA within all transformants and retained its original sequence organization. Using RNA blotting technique, ovalbumin mRNA was identified from transformant cells. A protein identified as chicken ovalbumin by a solid-phase immunoassay was detected both within the cells and also in the medium. Our preliminary results indicate a 3-5 fold increase in the amount of ovalbumin mRNA in cells cultured in 10^{-8} M estradiol. We also constructed a hybrid gene containing the 5' flanking sequence and the first exon of the ovalbumin gene which was linked to the XGPRT gene such that expression of this bacterial gene would be promoted and regulated by the chicken's sequences. After introduction of this hybrid gene into MCF-7 cells, we observed that the survival of the transformed cells in our selection medium is highly dependent on the presence of estradiol. Our results suggest that we may have a useful model system for the analysis of eucaryotic gene expression and its regulation by steroid hormones.

0996 GENE TRANSFER AND EXPRESSION OF CLONED HLA GENOMIC SEQUENCES IN MOUSE RECIPIENT CELLS
J. A. Barbosa, M. E. Kamarck, A. Biro, S. Orkin, H. Ploegh, J. Strominger, S. Weissman and F. H. Ruddle, Yale University, New Haven, Conn 06511, Harvard Univ., Cambridge, MA. 02138

Human genomic sequences which hybridize to HLA cDNA clones have recently been cloned in Charon phages. We have used DNA-mediated gene transfer (DMGT) to cotransfer these genomic clones with an HSV-TK containing plasmid into mouse Ltk- recipient cells. The cell surface expression of an HLA-A,B,C determinant was examined after DMGT by indirect immunofluorescence with monoclonal antibody W6/32 using the Fluorescence-Activated Cell Sorter (FACS). Expression detected at 60 hours post-transfer provided a rapid method for screening large numbers of genomic clones for their ability to direct the synthesis of HLA-A,B,C surface antigens. HAT resistant mass populations could be analyzed two weeks after DMGT. Using particular genomic clones high levels of HLA surface expression was detectable on 20-40% of the cells. These mouse cells expressing HLA were then viably isolated using the sorting capability of the FACS and are presently being used for biochemical studies. HAT resistant clonal populations were also isolated, and displayed varying frequencies and levels of HLA surface expression. These clonal lines are being analyzed by Southern blot hybridization to assess the stability of the transferred sequences. A genomic clone which did not direct HLA surface expression was shown by DNA sequencing analysis to represent a pseudogene. The bioassay we have developed provides a system for the correlation of surface antigen gene structure with expression.

0997 METHYLATION OF SV40 Hpa II SITE AFFECTS LATE BUT NOT EARLY VIRAL GENE EXPRESSION,
Anny Fradin, Carol Prives and James L. Manley, Department of Biological Sciences, Columbia University, New York, NY 10027

A number of studies have demonstrated a correlation between DNA methylation and gene expression: Methylated DNA is frequently transcriptionally inactive while expressed genes are often undermethylated. However, evidence that DNA methylation plays a causal role in regulating gene expression has been lacking. The fact that microinjection of SV40 into the *nucleus of X laevis oocyte results in the synthesis of both early and late gene products* has provided us with a means to test whether methylation at a specific site can affect gene expression. Using HpaII methylase, the single SV40 Hpa II site (0.72 m.u.) was specifically methylated. When this DNA was injected into oocytes, a drastic reduction in the synthesis of late viral proteins (VP-1, VP-2 and VP-3), relative to the synthesis obtained from an unmethylated control, was observed. However, production of the early proteins (the large and small T antigens) was not affected. We therefore conclude that methylation at a single site on the viral DNA located near the 5' end of the late region, can specifically repress synthesis of late gene products. Since the Hpa II site falls within the limits of the nucleosome free "gap" which exists in SV40 minichromosomes, and since this region is extremely rich in the methylatable dinucleotide CG relative to the rest of the viral genome, we speculate that DNA methylation might inhibit late viral transcription by altering the structure of the mini chromosome.

0998 REGULATION OF RAT GROWTH HORMONE GENE EXPRESSION STUDIED BY GENE TRANSFER AND SOMATIC CELL HYBRIDISATION,
Michael D. Walker, Peter Kushner, and Howard M. Goodman, Department of Biochemistry and Biophysics, University of California, San Francisco, Ca 94143, and Department of Molecular Biology, Massachusetts General Hospital, Boston, Ma 02114.

We have introduced an 11 kb EcoRI fragment of rat genomic DNA containing the growth hormone (GH) gene into mouse TK^- L cells by co-transformation with the Herpes Simplex Virus TK gene. Synthesis of GH RNA was detectable in several independent transformants but not in the parental L cells. The transcript was about 200 nucleotides shorter than GH mRNA from GH_3 cells (a cell line derived from rat pituitary). In 3 out of 5 transformants examined, the level of GH RNA was increased (2-4 fold) following treatment of the cells with glucocorticoid and thyroid hormone. This indicates that some of the signals that enable the gene to respond to hormones lie close to the gene itself.

A cell line $LXGH_3$-Cl was constructed by hybridising L cells with GH_3 cells. Although GH_3 cells synthesize significant quantities of GH, the hybrid (which contained both rat and mouse GH genes) did not synthesize detectable amounts of GH RNA. These results indicate that the mouse fibroblast can extinguish expression of the rat GH gene at the level of RNA synthesis when the gene is contributed by cell fusion but not when the gene is contributed by co-transformation.

0999 STRUCTURE AND EXPRESSION OF HAEMOGLOBIN GENES, Frank G. Grosveld, D. Kioussis, S. Wright and R. A. Flavell, Laboratory of Gene Structure and Expression, National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

The entire β-globin locus with its surrounding regions (in total ~ 150kb) has been isolated as a series of overlapping cosmid DNA recombinant clones. These cloned DNA's have been characterized for the presence of families of repeated sequences and compared to similar families present in the rabbit β-globin locus. The expression of these clones has been studied in vivo by introduction in TK⁻ cell lines (L-cells, Friend cells). In addition, clones from different β thalassaemic patients have been isolated and studied by similar methods.

1000 STRUCTURE AND EXPRESSION OF SIMIAN SARCOMA VIRUS ONC GENE ANALOGUE IN HUMAN CELLS. Alessandra Eva, Keith C. Robbins, Steven R. Tronick and Stuart A. Aaronson. Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205.

We have investigated whether human cellular analogues of retroviral transforming (onc) genes are transcribed in human cancer cells. These studies have revealed that poly A-containing RNAs from human solid tumor-derived cell lines contain transcripts that hybridize with probes specific to the onc genes of retroviruses including BALB-MSV, Abelson-MuLV, SSV and MC29 virus (A. Eva et al, Nature in press). The SSV onc gene, sis, showed the most striking specificity of expression, in that sis transcripts were detected only in some sarcomas and bladder carcinomas but not in the large majority of carcinomas or normal cells analyzed. We first investigated the organization of the sis locus in normal human cells. Our studies reveal that sis is uniquely represented and spans a region of approximately 12 kbp as compared to its 1 kbp length within the SSV genome. We cloned the sis locus from normal human cells (c-sis) and demonstrated that it contains at least 5 noncontiguous regions compared to SSV the sis (v-sis) locus. The molecular cloning of the v-sis and human c-sis genes allowed us to prepare DNA fragments comprising specific regions of these two genes. These DNA fragments were used to study the structure of the locus and its 4.2 kb sis-related transcript in human tumor cells. The results of these studies will be discussed with respect to the structure and control of expression of this onc gene in human cells.

1001 INDUCTION OF HUMAN α-GLOBIN AND VIRAL TK GENE TRANSCRIPTION USING MOUSE METALLOTHIONEIN GENE PROMOTER SEQUENCES. Yun-Fai Lau*, Yuet Wai Kan* and Richard D. Palmiter†, Howard Hughes Medical Institute Laboratories, * Department of Medicine, University of California, San Francisco, CA 94143, and † Department of Biochemistry, University of Washington, Seattle, WA 98195

The expression of the metallothionein genes are inducible with heavy metals, e.g. Cd^{++}, and glucocorticoids both in vivo and in vitro. We have constructed several plasmids containing hybrid genes between the 5' promoter region (0.65-1.8 kb) of the mouse metallothionein (MT-I) gene and the coding sequences of either the human α2-globin gene (pMα2) or the TK gene of Hepes Simplex virus (pMK), and have studied the effects of MT-I promoter on their expression during acute DEAE dextran-mediated DNA transfections in mouse Ltk⁻ cells. RNAs from transfected cells were analyzed with Northern hybridization and S_1-mapping techniques. We observed the following : 1) The natural α-globin and TK genes did not response to Cd^{++} induction. Little or no globin nor TK RNAs were detected in the cytoplasm, but small amount were present in the nuclei. 2) Hybrid genes, under non-induced conditions, behaved similarly. After induction, transcriptions of globin and TK RNAs increased 6-10 folds in both the nuclei and cytoplasm. 3) Most nuclear RNAs are results of "read-through" transcriptions. However, some cytoplasmic RNAs appeared to be correctly processed mature RNAs. Experiments are in progress to explore the possibility of α-globin chain synthesis in both acutely and stably transformed cells.

1002 ABELSON VIRUS TRANSFORMED CELLS AS A MODEL SYSTEM FOR THE STUDY OF B-LYMPHOID CELL DIFFERENTIATION, Frederick W. Alt, Susanna Lewis, Naomi Rosenberg, and David Baltimore, Massachusetts Institute of Technology, Cambridge, Mass. 02139

Infection of murine bone marrow or fetal liver with Abelson Murine Leukemia Virus (A-MuLV) transforms a small fraction of the cells into clonal, continuous cell lines. Various properties of these lines have indicated that they were generated from cells of the B-lymphoid lineage, the majority being related to the most immature cell known in this pathway- the pre-B cell. Surveys of the structure and expression of immunoglobulin (Ig) genes in large numbers of such lines has proven extremely useful for defining the pre-B differentiation stage at a molecular level. In addition, these studies have also defined several sub-classes of A-MuLV transformants which exhibit a number of unique and useful immunodifferentiaive properties including heavy and light chain gene rearrangememt and heavy chain class switching during growth in culture. Studies of lines which undergo the recombination processes associated with the formation of complete light or heavy chain variable regions (e.g. V-J joining) during growth in culture have provided new insight into the mechanics and regulation of these processes. Analysis of lines which switch from μ to γ during growth in culture has suggested that the μ to γ class switch can occur, at least in part, by an RNA processing mechanism. Finally, analysis of variants of certain A-MuLV transformants which lose constitutive μ chain synthesis while acquiring LPS inducible synthesis of that chain offers the possibility of studying the regulation of heavy chain gene trancscription. This variation is associated with the expressed allele and correlated with deletions in the J_H-C_μ intron.

1003 DIFFERENT EXPRESSION OF SV40 LARGE T FRAGMENTS AND SMALL t BY TRUNCATED 5'-EARLY SV40 DNA. Jürgen Horst, Günter Assum, Christine Weckler, [+]Elard Jacob and [++]Wolfgang Deppert. [+]Abteilung Humangenetik and [++]Abteilung Biochemie, Universität Ulm, 79 Ulm. [+]Max Planck Inst. f. Virusforschung, 74 Tübingen, FRG.

Recombinant plasmids, based on pBR322 have been constructed which carry about 50 % and 70 %, respectively, of the replicator proximal early region of SV40 DNA. The cloned DNA fragments include the viral origin of replication; they lack, however, a part of the large tumour (T)-antigen 3'-coding region, the large T termination codon and the polyadenylation recognition site. The recombinant plasmids were transferred together with the herpes simplex virus thymidine kinase (TK) gene as a selectable marker into mouse LTK cells. Integration of DNA was analyzed by DNA blotting and hybridization. TK^+ cell clones were investigated for the presence of SV40 T-antigen related proteins by immunofluorescence and immunoprecipitation analysis. SV40 small t-antigen could only be detected in clones derived from transformation with the larger early SV40-DNA fragment. However, large T-antigen related proteins were expressed in both types of transformants containing either the longer or the shorter SV40-derived DNA fragment. This might indicate that small t-antigen expression depends on defined nucleotide sequences present in the larger SV40-DNA fragment but missing in the smaller fragment.

1004 ENHANCEMENT OF TK TRANSFORMATION EFFICIENCY BY A HUMAN DNA SEQUENCE HOMOLOGOUS TO THE CONTROL REGION OF SV40 DNA, Susan E. Conrad and Michael Botchan, University of California, Berkeley Ca. 94720.

We have previously reported the isolation of human DNA sequences homologous to the region surrounding the SV40 origin of replication(SV40 control region). One of these sequences, (OL29),was shown to enhance the ability of a plasmid containing the cloned chicken thymidine kinase (CHTK) gene to transform human TK^- cells to a TK^+ phenotype by about ten fold. The SV40-hybridizing region of this DNA has been sequenced and shown to contain regions that are homologous to the T-antigen binding sites, the twenty one base pair repeats, and the seventy two base pair repeats of SV40.The seventy two base pair repeats of SV40 are required for efficient early gene expression in vivo, and seem to enhance the expression of several other genes in DNA transfection experiments. We are currently testing whether a sequence in OL29 has a function analogous to the seventy two base pair repeats.The region responsible for the TK "enhancement" is being mapped in greater detail. We are also testing whether this sequence can substitute for the seventy two base pair repeats in allowing for SV40 early gene expression. The results of these experiments will be presented.

1005 A GENE TRANSFER METHOD FOR IDENTIFYING RECOMBINANT COSMIDS CONTAINING THE ENTIRE DIHYDROFOLATE REDUCTASE GENE, Jeffrey D. Milbrandt and Joyce L. Hamlin, University of Virginia, Charlottesville, VA 22908

We have developed a methotrexate-resistant cell line (CHOC 400) that contains 1000 copies of a 135 kb sequence which includes the gene for dihydrofolate reductase (DHFR). We have constructed a genomic library from this cell line in a cosmid vector, with average insert sizes in the range 30-40 kb. A single cosmid could therefore include the entire DHFR gene, which is expected to be 32 kb long. In order to identify such a cosmid, we have utilized a spheroplast fusion method to introduce recombinant cosmids into DHFR$^-$ CHO cells.

The genomic library was screened with murine DHFR cDNA, and positive colonies were pooled into eleven groups of six each. Spheroplasts were prepared from each pool by lysozyme/EDTA treatment and were fused to DHFR$^-$ CHO cells with polyethylene glycol. Cells were transferred into selective medium (MEM) 24 hours after fusion. One of the eleven pools gave rise to three CHO colonies after two weeks of culture. The clone containing the entire DHFR gene was identified by subdividing this pool and repeating the fusion experiment. Mapping data on this recombinant cosmid and the physical state of the transferred DNA will be presented.

Since only one out of sixty-six colonies containing DHFR coding sequences was able to rescue a DHFR$^-$ CHO cell by this method, we assume that recombination of the cosmid with the endogenous gene is not a mechanism for rescue. Our data suggest that the entire DHFR gene must be transferred intact to convert the cell to the DHFR$^+$ phenotype. Thus the use of the spheroplast fusion method in combination with cosmid libraries makes it possible to identify bacterial clones containing entire eukaryotic genes in the size range 20-40 kb.

1006 TRANSCRIPTIONAL AND TRANSLATIONAL CONTROL OVER ENDOGENOUS MOUSE MAMMARY TUMOR VIRUS GENE EXPRESSION, A.B. Vaidya, N.E. Taraschi, S.L. Tancin, and C.A. Long, Dept. of Microbiology, Hahnemann Medical College, Philadelphia, PA 19102.

Two strains of mice, BALB/c and C57BL, do not contain any detectable MuMTV polypeptides in their lactating mammary glands (LMG). The BALB/c mice have a barely detectable amount of MuMTV RNA, whereas the C57BL mice contain a substantial amount of viral RNA. To understand the mechanisms for the control of endogenous MuMTV gene expression, we have carried out a number of experiments and our findings are: 1) Mendelian segregation analysis suggests that there are three unlinked, independently segregating genes that are responsible for the presence of MuMTV RNA in LMG of C57BL mice; 2) These genes are probably the endogenous MuMTV proviruses of C57BL mice; 3) Two of the proviruses of BALB/c mice are indistinguishable (with regard to their integration sites and internal structure) from two of the proviruses in C57BL mice, but control over the expression of these proviruses appears to be different; 4) mRNA isolated from C57BL LMG can direct the synthesis of MuMTV proteins in a cell-free translation system; 5) MuMTV RNA in C57BL LMG co-purifies with EDTA-sensitive, polysomes, but no viral proteins are detectable; 6) No MuMTV proteins are precipitable when polysomes from the C57BL LMG are run-off in vitro; 7) After the run-off of polysomes, MuMTV RNA sediments as if it is still on the polysomes. These results suggest a novel form of translational control in which some eukaryotic mRNA's are processed and transported to the cytoplasm, but are kept in a "holding pattern." An analogous situation may be the fate of normal mRNA's in heat-shocked Drosophila cells where these mRNA's are not translated, but are present on polysomes.

1007 CHANGES IN mRNA ABUNDANCE DURING FLAGELLAR REGENERATION IN CHLAMYDOMONAS: CLONED cDNA PROBES. Jeffery A. Schloss, Carolyn D. Silflow and Joel L. Rosenbaum, Yale University New Haven, CT 06511

When the flagella of the unicellular alga Chlamydomonas reinhardii are removed, the cell undergoes rapid alterations in biosynthetic character. Superimposed over constitutive protein synthesis, the production of a set of flagellar proteins increases, relative to their levels in control cells, within 15 minutes. Flagellar protein synthesis peaks at 40 to 60 minutes and then decreases to control levels over the next 3 hours. In vitro translation of mRNA isolated from regenerating cells demonstrates that the alterations in protein synthesis are closely paralleled by changes in mRNA abundance. We are characterizing a cloned cDNA bank whose members correspond to sequences that are regulated during regeneration. Included are nearly-full-length cDNAs corresponding to 2 alpha- and 2 beta-tubulin (the major flagellar protein) mRNAs, that are coordinately regulated. Some 20 other different sequences have been identified, whose abundances increase and then decrease during the regeneration process. In addition to cDNAs to individual message species, several clones hybridize to different nearly continuous arrays of RNA bands in Northern transfer experiments using total RNA isolated from cells at a series of time points during regeneration. These RNAs are polyadenylated, non-polysomal, coordinately regulated during regeneration, and present in abundance similar to that of the regulated mRNAs described above. We are currently studying the properties of these sequences in the genome, to determine whether they might play a role in regulating the expression of flagellar protein genes. (Supported by funds from the NIH)

1008 THE DEVELOPMENT OF ADENOVIRUS AS A VECTOR SYSTEM FOR THE EXPRESSION OF FOREIGN GENES, Carl S. Thummel, Terri Grodzicker*, and Robert Tjian, Dept. of Biochemistry, Univ. of Calif., Berkeley, CA 94720; *Cold Spring Harbor Lab, CSH, NY 11724.

In order to increase the amount of SV40 large T antigen produced by constructed adenovirus-SV40 hybrid viruses, we have positioned the SV40 A gene directly adjacent to the adenovirus major late promoter. This was achieved by first inserting into an adenovirus vector a fragment containing the adenovirus late promoter fused to the SV40 A gene. Upon passaging this recombinant, the tandem duplication of late promoter sequences is removed by recombination to create a defective hybrid virus containing one copy of the late promoter. This recombinant, Ad-SVR26, produces two T antigen-related proteins: one 5,000 daltons larger than the wild-type protein, and one missing a few amino acids from the amino-terminus of T antigen. Analysis of the R26-encoded transcripts reveals two RNA species with unique splicing patterns. The larger transcript contains an adenovirus AUG codon that would allow translation of a fusion protein 50 amino acids longer than T antigen, whereas the smaller RNA contains an internal SV40 AUG triplet as the initiation codon. Although R26 promotes very efficient transcription of the SV40 DNA, the amount of T antigen produced is not proportionately high, possibly because of inefficient translation due to the absence of any leader sequences in the mRNA. We are currently constructing several similar recombinant viruses, in collaboration with Shiu-Lok Hu, that will include different lengths of adenovirus DNA, extending through each of the tripartite leaders. Our current effort to use adenovirus as a vector to express human β globin gene will also be presented.

1009 GLUCOCORTICOID-INDUCED PROTEINS AS INITIATORS OF RAPIDLY-EVOLVING METABOLIC HORMONE EFFECTS. Donald A. Young and Edward V. Maytin, E. Henry Keutman Laboratories, Endocrine-Metabolism Unit, Departments of Medicine and of Radiation Biology & Biophysics, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642.

The availability of very high-resolution separations of cellular proteins on giant two-dimensional gels (Voris and Young, Anal. Biochem. 104, 478, 1980) has allowed the detection of several glucocorticoid-induced proteins that appear in time to account for the most rapidly evolving metabolic effects of steroid hormones (Voris and Young, J. Biol. Chem. 256, 11319, 1981). The early changes observed in these putative "regulatory" proteins lend support to the generally accepted but still unproven hypothesis that steroids initiate their biological responses by altering gene expression.

Results of our continuing studies in which comparisons are made between the different metabolic glucocorticoid responses of individual tissues and the appearance of specific glucocorticoid-induced proteins in that tissue will be presented.

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1010 SELECTIVE CONTROL OF EXPRESSION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR AND OF ACETYLCHOLINESTERASE DURING ONTOGENESIS OF TORPEDO ELECTRIC ORGAN. Hermona Soreq, Ruti Parvari, Daniel Bartfeld, Israel Silman and Sara Fuchs. Weizmann Institute of Science, Rehovot 76100, Israel.

mRNA was prepared from electric organ of Torpedo ocellata embryos at the electroblast stage (I), when the electrocytes acquire columnar shape (II) and when they display electroplate morphology (III); it was translated in vitro and in Xenopus oocytes. Poly(A)-containing RNA decreases in concentration 10-fold at stage III as compared to I and II, whereas protein accumulates throughout ontogenesis. The concentration of mRNA for nicotinic acetylcholine receptor (AChRmRNA), monitored by immunoprecipitation of in vitro translation products with rabbit antibodies, is low at I, reaches maximal levels (0.4% of total mRNA) at II, the onset of synaptogenesis, and remains apparently unchanged thereafter. The concentration of AChR protein, determined by ^{125}I-α-Bungarotoxin binding, is also low at I, reaches maximal levels at II and remains unchanged at III, at 35 pmol binding sites/gr tissue. The concentration of the mRNA for acetylcholinesterase (AChEmRNA), monitored by a Xenopus oocyte bioassay (1), is relatively high at I and II (0.07% of total mRNA) and decreases 4-fold by III. Active AChE protein increases in concentration 10-fold between I and II and 1.5-fold by stage III, to reach an activity of 46 mmole ^{3}H-ACh degraded/hr/gr tissue. Thus, the net degradation rate of AChE is much slower than that of AChEmRNA throughout ontogenesis, and the patterns of expression of both AChE and AChR differ from that of total electrocyte protein.

(1) Soreq, H., Parvari, R. and Silman, I. Proc. Natl. Acad. Sci. USA, in press.

1011 ABERRANT AND NON RANDOM METHYLATION OF CHROMOSOMAL DNA-BINDING PROTEINS BY THE CARCINOGEN 1,2-DIMETHYLHYDRAZINE.L.C.Boffa,R.J.Gruss,and V.G.Allfrey,The Rockefeller University,New York NY 10021.

The alkylating carcinogen 1,2-Dimethylhydrazine(DMH) is a very effective tissue specific colon carcinogen. The sites of in vivo modifications of nuclear macromolecules were studied using [^{3}H-methyl]DMH. The carcinogen methylates not only nucleic acids,but histones and other DNA-binding proteins in target cells. DMH-modified histone H1 contained methylated lysine, arginine and histidine residues not normally detected and other histones had abnormal methyl-arginine contents. In the same cells high-mobility-group proteins (HMG)and other nuclear proteins contained methyl-lysine residues not normally detected. Proteins known to be associated with template-active and more accessibleDNA sequences such as HMG and multiacetylated forms of histone H3 and H4 were preferentially damaged after exposure to[3H]-DMH.Differential sensitivity to nucleases of highly carcinogen-methylated chromatin elements were also detected These results are in agreement with a carcinogen-induced non random chromosomal damage that may selectively affect proteins in the actively transcribing or replicating genes in the target cells.

1012 CHANGES IN METHYLATION AND MELTING OF DNA ACCOMPANYING CHEMICAL INDUCTION OF DIFFERENTIATION IN A FRIEND ERYTHROLEUKEMIA CELL (FELC) SYSTEM, Herman S. Shapiro and Christian P. N. Reboulleau, Dept. of Biochemistry, CMDNJ-New Jersey Medical School, Newark, NJ 07103

We have studied a correlation of the transcription process and the level of methylation in DNA upon induction of hemoglobin synthesis in FELC. Although ethionine is a weaker inducer than either DMSO or sodium butyrate, all three compounds were found to interfere with DNA methylation. The percentage of cytosine that is found as methylcytosine was 4.4%, 3.9%, 3.3% and 4.3% for DNA of nontreated cells, DL-ethionine, butyrate and DMSO treated cells, respectively. An examination of the melting profiles of the DNA in either SSC or SSC/10 indicated a greater hyperchromicity; 1.65 (compared to 1.55), accompanied by early melting of certain populations of genome areas; of hypomethylated DNA. The T_m changes were not, however, significantly different among the several DNA preparations (71.5^{0} at SSC/10). These changes coincide with the proposed influence of methylation on differential gene transcription.

1013 MOLECULAR PROBES TO STUDY THE FIRST DIFFERENTIATION IN THE MOUSE EMBRYO, Philippe Brûlet, Yi-Sheng Xu and François Jacob, Institut Pasteur, 75015 Paris, France

A cDNA sequence encoding a trophectoderm specific marker has been isolated from a trophoblastoma cDNA library. The complementary mRNA directs the synthesis of an intermediate filament protein which binds to TROMA 1, a monoclonal antibody which stains trophectoderm cells but neither embryos at the 8-cell stage nor ICM cells.

An other cDNA sequence detects a 29 S mRNA in undifferentiated embryonal carcinoma cells but not in various differentiated cells. A large part of the mRNA sequence is repeated about 3000 times in the genome. Several genomic clones are analyzed.

A monoclonal antibody has been made which recognizes a nuclear protein that is synthesized in ICM cells but not in the trophectoderm cells.

1014 DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS IN RESPONSE TO SODIUM BUTYRATE, Bennett N Cohen, Peter A. McCue and Michael I. Sherman, Roche Inst. of Mol. Biol. Nutley NJ

Embryonal carcinoma (EC) cells, the malignant stem cells of teratocarcinomas, can differentiate *in vivo* and *in vitro* to a variety of cell types which are characteristically benign. In culture, EC cells have been induced to differentiate in response to several stimuli; the most effective of these, retinoic acid (RA), is active at concentrations as low as 10^{-9} M. RA has been postulated to act via complex formation with a cellular RA binding protein (cRABP) which might translocate into the nucleus and activate specific genes (Jetten, A.M. and Jetten, M.E.R., Nature 278:180, 1979). In support of this hypothesis we have isolated mutant EC cell lines which lack cRABP. These cells fail to differentiate not only in response to RA, but also to other stimuli which normally promote differentiation of EC cells (Schindler, J. *et al.*, PNAS 78:1077, 1981). Sodium butyrate can promote alterations in gene expression in several different cell types. This agent is thought to act by inhibiting histone deacetylation, thus leading to the production of hyperacetylated histone core particles. We have found that at appropriate concentrations sodium butyrate promotes morphological differentiation not only of "wild-type" EC cells but also of mutant EC cells. As an initial test of the hypothesis that sodium butyrate-induced differentiation of EC cells is related to generalized histone hyperacetylation, we have analyzed histone core proteins in these cells. Our studies reveal that histones from both normal and mutant EC cells are hyperacetylated in the presence of sodium butyrate at concentrations which promote differentiation. We are presently determining the state of histone protein acetylation in wild-type and mutant EC cells exposed to RA.

1015 ASSEMBLY OF NUCLEAR RIBONUCLEOPROTEIN PARTICLES DURING IN VITRO TRANSCRIPTION, Ioannis V. Economidis and Thoru Pederson, Worcester Foundation for Experimental Biology, 222 Maple Avenue, Shrewsbury, Massachusetts 01545

The assembly of heterogeneous nuclear RNA (hnRNA) into ribonucleoprotein particles has been investigated during in vitro transcription using the system of Manley et al. [J. Mol. Biol. 135:171:197 (1979)]. Approximately 80% of the in vitro transcription in induced mouse Friend erythroleukemia cell nuclei is inhibited by a low concentration of α-amamitin (0.50g/ml) and by this criterion reflects the activity of RNA polymerase II. In vitro hnRNA transcripts are assembled into particles having the same properties as the nuclear ribonucleoprotein particles (hnRNA) in which Friend cell hnRNA is found in vivo [Pederson, T. and Davis, N.G., Cell Biol. 82:42-54 (1980)]. In sucrose gradients, the in vitro hnRNA transcripts (labeled with α-^{32}P-UTP) reside in structures whose sedimentation velocity is similar to that of hnRNP particles synthesized in vivo (labeled in whole cells with ^{3}H-Uridine). In Cs_2SO_4 density gradients the in vitro hnRNA transcripts band at 1.36g/cm^3, which corresponds to the density of native hnRNP. Direct contact of hnRNP proteins with newly-transcribed hnRNA was demonstrated by nuclease protection experiments, and by the covalent transfer of ^{32}P nucleotides from α-^{32}P-UTP-labeled hnRNA transcripts to specific proteins by RNA-protein cross-linking, followed by nuclease digestion and electrophoresis to display the nucleotide-bearing proteins. The availability of an in vitro system for hnRNP assembly opens a new route for investigating the functional relationship between nuclear structure and mRNA processing.