GENE EXPRESSION

Dean Hamer and Martin Rosenberg, Organizers

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Plenary Sessions

Poster Sessions

The Structure of DNA

0203 LEFT-HANDED DNA HELICES, SUPERCOILING, AND THE B-Z JUNCTION, Robert D. Wells, Department of Biochemistry, School of Medicine and Dentistry, University of Alabama in Birmingham, Birmingham, Alabama 35294

Biological and physical studies on recombinant plasmids and restriction fragments containing tracts of (dC-dG) sequences which are 58, 32, 26, and 10 bp in length reveal the following: A) Left-handed Z-DNA can neighbor right-handed regions of DNA in close proximity on the same chain (1-7). B) Negative supercoiling (density greater than 0.072 is sufficient to convert the 58 bp (dC-dG) regions in pRW751 into a left-handed structure under physiological ionic conditions (200mM NaCl). Thus, left-handed DNA probably exists in vivo (6). C) Single-strand specific nucleases recognize and cleave aberrant structural features at the B-Z junctions (6,8). D) The B-Z junction is very short (several bp) as judged by ^{31}P-NMR, Raman spectroscopy, extent of relaxation of supercoils, and the sharpness of the products of single-strand specific nucleases (1-7). E) The left-handed conformation perturbs the right-handed backbone structure neighboring the B-Z junction for 3-4 helical turns as judged by Raman spectroscopy (5). F) (dC-dG) sequences exist in a family of left-handed conformations as stabilized by different conditions. Furthermore, spectroscopically identifiable conformational intermediates exist between the B and Z structures (7). G) A cloned segment of immunoglobulin gene containing $(dT\text{-}dG)_{31}\cdot(dC\text{-}dA)_{31}$ adopts a left-handed conformation under the influence of negative supercoiling (8). Also, the AAF-reacted polymer is left-handed in high salt solutions (4). H) The stabilizing effect of methylation on the Z-form in fragments and plasmids approximately offsets the free energy contributions of the B/Z junctions (9). I) The (dC-dG) tracts are specifically and highly susceptible to suffer deletions in vivo. Also, these tracts seem to enhance rec A mediated recombination (1,2).

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Transcription Initiation

0204 TRANSCRIPTIONAL ACTIVATION BY THE PHAGE λ REGULATORY PROTEIN cII, Martin Rosenberg, Yen-Sen Ho and Daniel Wulff*, Department of Molecular Genetics, Smith Kline & French Laboratories, Philadelphia, PA 19101 and *Department of Biological Sciences, State University of New York at Albany, Albany, NY 12222*

The phage λ regulatory protein, cII, is a positive effector of RNA transcription. This regulator activates transcription from two different promoter signals positioned far apart on the phage genome. These two promoters coordinately control gene expression of the two functions required for phage lysogenic development. We have been able to overproduce and obtain large quantities of purified cII protein which allows us to characterize both its physical and chemical properties. We have demonstrated that cII is a DNA binding protein and that its interaction with DNA is both necessary and sufficient to activate RNA polymerase to initiate transcription from both promoter signals. We have used a variety of chemical and enzymatic probe techniques to study the direct interaction of cII with DNA. These protection studies indicate that cII binds specifically in the -35 region sequence of the promoter signal. Additional studies carried out with single base pair promoter mutations implicate that a tetranucleotide repeat sequence positioned in the -35 region of the promoter signal is important in the interaction of cII with DNA. More detailed probe experiments indicate that cII interacts with this tetranucleotide repeat on only one face of the DNA helix and predominantly within the major groove. In addition to characterizing the normal cII activator protein, we have obtained and begun to examine a variety of cII variants which differ by only single amino acid substitutions. Each of these variant proteins is being overproduced, isolated, and characterized with respect to their ability to interact with DNA and activate the transcription event.

0205 REGULATION OF YEAST HISTONE MRNA SYNTHESIS Lynna Hereford and Mary Ann Osley, Sidney Farber Cancer Institute and the Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Ma., 02115.

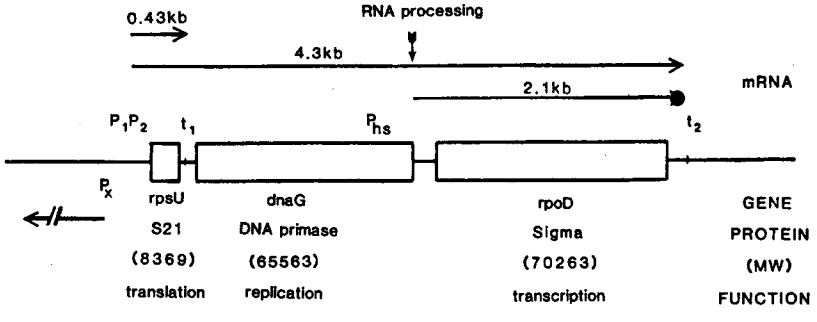
Histone mRNA levels are tightly controlled during cell division such that they are maximally accumulated during the S phase of the cell cycle. This control is exerted at two distinct levels: one transcriptional and one post-transcriptional. Transcriptional regulation is mediated via ARS sequences located at the 3' ends of the H2B genes, leading to the conclusion that transciption is intimately related to the replicative state of the DNA.

Complex Gene Systems

0206 THE STRUCTURE AND REGULATORY FEATURES OF THE rpsU-dnaG-rpoD OPERON IN E. COLI. Richard R. Burgess[1], Zachary F. Burton[1], Carol A. Gross[2], and Wayne E. Taylor[1]. [1]McArdle Laboratory and [2]Dept. of Bacteriology, Univ. of Wis., Madison, WI 53706.

Genes for the ribosomal protein S21 (rpsU), DNA primase (dnaG) and RNA polymerase sigma subunit (rpoD) have been sequenced. They are clustered on the E. coli genome at a map position of 66 min and are transcribed in the same direction. Low resolution S1 mapping of in vivo transcripts identifies a 4.3 kb transcript which codes for S21, primase, and sigma. Thus these three genes are within an operon. In addition we observe a 0.43 kb transcript coding for S21 and a 2.1 kb transcript coding for sigma. High resolution S1 mapping allows us to locate precisely 5' and 3' ends of RNA from this region and to identify some of the regulatory features of the operon. These features include tandem promoters (P1 and P2), terminators (t1 and t2), an RNA processing site (↓) between dnaG and rpoD, and a promoter (Px) located very near P1 but oriented in the opposite direction.

Although the three genes in the sigma operon can be co-transcribed, the levels of the three proteins in the cells are very different; the molar ratio of S21:primase:sigma is approximately 1000:1:60. The low level of dnaG expression is posttranscriptionally regulated by mRNA instability and possibly by inefficient translation. The regulatory features which we identify may explain the discoordinate regulation of operon genes that occurs under some growth conditions. All of the genes for RNA polymerase subunits have now been shown to be located in operons which also encode ribosomal proteins. The genetic environment of rpoD is very similar to that of beta (rpoB) and beta' (rpoC).



DNA-Protein Interactions

0207 REPRESSOR STRUCTURES---MECHANISM OF DNA RECOGNITION AND POSITIVE CONTROL, Mark Ptashne, Ann Hochschild and Nina Irwin, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

The repressors of phage lambda and P22 are both positive and negative regulators of gene transcription. Structural and genetic experiments indicate that in both cases (and perhaps in many others as well) a structure comprised of two alpha helices directs specific DNA binding. Moreover, each of the two-helix structures in the lambda and P22 repressors also mediates positive control of transcription by contacting RNA polymerase bound to the adjacent promotor. In the two cases, different regions of the helix pair evidently contact the same surface of DNA-bound polymerase.

Hochschild, A., Irwin, N. and Ptashne, M. (1983) Repressor structure and the mechanism of positive control. Cell (Feb), in press.

0208 CRO REPRESSOR AND ITS INTERACTION WITH DNA, W.F. Anderson[1], D.H. Ohlendorf[2], M. Cygler[1], Y. Takeda[3] and B.W. Matthews[2], [1]MRC Group on Protein Structure and Function, University of Alberta, Edmonton, Alberta T6G 2H7; [2]Institute of Molecular Biology, University of Oregon, Eugene, OR 97403; [3]Chemistry Department, University of Maryland, Baltimore County, Catonsville, MD 21228.

The structure of the bacteriophage λ Cro repressor suggests that the protein binds to specific base sequences of DNA with a pair of two-fold related α-helices in successive major grooves of right handed B form DNA[1]. Using the known three dimensional structure of the Cro repressor, model building and energy refinement have been used to explore the apparent interactions between the repressor and its operators[2]. Recognition of the specific DNA binding site appears to occur, in a large part, by hydrogen bonds between the amino acid side chains of the protein and the exposed parts of the base pairs in the major grooves. Most of the sequence specific interactions between Cro and DNA, as well as a number of sequence independent ones, are mediated by a structural unit of the protein composed of two α-helices.

Comparison of the structure of the Cro repressor with the λ repressor[3] and the E. coli catabolite gene activator protein[4] together with amino acid sequence comparisons for a number of base sequence specific DNA binding proteins suggests that a common α-helical DNA binding region occurs in many proteins which regulate gene expression[5,6,7].

Complexes of the Cro repressor with two different DNA oligomers $\frac{\text{ATCACC}}{\text{TAGTGG}}$ and $\frac{\text{ACCGCAAGG}}{\text{TGGCGTTCC}}$ which correspond to different portions of the λ O_R[3] operator have been crystallized. Analysis of dissolved crystals indicates that there are two of the six base pair duplexes per Cro dimer and one of the nine base pair duplexes per Cro dimer. The space group of both complexes is $C222_1$ with cell dimensions of approximately a=81.3Å b=89.2Å c=80.1Å. The crystals diffract to about 3Å resolution and the crystallographic analysis of these crystals is in progress. The structures of these complexes will provide further information about Cro repressor-DNA interactions.

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0209 ISOLATION OF TRANSCRIPTION FACTORS THAT DISCRIMINATE BETWEEN DIFFERENT PROMOTERS RECOGNIZED BY RNA POLYMERASE II, William S. Dynan and Robert Tjian, University of California, Berkeley, CA 94720.

A new and efficient fractionation procedure separates a whole-cell HeLa extract into three components required for accurate in vitro transcription. One component (Sp1) is a novel promoter-specific factor required for transcription of the SV40 early and late promoters, but not for transcription of other promoters we have tested. The second component (Sp2) is a general factor required for transcription of the SV40 promoters and a series of others, including the adenovirus 2 major late promoter, the human β-globin promoter, and the avian sarcoma virus promoter. The third component is a fraction containing the endogenous RNA polymerase II. SV40 early promoter transcripts synthesized in vitro using the purified system were mapped by primer extension and found to originate at the same nucleotides as early transcripts isolated from SV40 infected cells. When SV40 and adenovirus templates were present simultaneously in an in vitro transcription reaction, addition of the Sp1 factor stimulated SV40 early promoter transcription 40-fold and inhibited adenovirus major late promoter transcription by 40%. This finding suggests that Sp1 is involved in promoter selection and is not merely a general transcription stimulatory factor. The requirement for an SV40 promoter-specific transcription factor may be linked to the distinctive structure of the SV40 promoters.

Inducible Gene Systems

0210 THE SOS SYSTEM OF *Escherichia coli*, David. W. Mount, John W. Little, Ken Wertman, Bruce Markham, and Joan Harper, Departments of Molecular and Medical Microbiology and Biochemistry, University of Arizona, Tucson, Arizona, 85724.

Following DNA damage, *Escherichia coli* displays a complex response, called the SOS response, including an enhanced capacity for DNA repair and increased mutagenesis. This response is regulated by two proteins: LexA repressor and RecA protease. In normal, undamaged cells, LexA repressor limits transcription of at least 11 unlinked genes (1,2). In damaged cells, the RecA protease is activated and cleaves LexA repressor. The products of the target genes are then expressed at much higher levels, and the secondary SOS functions are expressed. When the damage is repaired, the protease activity declines and LexA repressor can accumulate and reestablish repression, including autorepression of its own gene.

Purified LexA protein inhibits *in vitro* transcription from the *recA*, *lexA*, *uvrA*, *uvrB*, *dinA* and *dinB* promoters. Nuclease-protection experiments show that LexA protein binds to specific sequences in the control regions of *recA*, *lexA*, *uvrA* and *uvrB*. In all but the *lexA* operator, the protected region is about 25 bp in length; in *lexA* it is about 45 bp long. Comparison of the operator sequences reveals considerable homology among them, and shows that the *lexA* control region contains two adjacent and closely similar 20 bp sites (1, 3).

As in the case of phage lambda repressor, cleavage separates LexA repressor into two fragments of roughly equal size at an ala-gly bond (1, 4). Cleavage also occurs *in vivo*. We are presently characterizing mutant forms of LexA repressor that are resistant to cleavage.

Derepression of *lexA* target genes occurs within 5 minutes of induction, the ratio of induced to repressed levels varies and re-establishment of *lexA* repression is delayed the longest, as expected from the weak binding of LexA repressor to *lexA* operator (2).

We are presently using *in vivo* and *in vitro* methods of mutagenesis to analyze the interaction between LexA repressor and RecA protease, and between LexA repressor and its target operators.

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0211 REGULATION OF TRANSCRIPTION BY GLUCOCORTICOIDS AT AN EPISOMAL PROMOTER, Gordon L. Hager, Helene Richard-Foy, Alex C. Lichtler and Michael C. Ostrowski, Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, MD 20205

The long terminal repeat (LTR) of mouse mammary tumor virus has been shown to contain a transcriptional promoter that is sensitive to glucocorticoid regulation by fusion of the viral LTR to a transformation gene (v-ras) from Harvey murine sarcoma virus (1). A transfection assay has been developed in which the production of transformed foci with the v-ras gene driven by the MMTV promoter is dependent on the presence of glucocorticoids. Mutational evidence defining the position of the regulatory sequence will be discussed.

The MMTV LTR-v-ras fusions have been mobilized on the episomally replicating bovine papilloma virus (BPV) in order to (a) analyze hormone regulation in the absence of position effects that occur in the integrated state, and (b) to develop a well-defined minichromosome state where hormone-responsive promoters can be amplified and isolated as chromatin. Transformed cell lines have been developed with BPV LTR-v-ras fusions replicating as extrachromosomal circular elements at approximately 200 copies per cell. These cell lines are stable, the fusion DNA is unrearranged, and the number of episomal copies is unaffected by hormone. The extrachromosomal DNA is organized into a repeating nucleosomic structure as evidenced by the micrococal nuclease digestion pattern. The extrachromosomal elements can be selectively extracted as nucleoprotein from nuclei by a variety of techniques. This single-step extraction results in a chromatin preparation that is approximately 10,000 fold enriched for the BPV LTR-v-ras fusion with respect to a single-copy gene. At this point, the fusion is estimated to represent 2-3% of the total nucleoprotein. When isolated minichromosomes are introduced into an *in vitro* transcription cocktail, and transcription complexes that have been initiated *in vivo* are measured by "run-off" transscription, the activity of the LTR promoter on episomes from cells treated with hormone is found to be increased by 25 fold, whereas the number of complexes initiated from the BPV promoter is unaffected. This evidence indicates that the extrachromosomal LTR is normally regulated by hormone, and that the regulation is a direct transcriptional effect. The efficacy of this system for the analysis of hormone action will be discussed.

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RNA Transcription and Processing

0212 CONTROL OF λ INT GENE EXPRESSION BY RNA PROCESSING. Donald Court*, Ursula Schmeissner¶, Martin Rosenberg¶, Amos Oppenheim+, Gabriel Guarneros#, Cecilia Montanez#. *Department of Molecular Oncology, ¶Department of Biochemistry, NCI, NIH, Bethesda, Md. 20205; +Hebrew University, Jerusalem, Israel. #Departamento de Genetica y Biologia Molecular, Centro de Investigacion y de Estudios Avanzados, A.P. 14-740 Mexico, D.F. Mexico.

The int gene on phage λ DNA codes for a product that is required for integrative recombination during lysogeny by this temperate phage. Transcription of int occurs from either of two positively regulated promoters, p_L and p_I. The p_L transcript of phage λ includes int and as many as 15 other genes. A great amount of this transcript is made, but very little Int protein is produced. In contrast to the low level of int expression from the p_L transcript, high levels of Int are made from the p_I transcript. λ sib mutants were isolated which produce high levels of Int from the p_L transcript. These mutations inactivate an inhibitor of Int expression and map beyond the int gene. The sib inhibitor is not a diffusible factor, but is rather a site beyond int which inhibits int expression. In addition to λsib mutants, a host mutant has also been shown to increase int levels from p_L transcripts. This mutant is defective in the endoribonuclease, RNaseIII. The RNA in the region around the sib mutations can adopt a secondary structure that is similar in form to known RNaseIII sites. All sib mutations decrease the stability of this structure. RNA within the secondary structure of sib is processed both in vivo and in vitro. In vivo processing requires an intact site, i.e., sib+ phage DNA, and normal RNaseIII activity of the host. This processing at a site some 260 bp from the int gene, inactivates int gene expression. Inactivation may occur because the endonuclease cut beyond int leads to increased nuclease sensitivity of int RNA from the 3' end . In support of such a nonspecific effect, if another gene replaces int adjacent to the RNaseIII site at sib, it also becomes reduced in its expression. The p_I - trancript, unlike that of p_L, terminates at a trancription stop site encountered beyond the int gene within the sib site; RNaseIII does not process the terminated transcript, and high levels of int are made.

0212A DECAY OF mRNA IN E.COLI: THE FATE OF SPECIFIC SEGMENTS OF TRANSCRIPTS J.G. Belasco, A. von Gabain, J.L. Schottel, A.C.Y. Chang and S.N. Cohen, Departments of Genetics and Medicine, Stanford University, School of Medicine, Stanford, California 94305

The fate of specific segments of gene transcripts in E. coli and the directionality of mRNA decay were investigated by a novel assay that employs mRNA protection of segments of DNA cloned in bacteriophage M13. Decay of mRNA encoded by the β-lactamase (bla) and ompA genes, of hybrid transcripts, and of segments of eukaryotic RNA introduced into bacterial mRNA species having different decay times was examined. Our results indicate that the half-life characteristics of a bla and ompA mRNA are determined primarily by an initial rate-limiting cleavage of these messages. Once the initial cleavage has occured, relatively rapid degradation of the transcript ensues, the segments at the 5'-end of the ompA message decaying last. No differential degradation of translated versus untranslated segments of mRNA was observed.

0213 *IN VITRO* SPLICING OF THE RIBOSOMAL RNA PRECURSOR OF *TETRAHYMENA*, Thomas R. Cech, Paula J. Grabowski, Arthur J. Zaug, Kelly Kruger and Brenda L. Bass, University of Colorado, Boulder CO 80309

In the macronuclear ribosomal RNA genes of *Tetrahymena thermophila*, a 413 base pair intervening sequence (IVS) interrupts the 26S rRNA coding region. The IVS is transcribed as part of the rRNA precursor and subsequently deleted by RNA splicing. Splicing requires a guanosine cofactor, which becomes covalently bound to the 5' end of the IVS during its excision.[1,2] We propose that this is the first of two concerted cleavage-ligation reactions, or phosphoester transfers, that result in excision of the IVS and ligation of the exons. Following its excision, the IVS RNA is covalently cyclized in a cleavage-ligation reaction that is analogous to the first step in pre-rRNA splicing. Both the splicing and the cyclization reactions are unusual in that they are catalyzed by the RNA molecule and require no enzyme or other protein. The lack of a protein requirement was shown by synthesizing a shortened rRNA precursor *in vitro* using a purified plasmid DNA template and *E. coli* RNA polymerase. After deproteinization, this RNA underwent the following reactions: IVS excision, G-addition, exon ligation, and IVS cyclization.[3] Although no enzyme is required, the RNA itself has several enzyme-like properties. We therefore call the RNA a ribozyme - an RNA molecule that lowers the activation energy for specific bond cleavage and formation reactions. The best characterized of the RNA's enzyme-like characteristics is its specific binding of the guanosine cofactor. Analysis of the reaction rate as a function of guanosine concentration gives an apparent K_M of 8 μM and shows saturation at high concentrations. Guanosine analogs with altered functional groups on the guanine base (inosine, 2-aminopurine ribonucleoside, N^2-methylguanosine) are active in the reaction but have higher apparent K_M's, consistent with the loss of hydrogen bonding interactions. Future work will be directed toward identifying the guanosine binding site and characterizing the mechanism of the cleavage-ligation reaction.

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Gene Structure and Function

0213A **COOPERATIVE ENHANCEMENT OF TRANSCRIPTION INITIATION AT TANDEM PROMOTERS.** G. Wesley Hatfield and Craig W. Adams, Department of Microbiology, University of California, Irvine, CA 92717

Classical genetic techniques and recombinant DNA methodologies have defined DNA sequences necessary for proper transcription and initiation of prokaryotic and eukaryotic genes. In eukaryotes the TATA box region located 20 to 30 base pairs upstream of the start site of type II genes is involved in fixing initiation precisely but is dispensable for gene expression while sequences located more than 150 bases upstream are indispensable. In prokaryotes two regions located approximately 10 and 35 bases upstream of the transcription start site are required for transcription. There are no reports which demonstrate that DNA sequences farther than 50 bases upstream are required for the proper function of prokaryotic promoters not regulated by trans acting elements. The IlvGEDA operon of Escherichia coli K12 is preceded by tandem promoters, P1 and P2, located 72 bases apart. Both promoters are transcriptionally active in vitro but in vivo greater than 99% of the transcription from this region is initiated from the downstream promoter, P2. In order to assess in vivo interdependencies between these promoters they were cloned together and separately into a galK transcription fusion vector. Deletion fragments with end points at many sites within the tandem promoter region were also inserted into this vector. Analysis of the ilv promoter activity in these constructions shows that the upstream promoter, P1, stimulates transcription from the downstream promoter, P2, greater than tenfold. Further analysis shows that this stimulation of transcription is not involved in the regulation of operon expression. Other evidence suggests that this enhancement of transcription is mediated by protein-protein interaction between two RNA polymerases at the tandem promoter sites. Thus, RNA polymerase functions as a positive activator of the ilv P2 promoter.

Termination and Post-Transcriptional Controls

0214 REGULATION OF THE SYNTHESIS OF RIBOSOMES AND RIBOSOMAL COMPONENTS IN ESCHERICHIA COLI, Masayasu Nomura, Institute for Enzyme Research, University of Wisconsin, Madison, WI 53706.

The rate of ribosome synthesis in Escherichia coli is regulated and is directly proportional to cellular growth rate. In addition, the synthesis rates of all the ribosomal components are balanced and, like ribosomes, respond coordinately to changes in environmental conditions. Based on experiments to analyse gene dosage effects on the rates of synthesis of ribosomal proteins (r-proteins) and their mRNA, we have previously proposed a model for the feedback regulation of r-protein synthesis which explains the coordinate and balanced synthesis of all the ribosomal components (1). The model proposes that r-protein synthesis and ribosome assembly are coupled such that when r-protein synthesis exceeds the rate of ribosome biosynthesis certain key r-proteins act as inhibitors that prevent further translation of their own mRNA (1, 2). Subsequent experiments both in vitro and in vivo carried out in our laboratory as well as in others have shown that the model is basically correct, and have identified specific r-proteins, such as L1, L4, L10, S4, S7, S8 and S20, as translational repressors (for a review, see 3). Experiments relevant to some essential features of the model will be discussed. In addition, some new experiments will be discussed which addresses the question what determines the overall rate of ribosome synthesis. We suggest the presence of another level of feedback regulation which might be involved in the regulation of ribosome accumulation.

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0215 ANTITERMINATION BY PURIFIED LAMBDA Q GENE PROTEIN IN VITRO, Jeffrey W. Roberts and Elizabeth J. Grayhack, Section of Biochemistry, Molecular and Cell Biology, Wing Hall, Cornell University, Ithaca, NY 14853

The product of the early lambda gene Q is required for phage late gene expression. Lambda late genes constitute a single operon, the promoter for which is adjacent to gene Q. This promoter is followed after 200 nucleotides by a terminator, so that most transcription by purified RNA polymerase in vitro stops before the late gene coding sequences. The existence of this terminator suggested that Q protein, like the early regulator encoded by gene N, is a transcription antiterminator; we have shown that this is true. Furthermore, purified gene Q protein antiterminates transcription in vitro, requiring in addition only DNA containing the late promoter, RNA polymerase, and L-factor (the product of the nusA gene) (1). A corresponding regulatory encoded by the lambdoid phage 82 (2) functions similarly in a purified transcription reaction, but is instead specific for 82 DNA (K. Verner, J. Goliger, E.J. Grayhack and J.W. Roberts, unpublished). We suggest that Q protein acts at a specific site before or within the first 17 nucleotides of the late transcript coding sequence (C. Hart, X. Yang, E.J. Grayhack and J.W. Roberts, unpublished), modifying the transcribing complex so that it traverses the 20 kilobase late region without attenuation.

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0216 HISTONE GENE TERMINATION, Daniel Schümperli, Carmen Birchmeier, Hendrik G. Stunnenberg, Erika Lötscher, Max L. Birnstiel, Institut für Molekularbiologie II, Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland

Histone mRNAs of higher eukaryotes terminate in a characteristic 23 base pair conserved sequence 5'AACGGC$^{C}_{U}$CUUUUCAG$^{C}_{A}$GCCACCA3'. This sequence motif contains a dyad symmetry and thus resembles prokaryotic termination signals. We have used two different expression systems, frog oocyte injection and cell culture transfection, to study what sequences and what additional factors are required to generate faithful 3' ends. Injection of the sea urchin histone DNA clone h22 into X.laevis oocytes leads to efficient transcription of the H2A, H2B, and H3 genes. However, transcription termination is efficient for H2A and H2B but very inefficient for H3. In vitro constructed deletion, transposition and point mutants of the H2A terminator region were tested for the production of RNA with faithful 3' ends. H2A coding sequences are dispensable but small deletions or point mutants within the dyad symmetry element elicit partial or complete readthrough transcription. In addition, a pseudorevertant of a point mutant which restores the dyad symmetry by a symmetrical mutation also restores the amount of faithful H2A transcripts to roughly wild-type level. However, additional spacer sequence within the first 50 nucleotides following the conserved motif appear to be required as well. The unusual behaviour of the H3 gene was exploited to search for regulatory factors which can complement the frog oocyte to generate correctly terminating H3 mRNA. Chromosomal salt wash fractions from cleavage stage sea urchin embryos were found to fulfill this function. The active component was partially purified by selective salt extraction, ammonium sulfate precipitation, column chromatography and sucrose gradient centrifugation. Heparin binding and sedimentation at about 12S suggest a nucleic acid binding protein and a molecular weight of 200.000 - 250.000. The mouse H4 gene terminator, fused to the early promoter of SV40, was similarly analysed after DNA transfection into mammalian cells. An indicator gene, E.coli galactokinase (galK), was positioned downstream to allow easy detection of readthrough transcription. Preliminary findings suggest that stable, translatable galK mRNA is produced, regardless of whether the H4 terminator is present or not, but that galK expression is somewhat reduced by the terminator. The reasons for this apparently low termination efficiency are being investigated.

0217 GENETIC AND PHYSIOLOGICAL STUDIES ON THE SECRETORY APPARATUS OF E. COLI, D. Oliver, C. Kumamoto, E. Brickman, S. Ferro-Novick and J. Beckwith, Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, MA 02115

The mechanisms of secretion in prokaryotes and eukaryotes have many features in common. The similarities suggest that prokaryotes and eukaryotes may have a comparable secretion machinery. Studies based in genetics of the prokaryote, Escherichia coli, offer the prospect of providing insight into the mechanism of secretion with applications for a wide range of species.

We have developed a selection for mutants of E. coli with defects in its secretion machinery. This selection utilizes a strain in which the signal sequence of maltose binding protein, a periplasmic protein, is attached to the normally cytoplasmic ß-galactosidase, resulting in a membrane location of the hybrid protein. The membrane localization of the hybrid protein results in an inactive ß-galactosidase molecule and a Lac^{-} phenotype; selection for Lac^{+} revertants yields mutants with defects in secretion which result in a cytoplasmic location for the hybrid protein. Some of these mutants exhibit only slight defects in the secretory apparatus. The small amount of cytoplasmic hybrid ß-galactosidase in such mutants has enough enzymatic activity to allow growth on lactose. The characterization of these mutants has led to the identification of two new genes, secA and secB. These genes appear to code for components of the secretion machinery. The gene product of secA is a peripheral cytoplasmic membrane protein, the synthesis of which is regulated by the secretion needs of the cell. In the absence of secA protein (which is lethal to the cell), the export of a number of proteins, but not all, is prevented. Further, in the absence of secA protein, the synthesis of at least one periplasmic protein is inhibited. These results suggest 1) that there may be different membrane complexes for the export of different proteins and 2) that secretion and synthesis of proteins can be coupled.

Beginning with a secA^{ts} mutant, we have isolated unlinked suppressors which reverse the temperature-sensitive phenotype. One of these maps in a previously identified gene involved in secretion, prlA. In addition, new genes which may code for additional components of the secretory apparatus have been identified by this selection. Thus, this approach may allow the identification of all the genes and proteins required for secretion in E. coli and ultimately the in vitro reconstitution of a secretion system.

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Prokaryotic and Eukaryotic Gene Regulation

0218 ATTENUATION CONTROL OF trp OPERON EXPRESSION, Charles Yanofsky, Anathbandhu Das, Robert Fisher, Roberto Kolter and Vivian Berlin, Department of Biological Sciences, Stanford University, Stanford, CA 94305

Transcription of many operons concerned with amino acid biosynthesis and utilization are regulated at a transcription termination site preceding the structural genes of the operon. Regulation at this site appears to be accomplished in response to ribosome movement over a short peptide coding region in the initial segment of the transcript. Deficiency of a particular amino acid is believed to result in ribosome stalling over the corresponding codons of the leader segment of the transcript resulting in the formation of a particular RNA secondary structure termed the antiterminator. When this structure forms it precludes formation of a second RNA structure, the terminator, that is the transcription termination signal recognized by the transcribing polymerase. Many of the essential features of this model have been substantiated in studies with amino acid operons regulated by attenuation. Our recent studies have been concerned with features of the model that require experimental verification as well as with several unanticipated aspects of attenuation. We have succeeded in demonstrating - for the first time - that a trp leader peptide is synthesized; as expected, the peptide is extraordinarily labile (A. Das and C. Yanofsky, in preparation). We have also obtained evidence consistent with transcriptional pausing in the leader region of the trp operon playing a role in synchronizing transcription and translation in regulation by attenuation (R. Fisher and C. Yanofsky, unpublished). The dependency of pausing on RNA secondary structure formation was demonstrated. It was also shown that RNA pause and termination signals probably are recognized at the same site in the transcribing polymerase molecule (R. Fisher and C. Yanofsky, in preparation). In other studies we found that no accessory factors are required in vitro for dissociation of polymerase, template and transcript at the trp operon attenuator (V. Berlin and C. Yanofsky J. Biol Chem., in press). Thus the termination complex appears to dissociate spontaneously. We also observed that transcription termination at the trp attenuator is increased 5-10 fold in vivo when initiation of synthesis of the leader peptide is prevented (superattenuation). Thus the steady state level of trp operon expression in cells growing with excess tryptophan is 5-10 fold elevated over the level obtained under conditions of non-translation. Studies with leader mutants suggest that the steady-state level is set by ribosome dissociation at the leader peptide stop codon and the subsequent formation of either the termination or read-through RNA structure (R. Kolter and C. Yanofsky, unpublished).

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Transposable Genes and Elements

0219 RECOMBINATIONAL PROCESSES OF BACTERIAL TRANSPOSONS, Nigel D.F. Grindley, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06510.

Most bacterial transposable elements that have been characterized in detail fall into one of two groups: (a) the Tn3 family of transposons and (b) the IS (insertion sequences) together with their related composite transposons. A distinctive feature of transposons of the Tn3 family is that they encode two separate recombinational mechanisms, both of which are required for normal tranposition. Inter-replicon transposition of these transposons is a two step process. First, donor and target replicons fuse to form a single molecule called a cointegrate. This contains two copies of the transposon in the same orientation, one at each junction between the two replicons. Replicon fusion is dependent on the transposase, product of the transposon tnpA gene. To complete the transposition a cointegrate is "resolved" by site-specific recombination between the two transposon copies. This step is mediated by the tnpR gene product, resolvase, acting at the res site of each transposon. The first step clearly involves a replicative recombination process since the transposon is duplicated. By contrast, the second step occurs by a conservative mechanism that involves simple breakage and reunion of the two res sites without further DNA synthesis.

Considerable information has been obtained from studying cointegrate resolution in vitro. We have shown that the recombination involves formation of an intermediate in which resolvase has cleaved the DNA at the point of recombination and has become covalently joined to the 5-phosphoryl ends. Since no high energy nucleotide cofactor is required for the recombination, the protein-DNA linkage presumably conserves the energy of the broken phosphodiester bond. Substrate requirements for both the cleavage reaction and complete recombination are the same, namely a supercoiled DNA molecule with two res sites in the same orientation. Deletion analysis shows that a fully functional res site spans about 120 bp of the tnpA-tnpR intercistronic region. Three sites of 30-40 bp that specifically bind resolvase have been identified within this region. Interaction of resolvase with these sites effects both resolution of a cointegrate and regulation of the divergently transcribed tnpA and tnpR genes.

0220 GENE REARRANGEMENTS CONTROLLING THE EXPRESSION OF SURFACE ANTIGEN GENES IN TRYPANOSOMES, P.Borst, A.Bernards, L.H.T.van der Ploeg, P.A.M.Michels, A.Y.C.Liu and T.De Lange, Section for Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam, The Netherlands.

The surface coat of African trypanosomes, like Trypanosoma brucei, consists of a single protein, the Variant Surface Glycoprotein (VSG). By switching from the synthesis of one VSG to the next, trypanosomes change the antigenic nature of their surface and escape immune destruction (see ref. 1). We have shown that one route for VSG gene activation involves the duplication and transposition of a silent Basic Copy (BC) gene into an expression site, yielding an expression-linked copy, that is transcribed [2-8]. The expression site is located at the end of a chromosome [4,5,9]. The 5' 35 nucleotides of the mature mRNA are not encoded in the transposed segment and must be derived from a mini-exon in the expression site [7]. The DNA sequence of one of the circa 100 mini-exons shows that pre-mRNA splicing follows the GT/AG rule.

A minor group of VSG BC genes is activated by a second route that does not involve gene duplication [1]. Like the VSG gene expression site, such genes are at the end of a chromosome [1,10,11]. We interpret our recent results to mean that these genes are transposed into the expression site by chromosome end exchange [10,12]. A single expression site could, therefore, control expression of the 1000-odd VSG genes [8] in a mutually exclusive fashion.

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0221 **MATING TYPE SWITCHING AND CONTROL OF GENE EXPRESSION.** **J. Hicks, J. Strathern, A. Klar, J. Abraham, K. Nasmyth, J. Ivy, J. Feldman and *J. Broach. Cold Spring Harbor Laboratory, New York, *State University of New York, Stony Brook, New York.** **The genes responsible for the control of cell type in Saccharomyces yeasts reside on transposable elements (cassettes) located at an expressed locus (MAT) and two silent storage loci (HML and HMR) on chromosome III. The HML and HMR cassettes are under negative transcriptional control by the products of four unlinked genes known as MAR or SIR. Cassettes are of two types (a and α) and consist of two conserved DNA segments (homologous at all three loci) flanking a core region which contains information unique to the a or α cell type. Alternate expression of a and α cell type occurs during sexual development by sequential replacement of the expressed cassette at MAT with a cassette of the opposite type from HML or HMR. This transposition is under the control of the HO (homothallism) gene.**

By in vitro mutagenesis of recombinant plasmids we have determined that cassettes at the silent HML and HMR loci are located between two control sites which apparently interact with products of the MAR/SIR genes to: (1) alter the state of the chromatin at that locus; (2) turn off transcription of the cassette; and (3) determine its role as a donor in the transposition reaction. It is a novel feature of this transcriptional control mechanism that the control sites are located distal to the 3' ends of the two divergent transcripts from each cassette, at a distance of more than 1 kbp from the transcription initiation sites.

Thus, the control sites at HML and HMR define a region of the chromosome that is both transcriptionally inactive and designed to perform a specific role in transposition. Possible mechanisms for this control will be discussed.

We have also defined a site within the cassette which is required for transposition and is subject to cleavage by a specific nuclease under control of the HO gene. It is proposed that this cleavage reaction is the initial event in transposition, followed by invasion of the donor molecule by both recipient strands and eventual resolution of the resulting crossover structure.

0222 TRANSPOSABLE ELEMENTS IN DROSOPHILA, Gerald M. Rubin, Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210

The effects on gene expression of three distinct classes of Drosophila transposable elements will be discussed (1-7). The results of recent studies on the regulation of the white and singed loci and its modulation by transposable element insertion will be presented (K. O'Hare, R. Levis, T. Hazelrigg, H. Roiha and G. Rubin, unpublished). Studies of the effects of chromosomal position on gene expression will also be presented (A. Spradling, G. Rubin, unpublished).

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Viral Gene Regulation

0223 ORGANIZATION AND EXPRESSION OF BACTERIOPHAGE T7 DNA, F. William Studier and John J. Dunn, Biology Department, Brookhaven National Laboratory, Upton, New York 11973

Bacteriophage T7 contains a single molecule of linear, double-stranded DNA that is 39,936 base pairs long. The entire nucleotide sequence of T7 DNA has been determined, and the T7 genes and genetic signals have been located within the sequence (1). T7 DNA carries genetic information very efficiently. Coding sequences for 50 genes are closely packed but essentially not overlapping, and occupy almost 92% of the sequence. This arrangement suggests that all 50 of these genes are expressed, although genetic or biochemical evidence for the expression of only 38 of them is so far available. Five potential genes whose coding sequences overlap those of the above genes in different reading frames have also been identified in the nucleotide sequence, but expression of only one of these potential overlapping genes has been detected so far. Where small gaps between coding sequences occur, they usually contain transcription signals, RNase III cleavage sites, or origins of replication. The longest stretches of sequence that do not code for any protein are found at the two ends of the DNA. The molecule begins and ends with a perfect direct repeat of 160 base pairs. Adjacent to the terminal repetition, at both ends of the DNA, lie very similar arrays of 12 imperfect copies of a 7-base sequence, spread across 160 base pairs or so. The terminal repetition and the adjacent arrays of short repeated sequences seem likely to have a role in concatemer formation, maturation, or packaging.

T7 genes are arranged in the DNA, from left to right, in the approximate order in which they function during infection: first come the early genes, then the DNA metabolizing genes, and finally the structural and assembly genes. Transcription is also from left to right, first by the host RNA polymerase and then by newly made T7 RNA polymerase. The initial transcription of T7 DNA proceeds in a wave that takes almost 40% of the latent period to reach the right end of the DNA, and which may be coupled to entry of the DNA into the cell. T7 RNA polymerase is very specific for its own promoters, 17 of which are distributed across the T7 DNA molecule. These promoters share a highly conserved sequence of 23 continuous base pairs that includes the start site for the RNA. This recognition sequence is large enough that it is unlikely to be found in host DNA, which allows T7 to switch all transcription in the cell from host DNA to its own DNA simply by inactivating the host RNA polymerase after its own RNA polymerase has been made. The primary transcripts from T7 DNA are cut at specific sites by a host enzyme, RNase III, to produce the mRNAs observed in vivo. Most T7 mRNAs are polycistronic, but few polar effects have been identified, and most T7 proteins would appear to be initiated independently, each from its own ribosome-binding and initiation site.

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0224 EXPRESSION OF THE ABELSON MURINE LEUKEMIA VIRUS GENOME AND OF TRANSFECTED κ IMMUNOGLOBULIN GENES, David Baltimore, Jean Y.J. Wang, J. Gordon Foulkes, Ron Prywes, Fred Ledley, Rosalind Lee, Leon Shiman and Douglas Rice, Massachusetts Institute of Technology, and Whitehead Institute for Biomedical Research, Cambridge, MA 02139

Abelson murine leukemia virus (A-MuLV) is a defective retrovirus able to transform fibroblasts and immature B-lymphoid cells. Its transforming potential derives from the v-abl sequence, a sequence captured from the normal c-abl cellular gene. The c-abl gene consists of at least 10 apparent exons spread over more than 30 kb of cell DNA; the v-abl sequence is a cDNA copy of the central exons. The normal c-abl gene is expressed in many cell types as two poly(A)-containing RNAs of 5.5 and 6.5 kb which differ at their 3' ends. A-MuLV encodes a single protein with retroviral gag sequence at its N-terminal end followed by v-abl-encoded sequence. Of the 130,000 molecular weight of protein encoded by the v-abl sequence, at least 85,000 molecular weight of the C-terminus can be deleted without loss of fibroblast transforming activity. The remaining sequence is highly homologous to the v-src, v-fps, v-fes and v-yes sequences indicating that all transforming genes with tyrosine-specific protein kinase activity transform cells by virtue of highly homologous protein domains. The transforming segment of v-abl when expressed in E. coli causes extensive phosphorylation of the bacterial protein on tyrosine residues. The minimal transforming A-MuLV causes a 5-fold rise in overall cellular phosphotyrosine in transformants.

The A-MuLV lymphoid transformants are excellent models for studying the immunodifferentiation program. To facilitate such studies we have constructed a plasmid containing a rearranged κ immunoglobulin gene and gpt. This plasmid can be established in A-MuLV transformants where it will direct the regulated synthesis of κ protein.

0225 THE UNIQUE INTERACTION OF INFLUENZA VIRAL RNA TRANSCRIPTION WITH THE HOST CELL TRANSCRIPTIONAL MACHINERY. Robert M. Krug, Ismo Ulmanen, Janet Braam, Ann Beaton, Michael G. Katze, Barbara M. Detjen and Stephen J. Plotch, Molecular Biology and Virology Division, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Influenza viral mRNA synthesis in the infected cell requires the continuous functioning of the host nuclear RNA polymerase II. Viral mRNA synthesis occurs in the nucleus, and newly synthesized capped (m^7GpppNm-containing) cellular RNAs in the nucleus, i.e. most likely heterogeneous nuclear RNAs, which are synthesized by RNA polymerase II, provide the primers needed for the initiation of viral mRNA chains. A viral cap-dependent endonuclease cleaves these cellular transcripts to generate capped fragments 10 to 13 nucleotides long. As initially revealed by studies of viral mRNA synthesis in vitro catalyzed by the virion-associated transcriptase, those capped fragments containing an A residue at their 3' ends are preferentially used as primers to initiate transcription via the initial incorporation of a G residue directed by the penultimate C residue of the virion RNA template (3' UCG...). The viral mRNA chains are then elongated. Experiments employing ultraviolet (UV)-crosslinking and temperature-sensitive virus mutants to analyze the in vitro reaction have established that one of the three viral P proteins associated with the viral nucleocapsids, the PB2 protein, functions as the cap-recognizing component of the endonuclease reaction. UV-crosslinking experiments have also indicated that: (i) a different viral P protein, the PB1 protein, most likely catalyzes the initiation of transcription via the initial incorporation of a G residue; and (ii) mRNA chain elongation is catalyzed at least in part by the third viral P protein, the PA protein. The interaction with host nuclear functions apparently continues after the viral mRNAs are synthesized: the viral mRNAs contain internal m^6A residues, and several of the viral mRNAs appear to be generated by splicing. These processes are most likely carried out by cellular RNA processing enzymes in the nucleus. The m^6A residues in the viral-coded region of the viral mRNAs are in GAC and AAC sequences, the same sequences m^6As are found in DNA-directed RNA polymerase II transcripts. This strongly suggests that this internal methylation is catalyzed by host enzymes. All eight of the major viral mRNAs, both those that do and those that do not undergo splicing, contain internal m^6A residues.

Gene Transfer

0226 EXPRESSION VECTORS FOR FUSIONS OF THE E.COLI GALK GENE TO YEAST GENE REGULATORY SEQUENCES, Richard S. Zitomer, Brian C. Rymond and Charles V. Lowry, Department of Biological Sciences, State University of New York at Albany, Albany, N.Y. 12222

We have constructed a series of yeast-E.coli transformation vectors containing fusions between the transcriptional and, in some cases, the translational initiation signals of the yeast CYC1 gene, encoding iso-1-cytochrome c, and the E.coli galactokinase gene, galK. When gal1$^-$ (galactokinase deficient) yeast mutants were transformed with such fusion plasmids, gal$^+$ transformants were obtained, and galactokinase function in these transformants was regulated in an identical fashion as that for cytochrome c. Furthermore, mutations which altered CYC1 transcription also altered expression of galactokinase. The fusions constructed were of two types: protein coding sequence and mRNA leader sequence fusions. In those fusions which direct the formation of a fused protein with the amino terminus of cytochrome c and the bulk of the galactokinase sequence, the complete 5' transcriptional and translational signals of CYC1 remain intact. These fusions express the galK functions in yeast and indicate that the amino terminus of the galactokinase enzyme is not essential for function. Fusion between the 5' portion of the coding sequence of the yeast LEU2 gene and the coding sequence of the galK gene have been constructed to determine how much protein can be placed on the amino terminus of the galactokinase enzyme without destroying its activity. The mRNA leader sequence fusions contained the transcriptional regulatory sequences and part or all of the 5' mRNA leader sequence of the CYC1 gene joined to various lengths of the sequence 5' to the galK ATG initiation codon. These fusions expressed the galK function as well as the protein coding fusions suggesting that bacterial sequences can substitute for the sequences immediately surrounding the CYC1 ATG initiation codon without a drastic loss in translational efficiency. Only in those cases in which the CYC1 ATG initiation codon remained and was followed by an out-of-frame galK initiation codon and coding sequence was galK expression seriously impaired.

These plasmids can be used to select for regulatory mutations in cases where no selection is available using the intact gene. For example, we have used a CYC1/galK fusion plasmid to select for mutants that could grow on galactose in the absence of oxygen, conditions under which the CYC1 gene is normally repressed. The selection for and the nature of such mutations will be discussed. We believe that these fusion plasmids can be of general use for the construction of other yeast gene/galK fusions at the levels of protein coding fusions, mRNA leader sequence fusions, or fusions substituting different upstream regulatory sequences for those of CYC1. The construction of and the requirements for such fusions will be discussed. Such fusions could be used to isolate regulatory mutants taking advantage of the strong selections both for and against galactokinase expression in yeast cells.

0227 METALLOTHIONEIN GENE REGULATION, Dean H. Hamer, Anthony D. Carter, Barbara Felber, Marie-France Jubier, George N. Pavlakis, Carl J. Schmidt and MaryJane Walling, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

The metallothioneins are ubiquitous heavy metal binding proteins whose synthesis is inducible by both heavy metals and glucocorticoids. We have cloned and initiated a structural and functional analysis of metallothionein genes from mouse, monkey and man. The mouse metallothionein-I gene is transcriptionally regulated by cadmium when introduced into cultured cells on SV40 and BPV vectors. Analysis of deletion and linker-scanning mutants, using a convenient E. coli galactokinase read-through assay, shows that the regulatory sequences lie within a small region close to the 5' end of the gene. The cloned mouse metallothionein-I gene does not respond to dexamethasone treatment, even though the chromosomal genes in the same cells retain their inducibility, indicating that heavy metals and glucocorticoids regulate this gene by independent mechanisms. Metallothionein genes can also be used as dominant selective markers, by conferring resistance to cadmium, and to obtain the efficient and regulatable expression of other gene products such as human growth hormone.

0228 Cellular Transforming Genes

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A variety of different types of neoplasms contain activated transforming genes which can be detected by transfection of tumor DNAs. These neoplasms include several different types of chicken, mouse and human lymphocyte neoplasms and mouse and human carcinomas of bladder, colon, lung and mammary origin. The transforming genes of some of these neoplasms have been characterized by molecular cloning and/or by homology with transforming genes of retroviruses. The current status of characterization of these tumor transforming genes will be discussed.

Gene Regulation In Development

0229 DEVELOPMENTALLY REGULATED PROCARYOTIC GENES AND THEIR PROMOTERS, Richard Losick, W. Charles Johnson, Peter A. Zuber, Michael A. Stephens, Department of Cellular and Developmental Biology, The Biological Laboratories, Harvard University, Cambridge, MA 02138

Bacillus subtilis is a differentiating, Gram-positive bacterium, which produces a dormant cell form known as the endospore in response to nutrient depletion. The initiation phase of this differentiation process is controlled by a class of regulatory genes known as the spo0 loci; mutations in any one of these eight regulatory genes block development before the earliest morphological events of spore formation. To study the control of gene expression during spore formation, we previously cloned in E. coli B. subtilis genes whose transcription is developmentally regulated. One of these genes, which is known as spoVG, is activated at the onset of sporulation and its transcription is controlled by the products of at least five of the eight known spo0 loci (ref. 1). A curious feature of spoVG is that its transcription in sporulating cells is initiated from two closely space (10 bp) startpoints known as P1 and P2 (ref. 2). The upstream startpoint P1 is utilized in vitro by a modified form of B. subtilis RNA polymerase containing a 37,000 dalton species of B. subtilis sigma factor known as σ^{37} (ref. 3). [The principal form of B. subtilis RNA polymerase contains a sigma of 55,000 daltons (σ^{55})] We will report on the isolation of a 32,000 dalton species of sigma factor (σ^{32}), which exclusively dictates utilization of the downstream promoter P2 and the use of a fusion of spoVG to the E. coli lacZ gene to study spoVG regulation in vivo. We will also report on the structure of another developmentally regulated promoter whose activation is delayed until four hours after the onset of sporulation (ref. 4).

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0230 REGULATION OF DICTYOSTELIUM DISCOIDEUM mRNAS SPECIFIC FOR PRESPORE OR PRESTALK CELLS
Harvey F. Lodish, Eric Barklis, Rex Chisholm and Brian Pontius, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

Prespore and prestalk cells in Dictyostelium discoideum aggregates can be separated by density gradient centrifugation. Using poly (A^+) RNA from the fractionated cells to probe a cDNA library of mRNAs from 15 hr and 22 hr postaggregation cells, we were able to identify 14 cDNA clones representing RNAs enriched in prespore or prestalk cells. Remarkably, transcripts of 6 of 7 cDNA clones, previously selected to encode mRNAs present in postaggregating cells but low or absent in growing cells, also are enriched in RNA from either prestalk or prespore cells. These 21 aggregation-stage mRNAs fit into five different co-regulated classes.

Class I mRNAs enriched in prespore cells are totally absent in vegetative cells and appear between 4-8 hr of differentiation. Those in Class II appear between 8 and 12 hr. All prespore mRNAs disappear from slugs when disaggregated, but the level of mRNA is maintained in disaggregated cells if cAMP is added. We have shown that in disaggregated cells cAMP both stimulates the transcription of these mRNAs, and also acts to stabilize them against decay.

mRNAs enriched in cells programmed to become stalk cells fall into two classes: Prestalk Class I mRNAs are detectable at low levels in vegetative cells but present at 20-50 fold higher levels late in development. The levels of these mRNAs are less sensitive to disaggregation and/or cAMP than are prespore mRNAs. mRNAs in pre-stalk-specific Class II appear late in aggregation - between 12 and 15 hours. Their level is dependent on continued cell-cell contact but remain relatively unaffected by addition of cAMP to disaggregated cells. RNA species which are present at significant levels both in vegetative cells and throughout development, such as actin and discoidin mRNA, are not specific for either cell type. We have found only one cloned mRNA whose level is increased in aggregated cells, but which is not localized to prespore or prestalk cells.

Further evidence for the co-regulation of these classes of mRNAs comes from studies on cells which are starved, to initiate the developmental program, but shaken in suspension to disrupt cell-cell signalling and cell-cell contact. In the absence of cAMP, only Class I prestalk mRNAs are induced and these only weakly; as we have shown previously, no other aggregation-stage mRNA is induced. In other studies, the cells were subjected to pulses of 50 nM cAMP every 5.5 minutes, to mimic cell-cell signalling with cAMP. This treatment induces Class I prespore mRNAs, and elevates the level of Class I prestalk mRNAs but has marginal effects on Class II prestalk or Class II prespore mRNAs.

These studies suggest that cAMP is the predominant if not the only inducer of Class I prespore and Class I prestalk mNRAs. Induction of Class II prespore mRNAs requires cell-cell contact as well as cAMP, and Class II prestalk mRNAs appear to require only cell-cell contact.

Transcription and Processing

0231 PROCESSING/POLYADENYLATION SIGNALS AND GENE REGULATION.
Charles N. Cole and George M. Santangelo. Department of Human Genetics, Yale University School of Medicine, New Haven, Ct. 06510. Most genes transcribed by RNA polymerase II contain the hexanucleotide, 5'-AAUAAA-3', just upstream from the polyadenylation site. We have examined the signal requirements for processing/polyadenylation. The herpes virus thymidine kinase gene was resected from its 3' end, and a plasmid isolated, pTK206, which lacks the AAUAAA signals normally found at the 3' end of the tk gene. This resected gene, and the wildtype gene, were transferred to pSV010, an SV40-origin-containing plasmid, allowing replication and analysis of transcription in cos-1 cells. Fragments of DNA containing processing/polyadenylation signals from SV40 and polyoma were inserted into the 3' end of the resected gene, pTK206. We find that tk gene expression requires a processing/polyadenylation signal, that signals from SV40 and polyoma can substitute for the hsv-tk signal, and that very large differences in levels of tk mRNA are found in cos-1 cells transfected by different gene constructs. These differences may be due to differential utilization of the signal, differential stability of the tk mRNAs or both. In addition, tk gene expression was restored to a low level by insertion of an 88 bp fragment of SV40 DNA, containing an AAUAAA, but normally not involved in polyadenylation. TK gene expression was also restored by 83% of fragments (3.5-18 kb) of African green monkey DNA inserted into pTK206. Restoration of tk gene expression was always associated with the production of polyA+ mRNA. The frequency with which inserted fragments restored gene expression suggests that the minimal signal for processing/polyadenylation is a hexanucleotide (AAUAAA or similar). Experiments to test this hypothesis will be discussed.

0232 NATURAL AND SYNTHETIC SEQUENCES WITH Z-DNA POTENTIAL INHIBIT tRNA GENE TRANSCRIPTION IN VITRO, R.A. Hipskind and S.G. Clarkson, Dept. Microbiology, U. Geneva School of Medicine, 1205 Geneva, Switzerland

A variant $tRNA_1^{Met}$ gene of Xenopus laevis is poorly transcribed in a homologous S-100 extract due to DNA in its 5' flanking region. Using BAL31 nuclease, 24 deletions ranging from 1-91 bp have been created in this region, starting from a Pvu II site located just upstream of the two major initiation sites. These deletions influence the choice between these two sites and also cause large changes in transcription efficiency, the most dramatic of which is the 6-fold stimulation of transcription resulting from the elimination of the sequence d(TGCGCGTGC). To determine whether the inhibitory influence of this tract can be mimicked by other d(G+C)-rich stretches of alternating purine-pyrimidine residues, various synthetic DNA octamers have been inserted at a single site within the 5' flanking region of a deletion mutant that lacks this tract. Transcription is indeed inhibited by octamers that have been shown to crystallize as left-handed Z-DNA, and is most severely reduced by the insertion of the sequence d(CGCGCGCG). This effect is not simply due to the high d(G+C) content of this octamer because transcription is much more efficient with inserts of d(CCCCGGGG), d(CCGGCCGG), or d(GGCCGGCC). These results thus demonstrate a strong correlation between the inhibition of transcription of this tRNA gene and the potential ability of sequences upstream to form Z-DNA.

0233 ACCURATE IN VITRO SPLICING OF PURIFIED ADENOVIRUS 2 PRECURSOR RNAS, Carlos J. Goldenberg and Scott Hauser, Washington Univ. Medical School, St. Louis, MO 63110

We have used nuclear extracts from MOPC-315 mouse myeloma cells to study in vitro splicing of adenovirus 2 early region 2 (E_2) mRNA precursors. Using purified and deproteinized nuclear E_2 precursor RNAs isolated from cells infected early during infection, we have shown that approximately 80% of the precursor RNA is converted in vitro to a mature form. The nucleotide sequence of the splice junction in the E_2 RNAs processed in vitro was investigated by performing primer extensions with the four dideoxynucleotides and direct sequencing on polyacrylamide gels. We conclude that the in vitro splicing has the same precision as what occurs in vivo. Some properties of the in vitro splicing reaction will also be discussed.

0234 COMMON ELEMENTS IN THE PROMOTER OF POLIII TRANSCRIPTIONAL UNITS, R.Cortese G.Ciliberto, G.Raugei, F.Costanzo, L.Dente, EMBL, Heidelberg, West-Germany. The 34 bp internal control region of the somatic 5S RNA gene from Xenopus borealis can be split in two separable components: a maxigene carrying an insertion between nucleotide 71 and 74 of the coding region is actively transcribed in the nucleus of X. laevis oocytes, giving rise to a maxitranscript with initiation and termination points identical to those of the wild-type transcript. The first 11 nucleotides of the 5S RNA gene promoter are shown to be structurally and functionally homologous to the first component (Box A) of tRNA genes promoter. This was shown by constructing hybrid 5S RNA/tRNA and tRNA/5S RNA genes which were efficiently transcribed. Initiation of transcription appears to be a complex phenomenon in which both components of the internal promoter play a role.

0235 CONTROL OF RNA ACCUMULATION DURING SPORULATION IN YEAST, David B. Kaback, Elizabeth G. Ninfa and Larry Feldberg, Dept. of Microbiology, NJ Medical School, Newark, NJ 07103

To investigate the control of gene expression during meiosis and sporulation we examined the temporal accumulation of RNA complementary to several cloned genes by "Northern" blot hybridizations. During these studies several surprising observations were made. The genes encoding histones H2A and H2B were examined as examples of genes necessary for the completion of meiosis. Poly(A)+ RNA species for H2A and H2B appeared as expected during premeiotic DNA synthesis. Unexpectedly, these RNA species persisted after the cessation of DNA synthesis and were ultimately found in mature spores. This persistance is in contrast to vegetative cells which lack detectable histone RNA in the absence of DNA synthesis. The histone RNA found in mature spores may be analogous to the stored maternal histone RNA in sea urchin eggs. We also obtained surprising results when we tested whether genes not required for sporulation were transcribed or repressed. CDC10 is a gene that is required for mitotic growth but is thought not to be required for sporulation. Strains with ts mutations mapping in cdc10 fail to complete mitotic cytokinesis but are able to sporulate at the restrictive temperature. Unexpectedly, RNA complementary to this gene was found to accumulate at 20 fold higher levels in sporulating a/a cells than in nonsporulating a/a and a and vegetative cells. In addition, transcripts complementary to the inducible galactose catabolic genes (GAL7,10,1) were also found in cells incubated in sporulation medium that contains no inducer. These transcripts were not detected in uninduced vegetative cells. The GAL genes serve no apparent function in meiosis since homozygous mutants unable to catabolize galactose also sporulate. The appearance of the CDC10 and GAL10 transcripts and the H2A and H2B transcripts (after DNA replication) suggest that transcriptional controls on some genes may be partially relaxed when cells are put in sporulation medium.

0236 Oocyte Extracts Reactivate Developmentally Inert Xenopus 5S Genes in Somatic Nuclei, Laurence Jay Korn.

In Xenopus and other amphibians, there are two major classes of 5S RNA genes. One class, which codes for somatic-type 5S RNA, is expressed in both oocytes and somatic cells. The other class, which is 50 times more abundant and which codes for oocyte-type 5S RNA, is expressed in oocytes but not in somatic cells. The developmentally inactivated oocyte-type genes are reactivated when erythrocyte nuclei are injected into most oocytes (activating oocytes). Since the injected nuclei are induced to take on the natural activity of the host cell, the activation of oocyte-type 5S genes may represent a normal control mechanism. A special opportunity to investigate this mechanism is provided by the existence of certain individual animals whose oocytes consistently fail to activate 5S genes (non-activating oocytes). We have injected extracts of activating or non-activating oocytes, together with erythrocyte nuclei, into oocytes of each kind. We show that the transcription of 5S genes is induced by extracts of activating oocytes (positive regulation) and is not inhibited by similar extracts of non-activating oocytes. The activating factor is sensitive to protease and to heat treatment, suggesting that a protein is involved. We are currently attempting to purify this factor(s) in the hope that it will give us further insight into the mechanism by which transcription is regulated during development.

0237 POINT MUTATIONS IN A PROKARYOTIC PROMOTER SIGNAL CARRIED ON THE SIMIAN VIRUS 40 GENOME, Ana B. Chepelinsky, Tikva Vogel, Don Court* and Martin Rosenberg Laboratories of Biochemistry and *Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

We have used the galK fusion vector system, pKO[1], to characterize a promoter signal recognized by E. coli RNA polymerase which occurs on the SV40 genome. This promoter signal overlaps structurally with the SV40 polyadenylation signal regulating late gene expression. A small DNA fragment from this region of SV40 was inserted into the pKO vector system and shown to activate expression of the galactokinase gene.

The following procedure was used to select down mutations in this promoter signal. Cells containing this plasmid were exposed to a mutagen. Mutants were selected by transfecting mutagenized plasmids into a gal$^-$ cell and selecting survivors on TB gal media. Mutants were then pooled and the promoter fragment reisolated from the mixture of mutated plasmids. This fragment was cloned into a new pKO vector, and subjected to the same selection as described above, to discriminate promoter down mutantions from mutations in the kinase gene. The mutants obtained carried point mutations in the -35 or the -10 region of the promoter.

Examination of one of these mutants indicates discrepancies between in vivo galactokinase activity and in vitro run off transcription. These results may be explained by effects of DNA supercoiling on promoter activity.

[1] McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C. and Rosenberg, M.: Gene Amplification and Analysis, Vol II: Analysis of Nucleic Acids by Enzymatic Methods (Elsevier-North Holland, J.C. Chirikjian and T.S. Papas, Editors) pp. 383-415, 1982.

0238 HORMONE INDUCED EXPRESSION OF KERATIN GENES IN VAGINAL EPITHELIUM. Dennis R. Roop, Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, NIH, Bethesda, MD. 20205. Mark Kronenberg and James H. Clark, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Using cDNA clones corresponding to the major keratins synthesized in newborn mouse epidermis of 55, 59 and 67 kilodaltons (kd), we have examined the expression of these keratin genes in rat vaginal epithelium. It was of interest to investigate the expression of keratin genes in vaginal epithelium since the degree of keratinization in this tissue is hormonally regulated. Total RNA was isolated from vaginal epithelium obtained from ovariectomized rats and at 24, 48 and 72 hr after exposure to estradiol benzoate. Northern blot analysis was performed on these RNAs using cDNA clones for the 55, 59 and 67 kd epidermal keratins as probes. On the basis of size and degree of homology, the mRNAs coding for these keratins in the internal keratinizing epithelium of the vagina appear to be similar if not identical to those synthesized in mouse epidermis. The concentration of the mRNAs coding for these keratins is very low in vaginal epithelium obtained from ovariectomized animals. However, 24 hr after exposure to estradiol, the concentration of the mRNA coding for the 55 kd keratin is dramatically increased to maximal levels. A moderate increase in the concentration in the mRNA coding for the 59 kd keratin is observed at this time with maximal induction occuring at 48 hr. The mRNA coding for the 67 kd keratin only becomes apparent 72 hr after exposure to hormone. The induced expression of these keratin genes after exposure to estradiol correlates with the observed morphological changes in this epithelium of stratification and keratinization.

0239 THE USE OF COMPUTERIZED IMAGE PROCESSING TO IDENTIFY AND QUANTITATE THE DOMAIN OF RNAS AFFECTED BY A SPECIFIC STIMULUS, Floyd E. Taub,* James De Leoł and E. Brad Thompson*, *Laboratory of Biochemistry, National Cancer Institute and ŧDivision of Computer Research and Technology, National Institutes of Health, Bethesda, MD 20205

A method to simultaneously analyze shifts in the relative abundance of many specific RNAs following any stimulus is presented. Hybridizations of two, complex "total" cDNA probes (from the pre- and post-stimulus states) to each member of a cDNA library are quantitatively analyzed and mathematically compared. The method utilizes computer assisted image processing and statistical analysis of sequential hybridizations. Experiments indicate that shifts in abundance between two states can be identified and reproducibly quantitated without purified probes. Direct isolation of recombinant cDNA colonies containing inserts for regulated RNAs is possible. The use of this system is demonstrated for partial analysis of *in vivo* response of rat liver to glucocorticoids. Application to other biologic systems where a shift between two states occurs is discussed.

0240 mRNA BIOSYNTHESIS: INITIATION OF TRANSCRIPTION AND POST-TRANSCRIPTIONAL MODIFICATION, Jerry M. Keith, Diana Galer, and Larry Westreich, New York University, New York, NY 10010

Post-transcriptional modification of eukaryotic mRNA occurs in a complex series of enzymatically catalyzed reactions. An early event in this modification process is the formation of the capped 5'-end of the mRNA. In its simplest configuration, the cap consists of a 7-methylguanine residue linked from its 5'-position through a triphosphate bridge to the 5'-position of the penultimate nucleoside in the RNA molecule. We have purified two enzymes involved in synthesis of the methylated cap in wheat germ mRNA. Characterization of the capping activity revealed that an enzyme-GMP covalent intermediate is formed during the capping process. Chemical studies and analysis of purified enzyme-GMP complex on SDS polyacrylamide gels indicate that two polypeptides with molecular weights of approximately 90K are covalently linked through a phosphoamide bond to GMP. Partial V8 digests of the two polypeptides suggest that they are very dissimilar; however, the GMP residue in both polypeptides can be transferred to an acceptor molecule to form a capped 5'-end. Further studies indicate that the enzyme-GMP complex can be formed even after partial trypsin digest of the purified enzyme. The molecular nature of this protein-nucleic acid bond is being investigated.

The other enzyme activity isolated specifically catalyzes the methylation of the 6-position of the terminal guanosine residue in the capped 5'-end of mRNA. This activity, as well as the RNA polymerase activity are both clearly separated from capping activity during the purification scheme. However, the relationship between the RNA polymerase and the capping and methylating activities is being investigated in crude lysates to determine if an *in vivo* association may exist.

0241 COMPARISON OF ANTITERMINATION PROTEINS FROM THREE LAMBDOID BACTERIOPHAGES. Naomi C. Franklin. Biology Dept., University of Utah, Salt Lake City, Utah 84112.

The antiterminating N protein of bacteriophage lambda is required early in that phage's development in order to prevent termination of transcription by host RNA polymerase at various termination signals in the lambda genome. Genetic evidence has shown that the N protein makes a long lived impression on transcription complexes initiated at the phage promoters. This effect depends upon the presence of specific N recognition sites downstream from the phage promoters. Phages related to lambda practise the same life style, but for the most part fail to complement each other in the N system, ostensibly because the different N proteins have specificity for their own recognition sites.

We have now sequenced the DNA for the N genes and their environs in three related phages: lambda, 21 and P22. The N proteins deduced from those DNA sequences are all small and basic, but otherwise highly variant. The promoters and leaders regulating N translation can also be compared, as well as the putative sites of N recognition. The DNA analysis, furthermore, makes possible the cloning and mixing of elements from the three differentiated phages, so that the seats of their specificity may eventually be comprehended.

0242 PROTEIN BINDING SITES ARE CONSERVED IN SMALL NUCLEAR RNA U1 FROM INSECTS AND MAMMALS. Eric D. Wieben, Steven Madore and Thoru Pederson, Cell Biology Group, Worcester Fdn. for Exptl. Biology, Shrewsbury, MA 01545.

To gain insight into the ribonucleoprotein (RNP) structure of small nuclear RNA's, HeLa cell poly(A)$^+$ mRNA was translated in a reticulocyte lysate, and the in vitro binding of ^{35}S-labeled proteins to individual small nuclear RNA species was examined using human autoimmune antibodies. Addition of human small nuclear RNA U1 to the translation products leads to the formation of a specific U1 RNA-protein complex that is recognized by a human autoimmune antibody specific for U1 snRNP. This antibody does not react with U1 RNA itself or free proteins. Addition of a 10- to 20-fold molar excess of transfer RNA instead of U1 RNA does not lead to the formation of an antibody-recognized RNP. The proteins forming the specific complex with U1 RNA correspond to the A, B_1, and B_2 species (32,000, 27,000 and 26,000 mol. wt. respectively) observed in previous studies with U1 snRNP obtained by antibody-precipitation of nuclear extracts. Similar translation-assembly experiments with Drosophila poly(A)$^+$ mRNA reveal that a 26,000 mol. wt. protein previously identified in Drosophila U1 RNP (Wieben and Pederson, Mol. Cell. Biol. 2:914-920, 1982) also binds to U1 RNA in vitro. When the translation products of HeLa or Drosophila mRNA are presented with U1 RNA of the other species, the 32,000 and 26,000 mol. wt. proteins recognize binding sites on the heterologous U1 and in both cases form complexes recognized by RNP antibody. These results establish that the U1 RNA binding site for the mammalian 32,000 mol. wt. snRNP protein and a 26,000 mol. wt. homologue in Drosophila has been highly conserved in evolution. This site may be the identical 13 nucleotides at the 5' ends of human and Drosophila U1 RNA, or a highly conserved aspect of U1 secondary structure.

0243 RNA SPLICING: STUDIES USING CLONED GENES INTRODUCED INTO MAMMALIAN CELLS, Katharine M. Lang, George A. Martin, and Richard A. Spritz, University of Wisconsin, Madison, WI 53706

The mechanism of excision of intervening sequences from the RNA precursor, in particular those factors which govern selection among multiple potential splice sites, is largely unknown. In order to investigate the factors which influence the RNA splicing process, we have utilized systems capable of expressing cloned eukaryotic genes in mammalian cells. Directionality of the splice site recognition process was assessed by the construction of human G_γ-globin genes containing small tandem duplications which embed the 5' (donor) or 3' (acceptor) splice junctions of the second intervening sequence (IVS2). RNA transcribed from the gene with duplicated donor splice sites was spliced exclusively at the upstream (5') donor site. Most RNA from the gene with duplicated acceptor splice sites was spliced at the upstream acceptor site, although a small amount was spliced at the downstream (3') acceptor. These data appear to support a model in which the recognition of donor, and probably also acceptor, coding-IVS2 junctions in the human G_γ-globin mRNA precursor occurs by a 5'→3' processive process, at least locally along the RNA molecule. However, it is likely that other factors such as specific RNA sequence, secondary structure, and protein binding can modify the recognition process in vivo. We have also constructed a complete mouse immunoglobulin μ heavy chain gene and have introduced this gene into mammalian cells. This gene contains both the μ_s and μ_m 3' termini and therefore should prove useful in investigations of alternate transcriptional termination and/or splicing of μ RNA during lymphoid cell differentiation.

0244 RHO-DEPENDENT TERMINATION IN THE TRP OPERON, Terry Platt, Peggy J. Farnham, Jill L. Galloway, John E. Mott, and Janice A. Sharp, Yale University, New Haven, CT 06510.

The trp t site in the E. coli tryptophan operon corresponds to the observed endpoint of trp mRNA in vivo, 36 nucleotides past the last structural gene. A second site, trp t', is located about 250 nucleotides downstream, in a region rich in AT-base pairs with little potential for secondary structure. In vivo, readthrough transcription occurs at trp t in rho$^-$ strains, or when trp t' has been deleted. In vitro, trp t does not respond to added rho, whereas termination at trp t' is completely rho-dependent. Further analysis of this region demonstrates that (1) the signal specifying termination is some distance upstream from the point(s) of termination. (2) Termination at trp t' is tightly coupled to the ATPase activity of rho factor: the non-hydrolyzable analog ATP-γ-S blocks both hydrolysis and termination, and when transcription is carried out with templates lacking the trp t' region (but otherwise similar) so that rho-dependent termination does not occur, no significant amount of ATPase activity is detectable. (3) Termination is abolished, as is the ATPase activity of rho (though transcription is not affected) by the presence of T1 RNase during transcription. Thus a substantial continuous portion of the RNA transcript must be required for termination to occur. (4) Galactokinase levels regulated by trp t and trp t' separately and in their normal tandem configuration are consistent with the previously observed rho-dependence of these two terminators in vivo, indicating that trp t' is required for trp t "termination" in vivo. We hypothesize that the primary event in vivo is rho-dependent termination at trp t', followed by rapid exonucleolytic degradation of the transcript that is arrested when the exonuclease (RNase II?) encounters the stable hairpin in the mRNA corresponding to the trp t site.

0245 MUTATIONAL ANALYSIS OF A rho INDEPENDENT TRANSCRIPTION TERMINATION SITE RECOGNIZED BY E.coli RNA POLYMERASE. Keith McKenney, Laboratory of Molecular Biology, MRC, Hills Road, Cambridge CB2 2QH England.

A detailed study of the rho independent lambda t_o terminator has been carried out by analyzing a number of deletions and single base mutations in the t_o site. Mutations were isolated and in vivo function quantitated using a gene fusion vector system based on the E.coli galK gene[1] that was modified to incorporate poly cloning sites and primer sequences of M13 phage derivatives mp8 and mp9[2]. A technique for rapid sequence analysis was developed using the Sanger dideoxy method on double stranded DNA templates. Mutations were isolated using 1) classic genetic selections and 2) in a directed fashion using synthetic oligonucleotides to introduce mutations not obtainable by classic techniques. Mutations were found in a hyphenated dyad symmetry element (stem and loop) preceding the termination site, that change the stem stability and the base composition of the loop. Specific single mutations were recombined in vitro to construct double mutations and quantitated for in vivo function. Deletion analysis suggests that sequences within 8 bp distal to the termination site are sufficient for normal t_o function.

1. McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C., and Rosenberg, M. (1981) in Gene Amplification and Analysis, vol.II, Chirikjian J.G. and Papas, T.S.,eds. Elsevier-North Holland, New York, pp. 383-415.

2. Messing, J. and Viera, J. (1982) Gene in press.

0246 REGULATION OF YEAST MITOCHONDRIAL SPLICING, Susan G. Bonitz and Alexander Tzagoloff, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

The regulation of yeast mitochondrial genes is not understood. Most of the molecules controlling the expression of yeast mitochondrial genes are transcribed from yeast nuclear DNA. These transcripts are translated in the cytoplasm and these nuclear-encoded gene products are then transported into mitochondria. By studying yeast nuclear mutants that affect mitochondrial functions, the nuclear genes and gene products regulating mitochondrial genes can be studied.

I have begun characterizing yeast nuclear mutants specifically affecting a mitochondrial gene, the subunit 1 gene of cytochrome oxidase. From DNA sequencing studies, it has been determined that the subunit 1 gene of cytochrome oxidase is a split gene spanning 10,000 nucleotides. The gene contains 7-8 exons and 6-7 introns. The subunit 1 protein has a molecular weight of 55,000. The entire subunit 1 gene is transcribed as a 10 kb precursor. Removal of the introns occurs in several steps resulting in a mature 2 kb mRNA. Most of the molecules responsible for removal of these introns are encoded in yeast nuclear genes. By characterizing yeast nuclear mutants blocked at various stages of subunit 1 splicing, I hope to isolate the nuclear genes and gene products responsible for this process.

0247 SPLICING OF MESSENGER RNA IN YEAST, Dieter Gallwitz, Wolfgang Nellen and Christopher Langford, Department of Physiological Chemistry I, University of Marburg, Lahnberge, D-3550 Marburg/Lahn, FRG.

The splice junction sequences in split genes of the yeast S.cerevisiae transcribed by RNA polymerase II have been shown to conform to the "GT-AG rule". To investigate whether foreign intervening sequences are correctly excised from transcripts formed in transformed yeast cells, we have constructed several chimaeric genes by inserting intron-containing DNA fragments from genes of higher eukaryotes into the coding region of the split yeast actin gene. It was shown that the splicing of the chimaeric transcripts is selective in the sense that the yeast but not the foreign intervening sequences were correctly excised from such hybrid transcripts. The insertion of a second yeast intron into the coding region of the actin gene led to an mRNA from which both intervening sequences were correctly removed eliminating the possibility that only one splicing event can occur during the processing of mRNA in yeast.
By extensive deletion mapping of the yeast actin gene intron we have identified a short region within the intervening sequence which is absolutely required for splicing to occur. Inserting an intron fragment containing this sequence into the coding region of the actin gene resulted in the use of cryptic splice sites and the excision of RNA fragments of different lengths. These experiments suggest that the intron of the yeast gene contains a signal sequence which is recognized by factor(s) of the splicing machinery and from which the transcript might be scanned for potential splice sites.
(This work was supported by grants from the Deutsche Forschungsgemeinschaft to D.G.)

0248 RHO FACTOR-RNA INTERACTIONS: PHYSICAL STUDIES, J.A. McSwiggen, D.G. Bear, D.M. Feinstein, J.D. Singer, W.D. Morgan, T. Platt, & P.H. von Hippel, Univ. of Oregon, Eugene, OR 97403

The rho protein of E. coli is required for proper termination of transcription at some termination sites. Binding of RNA to rho activates its ATPase activity; rho may then interact in some way with paused RNA polymerase to stimulate release of the RNA transcript. The following physical studies using synthetic ribopolymers help define the mechanism and site of rho interaction with RNA. (i) Electron micrographs indicate that rho exists as a hexamer in solution and maintains its hexameric form upon binding to poly C; some binding cooperativity is also seen. (ii) Ethenoadenosine (εA), a fluorescent analog of A, can be used to monitor binding of rho to random (εA-containing) copolymers, in either the presence or absence of competing poly C or poly U. Using εA:C or εA:U (1:4), we find that rho interacts with, and excludes from further binding, a region of about 12 nucleotides per rho monomer. Binding is moderately cooperative (ω=200) with rho favoring poly C binding ~100x over poly U. (iii) Experiments in which rho protects poly C from nuclease digestion yield six RNA size classes, whose lengths are multiples of 12 nucleotides. This further supports the suggestion that each rho subunit binds ~12 nucleotides, or ~72 nucleotides per hexamer. (iv) Trypsin digestion of rho yields 2 major fragments, of molecular weight 31 kD (f1) and 15 kD (f3), which add up to the intact rho monomer of 46 kD. N-terminal sequencing indicates that f1 is the N-terminal fragment, of rho, and that f3 begins 135 residues from the C-terminus. UV-activated crosslinking of azido-ATP to rho labels both f1 and f3; since only f3 labelling is competed with cold ATP, f3 may contain the ATP binding site. (Supported by NIH & NSF Research Grants.)

0249 NONHISTONE PROTEINS BINDING SPECIFICALLY TO THE MOUSE ALPHA-FETOPROTEIN GENE, Barry H. Lesser and Helen W.H. Chan, University of Calgary, Calgary, Alberta T2N 1N4 Canada.

This existence of sequence-specific DNA-binding proteins has been well established in prokaryotes, and evidence is accumulating that site- or sequence- specific DNA binding proteins also occur in eukaryotes. We have developed methods for the detection and isolation of such proteins that depend on their higher affinity for particular DNA sequences relative to their affinity for DNA in general. Mouse liver nonhistone proteins are allowed to equilibrate between an excess of bacterial DNA, which sequesters nonspecific binding proteins, and trace quantities of cloned mouse DNA fragments. Formation of specific protein-DNA complexes is measured by retention of labeled mouse DNA on nitrocellulose filters, and such complexes are separated from unbound proteins and from nonspecific complexes between proteins and bacterial DNA by sucrose density gradient centrifugation. We have examined the interaction between nonhistone proteins isolated from 18-day fetal mouse liver or adult mouse liver, where the α-fetoprotein (AFP) gene is transcriptionally active or inactive, respectively, and DNA fragments coding for the 5'end or the 3' region of the mouse AFP gene. Both tissues contain nonhistone proteins that bind specifically to either DNA fragment. Proteins binding specifically to the 3' region of the gene comprise only about 0.1% by weight of the nonhistones, and consist of only 5-6 polypeptides out of the hundreds present in the nonhistone fraction. Similar specific binding proteins are present in both fetal and adult liver and have the same binding activity. Proteins binding specifically to the 5' end are currently being isolated. These studies are among the first to identify specific DNA-binding proteins in eukaryotes. (Supported by NSERC, Canada, and the Provincial Cancer Hospitals Board, Alberta).

0250 EFFECT OF DEXAMETHASONE ON GLOBIN GENE CHROMATIN STRUCTURE, Richard D. Smith, Ronald L. Seale and John Yu, Research Institute of Scripps Clinic, La Jolla, CA 92037

The glucocorticoid hormone, dexamethasone, is known to inhibit the expression of βmajor globin mRNA during the chemically induced erythroid differentiation of murine erythroleukemia cells. We present evidence that dexamethasone may inhibit βmajor transcription during differentiation by preventing the maturation of βmajor gene chromatin to an active conformation. During chemically stimulated differentiation, βmajor gene chromatin becomes increasingly sensitive to micrococcal nuclease and low levels of DNAse I. Micrococcal nuclease was able to distinguish the chromatin conformations of the transcribed βmajor gene in induced cells, the potentially transcribed βmajor genes in uninduced cells and the non-transcribed embryonic globin gene (εY3). Furthermore, two distinct DNAse I hypersensitive sites were found on the 5' side of the βmajor globin gene following chemical induction and their appearence coincides with the commitment of these cells to differentiate. We found that dexamethasone not only inhibits the increase in micrococcal nuclease and DNAse I sensitivity of βmajor gene chromatin during chemical induction but prevents the formation of the two DNAse I hypersensitive sites as well. We demonstrate that once βmajor gene chromatin adopts its transcriptional conformation it becomes insensitive to the effect(s) of dexamethasone. We also show that DNAse I hypersensitive sites are propogated to daughter cells in the absence of chemical inducer.

0251 SPECIFIC PROTEIN DNA INTERACTION AT THE 5' END OF THE RAT ALBUMIN GENE, J.S. Sevall and S.L. Berent, Wadley Institutes of Molecular Medicine, Dallas, TX 75235

The 5' end of the 15-exon rat albumin gene was screened with two nitrocellulose filter assays for specific recognition by nuclear DNA-binding proteins from rat liver nuclei. Nuclear extracts made by successive ten millimolar Tris pH8.0, 0-.35M salt, and .35M-1.0M salt washes, as well as histone H-1 and core histone extracts, were used to identify binding activity. Only the 0.35-1.0M salt extract contained a heat-stable protein(s) with preferential binding to a region of the rat albumin gene. The region includes a 1400-1500 bp fragment that includes 441 nucleotides upstream from the first (Z exon) to the 3' side of the second exon. This was shown by the enrichment of this Hind III - RI restriction fragment using a filter-binding assay. The specificity was confirmed by a competition-binding assay using subcoloned fragments of the albumin gene which were of approximately equal length. Nuclear extracts from the spleen and brain had a substantial decrease in the sequence preference shown by the liver extract. The preferential binding activity is independent of pH (7-9) and shows increased preference at 250mm KCl (0-250mm KCl range tested). Molecular sieve chromatography of the binding activity indicated a molecular weight greater than 70,000. The proteins are heterogeneous with pIs between 7.0-5.0 and denatured molecular weight greater than 68,000.

0252 FUNCTIONAL CHANGES IN REPRESSOR BINDING AFTER COVALENT MODIFICATION OF THE LACTOSE OPERATOR BY EPOXIDES, G. Stöhrer, Sloan-Kettering Institute, Rye, NY 10580

Chemical carcinogenesis is widely believed to be due to mutations induced by carcinogens which are expressed in the progeny of the exposed cell. We have considered a direct action that carcinogens might exert on regulatory units of the exposed cell and have chosen the bacterial lactose control system as an example of the more general DNA-protein interactions known to be involved in bacterial and eukaryotic gene control. Here we report a sensitive response of the lactose operator to covalent modification. Cloned operators on plasmids pMB9-lac21 and pBR345 were labeled by nick-translation and modified by 1-pyrenyloxirane and benzo(a)pyrene diol epoxide. Repressor-binding was determined by the filter-binding assay. Covalent modification alone causes a concentration-dependent binding of DNA to the nitrocellulose filters at high degrees of modification and in the absence of repressor. At modifications up to 5%, this nonspecific binding is less than 10%. Operator DNA, modified up to 8 adducts per 100 base pairs, showed a reduction in repressor-binding proportional to the degree of modification. This inactivation was from 40 to 70% for the two epoxides when one base in the 21-base pair operator was modified. Analysis of dissociation of the operator-repressor complexes revealed the presence of a fraction within the modified operator with increased affinity for the repressor. These results indicate a sensitive response of the lactose operator to carcinogenic modification and suggest a role for such modification in protein-mediated transcriptional control systems.

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0253 EXPRESSION OF HUMAN β-GLOBIN GENES. Dimitris Kioussis, Stephanie Wright, Ernie DeBoer and Frank G. Grosveld. National Institute for Medical Research, Mill Hill London NW7 1AA. U.K.

The natural site of globin mRNA synthesis is the erythroid cell. These cells differentiate from the haematopoietic stem cell by a number of cell divisions and in the final stages, globin mRNA's are synthesized. The MEL cell line provides a useful model for the late stages of erythroid differentiation, since they can be induced (e.g. DMSO or HMBA) to synthesize haemoglobin mRNA and protein. We have examined the expression of human globin genes in MEL cells before and after induction. The RNA's were examined for ε- γ- and β-globin mRNA by S1 mapping. All the globin genes are expressed constitutively at a low level, however only the β-globin gene can be induced to a higher level (3-20 fold) by the addition of HMBA. The results indicate that only a short region around the β-globin gene is involved in the response to a presumably transacting signal in the MEL cell. We are presently determining the exact nature and location of these DNA sequences. A similar study is in progress to determine the sequences involved in the induction of the ε- and γ-globin genes using the human cell line K562 as the model system. In addition, we are currently analyzing the expression of the human β-like globin genes in long term bone marrow cultures.

0254 REGULATION OF THE ilvHI OPERON OF ESCHERICHIA COLI BY LEUCINE. George W. Haughn[1], Charles H. Squires[2], Maurilio De Felice[3], and Joseph M. Calvo[1].

1. Cornell University, Ithaca, N.Y. 2. Synergen, Boulder, CO. 3. CNR, Naples, Italy

The ilvHI operon of some strains of E. coli is repressed more than five fold by exogenous leucine. Leucine is known to decrease the rate of synthesis of ilvHI-specific mRNA. To investigate this phenomenon further, we sought to identify the ilvHI promoter. Two structural genes in the operon were sequenced (C. Squires, M. DeFelice, and J. Calvo, unpublished). A 360 base pair HaeIII fragment located immediately upstream from one of these genes provided a promoter function for a galK gene on a plasmid provided by K. McKenney. In a strain carrying this hybrid plasmid, galactokinase production is repressed by leucine. Three types of experiments were carried out to locate the promoter; S1 mapping employing 5' end-labeled DNA fragments, DNA sequence analysis of mutants having elevated levels of ilvHI gene products, and analysis of galactokinase specified by plasmids carrying deletions within the cloned 360 base pair HaeIII fragment. Results of the first two approaches tentatively define the ilvHI promoter. The third approach, however, suggests that DNA sequences several hundred base pairs upstream are required for function of the promoter.

0255 STRUCTURE OF RNA POLYMERASE II INITIATION COMPLEXES. Martha Simon, *David Dignam, Iris Mastrangelo, James Hainfeld, Joseph Wall, *Robert Roeder and Paul Hough, Brookhaven National Laboratory, Upton, NY 11973. *Washington University, St. Louis, MO 63110. The SmaI F fragment of adenovirus 2 which contains the major late promoter is incubated with purified RNA polymerase II and partially purified initiation factors, under conditions known to give selective and accurate initiation of transcription at the major late cap site. ATP and CTP are provided, but not GTP or UTP. After incubation, the reaction mix is passed over a Sepharose CL 4B column. DNA-protein complexes elute in the void volume and electron microscope grids are prepared by diffusion from droplets from that fraction. The complexes have been lightly fixed by passing through a low concentration of glutaraldehyde in part of the column. Grids are not stained or shadowed and are observed in a scanning transmission electron microscope. Scans of picture elements, 0.5 nm x 0.5 nm, sample the projected mass distribution by digitally recording the number of scattered electrons; a real resolution of about 1 nm is obtained. Large DNA-associated protein complexes are found, many within a few tens of base pairs upstream of the cap site and frequently covering several tens of base pairs. The protein complexes have a range of molecular weights, presumably from variable fragmentation or assembly. The largest molecular weights observed are about 1×10^6. Occasionally a bipartite structure is seen: one portion of M. W. about 1×10^5 is found several tens of base pairs downstream of the main complex. The main complexes show structural detail suggesting that they are composed of subunits; some of these may occur in association with known conserved sequences of eukaryotic promoters.

0256 Studies of the Binding of a Purified Transcription Factor to Wild-type and Mutant Xenopus 5S RNA Genes. Daniel F. Bogenhagen, Jay S. Hanas and Cheng-Wen Wu. State University of New York at Stony Brook.

We have studied the binding of a purified positive transcription factor to the intragenic control region of Xenpus 5S RNA genes using the DNase I footprinting method. A direct determination of the stoichiometry of binding suggests that two protein molecules bind per 5S RNA gene. Under equilibrium binding conditions, a titration of the 5S RNA gene with the transcription factor results in a sigmoidal binding isotherm suggesting a cooperative interaction; half-saturation of binding occurs at a free protein concentration of 1×10^{-9} M. Analysis of the binding of the transcription factor to a variety of deletion and linker substitution mutants is consistent with a two domain structure for the control region.

0257 USING THE E. COLI GALACTOKINASE GENE TO STUDY EUKARYOTIC TRANSCRIPTIONAL AND TRANSLATIONAL CONTROLS, Hanne Johansen, Dani Schumperli, Mitchell Reff and Martin Rosenberg, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205 and Smith Kline and French Research Laboratories, Philadelphia, PA 19101

We have constructed a hybrid fusion vector, pSVK-gpt carrying two independent transcription units each of which controls the expression of a different assayable, selectable gene; one being galactokinase (galK) and the other xgprt. The modular design of the vector allows each transcriptional element controlling galK expression to be removed and replaced with other DNA segments. Variations in galK expression resulting from these alterations can be accurately measured using xgprt expression as an internal standard. We have used the system to characterize and compare a variety of transcriptional regulatory signals. We have also studied the effects on galK expression which result from varying the size and sequence of the 5'-noncoding leader region. In particular, the effect of inserting ATG codons upstream of the galK initiation codon was examined. Our results indicate that the sequence immediately surrounding the ATG insert plays an important role in determining its effect on galK translation.

0258 NMR OBSERVATIONS OF GENE REPRESSORS AND THEIR INTERACTION WITH DNA, Kim Arndt, Frank Boschelli, Susannie Cheung, Ponzy Lu, Harry Nick, Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104, Yoshinori Takeda, Chemistry Department, University of Maryland, Baltimore County, Catonsville, MD 21228 and John Sadler, Department of Chemistry Department of Biophysical Genetics, University of Colorado, 80262.

We have examined, by NMR spectroscopy, the interaction of the lac repressor of Escherichia coli and the cro repressor of its lysogenic phage λ with both random sequence DNA and their respective operator DNA sequences. The resonances from the aromatic region of the ^{1}H-NMR spectrum of the cro repressor, the lowfield region of a number of λ operator, O_R3, DNA sequences, as well as those in the ^{19}F-NMR spectra from 3-fluorophenylalanine or 3-fluorotyrosine substituted cro repressor were used as probes for the complex formation of the cro repressor with DNA. The NMR data offer direct support for the model of the cro repressor-DNA complex proposed from X-ray structure analysis on the protein alone.

^{19}F-NMR spectroscopy on 3-fluorotyrosine and 5-fluorotryptophan substituted lac repressor was used to examine the interaction with lac operator DNA. Inducer and salt concentration effects of the lac repressor-operator complex lead to a scheme for the interactions between the repressor, operator and inducer, in both binary and ternary complexes. The complex between the tetrameric repressor and one 36 base pair operator DNA fragment results in the simultaneous broadening of the resonances from all four N-terminal DNA binding domains. When the inducer binds to the tetrameric lac repressor, the increased N-terminal resonance intensity in the complex is transmitted to repressor subunits that have not yet bound an inducer molecule.

0259 Regulation of Globin Genes After DNA Transfomation into Mouse Erythroleukemia Cells. Pamela Mellon*, Moses Chao#, Patrick Charnay*, Richard Axel# and Tom Maniatis*. Harvard University, Cambridge, MA 02178* and Columbia University, New York,NY,10032#

We have transferred cloned mouse and human beta globin genes into mouse erythroleukemia cells (MEL) to study the regulatory sequences involved in induction of globin expression during differentiation of these cells in culture. The genes were cotransferred with the hamster APRT gene into APRT deficient MEL cells and stable APRT positive clones were isolated. The transferred genes are present at 1 to 200 copies per cell. Upon induction the transferred gene RNA transcripts increase 5 to 50 fold in 90% of the cell lines examined. The amount of RNA loosely correlates with the DNA copy number and appears to be correctly initiated. The mRNA of the transferred mouse beta major gene can be distinguished by nuclease S1 analysis from the endogenous transcripts by virtue of the substitution of a human third exon in the cloned gene. Nuclear transcription experiments demonstrate that the transferred gene is activated at the transcriptional level.

In order to more pecisely define the sequences required for induction, we have analysed a series of truncated plasmids which contain from 1.2 kb to no 5' flanking sequence. This far genes with as little as 78 bp of 5' flanking sequence remain fully inducible and genes with 53 bp are constituitive or fail to transcribe. Thus we nave localized sequences required for induction to within 78 bp of the 5' end of the mouse beta major globin gene.

0260 NUCLEOTIDE SEQUENCE OF THE ilvB MULTIVALENT ATTENUATOR REGION OF ESCHERICHIA COLI K12: ENDPRODUCT AND SUBSTRATE ATTENUATION, Craig A. Hauser and G. Wesley Hatfield, Department of Microbiology, College of Medicine, University of California, Irvine 92717

The ilvB gene of Escherichia coli K12 has been cloned into a multicopy plasmid. The regulation of the cloned gene by valine or leucine limitation and by catabolite repression is the same as for the chromosome encoded gene. The nucleotide sequence of a regulatory region preceding the ilvB structural gene has been determined. This DNA sequence includes a promoter, a region which codes for a putative 32 amino acid polypeptide containing tandem valine, leucine, alanine, and threonine codons, and a transcription termination site. In vitro transcription of this region produces a 184 nucleotide terminated leader transcript. Mutually exclusive secondary structures of the leader transcript are predicted. On the basis of these data, a model is presented for the multivalent endproduct attenuation of the ilvB operon by valine and leucine and substrate attenuation of this operon by alanine and threonine, the amino acids which reflect the levels of the substrates (pyruvate and α-ketobutyrate) of the ilvB gene product.

0261 POSITIVE CONTROL OF *Eco*RI ENDONUCLEASE EXPRESSION BY THE *Eco*RI METHYLASE, Stephen Yanofsky, Herbert Boyer and Patricia Greene, University of California, San Francisco, San Francisco, CA 94143

The presence of a wild type *Eco*RI endonuclease in cells lacking the cognate methylase is lethal. We have isolated a mutant endonuclease in which serine replaces arginine at residue 187. The Ser 187 enzyme retains RI specificity but has reduced in vivo activity (as measured by ability to restrict growth of lambda phage). Cells carrying the Ser 187 endonuclease can survive when the methylase is deleted. Deletion of the methylase results in drastically reduced endonuclease production but levels can be restored to near normal by the presence of the methylase provided in trans on pSC101, a compatible low-copy number plasmid. We have constructed a number of strains in which the methylase gene is truncated at different sites. Analysis of these strains has allowed us to define a domain within the methylase gene which activates endonuclease production. In some of the methylase-deficient strains we are able to increase endonuclease production by induction of a linked *lac* promoter. This induction is lethal to the cell, and it is clear that survival depends upon both low production of the Ser 187 endonuclease as well as its reduced in vivo activity. This positive control of endonuclease production by methylase provides a mechanism for establishment and maintenance of this system in a cell without damaging cellular DNA.

0262 TOPOLOGICAL CONSIDERATIONS OF SV40 EARLY PROMOTER ENHANCEMENT, Thomas R. Kadesch and Paul Berg, Stanford University, Stanford, CA 94305

We have carried out a series of experiments designed to further define the behavior of the SV40 enhancer sequence and to elucidate its mode of action. The DNA vectors designed for these studies contain from one to three genes located in tandem, each under the control of either an intact or an enhancer-deleted SV40 early promoter. The 72 base repeat element has been placed in a number of different positions with respect to the genes in question and the resulting levels of transcription of each gene have been determined after transfection of monkey kidney cells. The results indicate that instead of simply acting as an "on-off" switch for all promoters present on the same DNA molecule, the 72 base repeat sequence enhances gene expression to varying levels, depending upon the "transcriptional environment" of that gene. In general, active transcription of an upstream gene reduces the level to which expression of a downstream gene can be enhanced. Results obtained from the study of vectors that contain three transcription units, situated in tandem, suggest that the 72 base repeat can enhance the expression of a gene only if it is located immediately 5' or 3' to that gene and cannot enhance the expression of that gene if it is located on the opposite side of a flanking transcription unit. The latter result is consistent with the hypothesis that the enhancer serves as an entry signal for RNA polymerase.

0263 EXPRESSION OF THE PRO-OPIOMELANOCORTIN (POMC) GENE, Jacques Drouin, Peter Burbach, Jean Charron and Jean-Pierre Gagner, Institut de Recherches Cliniques de Montreal, 110 avenue des Pins, ouest, Montreal H2W 1R7.

Pro-opiomelanocortin (POMC) is the pituitary precursor to ACTH, α-, β-, γ-MSH, β-lipotropin and β-endorphin. The processing of this precursor is different in different tissues where it is expressed (e.g. the anterior and intermediate lobes of the pituitary) and so seems to be the regulation of the gene encoding it. For example, glucocorticoids only decrease POMC mRNA levels in the anterior lobe of the pituitary and do not affect these levels in the intermediate lobe. We are studying the expression of the POMC gene in three distinct tissues where it is expressed: the anterior and intermediate lobes of the pituitary and the arcuate nucleus of the hypothalamus. We have isolated by cloning from a rat genomic DNA library in bacteriophage λ1059 about 25 kb of rat DNA containing the entire POMC gene. The three exons of the gene and about 500 bp of upstream sequences have been sequenced. Southern blot analysis suggest that there is only one POMC gene in the rat; this is born out in the fact that eight independent isolates of this gene contain the same genomic sequences. Northern blot analysis indicate that POMC mRNAs of similar size are found in pituitary lobes but that the arcuate nucleus mRNA is slightly bigger. This size difference can be accounted for by different lenghts of polyA tails. Primer-extension studies suggest that the 5'-ends of POMC mRNAs from the two pituitary lobes are similar. The effect of glucocorticoid treatment on POMC transcription rates was measured by incorporation of labelled UTP into run-off transcripts in isolated pituitary nuclei. These experiments suggest that, in accordance with their effect on mRNA levels, glucocorticoids decrease POMC transcription rates in the anterior pituitary while they have little effect in the intermediate lobe. This anterior lobe-specific effect of glucocorticoids is being further characterized and the glucocorticoid responsiveness of the POMC gene is under study by gene transfer experiments.

0264 METHYLATION AND GLOBIN GENE EXPRESSION. Frank G. Grosveld, Meinrad Busslinger, Dimitris Kioussis, Titia deLange and Richard A. Flavell. National Institute for Medical Research, Mill Hill, London NW7 1AA. U.K.

When the adult globin genes are expressed as in erythroblasts in the adult, the δ and β globin genes are hypomethylated, whereas the γ-globin gene region is hypermethylated. The reciprocal is true when the foetal globin genes are expressed early in development. This suggests that DNA methylation might play a role in the developmental regulation of globin gene expression. To establish that DNA methylation is the cause rather than the effect of gene expression, we have used a second strand synthesis of the γ-globin gene cloned in M13 in the presence of $d^{m}CTP$. This hemimethylated DNA is used to transform Ltk^{-} cells together with an unmethylated β-globin control and the HSV tk gene. The results obtained to date indicate that the methylation pattern is inherited at ^{m}CpG residues, including the MspI resistant methylation $^{m}C^{m}CGG$ which occurs at specific sites in vivo. A recognition sequence for the latter methylation pattern has been established. By narrowing down the methylated area of the substrates, preliminary results indicate that only a short region at the 5' side of the gene is responsible for the suppression of γ globin gene expression, when this region is methylated. Secondly, we have studied a Dutch case of γβ-thalassaemia. In this mutation a large part, 5' of the β globin gene has been deleted. We have cloned this defective locus and shown that the β-globin gene is normal and can be expressed in transformation experiments. Preliminary data indicate that the β-globin gene from the defective locus is hypermethylated in vivo and we are presently analyzing the transposed DNA to determine whether this DNA is responsible for inactivation of the β-globin gene on the mutant chromosome.

0265 CONTROL OF EXPRESSION OF THE MOUSE METALLOTHIONEIN-I GENE DURING DEVELOPMENT OF THE LIVER AND AFTER INDUCTION WITH HEAVY METALS, James Koropatnick and J.D. Duerksen, Department of Biology, University of Calgary, Calgary, Alberta, Canada T2N 1N4

Metallothionein (MT) is a cysteine-rich, inducible protein involved in resistance to heavy metal toxicity and zinc homeostasis. We have shown that: 1) the murine MT-I gene is constitutively more sensitive to nuclease attack than surrounding chromatin, and 2) treatment of mice with low levels of cadmium (1-5 mg per kg mouse) will induce an increase in micrococcal nuclease sensitivity concomitant with MT-I mRNA production.
We have now demonstrated that there appears to be an increase in the number of MT gene copies in mouse liver DNA by 4 hours following treatment with cadmium, and some portion of these copies appears to be resistant to nuclease attack. However, by 6 hours after heavy metal treatment, extra copies are no longer present and sensitivity to nuclease attack is restored. In addition, this increase in copy number appears to be associated with fetal development of mouse liver. 19-day fetal mouse livers have a 2-3 fold increase in MT gene concentration, rising to 4-5 fold by 6 days after birth, and falling to control levels by 30 days after birth. The increase in copy number is proportional to MT mRNA production. We suggest that this short-term amplification of the MT gene may play a role in regulation of production of the metallothionein protein.

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0266 FACTORS CONTROLLING EXPRESSION OF A DIHYDROFOLATE REDUCTASE cDNA UNDER CONTROL OF DIFFERENT PROMOTERS AND POLYADENYLATION SIGNALS, Randal J. Kaufman, Genetics Institute, Boston, MA 02115

In order to understand factors which affect efficient expression of a gene, modular genes containing the murine dihydrofolate reductase (DHFR) coding region have been constructed in bacterial plasmids and analyzed for expression after transient transfection into COS monkey cells which replicate these plasmids and by both transient transfection and stable transformation of Chinese hamster ovary (CHO) cells deficient in DHFR. Presently, comparisons have been made between the adenovirus 2 major late promoter with and without enhancer elements, the adenovirus early region 2 promoter with and without enhancer elements, the SV40 early promoter, the SV40 late promoter, and the drosophila heat shock promoter with and without enhancer elements. Results indicate the drosophila heat shock promoter, (even without enhancer elements) with heat induction drives the highest levels of DHFR synthesis in COS cells and any promoter with a SV40 enhancer element very efficiently transform CHO DHFR deficient cells to the DHFR+ phenotype. In addition, recent data suggests the COS cells are inefficient in expressing protein despite high mRNA levels.

The generation of 3' ends (cleavage and/or polyadenylation) has also been analyzed. Results indicate efficient polyadenylation is necessary for stable mRNA production and the metabolic state of the cell is important in determining either the efficieny of polyadenylation at or the stability of mRNA polyadenylated at various signals. Evidence indicates the particular polyadenylation signal utilized depends upon the particular promoter utilized.

0267 **HIMA MUTANTS OF E. COLI K-12 EXHIBIT A DEFECT IN ISOLEUCINE AND VALINE BIOSYNTHESIS, Rolf Sternglanz, Karen Voelkel, Thomas Newman, Philip Friden and Martin Freundlich, SUNY, Stony Brook, NY 11794**

The E. coli himA gene product is a subunit of integration host factor (IHF), a protein required for phage λ integration and excision. The himA gene product is also involved in regulating the synthesis of λ Int and CI proteins. In addition, E. coli strains with mutations in himA show defects in numerous systems that involve site specific recombination. We have found that a himA deletion strain will not grow in minimal medium containing leucine. This defect can be overcome completely by isoleucine plus valine or almost completely by isoleucine or by any of a number of isoleucine precursors. The following observations suggest that the himA mutation affects acetohydroxy acid synthase I (AHAS I), an enzyme in the ilvB operon. 1) Leucine is not necessary for growth inhibition in himA strains that contain mutations in ilvHI, the gene for AHAS III, an isozyme which is strongly repressed by leucine. In these strains, AHAS I is the only isozyme expressed, and its normal function is necessary for growth. 2) The level of AHAS I, as measured by enzyme activity and by the amount of protein made from ilvB - carrying plasmids in maxi-cells, is significantly lower in the himA mutant as compared to the parent strain. 3) The following palindromic sequence, which ends 20 bases before the start codon for the leader peptide, is found in the ilvB promoter-attenuator region: 5'-TGTATAAATATTGTTAAACA-3'. The underlined bases include a 7 base sequence that differs by one base from the IHF-DNA binding site consensus sequence 5'-ATTGATA-3' (Nash; Ann. Rev. Genet. 15:143, 1981). In addition, an overlapping sequence, differing by one base from the consensus but in the opposite polarity, is found at the beginning of the underlined sequence.

0268 REGULATION OF NITROGENASE (nifHDK) OPERONS IN KLEBSIELLA PNEUMONIAE AND RHIZOBIUM MELILOTI, V. Sundaresan, D.W. Ow, J.D.G. Jones and F.M. Ausubel, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

In K. pneumoniae, nif derepression occurs under NH_4^+ deficiency and low pO_2. Transcription of the nifLA regulon requires glnG and glnF; in turn nifA activates transcription of nifHDK. By contrast, R. meliloti only fixes N_2 in symbiosis with alfalfa inside root nodules. Previously, we cloned the K. pneumoniae and R. meliloti nifHDK genes (ref.1 for review). Recent results can be summarized as follows: 1. We sequenced the nifHDK promoters from both species (by S1 mapping the RNA from N_2 fixing cells/bacteroids) and found unexpected homologies including 8 bp at -30 (ACGGCTGG) and 5 bp at -12 (TGCAC). 2. We demonstrated the significance of these homologies by showing that K. pneumoniae nifA can activate both nifH promoters (utilizing nifH-lacZ translational fusions that we constructed. 3. We discovered that the glnF product is required in addition to nifA for activation of the nifH promoters. By comparing sequences of promoters activated by glnG (including nifLA which we sequenced), we find a consensus sequence TTTGCA at -12 which might be a recognition sequence for glnG. These results are consistent with other results from our lab which indicate that nifA is evolutionarily related to glnG (ref.2) and that R. meliloti probably has a "nifA-like" protein which is presumably under symbiotic control.

References: 1. Ausubel, F.M., Brown, S.E., deBruijn, F.J., Ow, D.W., Riedel, G.E., Ruvkun, G.B. and Sundaresan, V. (1982) In Genetic Engineering (ed. Setlow, J.K. and Hollaender, A.) Plenum Press, New York, pp. 169-198.
2. Ow, D.W. and Ausubel, F.M. (1982) Nature, in press.

0269 LONG DISTANCE REGULATION OF THE *DUR1,2* GENE IN *S. cerevisiae*. Expression of all 5 allantoin pathway genes in yeast is induced by allophanate or the gratuitous inducer, oxalurate. In the past we have reported the isolation of recessive, pleiotropic mutations which result in constitutive mRNA synthesis from all the allantoin pathway genes or alternatively result in loss of ability to induce expression of these genes. The complex locus controlling inducibility has been found to occupy at least 10 map units (recombinational distance) on chromosome IX.

We have also isolated several new mutants in which only one gene, *DUR1,2* is expressed constitutively. Other allophanate-inducible genes associated with allantoin degradation are completely unaffected. The mutated locus carried in these strains has been designated *DUR80* and is situated 4-6 map units distal to the *DUR1,2* gene near the *MET8* locus. Mutations in *DUR80* generate the *DUR1, 2* constitutive phenotype in a cis-dominant fashion. In view of these striking characteristics, we have isolated the entire region between the *MET8* and *DUR1, 2* genes from wild-type and *DUR80* mutant cells on recombinant plasmids. This work generates the conclusion that the mutated locus affects expression of a gene 4 to 5 kb away. The physical structure of this region is currently being altered to determine the mechanism by which the *DUR80* locus exerts its long distance control and transcriptional maps of the entire region will assess whether or not the effect is specific or occurs in a bidirectional fashion.

0270 DNA SEQUENCES INVOLVED IN THE ALTERED CHROMATIN STRUCTURE OF THE SV40 ORI REGION. J. Jongstra, T.L. Reudelhuber, P. Oudet and P. Chambon, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS et U. 184 de l'INSERM, Strasbourg, France.

Nuclease digestions of SV40 infected cell nuclei and electron microscopic observation of isolated SV40 minichromosomes have shown that a fraction of the SV40 minichromosomes present late in infection have a unique chromatin structure in the region containing DNA sequences involved in the regulation of transcription of the SV40 early and late genes, as well as the origin of replication (the Ori region). To determine the DNA sequences involved in the induction of this altered chromatin structure we have constructed double origin SV40 mutants in which a second intact or mutated Ori region (Ori B) was inserted at the HpaI site (bp 2666). Both Ori regions were then analyzed late in infection for the presence of DNAse I sensitive sites and a nucleosome gap. Our results show the existence of two regions of increased DNAse I sensitivity. Region I is centered over the origin of replication and the "TATA-box" and is induced by a DNA fragment containing the 21-bp repeat, the "TATA-box" and the origin of replication. Region II is centered over the 72 bp repeat previously shown to be indispensable for early gene transcription. DNA sequences sufficient to induce DNAse I sensitivity of the 72 bp repeat are contained within this repeat. However insertion of the 72 bp repeat at Ori B does not lead to the formation of a nucleosome gap over the insert. Deletion of DNA sequences within the 72 bp repeat which are required for efficient early gene transcription does not prevent the induction of DNAse I sensitive region I or II or the formation of a nucleosome gap.

0271 PURIFIED X. LAEVIS RNA POLYMERASE III ACCURATELY TERMINATES TRANSCRIPTION OF 5S RNA GENES, Stephen P. Gerrard and Nicholas R. Cozzarelli, U. of California, Berkeley, CA 94720 and Mark Schlissel and Donald D. Brown, Carnegie Inst. of Washington, Baltimore, MD 21210

A control region in the middle of the X. laevis 5S RNA gene is recognized by a positive transcription factor. This and other factors are required along with RNA polymerase III for in vitro transcription of 5S RNA genes. We find that none of these factors are required for correct termination of transcription; purified X. laevis RNA polymerase III is sufficent. The assay used a X. laevis 5S RNA gene fragment cloned in pBR322 as template. The transcripts were hybridized with the complete 5S RNA gene, and non-hybridized RNA was digested with RNAase T_1. 5S RNA transcribed by a X. laevis germinal vesicle extract resulted in a discrete fragment of RNA - the product of T_1 digestion at its 3' end and correct termination at its 5' end. Even though purified RNA polymerase III initiated randomly and made no correct 5S RNA transcripts, the same hybridization assay resulted in the very same discrete fragment of correctly terminated RNA. A complimentary hybridization assay for initiation showed correct initiation in the germinal vesicle extract, but RNA polymerase III together with transcription factor A was not sufficent to initiate 5S RNA transcription. We conclude that RNA polymerase III alone correctly terminates transcription at the 5S RNA termination signal, but can not correctly initiate transcription, even with transcription factor A.

0272 FORMATION OF STABLE PREINITIATION COMPLEXES BETWEEN EUKARYOTIC CLASS B (II) TRANSCRIPTION FACTORS AND PROMOTER SEQUENCES. Egly, J.M., Davison, B.L. and Chambon, P., Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS et Unité 184 de l'INSERM, Strasbourg, France.

We have developped an assay for the detection of stable preinitiation complexes between DNA fragments containing eukaryotic class B promoter sequences and initiation factors partially purified from HeLa cell extracts. Three partially purified fractions (P0.5, P1.0 and DE0.35), in addition to RNA polymerase B, were found to be essential for accurate initiation of transcription from the Adenovirus-2 major late and conalbumin promoters. Two of these fractions (P1.0 and DE0.35) were sufficient for the formation of stable preinitiation complexes at the TATA-box promoter region in the absence of RNA polymerase B. One of these fractions (DE0.35) however, contained factor activity displaying no general DNA-binding capacity. We propose that preinitiation complex formation involves induction of the specific DNA-binding potential of factor(s) contained in P1.0 by factor(s) contained in DE0.35. Experiments will be presented which relate to the mode of action of these factors in specific complex formation.

0273 VSG GENE ACTIVATION IN Trypanosoma brucei INVOLVES PROMOTER ADDITION, L.H.T.van der Ploeg, A.Bernards, P.A.M.Michels, A.Y.C.Liu, T.De Lange and P.Borst, Section for Med. Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam, The Netherlands.

T. brucei is a unicellular parasite of the mammalian vascular system. Its cell surface is covered with a single type of glycoprotein, the Variant Surface Glycoprotein (VSG). There are 100-1000 different VSG genes in T. brucei and these genes are clustered. By sequentially expressing different genes, the trypanosome evades the host's immune response. The process of antigenic variation has been studied on two types of VSG genes: genes that are expressed by means of a duplicative transposition of the silent gene to an expression site (transposition-activated (TA) genes) and genes that are activated without duplication and a detectable transposition (non-TA genes). The transposed segment of the TA VSG genes 117 and 118 consists of the VSG gene coding sequence and 1-2 kb of DNA located in front of the gene: the borders of these segments are similar among VSG genes (i.e. at the 5' side a number of 70-bp imperfect repeats and at the 3' site short homology blocks up to 14 nucleotides in length), which may have a function in the transposition event. The TA genes 117 and 118 and the non-TA gene 221 (Boothroyd, J.C. & Cross, G.A.M. (1982) Gene, in press) have in common that the mature VSG mRNA contains a block of 35 nucleotides at its 5'-end which is not contiguously encoded in the DNA. Sequence analysis of the transposed segment of the 118 VSG gene revealed that it does not contain the 35-nucleotide exon. Genomic clones have been obtained containing the 35-nucleotide exon. Evidence that they represent the promoter area of the TA and probably of the non-TA VSG genes as well will be presented.

0274 CHROMATIN STRUCTURE OF A TRANSCRIPTION INITIATION REGION AND ITS RELATION TO EXPRESSION OF THE GENE, Dennis E. Lohr, Arizona State University, Tempe, AZ 85287

The chromatin structure around the transcription initiation sites of the 35S gene in yeast was analyzed in detail. The region shows several interesting features:

1. Immediately upstream (and perhaps overlapping one) of the initiation sites, for about 230 bp, there is a region which is exceedingly resistant to staph nuclease at all times of digestion. DNase I does cleave in the resistant domain and those results suggest the region is nucleoprotein, but not nucleosome, associated.
2. The resistant domain is flanked on both sides by a few quite prominent, 20-30 bp spaced staph nuclease cleavage sites, some of which are hypersensitive as well.
3. These are chromatin structural features not DNA sequence artifacts because chromatin and naked DNA digestions behave oppositely; the strong chromatin cleavage sites are very weak sites in naked DNA while the weak chromatin sites are very strong in naked DNA.
4. The coding sequences, in the majority of copies at least, are contained in nucleosomes. The nucleosomal domain begins at one of two defined positions, i.e. shows limited positioning (a.k.a. phasing). Both positions correspond to above noted strong cleavage sites just downstream of the resistant region .
5. The most downstream 5' nucleosome locus is the major one during maximal gene expression, in early log phase, while in stationary phase, where the gene is much less active, the more upstream site becomes the major 5' nucleosomal locus. Thus there is a shift in position preference, associated with gene expression.

0275 ATTENUATION AND MODULATION OF mRNA SECONDARY STRUCTURE IN A FEEDBACK CONTROL SYSTEM REGULATING SV40 GENE EXPRESSION. Yosef Aloni, Nissim Hay and Hagit Skolnik-David, Weizmann Institute of Science, Rehovot 76100, Israel

In a recent report (Hay, N., Skolnik-David, H. and Aloni, Y. Cell 29, 183-193 (1982)) we showed that RNA polymerase initiating transcription in vivo at residue 243 can terminate transcription in vitro 94 nucleotides downstream, at a typical prokaryotic transcription-termination signal, suggesting that an attenuation mechanism resembling that in prokaryotes regulates SV40 late transcription. We have suggested that the decision whether the RNA polymerase will prematurely terminate and produce the 94 nucleotide aborted RNA or will continue and produce the full length primary transcript depends on which of the alternative conformations at the 5' end of the RNA transcript prevails. The 16S mRNA encodes information for two proteins: the leader protein (agnoprotein) and the capsid protein VP_1. We have observed at the 5' end of the 16S mRNA that in one of the two alternative conformations the initiation AUG of agnoprotein is sequestered and presumably not available for ribosome binding while in the alternative conformation the same AUG is accessible for ribosome binding. Consistent with these observations we have proposed a model in which attenuation and mRNA modulation are fundamental elements in a feedback control mechanism regulating the production of VP_1. In the nucleus, in conformation A RNA transcription is attenuated, in conformation B a full length transcript is synthesized. In the cytoplasm, in conformation A VP_1 is synthesized, in conformation B agnoprotein is synthesized. We now extend this model and suggest that the production of the other two capsid proteins, VP_2 and VP_3, is also regulated by a similar control mechanism.

0276 Antibodies as Probes of RNA Polymerase II-DNA Interactions. Sean B. Carroll and B. David Stollar, Tufts Univ. Sch. of Med., Boston, Mass. 02111

We have approached the problem of eukaryotic RNA polymerase II subunit function by developing specific polyclonal and monoclonal antibodies to the native calf thymus enzyme. These antibodies were used as specific inhibitors of the enzyme. One monoclonal antibody was found to inhibit polymerase activity on calf thymus DNA. Detailed analysis of the mechanism of inhibition by this antibody revealed that it blocked binding of the enzyme to DNA. This blockage depended upon the preincubation of antibody with the enzyme. When enzyme was mixed with antibody and DNA without preincubation, the enzyme readily bound DNA , most probably due to the more rapid binding of RNA polymerase to DNA than of antibody to antigen. No significant release of enzyme from complexes with DNA took place with incubation up to one hour in the presence of excess antibody. Antibody binding to enzyme was decreased in the presence of DNA , indicating that DNA either blocks or alters the antigenic site recognized by the antibody. We are currently exploring the use of bifunctional cross-linkers to map the subunit contacts of the inhibitory antibody and to determine its relationship to the DNA-binding site of the enzyme.

0277 SPECIFIC BINDING OF THE GLUCOCORTICOID-RECEPTOR COMPLEX TO SEQUENCES ON MOUSE MAMMARY TUMOR PROVIRAL DNA. Magnus Pfahl, The Salk Institute for Biological Studies, Regulatory Biology Laboratory, P.O. Box 85800, San Diego, California 92138

To elucidate the molecular mechanism by which steroid hormones exert their regulatory function, the interaction of a glucocorticoid-receptor complex with purified DNA fragments from cloned mouse mammary tumor (MMTV) proviral DNA has been investigated. A DNA-cellulose binding assay was developed which allowed us to compare binding of an activated ^{3}H-triamcinolone acetonide-receptor complex to unspecific calf thymus DNA versus a number of purified restriction enzyme fragments from MMTV and other sources. Rat as well as mouse glucocorticoid receptors were found to interact with a high affinity site in the promoter region of the MMTV proviral DNA. Another high affinity site was detected near the beginning of the env gene. A deletion analysis has now been carried out for the specific binding site in the promoter region. The assay allowed the use of unpurified as well as purified receptor, and made it therefore possible to also investigate the binding properties of mutant receptors. Two nuclear transfer (nt^-) receptors were found to have a decreased affinity for specific as well as unspecific DNA but are still capable of distinguishing between the two types of DNA. The existence of specific DNA binding sites for steroid-receptor complexes in or near the promoter region of hormonally responsive genes favor a particular mode of action for steroid hormone receptors. They would act in a manner comparable to that of some prokaryotic regulatory proteins, which recognize specific DNA sequences in or near the promoter regions of genes under their control, and by binding to those regions, modulate the activity of the adjacent promoter.

0278 MAPPING TRANSCRIPTIONAL REGULATORY SEQUENCES ON XENOPUS RIBOSOMAL DNA. Sharon J. Busby, Marietta Dunaway, Ronald H. Reeder, Hutchinson Cancer Research Center, Seattle, WA 98104; Barbara Sollner-Webb and Joanne Wilkinson, Johns Hopkins Medical School, Baltimore, MD 21205

The major promoter for RNA polymerase I transcription of X. laevis ribosomal DNA spans nucleotides -150 to +16 surrounding the initiation site. The boundaries of this region have been determined by assaying a graded series of 5' and 3' deletions by injection into oocyte nuclei, in oocyte nuclear homogenates, and in a mouse polymerase I transcription system. There are at least two domains within this promoter because a sequence of about 20 nucleotides located at the initiation site is sufficient for correct initiation when injected into oocyte nuclei. DNA footprinting confirms that specific protein-DNA interactions occur throughout the promoter sequence. An additional regulatory region has been elucidated by assaying deletion mutants in embryos. If cloned ribosomal DNA is injected into embryos prior to first cleavage, transcription from the injected DNA begins at the same time as endogenous ribosomal gene transcription (mid-late blastula). Ribosomal spacer sequences, located several kilobases upstream from the major promoter, enhance transcription of the ribosomal genes injected into embryos. Current work is focusing on the role these enhancing sequences play in ribosomal gene activation.

0279 REGULATION OF THE E. COLI UNC OPERON, Nicholas J. Gay and John E. Walker Laboratory of Molecular Biology, The MRC Centre, Hills Road, Cambridge CB2 2QH, U.K.

The unc operon of E. coli encodes the eight structural subunits of the proton-translocating ATPase (H^+ATPase). This enzyme mediates the terminal step of oxidative phosphorylation coupling the proton electrochemical gradient, generated during electron transfer to the synthesis of ATP. We have determined the DNA sequence of the unc operon. As well as providing the complete primary structure of the enzyme, the DNA sequence revealed a ninth, promoter proximal gene which does not correspond to a structural component of the enzyme. Its predicted amino sequence is very hydrophobic with a net positive charge of +11. Its codon usage resembles that of control proteins and differs from other genes in the operon. A strain bearing a site-directed deletion mutation of this gene has been constructed by a novel and simple procedure. This strain retains the ability to grow on non-fermentable carbon sources (unc phenotype). Membrane bound, DCCD sensitive ATPase activity is identical to that of a completely isogenic normal strain. The growth rate of this strain is identical to that of its normal sibling but its growth yield is 5-10% lower. This result suggests that the mutant makes inefficient use of the available energy resources and may point to a rôle for this gene in efficient biosynthetic assembly of the ATPase.

The promoter/control region of the unc operon has been defined by in vitro transcription studies. The unc operon has a very extensive (400 bp) 5' non-coding region adjacent to a canonical promoter sequence. Other operons encoding central metabolic processes also have extensive promoter regions. These extended promoter regions may be important for qualitative and temporal regulation of transcription.

0280 5S GENE TRANSCRIPTION FACTOR TFIIIA ALTERS THE HELICAL CONFIGURATION OF DNA, Wanda F. Reynolds and Joel M. Gottesfeld, Research Institute of Scripps Clinic, La Jolla, California 92037

Transcription factor TFIIIA binds the central promoter region of the 5S ribosomal gene and facilitates accurate transcriptional initiation (Engelke et. al., 1980). We have found that relaxation of Xenopus 5S plasmid DNA in the presence of TFIIIA reduces the linking number of the DNA. The extent of unwinding (0.2 - 0.4 helical turns/TFIIIA binding site) is not consistent with the complete denaturation of the 50 base pair factor binding site; however, it is consistent with a change in helix rotation, denaturation of a few nucleotides per binding site, or DNA wrapping about a protein core. Competition filter binding assays indicate that TFIIIA has a higher affinity for supercoiled rather than linear DNA, consistent with unwinding activity. We have shown that proteins other than TFIIIA, including BSA and RNase, have no effect on the linking number of DNA when present during relaxation, and that the unwinding activity associated with factor is heat labile. These results suggest that TFIIIA may facilitate transcription by unwinding 5S DNA.

0281 THE TWO TANDEM E. COLI rrnA RIBOSOMAL PROMOTERS ARE DIFFERENTIALLY REGULATED, Paolo Sarmientos, Gianni Chinali and Michael Cashel, NIH, Bethesda, MD 20205

The tandem P1,P2 promoter region of the rrnA ribosomal cistron has been fused to the T1,T2 terminator region from the rrnB cistron deleting most internal RNA structural elements. P1 and P2 promoted transcripts terminate in vivo as well in vitro predominately in the T1 terminator region. The abundance of the two cellular transcripts arising from these plasmid borne promoters was measured directly to avoid post-transcriptional effects of provoking the stringent response by amino acid starvation. Taking into account the rates of decay of these transcripts, we find that the two promoters are differentially regulated during amino acid starvation. The P1 transcript shows classical stringent control while the P2 transcript is inhibited less and equally in both relaxed and stringent strains. A P2 promoter deletion extending to the beginning of the 16S RNA gene does not affect stringent regulation of the P1 activity. Also a deletion of the P1 promoter does not affect the insensitivity of the P2 promoter to ppGpp levels. If these indications of differential control of the tandem promoters are valid than the in vitro coordinate effects of ppGpp on both promoters so far observed must be reassessed.

0282 IN VITRO TRANSCRIPTION OF THE DISPERSED 5S rRNA GENES OF NEUROSPORA. Brett M. Tyler and Norman H. Giles, University of Georgia, Athens, GA 30602 and Robert L. Metzenberg, University of Wisconsin, Madison, WI 53706.

The 5S rRNA genes of Neurospora crassa are dispersed individually throughout the genome and occur in several distinct families (α, β, γ, δ, and ε)[1]. Members of a particular family differ only in their flanking sequences while genes from different families also differ in their presumptive control regions (nucleotides 55 to 80) and at their 5' ends. Soluble extracts from Neurospora have been used to correctly transcribe a variety of cloned 5S genes in vitro as well as two cloned tRNA genes. Cloned 5S genes from the same family or from different families were recombined in vitro and used to dissect the roles of flanking and internal sequences in the differential transcription of the genes. Hybrid genes consisting partly of a pseudogene were used to identify the lesion in the pseudogene and to identify the sequences required for transcription of the 5S genes in vitro.

1. Selker, E., Yanofsky, C., Driftmier, K., Metzenberg, R.L., Alzner-DeWeerd, B. & RajBhandary, U.L. (1981) Cell 24, 819-828.

0283 DRB, AN INHIBITOR OF INITIATION OF TRANSCRIPTION, Roberto Weinmann, Barbara Mittleman, Steve Ackerman and Ruben Zandomeni, The Wistar Institute, Philadelphia, PA 19104

DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole) is an adenosine analogue widely used to analyze short promotor proximal RNAs in tissue culture cells. We have found that DRB, although inactive on the purified RNA polymerase II, inhibits specific initiation in in vitro systems which initiate faithfully, at the same concentrations it acts in vivo. We have demonstrated that the specific effect is at the level of transcription initiation. To understand better the mechanism of action of DRB we have isolated a DRB-resistant HeLa cell mutant. The DRB^R mutation is stable, is not a permeability defect and shows resistance to DRB in whole cells and in transcriptional extracts prepared from these cells. Both DRB^R and DRB^S components were detected in extracts of mutant cells, whether grown in the presence or absence of DRB. The mutation is readily complemented by α-amanitin-resistant mutations (located in RNA polymerase II) and can be transferred to other cells by DNA-mediated transfection. This is probably the first example of a eukaryotic mutant in the transcriptional machinery that does not directly affect RNA polymerase II. This mutation is now being used to isolate the gene in question. A model to explain our results and the previously described DRB-induced premature termination of transcription will be presented.

0284 REGULATION OF THE HUMAN METALLOTHIONEIN-II_A GENE AFTER TRANSFECTION INTO RAT FIBROBLASTS, Michael Karin and Heidi Holtgreve, Department of Microbiology, USC School of Medicine, Los Angeles, CA 90033

The regulation of the cloned human MT-II_A gene has been examined after transfection into Rat 2 TK^- cells. In contrast to the mouse MT-I gene the expression of the human gene was regulated by both heavy metal ions and glucocorticoids.

The HSV-TK gene can be rendered both heavy metal and glucocorticoid regulatable by fusion to the 5' flanking region of the human MT-II_A gene. We have ruled out mRNA stabilization as a possible regulatory mechanism. Most likely, the transfected MT-II_A and the chimeric hMTK genes are regulated at the transcriptional level by both inducers.

Selection of TK^+ colonies in the presence of dexamethasone yield 15-20 fold more colonies when the hMTK gene is used as a donor. We have used this assay to map the 5' regulatory signals. Both heavy metal and glucocorticoid inducibility are left intact in a mutant containing only 150 nucleotides of the human MT-II_A gene fused to TK.

0285 USE OF THE E. COLI GALACTOKINASE GENE TO STUDY AN INDUCIBLE MOUSE METALLOTHIONEIN PROMOTER, Anthony D. Carter and Dean H. Hamer, Laboratory of Biochemistry, National Cancer Institute, Bethesda, MD 20205

Metallothioneins are low molecular weight, cysteine-rich proteins that bind heavy metals such as cadmium and zinc. Metallothionein gene expression is inducible in vivo by either heavy metals or glucocorticoids. We have shown previously that the cloned mouse metallothionein-I gene retains its ability to be induced by cadmium, but not by glucocorticoids, when introduced into cultured cells on SV40 vectors.

In order to identify nucleotide sequences involved in cadmium induction, we have constructed a series of deletion and linker-scanning mutants in the suspected promoter/regulatory region of the mouse metallothionein-I gene. To facilitate the screening of these mutants, we have fused both the normal and altered metallothionein promoter regions to the E. coli galactokinase gene. Cadmium induces the synthesis of bacterial galactokinase in monkey kidney cells transfected with these recombinants, but not in cells transfected with control plasmids containing a viral promoter. Nuclease S1 mapping shows that the hybrid RNA is initiated at the 5' end of the mouse metallothionein-I gene and that the amount of this RNA is proportional to the amount of galactokinase produced. Analysis of the mutants shows that sequences far upstream from the gene are required for efficient transcription. However, the information necessary for cadmium induction appears to lie within a maximum of 80 base pairs from the initiation site.

0286 FIFTY NUCLEOTIDES PRECEEDING THE MMTV LTR RNA CAP SITE CAN CAUSE HORMONE INDUCIBLE TRANSCRIPTION, B.Groner, A.van Ooyen, N.Kennedy, P.Herrlich, H.Ponta and N.E.Hynes, Kernforschungszentrum Karlsruhe, Institute of Genetics and Toxicology, Fed.Rep.of Germany

The transcription of mouse mammary tumor virus (MMTV) proviral DNA is subject to glucocorticoid hormone control. Gene cloning and DNA mediated proviral gene transfer experiments have shown that a DNA sequence present within the provirus is responsible for the hormonal induction. We constructed a chimeric molecule containing the MMTV LTR ligated to an intact Herpes thymidine kinase (tk) gene and have transfected it into Ltk^-cells. tk^+ revertants synthesize both a chimeric LTR-tk RNA and an authentic tk RNA. S1 nuclease analysis has shown that both RNAs initiate correctly and that both species are regulated by glucocorticoids. Therefore, the LTR contains the information necessary for the hormone response. The sequence requirements for this response were determined. We have deleted nucleotides in a stepwise fashion from the LTR 5' to its RNA initiation site. These deletion molecules have been stably transfected into Ltk^- cells and their response to dexamethasone has been tested. RNA transcripts from molecules deleted to within 202 nucleotides of the LTR RNA initiation site appear to be as sensitive to dexamethasone stimulation as RNA transcribed from the control containing the entire LTR. Thus, the first 992 nucleotides of the LTR seem not to be necessary for hormone induction. Molecules deleted to within 137 and 50 nucleotides of the LTR cap site are transcribed with much lower efficiency but are initiated correctly and are inducible. Transcripts initiating at the adjacent tk gene are also hormone inducible under the influence from the MMTV LTR regulating sequence.

0287 5' AND 3' FLANKING REGIONS OF YEAST GENES WHICH CONTROL THE EFFICIENCY OF HETEROLOGOUS GENE EXPRESSION. Susan M. Kingsman, Melanie J. Dobson, Michael F. Tuite, E. Jane Mellor, Nicola A. Roberts and Alan J. Kingsman. Department of Biochemistry, Oxford, U.K.

We have sequenced the 5' and 3' flanking regions of the yeast PGK (phosphoglycerate kinase) gene and the 5' flanking region of the yeast TRP1 (5'-phosphoribosyl anthranate isomerase) gene and have identified putative sequences involved in the control of gene expression. These regions of DNA have been incorporated into vectors to direct the expression of heterologous genes. A "sandwich vector" containing both the 5' and 3' flanking regions from the yeast PGK gene directs the synthesis of human interferon-alpha as 5% of total cell protein. In addition levels of interferon are regulated thirty fold by altering the carbon source. Levels of interferon directed by the TRP1 5' region are low and possible seqence features which may limit gene expression from this "promoter" include a hairpin loop near the mRNA start and an upstream open reading frame. Sequences in the flanking regions which determine levels of gene expression are being defined by in vitro mutagenesis and in particular the CT-CAAG structure (Dobson et al., 1982, Nucleic Acids Research, 10, 2625) must be retained for maximum gene expression. Various synthetic oligonucleotides have been used to alter the translation initiation environment and ten fold variations in yield are obtained. The PGK based vectors direct high level expression of several heterologous genes including HSV thymidine kinase and E.coli β-galactosidase and neomycin resistance.

0288 REGULATION OF TRANSCRIPTION BY A MEMBRANE BOUND PROTEIN. Stanley Maloy and John Roth. Univ. of Utah, Salt Lake City, UT 84112

The put genes allow Salmonella to oxidize proline for use as a carbon and nitrogen source. The putP gene encodes a proline transport protein and the putA gene encodes a bifunctional enzyme that oxidizes proline to glutamate. The putA enzyme has a tightly bound FAD moiety and requires coupling to the electron transport chain. Both of the put proteins are membrane bound. The two put genes form divergent transcripts from a common central regulatory region. Previous work suggested that the putA gene product was also involved in regulation of the put genes. Analysis of put::lac operon fusions confirmed this finding and showed that the putA gene product negatively controls transcription of both the putA and putP genes. Transcription of the put genes is induced by proline. Maximal induction of the put genes also requires oxygen or alternative electron acceptors. Strains lacking a functional putA protein show maximal transcription of the put genes independent of the presence of an electron acceptor, suggesting that the regulatory effect of electron acceptors is mediated by the putA protein. Some evidence suggests that inducing conditions may promote association of the putA protein with the membrane, thereby removing it from the cytoplasm where it acts as a repressor.

0289 DNA REGIONS INVOLVED IN CONTROL OF rRNA SYNTHESIS, Richard L. Gourse*+, Michael J.R. Stark*, Albert E. Dahlberg* and Masayasu Nomura+, *Brown University, Providence, RI 02912 and +U. of Wisconsin, Madison, WI 53706.

It is now apparent that the accumulation of rRNA is the controlling factor in the expression of the other components of the translation machinery in E. coli. To elucidate the control of rRNA synthesis in vivo, we have been altering plasmid genes coding for rRNA. We have shown that rRNA is transcribed from plasmids and have defined specific nucleotides distant from the processing sites that nevertheless are required for maturation of ribosomes (Gourse, Stark, & Dahlberg (1982), J. Mol. Biol. 159, 397-416; Stark, Gourse & Dahlberg (1982), J. Mol. Biol. 159, 417-439).

It has been proposed on the basis of in vitro studies (Kingston & Chamberlin (1981), Cell 27, 523-531) that the regulation of rRNA synthesis after amino acid starvation (the stringent response) might be explained by ppGpp causing increased pausing of RNA polymerase. We have shown that rRNA encoded by plasmids is under stringent control but that deletion of these RNA polymerase pause sites does not weaken the stringent response in vivo (Gourse, Stark, & Dahlberg, submitted). The DNA regions involved in the stringent response are thereby limited to regions upstream of or within the promoter for rRNA. Present studies (Gourse & Nomura, in progress) should further elucidate the mechanism(s) of rRNA regulation in vivo.

0290 DNA SEQUENCES RESPONSIBLE FOR CADMIUM REGULATION OF TRANSCRIPTION FROM THE MOUSE METALLOTHIONEIN-I PROMOTER.

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Transcription of the mouse metallothionein-I (MT-I) gene is inducible by heavy metals and glucocorticoid hormones in many mouse tissues and cell lines. Using a hybrid gene consisting of the MT-I promoter joined to the coding region of the herpes viral thymidine kinase (TK) gene, the activity of the MT-I promoter can be conveniently assayed by measuring viral TK activity after microinjection of the DNA into mouse eggs. From an analysis of the activity of 5' and 3' deletions within the promoter, the Cd-regulatory function has been localized to a 50 bp region containing a 16 bp hyphenated palindromic sequence. The effects of further 5' and 3' deletions, combinations of these to generate short regions of nucleotide substitution within the otherwise intact promoter (linker scanning mutants) and single nucleotide substitution mutations, will be presented.

0291 ACANTHAMOEBA RIBOSOMAL DNA: TRANSCRIPTION START SITE, SELECTIVE TRANSCRIPTION IN VITRO AND MOLECULAR MECHANISM OF DEVELOPMENTAL REGULATION.

M. R. Paule, C. T. Iida, G. H. Harris, S. L. Brown, P. J. Perna and J. M. D'Alessio, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523.

Evidence has been obtained that rRNA transcription is regulated in a differentiating eukaryotic cell by covalent modification of RNA polymerase I (RNAP I). During the early stages of starvation-induced differentiation of Acanthamoeba, transcription of rRNA is terminated. When cloned DNA fragments bracketing the start site for this transcription unit are mixed with purified amoeba RNAP I, no specific runoff RNAs are produced. However, when crude transcription initiation factor (eTIF-I) is included in the incubation mixture, select amanitin-resistant runoff RNA is made which initiates at the in vivo start site (~1700 bp upstream of the 18S coding sequence). Using the in vitro transcription assay supplemented in excess with purified RNAP I, we have shown that the level of eTIF-I is constant during the developmentally regulated shutoff of rRNA transcription. However, when the assay is repeated using only the RNAP I endogenous to the S100 extract, transcription of rRNA ceases in parallel with the in vivo shutoff. These two assays comprise a complementation assay in which homogeneous RNAP I is the regulatory component. Therefore, the polymerase must be altered in some way to elicit regulation. Our early studies show that the level of RNAP I in differentiating cells is constant, and when assayed with calf thymus DNA, the eTIF-I extracts showing regulation have identical RNAP I activities. Though RNAP I from vegetative and from starved cells have identical subunit compositions, RNAP I purified from starved cells has lost the ability to complement the inactive extracts; this suggests a covalent modification of the polymerase.

0292 AN UPSTREAM REGION REQUIRED FOR THE REGULATION AND PROMOTION OF tyrT EXPRESSION,

Andrew A. Travers, Angus I. Lamond, Hilary A.F. Mace and Michael L. Berman, MRC Laboratory of Molecular Biology, Cambridge, England.

The rate of *in vivo* transcription from the *E. coli* tRNA and rRNA promoters depends on both aminoacid availability and cellular growth rate. We have suggested that the characteristic growth rate control is a consequence of the binding of RNA polymerase dimers to stable RNA promoters and monomers to mRNA promoters. As predicted by this model we show that RNA polymerase can strongly protect 70 bps of the $tRNA^{Tyr}$ (*tyrT*) promoter from DNase I digestion and only 50 bps of the *lacUV5* mRNA promoter. In *tyrT* the protected region extends 63 bp upstream of the transcription startpoint in contrast to 44 bp for *lacUV5*. By *in vitro* analysis of deletion mutants we show that a sequence between -52 and -95 is required both for optimal transcription and regulation by ppGpp, the effector of aminoacid control. The *tyrT* promoter, defined as the RNA polymerase binding site, is thus more extensive than the *lac* promoter and contains at least two sites determining ppGpp regulation. We show also that a similar region of the *tyrT* promoter is required for optimal activity *in vivo* and define a consensus sequence for upstream regulation by RNA polymerase.

0293 PROMOTER-PROXIMAL PAUSING BY RNA POLYMERASE II IN VITRO: CHARACTERIZATION BY TRANSCRIPTS AND PURIFICATION OF TRANSCRIPTION COMPLEXES, Donal S. Luse, Joseph A. Coppola, and Angela Field, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267

RNA was synthesized in vitro by RNA polymerase II from cloned Ad2 late promoter DNA, using HeLa cell nuclear extracts, A, C, and GTP at 50 μM and UTP at 0.2 μM. Under these conditions, RNAs 6, 7, 13 and 17 nucleotides long are synthesized. The production of these RNAs requires DNA templates and is sensitive to 0.5 μg/ml α-amantin. Partial sequence analysis confirms that the short RNAs are initiated at the Ad2 late promoter; these RNAs result from pauses before the addition of the 3rd, 4th, 5th or 6th U residues to the growing transcripts. All of the short transcripts may be quantitatively chased into long (>190 bases) run-off RNAs with excess UTP. Although run-off transcripts as short as 75 nucleotides are completely capped, the short transcripts (<20 bases) are neither capped nor 2'-O-methylated. Thus, capping is not required for the initiation of transcription by RNA polymerase II. We have begun to purify complexes of the paused RNA polymerase and plasmid DNA by sedimentation on sucrose gradients. Complexes containing transcripts longer than about 10 bases are quite stable during this purification; the short transcripts remain capable of being quantitatively elongated into full-length RNA. Full-length RNAs synthesized by the purified complexes are not capped; thus, the capping activity has been removed during sedimentation, as have over 99% of the total proteins present in the original transcription mixture. Preliminary DNA protection studies using the purified complexes will be presented.

0924 DE NOVO DNA METHYLATION INACTIVATES THE EXPRESSION OF GLUCOCORTICOID SENSITIVITY IN T-LYMPHOID CELL LINES, Suzanne Bourgeois, Thomas Ryden and Judith C. Gasson. The Salk Institute, Regulatory Biology Laboratory, San Diego, California 92138.

SAK8 is a glucocorticoid resistant mouse thymic lymphoma line. Complementation studies show that the glucocorticoid receptor of SAK8 cells is functional and, therefore, SAK8 cells are resistant to glucocorticoid hormones due to a defect in another locus (or loci) designated "l" for lysis. Glucocorticoid sensitive subclones were generated at a high frequency by treatment of SAK8 cells with 5-azacytidine. This result demonstrates that the "lysis" gene(s) in SAK8 cells is intact but is not expressed because of the hypermethylated state of the DNA at this glucocorticoid-resistant state of differentiation.

Glucocorticoid sensitive clones, derived from SAK8 by 5-azacytidine treatment, reverted to the glucocorticoid resistant phenotype over a period of 1 to 2 months in culture in the absence of hormone. Such revertants are killed by hormone in the presence of 5-azacytidine, indicating that the glucocorticoid resistance of this revertant is due to de novo remethylation of the gene(s) involved in the lytic process. HPLC analyses show about a 50% decrease in 5-methylcytosine after 5-azacytidine treatment, with a return to higher levels of 5-methylcytosine in the revertant.

This demonstrates a strong correlation between glucocorticoid resistance and DNA hypermethylation: Demethylation of DNA by 5-azacytidine treatment activates the lytic response, but the sensitive clones obtained revert to glucocorticoid resistance by spontaneous de novo DNA remethylation. (Supported by NIH grant #GM20868 and fellowship #AM06179 and by a grant from the Whitehall Foundation.)

0295 SEPARATION OF PROMOTER AND HORMONE REGULATORY SEQUENCES IN MMTV Deborah E. Dobson, Frank Lee* and Gordon M. Ringold, Stanford University, Stanford, CA 94305 and *DNAX Ltd, Palo Alto, CA.

We are investigating the interactions between promoter and hormone regulatory regions present in the mouse mammary tumor virus (MMTV) long terminal repeat (LTR). Chimeric plasmids were constructed by fusing a full length or deleted LTR to the E. coli XGPRT gene. The deletions range in length from 40 to 700 bp, starting approximately 100 bp. upstream from the transcription start site within the LTR. These plasmids were introduced by DNA-mediated transformation into several rodent cell lines. Stably transformed cells were selected and mRNAs from these cells were analyzed by Northern blot hybridization, dot blot hybridization and S_1 mapping techniques. Analysis of one recombinant (ΔA1; 700 bp deletion) indicates that the promoter and hormone regulatory regions are separable. The basal level of GPT mRNA was similar in cells containing either the ΔA1 or full length LTR recombinants. However, the addition of the synthetic glucocorticoid dexamethasone increased the level of GPT mRNA only in cells harboring the full length LTR recombinant. S_1 mapping of the 5' end of these mRNAs indicates that transcription initiates at the appropriate position in both the full length and ΔA1 LTR constructions in the presence and absence of hormone.

0296 A REGULATORY HEAT-SHOCK PROMOTER ELEMENT, Hugh R.B. Pelham and Mariann Bienz, M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

Many (if not all) organisms contain heat-shock genes which are rapidly activated in response to high temperature and other stresses. We have investigated the DNA sequences required for heat-induction of the Drosophila hsp 70 heat-shock gene in monkey COS cells and Xenopus oocytes. Deletion analysis has identified a short region 13-32 bp upstream of the TATA box which is required for regulation and appears to be an upstream promoter element that is active only in heat-shocked cells. Consistent with this is the observation that placement of SV40 late promoter sequences (including the 72 bp repeat) 200 bp upstream of the start site can force synthesis of correct hsp 70 transcripts at low temperature. The hsp 70 gene does not, however, require the 72 bp repeat "enhancer" sequences for inducible activity, nor respond to them when they are present at the 3' end of the gene.

Comparison of the required regulatory sequence with other Drosophila heat-shock genes reveals a symmetric consensus sequence CT-GAA--TTC-AG at a variable distance from the TATA box. Replacement of the normal upstream promoter element of the herpes virus thymidine kinase (tk) gene with synthetic sequences similar or identical to this consensus makes the tk gene heat-inducible in both monkey cells and Xenopus oocytes. Many of the heat-shock genes also have a larger imperfect dyad symmetry centered to the left of the AG of the consensus sequence. Evidence from synthetic constructs and mutated genes indicates that the presence of such a feature in this position is neither necessary nor sufficient for heat-inducibility in our assay systems, although a requirement for a short (10 bp) inverted repeat somewhere in the vicinity cannot be ruled out.

0297 Specific Yeast Minichromosome Proteins and Transcription, R. Sidhu and Arthur P. Bollon, Wadley Institutes, Dallas, TX 75235

A fast and highly efficient method is utilized to isolate a high copy number yeast minichromosome consisting of a vector containing bacterial plasmid components, yeast 2μ DNA and yeast leu 3 DNA. The yeast strain containing the vector has a double mutation in leu B requiring the expression of the vector and is 2μ minus. The high copy number permits monitoring the presence of the minichromosome during purification simply by gel electrophoresis without the need for hybridization. Proteins associated with different components of the vector are being analyzed by one and two dimension electrophoresis using the multicolor silver stain technique. The minichromosome is being utilized for transcription analysis of vector components and the effect of associated proteins on such activity. Another vector contains, in addition to the above components, the Hind IIIr_2 component of the yeast rRNA gene repeat unit which contains the 5S rDNA, untranscribed spacer DNA, and the promoters for 5S and 35S rRNA genes (Bollon, "Organization of Fungal rRNA Genes," in The Cell Nucleus, Vo. X, 1982). The use of these vectors permit the identification of proteins associated with specific vector components. Results on the specific proteins and transcription will be presented. (This work is supported by NIH GM 28090 and the Meadows Foundation).

0298 HORMONAL REGULATION OF YOLK POLYPEPTIDE GENE TRANSCRIPTS IN ADULT FEMALE DROSOPHILA, Paul D. Shirk[1], Parviz Minoo[2] and John H. Postlethewait[2], [1]Department of Zoology, Oregon State University, Corvallis, OR 97331 and [2]Department of Biology, University of Oregon, Eugene, OR 97403.

The three yolk polypeptides (YPs) found in mature oocytes and hemolymph of adult female Drosophila melanogaster have been shown to be hormonally regulated. During maturation of adult females, both 20-hydroxyecdysone (20HE), a steroid, and juvenile hormone (JH) play a role in stimulating YP synthesis in the fat body and the follicle cells of maturing oocytes. We have used three parameters, the rate of [^{35}S]-methionine incorporation into YPs synthesized in vivo, the amount of RNA-directed YP synthesis in a cell-free rabbit reticulocyte lysate translation system, and the quantities of YP gene transcripts hybridizable to cloned YP gene DNAs in a "Northern" blot assay, to measure the hormonal stimulation of YP synthesis in normal females and hormonally manipulated preparations. We found that all three parameters increased approximately 35-fold during normal maturation of adult females from 0 to 24 hr after emergence. When abdomens of freshly emerged adult females were ligated from the anterior endocrine organs, treatment of the isolated abdomens with either 20HE or a JH analogue (JHA) stimulated the accumulation of translatable YP gene transcripts as well as protein synthesis. If the body walls (which contain predominately fat body) of the hormonally treated isolated abdomens were examined, both 20HE and JHA had stimulated accumulation of YP mRNA and YP synthesis. However, the ovaries from these same preparations showed an accumulation of translatable YP gene transcript only in the JHA treated isolated abdomens; 20HE had no effect. This demonstrates a tissue specific response to 20HE and JHA which suggests that the two hormones are acting to stimulate YP synthesis through separate mechanisms.

0299 TRANSCRIPTIONAL REGULATION OF GROWTH HORMONE BY GROWTH HORMONE RELEASING FACTOR, Marcia M. Barinaga, Ronald M. Evans, Michael G. Rosenfeld, Jean Rivier and Wylie Vale, Salk Institute, San Diego, CA 92138

The hypothalamic releasing factors are small peptide hormones which are synthesized by the neurosecretory cells of the hypothalamus and exert their releasing activity on the pituitary. In our laboratory, a growth hormone releasing factor (GRF) has recently been purified from a human pancreatic islet tumor obtained from an acromegalic patient. The amino acid sequence of this 40 amino acid peptide has been determined, and synthetic peptides with this sequence are being used to study the effects of GRF on the production of growth hormone in primary cultures of rat anterior pituitary cells, as well as in several rat pituitary tumor cell lines. The primary effect of GRF on pituitary cells is to stimulate release of growth hormone which is stored in secretory granules in the cells. We have demonstrated that GRF also exerts a rapid transcriptional effect on the growth hormone gene. Within one hour of exposure to GRF, the rate of transcription of the growth hormone gene is doubled, as measured directly by incorporation of ^{32}P-UTP into nascent transcripts in isolated nuclei.

0300 TISSUE-SPECIFIC tRNA GENE EXPRESSION IN BOMBYX MORI, Lisa S. Young and Karen U. Sprague, University of Oregon, Eugene, OR 97403

We wish to understand the mechanism(s) responsible for tissue-specific accumulation of alanine tRNA in the silkworm, Bombyx mori. In this organism, one species of alanine tRNA ($tRNA_2^{ala}$) is produced constitutively in a variety of cell types. In contrast, the other major $tRNA^{ala}$ species ($tRNA_1^{ala}$) appears only in the silkgland. These RNAs differ by a single nucleotide substitution (C $\rightarrow$ U). To learn whether the silkgland-specific $tRNA_1^{ala}$ is encoded by distinct genes, or is instead derived from $tRNA_2^{ala}$ by post-transcriptional modification, we have searched for the predicted two types of genes among fragments of the Bombyx genome. Examples of constitutive $tRNA_2^{ala}$ genes have already been extensively characterized in our laboratory, and recently we have identified two different copies of $tRNA_1^{ala}$ genes. The existence of different genes corresponding to the two alanine tRNAs argues that post-transcriptional modification probably does not account for the appearance of $tRNA_1^{ala}$ in the silkgland. Rather, we are focussing on selective gene amplification or differential transcription to explain this phenomenon.

To examine the latter possibility, we are studying the properties of both genes in homologous in vitro transcription extracts prepared from silkgland and non-silkgland cells. We have observed marked differences between the two kinds of genes in vitro, and are investigating whether these differences reflect genuine tissue-specific transcription. We are also constructing hybrid $tRNA_1^{ala}/tRNA_2^{ala}$ genes in order to localize nucleotide sequences responsible for the distinctive properties of the intact genes.

0301 STUDIES ON THE REGULATION OF EXPRESSION OF YEAST HISTONE - β-GALACTOSIDASE GENE FUSIONS, Larry R. Karns and M. Mitchell Smith, University of Virginia, School of Medicine, Charlottesville, VA 22908

Recent studies have shown that histone mRNA accumulates in the cell during late G1 and early S phase of the cell cycle. To study the regulation of the increase of transcription of the histone H3 and H4 genes in yeast, gene fusions between histone genes and the bacterial β-galactosidase gene were constructed. These were transformed into yeast and transformants have been isolated with the gene fusions present within the cell on autonomously replicating plasmids or stably integrated into several different chromosomal loci. Preliminary results show that the expression of only some of these stably integrated gene fusions are regulated within the cell cycle and experiments are currently in progress to determine the reasons for these variable results. Deletions within the 5' sequences of the gene fusions have been made to study the role of promoter sequences on the level of expression and possibly on the regulation of expression within the cell cycle. Expression of these gene fusions on mini-chromosomes is also being investigated.

0302 FURTHER STUDIES ON THE ROLE OF ppGpp IN THE CONTROL OF THE *his* OPERON OF *SALMONELLA*, Kenneth E. Rudd and John R. Roth, University of Utah, Salt Lake City, UT 84112
The nucleotide ppGpp is a positive regulator of the *his* operon in *Salmonella*. We have pursued the mechanism of this regulation by: (1)isolation of mutants with altered ppGpp levels, and (2)isolation of mutants in the *his* control region with an altered response to ppGpp. A mutation in the *spoT* gene of *Salmonella* which over-accumulates ppGpp due to reduced ppGpp-ase activity has been isolated (Barry Bochner, unpublished results). This mutation is co-transducible with both *gltC* and *pyrE*. The *spoT1* mutant has elevated levels of the *his* enzymes in rich media, but is distinct from all other *his* regulatory mutants. Using localized mutagenesis, a *spoT-ts* mutant has been isolated which is ts-lethal in a Rel$^-$ backgroun and absolutely lethal in a Rel$^+$ background. Presumably this lethality is due to an over-accumulation of ppGpp. This mutant should permit experimental variation of ppGpp levels over a wide range and allow selection of mutants with reduced ability to synthesize ppGpp. Mutants of the *relA* gene have reduced ppGpp levels and fail to fully derepress the *his* operon. This failure can be detected as a sensitivity to aminotriazole(AT), an inhibitor of the *hisB* enzyme. AT-resistant derivatives of a *relA*::Tn*10* mutant have been isolated. This should include mutants with increased levels of ppGpp and mutations in the *his* control region which eliminate the ppGpp requirement for maximal expression.

0303 SPECIES SPECIFIC TRANSCRIPTION OF GENOMIC DNA BY RNA POLYMERASE III, John J. Furth and Chun-Yeh Su, University of Pennsylvania, Philadelphia, PA 19104
When bovine DNA is used as template for RNA polymerase III in a HeLa cell cytosol extract a single discrete RNA, 120 nucleotides, is transcribed together with multiple RNA species ranging in size from 200 to 800 nucleotides. No one RNA predominates in the larger RNAs. Similar results are obtained with RNA transcribed from calf thymus, fetal calf thymus, calf liver, and bovine lymphosarcoma DNAs. With mouse liver or mouse thymus DNA as template, large RNAs similar to bovine RNAs are transcribed. In addition, a prominant RNA of about 180 nucleotides is synthesized. With *Xenopus laevis* erythrocyte DNA as template, multiple discrete RNAs are transcribed. Although minor RNAs of 105 and 115 nucleotides are synthesized, most of the RNAs are greater than 200 nucleotides. Similar results are obtained with *Xenopus* liver and kidney DNA. Thus, transcripts of DNA from different tissues of the same species are qualitatively similar while each species gives a different RNA pattern. RNA 5S in size is a quantitatively prominant transcript only with bovine DNA and RNAs the size of tRNA or tRNA precursor are not prominant. The Polymerase III preparation used recognizes sites for initiation and termination of RNA synthesis with precision. 5S RNA genes, tRNA genes and alu-type genes inserted into plasmid DNAs are transcribed into RNAs of the anticipated size. These results suggest (i) all Polymerase III genes in genomic DNA are potentially transcribable, and (ii) which genes are transcribed *in vitro* depend on several factors including substrate concentration (notably the triphosphate required for initiation), the frequency the particular gene occurs in the DNA, and the affinity of Polymerase III transcription factors for a particular gene. (Supported by grant GM10390 from NIH USPHS).

0304 **MAR/SIR** REGULATION OF THE UNEXPRESSED MATING TYPE CASSETTES. J. Ivy, J. Hicks, L. Haas, J. Strathern, K. Nasmyth, and A. Klar. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

The budding yeast *S. cerevisiae* has three loci capable of determining mating type, yet only the **MAT** locus is active in wild-type cells. Expression of the silent loci, or "cassettes", **HML** and **HMR**, is prevented by the action of four "**MAR/SIR**" loci. These four loci are unlinked to each other and to the mating type cassettes, and a mutation in any one alone allows expression of both **HML** and **HMR**. We have selected and established the identity of cloned sequences which suppress mutations in the four **MAR/SIR** loci. Using the cloned genes and various mutants, we have identified interactions among the **MAR/SIR** genes. First, a **MAR/SIR** mutation (**sir4-35oc**) previously shown by Rine not to complement **sir1-1** is complemented by YEp13:SIR4 and maps to **SIR4**. Also, two other **SIR4** mutants exhibit only partial complementation of **sir1-1** and **mar1-1**. Second, a cloned sequence which lacks **SIR4** activity but which overlaps the **SIR4** clone has an activity antagonistic to normal **MAR/SIR** regulation. This dominant "Antimar" activity might represent loss of normal gene regulation or an altered activity. Finally, YEp13:SIR3 complements mutations in both **SIR3** and **SIR4**. Results of Northern blots investigating the possibility of transcriptional regulation among the **MAR/SIR** genes will be presented.

0305 ATP REQUIREMENT FOR SPECIFIC IN VITRO TRANSCRIPTION INITIATION: COPURIFICATION OF AN ATPASE WITH ONE OF THE RNA POLYMERMASE II TRANSCRIPTION FACTORS, M. Sawadogo, J.D. Dignam and R.G. Roeder, Washington University School of Medicine, St. Louis, MO 63110.

We are investigating the mechanisms involved in the transcription initiation by RNA polymerase II. The chromatographic fractionation of the soluble transcription system derived from HeLa cells nuclei (1) reveals that five factors (TFIIA, TFIIB, TFIIC, TFIID and TFIIE), besides the RNA polymerase II, are required for the specific transcription in vitro from the Adenovirus 2 major late promoter. The reconstituted system is five to ten times more efficient than the initial extract. TFIIE has been purified more than 6,000 fold. An ATPase activity copurifies with this factor through six chromatographic steps. This ATPase is DNA dependent and works equally well with dATP or ATP.

An energy requirement for specific transcription in vitro by RNA polymerase II has recently been shown by Burnick et al. using the non-hydrolysable analog AMP-PNP (2). This ATP dependence is also found in our reconstituted system. However, we find that in the presence of dATP as the energy source and AMP-PNP as RNA polymerase substrate, the system functions as well as when ATP alone is present. This result further implies that in vitro, the specific transcription occurs independently of the RNA capping event.

1) Dignam, J.D., Lebowitz, R.M., Roeder, R.G. submitted for publication.
2) Burnick, D., Zandomeni, R., Ackerman, S., Weissmann, R. (1982) Cell 29, 877-886.

0306 DIFFERENTIAL EXPRESSION OF SUBSETS OF SEA URCHIN LATE HIGSTONE GENES, Robert Maxson, Timothy Mohun, Glen Gormezano and Laurence Kedes, Stanford University and Veterans Administration Hospital, Palo Alto, CA 94304

The sea urchin possesses two major classes of histone genes which are distinct in both developmental timing of expression and genomic arrangement. One class is active early in development and organized in tandem repeat units; the second is expressed later in embryogenesis and is irregularly arranged. We report here that some members of the second (late) class of histone genes exhibit different temporal patterns of expression during late embryogenesis. Fragments of two different late H2B genes spanning the 5' termini of the two mRNAs were end-labeled and allowed to react with RNA prepared from embryos at different stages of development. The resultant DNA-RNA hybrids were treated with S1 nuclease and the digestion products resolved on gels. In each case, several sets of nuclease-protected fragments are apparent, demonstrating considerable heterogeneity within the class of late H2B mRNAs. Moreover, the relative ratios of some of these late H2B histone mRNA subtypes change markedly in development. Thus, individual members of the late histone gene class have distinct developmental patterns of expression. The nucleotide sequences of the genes corresponding to differentially regulated mRNAs translate into different protein sequences. Hence, variability in the patterns of late histone gene expression may be significant at the level of nucleosome structure.

0307 MECHANISM OF RNA CHAIN INITIATION BY E. COLI RNA POLYMERASE OPEN PROMOTER COMPLEXES, Agamemnon J. Carpousis and Jay D. Gralla, Molecular Biology Institute and Department of Biochemistry, University of California, Los Angeles, CA 90024

E. coli RNA polymerase:DNA complexes engaged in transcription initiation and elongation in vitro at the lac UV5 promoter were probed by DNase I footprinting. The protection pattern that is characteristic of the open promoter complex was not altered by the reiterative synthesis and release of short RNA chains up to four bases long. This result indicates that the open promoter complex is not disrupted by short RNA synthesis. Upon synthesis of an RNA chain nine bases long, a completely new footprint was obtained. It is likely that this is a footprint of a stable ternary elongation complex consisting of RNA polymerase, template DNA and nascent messenger RNA. RNA chain initiation in E. coli is best defined as the conversion of the open promoter complex to an elongation complex. That is, RNA polymerase "escapes" from promoter DNA by breaking the contacts that are characteristic of the open promoter complex and forming an elongation complex. Our results indicate that escape from the lac UV5 promoter occurs sometime between the addition of the fifth and tenth base to the nascent messenger RNA chain.

0308 THE ROLE OF cAMP IN REGULATING DEVELOPMENTALLY EXPRESSED DICTYOSTELIUM GENES, Rex L. Chisholm, Giorgio Mangiarotti, Scott Landfear and Harvey F. Lodish. MIT. Cambridge, MA 02139.

We have used the molecular cloning of mRNA and genomic DNA to purify nucleotide sequences representing developmentally regulated Dictyostelium discoideum genes. Using these as probes of gene expression during development, we have identified several patterns of regulation. Two major classes of mRNA appear about the time of cellular aggregation. The levels of both classes of messages are markedly reduced by disaggregation and are restored by exposure of the disaggregated cells to cAMP. Messages of the first class appear between 4 and 8 hours of development, and can be induced in starved suspension cultures pulsed with cAMP. Accumulation of the second class occurs between 8 and 12 hours. These genes are not induced in cAMP pulsed suspension cultures, implying another signal, perhaps cell-cell contact is required to induce their expression. Using transcription in isolated nuclei, we have shown accumulation of both classes of mRNA is under transcriptional regulation. In addition, we find that the half life of these mRNAs in disagregated cells is prolonged by cAMP treatment. Multicellular aggregates were pulse labelled with ^{32}P phosphate between 13 and 17 hours of development, washed free of label, disaggregated and shaken in suspension with, or without cAMP. RNA was prepared after various times and hybridized to cloned DNA bound to nitrocellulose. Messages corresponding to the cAMP dependent genes exist at levels similar to normally developed cells, in RNA prepared from cAMP treated cells, but decay rapidly in the absence of cAMP.

0309 ANALYSIS OF METABOLIC PROPERTIES OF MITOCHONDRIAL tRNAS IN HELA CELLS AS AN APPROACH TO STUDY REGULATION OF MITOCHONDRIAL GENE EXPRESSION, Michael P. King and Giuseppe Attardi, California Institute of Technology, Pasadena, CA 91125

The analysis of mitochondrial DNA transcription in HeLa cells has led this laboratory to propose a model whereby the tRNAs, rRNAs and mRNAs encoded in the heavy strand are transcribed in the form of a polycistronic transcript in which the tRNA sequences are nearly always contiguous to the rRNA and mRNA sequences. These tRNAs then provide the signal for processing to the mature species. Two types of heavy strand transcription of the mitochondrial genome in the rDNA region have been identified. One produces the polycistronic molecule which encompasses nearly the entire heavy strand. The other, which occurs at a rate 20 to 60 times higher, covers only the rDNA region and is responsible for the synthesis of the bulk of the rRNA. Three tRNA genes flank the rRNA genes—the $tRNA^{Phe}$ gene is immediately 5' to the 12S rRNA gene, the $tRNA^{Val}$ gene is between the rRNAs and the $tRNA^{Leu}$ gene is immediately 3' to the 16S rRNA gene. Evidence has been obtained for the occurrence of two initiation sites of heavy strand transcription, one located very near the 5' end of the 12S rRNA gene and the other 20 to 40 nucleotides upstream of the $tRNA^{Phe}$ gene. An analysis of the metabolic properties of the three tRNA species encoded in the rDNA region will elucidate which of these two sites is involved in rRNA synthesis. The steady state level of the three above mentioned tRNA species is being determined, and their metabolic properties are being analyzed by measuring their kinetics of accumulation using labeled RNA precursors. These data will be compared to those obtained for other heavy strand encoded tRNAs and will enable us to assign the two known initiation sites to the two transcription events. This analysis will also define the 3' end of the rapidly synthesized rRNA transcript.

0310 EXPRESSION OF THE NATIVE α AND β INTERFERON GENES IN HUMAN CELLS. P. B. Sehgal, The Rockefeller University, New York, NY 10021.

The current paradigm for the expression of the several intronless human IFN-α_S genes (which cross-hybridize IFN-α_1) and the IFN-β_1 gene is that the transcription unit across these genes extends from a promoter immediately upstream from the structural gene to several staggered (~200 nucleotides) polyadenylation sites immediately downstream resulting in the synthesis of the abundant 0.8-1.4 kb IFN-α_S and the 0.9 kb IFN-β_1 mRNA species. We have investigated IFN-related transcripts expressed in human lymphoblastoid (Namalwa) cells by electrophoresis of polyadenylated RNA through agarose-CH_3HgOH gels followed by blot-hybridization using an IFN-α_1 cDNA probe (a prototype α_S) and also by translation of the eluted RNA into biologically active IFN using the Xenopus oocyte assay. We have observed 1) that IFN-α produced 'spontaneously' by Namalwa cells appears to be derived from an mRNA of length 5 kb; 2) that the 1.8 kb IFN-α_L mRNA(s) has less than 60-70% nucleotide sequence homology with IFN-α_1 in the coding region; 3) that induction of Namalwa cells with Sendai virus leads to the appearance of IFN-α_1 hybridizable cytoplasmic RNA of lengths between 0.2-0.6, 2-3, 3.5-5 and 7-8 kb in addition to the expected 0.8-1.4 kb RNA; 4) that all of these molecules are translationally active; and 5) that the 7-8 kb RNA is at least as abundant as the 0.8-1.4 kb RNA in nuclear polyadenylated RNA preps. A similar complex motif is observed for RNA derived from the IFN-β genes. Thus, at best, the current paradigm describes only half of the transcription events that occur across the classical IFN-α and β genes. Some of these observations suggest that the 4-5 kb DNA homology unit within which each of the IFN-α_1-related genes is embedded may represent functional elements involved in the transcription of these genes in the native state.

0311 MACROMOLECULAR CONTACTS ON THE PATH OF NASCENT RNA THROUGH THE PROCARYOTIC TRANSCRIPTION COMPLEX, Michelle H. Palmer and Claude F. Meares, Chemistry Department, University of California, Davis, CA 95616

We have synthesized a cleavable dinucleotide photoaffinity probe, 5'(4-azidophenylthio)phosphoryladenylyl(3'-5')uridine (denoted N_3RSpApU), and used it to initiate transcription with E. coli RNA polymerase at the A1 promoter of bacteriophage T7 D111 DNA. Transcription complexes were prepared containing a range of RNA lengths, with N_3RSpApU at the RNA 5' end, ^{32}P at other locations in the nascent RNA, and a terminator at the RNA 3' end. Four reactions, each containing a base-specific chain terminator (A,G,C,U), were used so that the sequence of each transcript could be confirmed. The 5' end of the RNA was then covalently attached to DNA or RNA polymerase subunits by UV irradiation. Radiolabeled reaction components were separated by electrophoresis, after which the RNA oligonucleotides were cleaved off each component and analyzed for size and sequence. Results show that in the transcription complex, the DNA template is covalently labeled by the leading end of RNA oligonucleotides with lengths between 3 and 12 residues. The alpha subunit of RNA polymerase is only labeled by oligonucleotides between 37 and about 62 residues long. The beta and beta-prime subunits are not resolved by electrophoresis when they bear oligonucleotides; together they are labeled by RNA's from 3 to roughly 80 residues long. The sigma subunit is heavily labeled by the trinucleotide. Further experiments using other templates should reveal the effect of RNA secondary structure on its path through the transcription complex.

0312 IDENTIFICATION AND CHARACTERIZATION OF PROTEINS THAT BIND TO MAMMALIAN RNA POLYMERASE II, Jack Greenblatt and Richard Carthew, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6

Affinity chromatography on micro-columns containing immobilized calf thymus RNA polymerase II has been used to identify RNA polymerase II-binding proteins in cultured mammalian cells.

We detect three RNA polymerase II-binding proteins (polB72, polB38, polB30) in murine erythroleukemia cells, Y1 adrenocortical tumor cells, and NIH-3T3 cells. All are phosphoproteins and all have salt-sensitive interactions ($K_a > 10^7 M^{-1}$) with RNA polymerase II. PolB38 and polB30 are greatly altered in their patterns of phosphorylation when erythroleukemia cells are treated with a tumor promoter or are induced to terminally differentiate with DMSO. The concentrations of the RNA polymerase II-binding phosphorylated forms of polB38 are reduced in NIH-3T3 cells transformed by the Kirsten murine sarcoma virus.

Human HeLa cells also have three RNA polymerase II-binding proteins with the same electrophoretic mobilities as polB72, polB38, polB30. In addition, a new protein which binds tightly to RNA polymerase II is detected in HeLa cells late during infection by human type 5 adenovirus. The properties of this protein suggest it may be the smallest product of the adenovirus E1a regulatory gene.

Our experiments are consistent with the idea that proteins which associate directly with RNA polymerase II have an important role in regulating gene expression and cellular differentiation.

0313 TRANSCRIPTION KINETICS OF DROSOPHILA MELANOGASTER $tRNA^{Val}$ GENES IN A HOMOLOGOUS CELL-FREE EXTRACT. G.B. Spiegelman, B. Rajput, L. Duncan, R.C. Miller, Jr., R. MacKay, R. Greenberg, University of British Columbia, Vancouver, B.C. V6T 1W5

Transcription of seven independently isolated clones of Drosophila melanogaster $tRNA^{Val}$ genes has been studied in a homologous in vitro reaction derived from S-100 extracts of Schneider-II cell lines. The cloned genes are nearly, but not completely, identical in DNA sequence within the gene, but vary considerably in sequences flanking the precursor RNA. The split-promoter regions vary only in positions which by consensus have little effect on transcription. Transcription rates of the different clones varies over a wide range. Recombinant clones have demonstrated that rates of transcription depend on the 5' flanking sequences. A deletion which removes the "B" block of the split-promoter region but does not alter any other nucleotides, transcribes at 2% of the parent DNA. Template competition experiments have demonstrated that a stable transcription complex forms in the in vitro synthesis reaction. Formation of the complex is kinetically complex involving at least two steps. Plasmids which are transcribed at high rates undergo at least one of the assembly steps required for complex formation faster than plasmids which are transcribed more slowly. Products of the in vitro reaction are processed by the S-100 extract to a tRNA sized RNA. The processing takes place in at least three steps and the rates of conversion between the intermediates varies for different clones. Transcription of recombinant clones has demonstrated that an A to G transition at position 69 in the tRNA coding region leads to a five-fold increase in processing rate. Similarly, a G to A transition at position 57 increases the processing efficiency.

Cells, Viruses and Gene Transfer

0314 PURIFICATION OF A GUANINE NUCLEOTIDE EXCHANGE COMPLEX, RF•eIF-2, AND REGULATION OF ITS ACTIVITY DURING PROTEIN SYNTHESIS INITIATION, Brian Safer, Andrej Konieczny, and Rose Jagus, NHLBI, NIH, Bethesda, MD, 20205

A 390,000 dalton complex of eIF-2 (3 subunits, M_r = 38, 52, and 55 x 10^3) and RF (5 subunits, M_r = 26, 39, 58, 67 and 82 x 10^3) has been purified from rabbit reticulocyte lysate. The RF-eIF-2 complex is required for formation of the eIF-2•Met-tRNA•GTP ternary complex at physiologic concentrations of GTP and GDP. The basis of this effect appears to be an increase in the K_D^{GDP} of eIF-2 from 3.1 x 10^{-8} to 2.8 x 10^{-7} when eIF-2 is associated with RF. While the addition of free eIF-2 to hemin-deficient reticulocyte lysates allows the synthesis of an equivalent amount of globin, both free RF and RF•eIF-2 catalyze the synthesis of 30 - 40 pmol globin/pmol factor. Increasing the concentration of GTP to 2 mM also restores control rates of protein synthesis in hemin-deficient lysate by circumventing the enzymatic RF-mediated guanine nucleotide exchange mechanism. Translation in hemin-deficient lysates appears to result for the inhibition of RF-mediated eIF-2 recycling involving guanine nucleotide exchange. Phosphorylation of eIF-2α greatly increases the affinity of RF for eIF-2 in the presence of guanine nucleotides. Since the RF pool size appears to be 10 - 20% that of eIF-2 inhibition in hemin-deficient lysates appears to result from the sequestration of the smaller RF pool by the limited phosphorylation of eIF-2α.

0315 BASIS FOR THE SPECIFICITY OF PROMOTER RECOGNITION BY THE BACTERIOPHAGE T3 AND T7 RNA POLYMERASES, William T. McAllister, Jean N. Bailey, Nancy Horn and John Klement, Rutgers Medical School, Piscataway, NJ 08854

During infection of *E. coli*, the closely related bacteriophages T3 and T7 encode RNA polymerases that each consist of a single protein species of *ca* 110,000 daltons (880 amino acids). Although the genes that encode the two polymerases are remarkably similar, neither enzyme will transcribe the heterologous DNA efficiently. To explore the basis for the template specificities of these enzymes we determined the nucleotide sequences of six promoters recognized by the T3 RNA polymerase and compared them with the previously determined T7 promoter sequences. Like the T7 promoters, the T3 promoters consist of a highly conserved 16 base pair sequence preceded by an AT rich region. The fundamental difference between the two kinds of promoters is a change in a two base pair region at -10 and -11. Whereas all T7 promoters have GA at these positions, these are replaced by a single C at position -10 in the T3 promoters.

The nucleotide sequence of the gene encoding the T7 RNA polymerase (and hence the amino acid sequence of the enzyme)has been determined in other laboratories. We have determined the nucleotide sequence of the T3 RNA polymerase gene. The resulting amino acid sequence exhibits very few non-conserved changes when compared to the T7 sequence. Analysis of the data from both enzymes suggests features that may be important for polymerase function.

0316 POSSIBLE INVOLVEMENT OF POLY(A) IN PROTEIN SYNTHESIS, Allan Jacobson, Mitchell Favreau, Richard E. Manrow and Laura Steel, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01605

We have observed that, in early *Dictyostelium* development, specific mRNAs are excluded from polysomes and this exclusion is correlated with selective deadenylation of these mRNAs. This has led us to re-evaluate the possibility that poly(A) is involved in protein synthesis. We reasoned that if the poly(A) tract of mRNA did participate in some step in translation then purified poly(A) might competitively inhibit the *in vitro* translation of poly(A)$^+$ mRNAs, much in the same way as purified 5'-CAP structures competitively inhibit *in vitro* translation of capped mRNAs. We have found that poly(A) inhibits the initiation of translation of many different poly(A)$^+$ mRNAs and that comparable inhibition is not observed with other ribopolymers. Inhibition by poly(A) preferentially affects the translation of adenylated mRNAs and can be overcome by increased mRNA concentrations or by translating mRNPs instead of mRNA. Inhibition is inversely related to the size of the competitor poly(A) as well as to the translation activity which a lysate has for poly(A)$^+$ RNA. Our results suggest a model for poly(A) function in which the binding of poly(A) to the ubiquitous cytoplasmic poly(A)-binding protein (PABP) leads to an enhancement of the rate of initiation of protein synthesis. To test this hypothesis we have prepared polyclonal antibodies against the *Dictyostelium* PABP. Preliminary experiments indicate that the PABP is primarily found on post-polysomal mRNPs in growing cells. In early development, when the rate of protein synthesis is reduced by 70%, the PABP is severely reduced in the mRNP fraction.

0317 THE NUCLEOTIDE SEQUENCES INVOLVED IN BACTERIOPHAGE T4 GENE 32 TRANSLATIONAL SELF-REGULATION, H.M. KRISCH* and B. ALLET+, *Department of Molecular Biology, University of Geneva, Geneva 4 - Switzerland, +Biogen S.A. Route de Troinex 3, 1227 Carouge - CH

We have determined the nucleotide sequence of a cloned segment of the bacteriophage T4D chromosome which contains the regulatory sequences and the structural gene for the single-stranded DNA binding (gene 32) protein. The amino acid sequence predicted by translation of the stuctural gene agrees well with that published for gene 32 protein (Williams et al. , (1980) P.N.A.S 77 : 4614-4617). To localize the nucleotide sequence involved in translational self-regulation of gene 32, we have constructed a series of plasmids in which gene 32 is fused to an amino terminal deletion mutant of the B-galactosidase gene of E. coli. Expression of a B-galactosidase fusion protein which contains only the first 7 amino acids of gene 32 is still repressed by gp32. The ribosomal binding site of gene 32 is flanked by a repetitive A+T rich sequence. Preferential and co-operative binding of gp32 to this region of its m-RNA could inhibit translation initiation and would thus account for the autoregulation. G+C rich linkers sequences have been inserted within this region by a novel method of in vitro mutagenesis, and the sensitivity of these mutant plasmids to repression by gene 32 protein has been analyzed.

0318 QUANTITATIVE MODEL FOR THE AUTOGENOUS TRANSLATIONAL REGULATION OF THE T4 GENE 32 PROTEIN, Gary D. Stormo and Larry Gold, MCD Biology, Univ. of Colorado, Boulder, Colorado, 80309

The product of T4 gene 32 is a single stranded DNA binding protein that regulates its own synthesis at the level of translation. The primary ligand of the protein is the intracellular ss-DNA. When that ligand is saturated it next binds to its own mRNA so as to inhibit translation. The translation of other T4 genes is not affected until much higher concentrations of the protein exist than are necessary to inhibit its own synthesis. Using the gene 32 protein's nucleic acid binding parameters, measured in Peter von Hippel's lab, and the gene 32 nucleotide sequence, from Henry Krisch and Bernard Allet, we have determined that the "operator" site is a 63-nucleotide region which covers the translational initiation site. Using rules of secondary structure prediction we calculate that this region will be 90% saturated, and thereby repressed to the measured levels, at a gene 32 protein concentration of about 1.5 μM. This is in good agreement with the measurements made both in vivo and in vitro. Examination of 10 other known T4 gene beginnings confirms that none would be bound by the 32 protein at that concentration. Furthermore, examination of all known T4 sequences, amounting to about 5% of the entire genome, shows that the gene 32 translational initiation site is likely to be nearly unique in its ability to bind the gene 32 protein.

0319 POST-TRANSCRIPTIONAL BLOCK TO SYNTHESIS OF A HUMAN ADENOVIRUS CAPSID PROTEIN IN ABORTIVELY INFECTED MONKEY CELLS. Kevin P. Anderson and Daniel F. Klessig, University of Utah, Salt Lake City, UT 84132

In monkey cells abortively infected with human adenovirus (Ad2) the steady state levels of Late Ad2 mRNA species are significantly reduced. A parallel reduction in the synthesis of most proteins encoded by these messages has been noted. However, the drastic reduction in fiber protein synthesis in abortively infected monkey cells (>100 fold) cannot be accounted for by the modest decreases in fiber mRNA levels (5-20 fold) in these cells.

Our experiments show, however, that fiber mRNA from abortively infected CV1 cells serves just as efficiently as a template for fiber synthesis in vitro as fiber mRNA from productively infected cells. This was observed both in a nuclease treated rabbit reticulocyte lysate where purified fiber mRNA or cytoplasmic ribonucleoprotein complex was added as exogenous template for fiber synthesis, and in S10 extracts of infected CV1 cells utilizing endogenous message as a template. Since translation initiation inhibitors did not diminish synthesis of fiber in S10 extracts of abortively infected CV1 cells, fiber mRNA probably is associated with ribosomes in abortively infected CV1 cells. This conclusion was supported by northern blot analysis which showed that in both abortively and productively infected CV1 cells the same proportion of cytoplasmic fiber mRNA co-sedimented with polyribosomes. While the possibility of extremely rapid turnover of fiber protein in abortively infected monkey cells cannot be rigorously excluded, preliminary data suggest that this is not the case. Thus, these results imply that translation of fiber message in abortively infected monkey cells is blocked after formation of the mRNA ribosome complex.

0320 THE ADENOVIRUS DNA BINDING PROTEIN CAN OVERCOME THE BLOCK TO LATE GENE EXPRESSION IN MONKEY CELLS IN THE ABSENCE OF ITS DNA REPLICATION FUNCTION: S. Rice and D. F. Klessig, University of Utah, Salt Lake City, Utah 84132

Human adenovirus (Ad2) does not normally grow in cultured monkey cells due to a block to late gene expression. Ad2hr400 is a host range mutant which can overcome this block due to a mutation in the amino-terminal half of the 72K DNA binding protein (DBP) gene. Ad2hr400, however, is cold-sensitive for growth in monkey (CV1) cells at 32.5°. Cold resistant mutants have been obtained after nitrous acid mutagenesis. One such variant, ts400, was also found to be temperature-sensitive (ts) for growth at 39.5° in both CV1 and human (HeLa) cells. Physical mapping using marker rescue techniques has shown that the ts phenotype and the cold-resistant phenotype are due to independent mutations. The ts mutation is in the carboxyl-terminal half of the DBP gene, while the cold-resistant mutation corresponds to a second alteration located in the amino-terminal half of the DBP gene.

The ts mutation of ts400 is in the same complementation group as the well-characterized DNA-negative DBP mutant Ad5ts125. At 39.5°, ts400 behaves phenotypically like ts125, i.e. fails to replicate viral DNA and enter the late stage of infection. However, the ability of ts400 to express late genes normally in monkey cells is not temperature-sensitive. When CV1 cells are coinfected at 39.5° with ts400 plus Ad2 (Ad2 provides the DNA replication function but cannot express its late genes efficiently) normal late gene expression and viral growth occur. This result suggests that DBP contains separable functional domains (DNA replication and late gene expression) which can operate independently in the infected cell.

0321 THE FUNCTION OF THE 3' UNTRANSLATED REGION IN GENE EXPRESSION: EXCHANGE OF TERMINATORS BETWEEN TWO YEAST GENES, Hans M. Warrick and John A. Carbon, University of California, Santa Barbara, CA 93106.

Two cloned yeast genes, ARG4 and PGK1, were modified by exchanging their 3' untranslated regions and by making deletions of this region. Each construction was inserted into a vector, containing CEN3, ars2, and LEU2 sequences, which is capable of replicating stably at low copy number in yeast. The plasmids were transformed into yeast and then assayed for enzymatic activity and messenger RNA production. The Northern analysis showed that the PGK messenger RNA continued to be made at unmodified levels even though its terminator region had been replaced by a similar region from ARG4. The substitution of terminator region from the transcriptionally very active PGK gene for the less active ARG4 gene terminator region did not alter the level of expression of ARG4 messenger RNA. The following observations were made: 1) Sequences in the 3' untranslated gene region are required for effective termination of messenger RNA synthesis, resulting in RNA of normal length; 2) The length of the messenger RNA is in part determined by the location of sequences within the 3' untranslated region; 3) A terminator region from one gene can replace another gene terminator without functional problems; 4) Levels of gene transcription are not determined by sequences in the 3' untranslated region.

0322 MECHANISMS OF INTERNAL INITIATION OF TRANSLATION IN ANIMAL CELLS, David S. Peabody and Paul Berg, Stanford University, Stanford, CA 94305.

The mechanism by which eucaryotic ribosomes select the correct AUG for translation initiation is still unknown. One of the unresolved issues is whether initiation is restricted to the first AUG downstream of the 5' cap site. Using SV40 derived vectors to produce polycistronic mRNAs in animal cells we have examined factors which influence the ability of ribosomes to initiate at AUG's other than ones nearest the 5' end. The plasmid pSV2dhfr-gpt contains a dicistronic transcription unit comprised of the coding region for mouse dihydrofolate reductase (DHFR) followed by coding sequences for the E. coli. enzyme xanthine-guanine phosphoribosyl transferase (XGPT). Monkey cells transfected with this plasmid produce only the expected dicistronic mRNA and both DHFR and XGPT. When the translation terminator of DHFR is deleted, initiation at the downstream XGPT sequence is abolished, suggesting that in this case initiation at an internal AUG occurs by a termination-reinitiation mechanism. A related set of plasmid constructions suggests that an additional mechanism may be operative in some cases. When a segment of DNA containing several AUG's is placed upstream and in frame with the XGPT sequences, a series of electrophoretic variants of the enzyme is produced, each apparently corresponding to initiation at one of the upstream initiators. Some AUG sequences, therefore, may be inefficiently recognized as initiators, permitting ribosomes to scan past them and initiate translation at downstream AUG's. Experiments are in progress to evaluate this mechanism further and to define the structural features of strong and weak initiators.

0323 OVERPRODUCTION AND PURIFICATION OF PROTEIN p4, WHICH CONTROLS PHAGE Ø29 LATE TRANSCRIPTION, Rafael P. Mellado, Cristina Escarmís, José M. Lázaro, Juan A. García, José M. Sogo and Margarita Salas, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma, Canto Blanco, Madrid 34, Spain

The Hind III G fragment from Bacillus subtilis phage Ø29 DNA was inserted downstream from the bacteriophage λ P_L promoter carried by the pBR322 derivative plasmid pPLc28. Upon P_L induction, E. coli harbouring the recombinant plasmid gave rise to the synthesis of phage Ø29 protein p4, which controls late transcription and the Ø29 DNA 5'-terminal protein p3 modified at its carboxyl end (p3'). These proteins are the products of the Ø29 genes 4 and 3 respectively, as determined by the cloning of the equivalent regions from the genome of the appropriate nonsense mutants. The nucleotide sequence of the Hind III G fragment from the wild-type Ø29 DNA and from the sus4 and sus3 mutants was determined. The change of a CAA triplet into a UAA ochre triplet was found in the two nonsense mutants.

Under the best conditions used, the Hind III G-containing recombinant plasmids directed the synthesis of proteins p4 and p3' in amounts which accounted for 30% and 7%, respectively, of the total de novo protein synthesis in E.coli.

Details on the purification of protein p4 as well as on its action when added to a Ø29 DNA-dependent in vitro transcription system will be presented.

0324 TRANSCRIPTS OF THE dsRNA VIRUS-LIKE PLASMIDS OF KILLER YEAST. D.J. Tipper and K.A. Bostian. Microbiology Dept., U.Mass. Medical School, Worcester MA and Bio-Med Dept., Brown University, Providence, R.I.

In Vivo transcripts of the L (4.5 kb) and M_1 (1.9 kb) dsRNA plasmids of Type I killer yeast include full length (l, m) and partial length (l_a, 2.3 kb and m_a, 1.2 kb) species. Both l and la have mRNA activity for L-P1, a major product of translation of denatured L-dsRNA previously identified with ScV-P1, the major capsid protein of the Virus-Like Particles containing the dsRNA's. l but not la is bound to poly(U)-Sepharose. Both m and m_a have mRNA activity for M_1-P1, the 32 kd preprotoxin encoded by M_1-dsRNA. Both are bound to poly(U) Sepharose and m_a is probably a 5' terminal fragment of m terminating at the AU-rich "bubble" sequence of M_1 dsRNA. Full length, plus-stranded transcripts are presumed to be replication intermediates in a Reovirus-type pathway which may also involve polyadenylation. Production of partial length transcripts may control replication rate and toxin production by controlling gene expression. The control mechanisms have yet to be elucidated.

0325 INDUCTION AND MODULATION OF CELL-TYPE SPECIFIC GENE EXPRESSION IN DICTYOSTELIUM, MONA C. Mehdy and Richard A. Firtel, Dept. of Biology, U.C. San Diego, La Jolla, CA 92093

We are studying the requirements for induction and modulation of tissue specific gene expression in Dictyostelium. Probes to genes preferentially expressed in either prespore or prestalk cells have shown that a class of prestalk mRNAs are detectable before cell aggregation is complete while a class of prespore mRNAs are not detectable until after tight cell contacts are formed. Exogenous cAMP, in the absence of cell contact, is sufficient to induce prestalk gene expression while multicellularity is required for the induction of prespore specific gene expression. A gene expressed equally in both cell types, which has the same developmental kinetics as the prestalk genes, is induced in the absence of either cAMP or cell contact. Dissociation of cell aggregates results in the rapid loss of mRNA complementary to the tissue specific genes. These mRNA can be induced to reaccumulate with the addition of cAMP. Cycloheximide inhibits the reaccumulation of the prespore but not the prestalk mRNAs indicating differences in the modulation by cAMP. Neither cell dissociation nor cAMP affects the expression of the cell type non-specific gene and several genes transcribed throughout development. From these experiments, (M. Mehdy, D. Ratner, and R. Firtel, submitted), we show that there are substantial differences in the regulation of genes which are differentially expressed in the two cell types. We are undertaking a molecular analysis of the mechanisms by which cAMP and multicellularity regulate these two populations of genes. One approach includes using our DNA mediated Dictyostelium transformation system.

0326 REGULATION OF INSULIN/β-GALACTOSIDASE HYBRID PROTEIN GENE FUSIONS, David A. Nielsen, Joany Chou, Albert J. MacKrell, Donald F. Steiner and Malcolm J. Casadaban, The University of Chicago, Chicago, IL 60637

We are studying eukaryotic gene expression through the use of β-galactosidase (β-gal) gene fusions in order to take advantage of the great sensitivity and simplicity of the assay for this enzyme, as well as the wealth of biochemical and genetic information of this system. The control elements and amino terminal sequences of the rat insulin II gene (kindly provided by A. Efstradiadis) and the control elements of the SV40 early gene have been ligated to the β-gal structural gene to yield fusions which should produce hybrid proteins. When these are transfected into Cos 7 cells (a T-antigen producing monkey kidney-derived cell line) β-gal expression is obtained. The pH optimum of the β-gal activity of the transfected cell extract was found to correspond well to that of E. Coli β-gal. Radioautography after SDS-PAGE electrophoresis of immunoprecipitated ^{35}S-methionine labelled cell proteins have shown a protein band having the predicted molecular weight of the fusion product. Preliminary results have shown that the expression of the fused β-gal is not significantly altered by high glucose, insulin, epidermal growth factor or dexamethasone. These fusions are currently being used to investigate the regulation of the rat insulin II gene in both Cos 7 cells and in RIN 5F cells (an insulin-producing rat islet tumor derived cell line).

0327 EXPRESSION OF THE 70,000d HEAT SHOCK PROTEIN IN NORMAL AVIAN RED CELLS, Richard Morimoto and Eric Fodor+, Dept. of Biochem., Molec., Cell Biology, Northwestern University and +Dept. of Biochem., Molec. Biology, Harvard University.

We have identified the 70,000d "heat shock" protein as one of the major non-globin proteins in the normal avian red cell. Adult chicken blood cells respond to elevated temperatures (43-45°C) by the induced synthesis of four heat shock proteins (hsps) of 83,000, 70,000, 23,000 and 22,000d and the repression of pre-existing protein synthesis. This change in the pattern of gene expression is almost entirely due to the lymphocyte component of white cells. The synthesis of hsps in red cells was shown by fractionation of anemic blood into white cells and reticulocytes. As expected, the enriched lymphocytes respond to a heat shock (43°C) by the induction of all four hsps while reticulocytes induce hsp70 above a constituitive level and almost completely repress globin expression. The Coomassie blue staining pattern reveals that hsp70 is a normal component of the reticulocyte comprising 4% of total cellular protein. We have confirmed the identity of the 70,000d protein synthesized by reticulocytes in the absence of heat shock as the major hsp70 by comparison on two dimensional gel electrophoresis with the hsp70 from chicken embryo fibroblasts, reticulocytes and white cells and by comparison of the proteolytic pattern generated by protease V8. Hsp70 is found associated with the nucleus and is present only in nucleated red cells or reticulocytes and undetectable in human erythrocytes.

0328 AMPLIFICATION OF THE GENE ENCODING GLUTAMINE SYNTHETASE IN MOUSE 3T6 CELLS, Anthony P. Young and Gordon M. Ringold, Stanford University, Stanford, Ca 94305

The enzyme glutamine synthetase (GS) occupies a central role in nitrogen metabolism and serves a specialized function in metabolism of neurotransmitters in glial cells. Alterations in enzyme levels are linked to differentiation of embryonic retinal cells and preadipocyte cell lines. Several hormones regulate GS expression in tissue culture systems. A cloned gene probe would greatly facilitate studies of the regulation of GS gene expression in these experimentally tractable systems. Toward this end, we have amplified the gene encoding GS in mouse 3T6 cells.

A 3T6 subline which persists in glutamine-free medium has been cloned and exposed to a regimen of growth in the presence of increasing concentrations of the specific GS inhibitor, methionine sulfoxime. Cells which show a 150-200 fold increase in GS activity have been generated. Immune precipitation of extracts from cells pulse-labeled with ^{35}S-methionine indicates that the increase in enzyme activity is due to an increase in biosynthesis of GS. The enzyme constitutes several percent of the newly synthesized protein in the drug-resistant cells. Increased expression of the enzyme is lost upon transfer of the cells to non-selective medium--a finding consistent with the observation of double minute chromosomes in only the drug-resistant cells. Current efforts are focused on the identification and isolation of GS gene inserts from a cDNA library constructed using mRNA from the drug-resistant cells. The cloned gene will be used to probe the underlying biochemistry responsible for regulation of GS gene expression using the simplified tissue culture systems mentioned above.

0329 TIME OF ONSET OF ACTIVE GENE EXPRESSION IN THE DEVELOPING NEW MACRONUCLEUS DURING CONJUGATION IN TETRAHYMENA, Kristen A. Mayo and Eduardo Orias, Dept. of Biol. Sciences, Univ. of Calif., Santa Barbara, Calif. 93106.

The galA1 and tyrC3 mutations in T. thermophila determine the virtual loss of galactokinase (gal.K.) and phenylalanine hydroxylase (phe.H.) activities, respectively, in this ciliate protozoan. Heterokaryons have been constructed which are homogeneous for the galA1 and tyrC3 mutations in the (45N) somatic macronucleus, but which contain a gal+/+, tyr+/+ germline micronucleus, and are lacking both enzyme activities, since the micronuclear genes are not expressed. Conjugation of two of these heterokaryons to each other allows the differentiation of gal+, tyr+ new macronuclei against a gal.K.-, phe.H.- cytoplasmic background, and is therefore useful in studying the process of gene activation in the developing new macronucleus. (During conjugation, the micronuclei engage in meiosis and reciprocal fertilization. The new macro- and micronuclei of the exconjugants differentiate from initially identical mitotic descendants of the diploid fertilization nuclei; the old macronuclei are destroyed.) In the case of gal.K., enzyme activity appears and rises dramatically 12 to 14 hrs. after starting the cross, around the time of separation of the exconjugants, and 3 to 5 hrs. after the beginning of morphological differentiation of the macronuclear anlage. The conjugating cultures must be refed in order to express the information in the new macronucleus, but the time of appearance of gal.K. is independent of the time of refeeding, if they are refed between 4 and 12 hrs. into the cross. Similar studies on the behavior of the phe.H. enzyme marker during conjugation are now underway in order to assess the generality of the timing of this (gal.K.) gene activation event in the developing new macronucleus.

0330 CLONING OF THE A1, A2, AND A3 PROMOTERS FOR E. COLI RNA POLYMERASE FROM THE EARLY REGION OF BACTERIOPHAGE T7 DNA, Alan H. Rosenberg, John J. Dunn and F. William Studier, Biology Department, Brookhaven National Laboratory, Upton, New York 11973

The A1, A2 and A3 promoters of T7 DNA have been cloned in the BamHI site of pBR322, using defined linkers that allow the cloned fragments to be excised. The three promoters have been cloned individually on restriction fragments of T7 DNA that range in size from 80 to 286 base pairs, and together on a single restriction fragment of 322 base pairs. The promoters appear to be stably maintained in pBR322 without additional transcription termination sites. They have also been cloned in combination with the transcription termination signal for E. coli RNA polymerase found at the end of the early region of T7 DNA, which appears to function effectively. The recombinant plasmid DNAs have been transcribed in the linear form with purified E. coli RNA polymerase. Transcription is initiated much more efficiently at A1, A2 or A3 than at the promoters of pBR322, as would be expected from the known strengths of the promoters in T7 DNA. The strength of the three promoters relative to each other appears to be much the same whether they are located in T7 DNA or in the plasmid DNAs. Comparison of the efficiency of initiation at the A1 promoter in two different clones indicates that no specific sequence further than 56 base pairs upstream from the RNA initiation site is necessary for optimal functioning of this promoter.

0331 DIFFERENTIAL GENE EXPRESSION IN THE EARLY EMBRYO OF XENOPUS LAEVIS, Thomas D. Sargent and Igor Dawid, NICHD/NIH, Bethesda MD 20205

We are interested in the possible role played by the embryonic genome in the initiation of differentiation. Our approach to this problem has been to isolate the relatively few genes whose polyadenylated cytoplasmic RNA's appear for the first time after fertilization, but before gastrulation is completed. We accomplished this by application of a "hybridization enrichment" cloning scheme which has resulted in a library of cDNA clones consisting almost entirely of sequences that are absent in eggs and blastulae but present at various abundance levels in frog gastrulae. We have found that in addition, many of these putative mRNA sequences have become undetectable by the tadpole stage, suggesting that they may be associated with processes that occur only during early embryogenesis. Current efforts include attempts to elucidate the mechanism whereby these RNA species abruptly accumulate in the cytoplasmic polyA+ compartment, improved quantification of sequence concentrations and measurements of representation of various cloned RNA's on polysomes. Eventually we plan to begin dissecting "gastrulation" gene expression by use of antibodies directed against bacterially manufactured polypeptides encoded by selected cDNA clones.

0332 ANALYSIS OF THE WHITE LOCUS IN DROSOPHILA UTILIZING P-FACTOR MEDIATED GENE TRANSFORMATION, Tulle Hazelrigg, Robert Levis, and Gerald Rubin, Carnegie Institute, Dept. of Embroyology, Baltimore, MD. 21210

The white (w) locus is a gene which confers a brick-red eye color in Drosophila. Using the technique of P-factor mediated gene transformation in Drosophila, the work described here is concerned with defining the molecular sequences at the white (w) locus necessary for the normal functioning of this locus during development. There are several interesting phenomena associated with gene expression at the w locus. It is a complex locus in which regions can be differentiated with regards to apparent regulatory functions. Mutations in one part of the locus exhibit the phenomenon of dosage compensation, so that males and females are identical in phenotype, whereas mutations in another region of w do not show dosage compensation. Expression of w is dependent on the zeste (z)locus; the normal eye color is altered in the presence of z mutations only when there are two paired copies of the w locus present. Certain chromosomal rearangements in which the w locus is placed next to heterochromatin show position effect variegation, in which the w gene is turned off in some cells during development, but not in others. The molecular bases of these phenomena are not understood. We are exploring this by combining in vitro mutagenesis of cloned w DNA sequences with P-factor mediated gene transformation to assess the in-vivo expression of these altered sequences. The isolation of transormants has already defined a segment of w DNA which is sufficient for restoring pigmentation to w eyes. Analysis of transformants obtained with mutagenized w sequences is currently underway.

0333 AMPLIFICATION AND EXPRESSION OF HUMAN α-GLOBIN GENE IN CHO CELLS, Yun-Fai Lau and Yuet Wai Kan, Howard Hughes Medical Institute and University of California, San Francisco, CA 94143

We have studied the amplification and expression of human globin genes in CHO cells employing the DNA-mediated gene transfer method with the SV_2-cDNA dihydrofolate reductase gene as vector. The normal human α-globin gene and a hybrid gene (MαG) containing the 5' promoter-regulator region of the mouse metallothionein gene and the human structural α2-globin gene were covalently linked to the DHFR gene. The recombinant DNA molecules were introduced into DHFR CHO-K1 cells using the calcium phosphate precipitate procedure. After the initial selection to retain the DHFR and linked sequences, the cells were cultured in increasing concentrations of methotrexate up to 0.2 mM. Southern hybridization analysis of total cellular DNA showed that the number of copies of DHFR and human α2-globin genes increased approximately 500 to 1,000 The transcriptional activity of both DHFR and α-globin genes increased as copies of both genes increased within the cells. Furthermore, at an amplified state, the transcription of the hybrid MαG gene was also induced by Cd treatments. However, most of the inductions were larger transcripts than the mature mRNA. DHFR constituted about 10-15% of pulse-labelled total cellular proteins. However, the α-globin was not detected. In vitro translation of total poly A+ RNA from these cells showed that the α-globin mRNA was able to direct globin synthesis. Our data demonstrate that: (1) when linked to a DHFR gene marker, the human α-globin genes can be amplified together with the DHFR gene through methotrexte selection; (2) transcription of the globin genes increases as the gene copy number increases; (3) cadmium inducibility of the hybrid MαG gene is obvserved at the amplified state; (4) expression of the amplified α-globin gene is not observed in vivo, but is detectable in in vitro translation assays, suggesting that the human α-globin may be very unstable or poorly translated in CHO cells.

0334 ISOLATION OF MOUSE L-CELLS EXPRESSING HUMAN TRANSFERRIN RECEPTOR, 4F2-ANTIGEN AND HLA AFTER GENE TRANSFER WITH TOTAL HUMAN DNA , Lukas C. Kühn, James A. Barbosa, Michael E. Kamarck and Frank H. Ruddle, Yale University, New Haven, CT 06511

Cotransfer of high molecular weight human DNA from MOLT-4 cells together with an HSV-TK containing plasmid into mouse Ltk^- cells results in stable expression of a particular human cell surface antigen in 1 out of 2000 HAT-resistant recipient cell colonies. By indirect immunofluorescence with the monoclonal antibodies OKT-9, 4F2 or W6/32 in combination with repeated sorting on the Fluorescence-Activated Cell Sorter (FACS), we have isolated many independant primary transferants expressing human transferrin receptor, 4F2-antigen or HLA, respectively. Stable secondary transferants were obtained after an identical cotransfer with total DNA from primary transferants. Southern-blot hybridization with Blur-8, a plasmid containing a highly repeated human alu-sequence, reveals a very restricted amount of human DNA present in secondary transferants. Similarly, hybridization with cloned HLA probes indicate the presence of these genes in mouse recipients expressing HLA. This procedure provides the basis to the molecular cloning of genes coding for cell surface antigens and receptors.

0335 A EUKARYOTIC-PROKARYOTIC PLASMID VECTOR, Steven H. Larsen, JoAnn Hoskins, *Stephen G. Rogers, Indiana University School of Medicine, Indianapolis, IN 46223, *Monsanto Company, St. Louis, MO 63167

A plasmid which is selectable by dominant markers in both eukaryotes and prokaryotes has been constructed. It contains 3 active resistance genes for bacteria and one (G418) for eukaryotes and is still only 6.1 Kbp in size. It has now been adapted to grow as a plasmid in animal cells to facilitate easy recovery of cloned DNA fragments. Progress in varying plasmid copy number in animal cells will also be reported.

0336 RAT GROWTH HORMONE GENE EXPRESSION AFTER TRANSFECTION OF GH_3 x L CELL HYBRIDS. J. Strobl,* R. Padmanabhan,† B. Howard† and E. B. Thompson* Laboratories of *Biochemistry and †Molecular Biology, National Cancer Institute, NIH, Bethesda, MD 20205

Somatic cell hybrids between GH_3 rat pituitary cells and LB82 mouse fibroblast cells contain ~ 70% of both parental sets of chromosomes including both the rat and mouse growth hormone (gh) genes. In such cells, the hormone-inducible expression of the rat gh gene is extinguished by a mechanism involving either blocked transcription or rapid RNA degradation. Using calcium phosphate mediated DNA transfection to introduce exogenous copies of the rat gh gene we have shown that gh gene transcription and RNA translation occur in hybrid cells selected for expression of a bacterial gene present in the same plasmid as the rat gh gene. In 3/5 clones examined by Northern analysis, gh RNA transcription proceeds from the promotor utilized in GH_3 cells and RNA undergoes identical processing steps to a mature gh mRNA form of the size seen in GH_3 cells. In four additional clones, gh mRNA of the expected size was observed, but RNA precursor data is unavailable. The level of gh mRNA was generally < .1% of that in hormone-induced GH_3 cells, however, one clone expressed gh mRNA at ~ 7% the level seen in induced GH_3 cells. In at least one clone, gh mRNA is transcribed from an exogenous gene since both the rat and mouse endogenous genes are absent. The gh mRNA levels were not increased in any clones treated for 48 hours with .1 μM dexamethasone despite the presence of high affinity glucocorticoid receptors in 3-5-fold excess of that present in either parent cell line. We conclude that extinction of gh expression in the non-transfected hybrid cells results from a transcriptional block since stable RNA can be produced and expressed upon transfection with exogenous genes.

0337 STABILITY OF AN SV40/pBR322 RECOMBINANT IN PERMISSIVE MONKEY CELLS, Martin L. Breitman and Lap-Chee Tsui, Department of Genetics, Research Institute, The Hospital for Sick Children, Toronto, Canada, M5G 1X8

We have previously reported that simian COS-1 cell lines transformed by the SV40 recombinant plasmid pSV2-gpt contain multiple copies of the plasmid in superhelical and concatenated form [Tsui et al. Cell 30:499-508 (1982)]. In situ hybridization analysis of one such cell line revealed that approximately 4-8% of the cells contained between 10^3 and 10^5 copies of plasmid DNA; the remainder contained relatively few copies of the plasmid in an indeterminate state. This heterogeneous distribution of pSV2-gpt was also observed in subclones derived from the parental cell line, suggesting that plasmid replication occurs periodically and spontaneously in all cells of the population. These observations suggest that COS-1 cells are semi-permissive for the replication of SV40 origin-containing recombinant molecules. A rapid loss of pSV2-gpt DNA was observed following passage of the pSV2-gpt-transformed cell line in the absence of biochemical selection. This loss occurred at a comparable rate for both superhelical and high molecular weight concatenated forms of the plasmid and was accompanied by the emergence of Gpt^- cells. Cloning and subsequent analysis showed that these revertant cells were completely free of detectable plasmid sequences but retained the ability to produce SV40 large T antigen, a property of parental COS-1 cells. These observations indicate that plasmid sequences failed to stably integrate in cellular DNA. However, since cellular sequences have been detected in superhelical plasmid DNAs and their corresponding concatemers, it is probable that integration and subsequent excision of plasmid sequences occasionally occurs in pSV2-gpt-transformed COS-1 cells.

0338 EXPRESSION AND REGULATION OF HUMAN INTERFERON GENES IN HETEROLOGOUS HOSTS,Michael Innis,Frank McCormick,Peter McCabe,Georgette Cole,Meg Trahey and Gordon Ringold*, CETUS Corporation,Berkeley,CA 94710, *Dept. Pharmacology,Stanford University School of Medicine,Stanford,CA 94305

We have studied the expression and regulation of human IFN genes in heterologous hosts including Chinese Hamster Ovary (CHO), mouse fibroblast (Balb/c 3T3), and mouse myeloma (SP2) cells. Plasmids containing selectable markers such as the mouse dihydrofolate reductase gene and either β-1, α-61 or α-76 IFN genes were constructed for expression from various promoters. Transformants from plasmids transfected into dhfr- CHO cells were tested for IFN expression. Cells containing the human β-1 gene under its own promoter expressed 1000 U/ml/10^6 cells constituitively, and up to 6 x 10^5 U/ml/10^6 cells upon poly (rI:rC) superinduction. The level of constituitive production increased ten-fold in cells selected for growth in 10 nM methotrexate (a folate analog) which selects for amplification of the integrated plasmids. Cells containing the β-1 gene expressed from the SV40 early promoter produced similar levels constituitively and were not inducible with poly (rI:rC). Expression of α-IFN genes was detected at lower levels (300 U/ml) following induction with NDV. Expression levels of 3000 U/ml of α-IFN were achieved using the SV40 early promoter. Results of further constructions will be presented.

0339 EXPRESSION OF MOUSE IMMUNOGLOBULIN GENES FOLLOWING REINTRODUCTION INTO LYMPHOCYTES USING VIRUS VECTORS Robert J. Deans and Randolph Wall
Dept. of Microbiology and Immunology, Molecular Biology Institute, UCLA
Los Angeles, CA 90024

The finding that polyoma virus genes can be expressed in mouse lymphocytes following DNA transfection prompted the construction of pBR322/polyoma recombinants which serve as efficient vectors to assay immunoglobulin gene expression. The mature alpha heavy chain gene from the M603 B cell myeloma was placed under the transcriptional control of the polyoma late promoter as well as its own normal 5' regulatory sequences. This recombinant was introduced into mouse fibroblasts, a T cell lymphoma, and a B cell myeloma lacking heavy chain activity although producing kappa light chain. Expression of the alpha heavy chain gene was determined by indirect immunofluorescence and yields determined by an ELISA assay. Expression of both the secreted and membrane exons of the gene, as suggested by the immunological data, was confirmed by in vitro RNA transcription studies using viral transcription complexes isolated from transfected cells.

0340 DNA MEDIATED GENE TRANSFER OF THE GENE FOR 3-HYDROXY-3-METHYLGLUTARYL CoA SYNTHASE. Sherry S. Leonard and Michael Sinensky, Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado, 80262.

Studies in a variety of normal and mutant mammalian cell lines suggest a biosynthetic regulatory mechanism for the enzyme 3-hydroxy-3-methylglutaryl coenzyme A synthase 3-hydroxy-3-methylglutaryl-CoA acetoacetyl-CoA-lyase (CoA acetylating), EC 4.1.3.5. (HMGCoA synthase), an enzyme involved in the synthesis of mevalonate.

Gene transfer has been accomplished into a mutant, auxotrophic for mevalonate and lacking HMGCoA synthase activity. A transformation efficiency of approximately 10^{-5} is established using a Ca-P_i precipitate and glycerol shock.

Transformants contain human DNA and a restriction endonuclease digestion followed by Southern blot show the human DNA is present in specific sequences. In addition, the transformants have very few bands in common, one or more of which may represent the genomic sequence(s) of the synthase gene.

Progress in cloning the gene for HMGCoA synthase using these methods will be described.

0341 EXPRESSION OF IMMUNOGLOBULIN GENES INTRODUCED INTO MOUSE LYMPHOID CELL LINES, Douglas Rice, Yehudit Bergmann and David Baltimore, Massachusetts Institute of Technology, Cambridge, MA 02139

While recent studies have provided detailed information about the structure of immunoglobulin (Ig) genes, little is currently known about the molecular mechanisms which control Ig gene expression. Our approach to study such controls is to introduce Ig genes into mouse lymphoid cell lines representing different stages of B-cell differentiation. Using the calcium-phosphate coprecipitation technique and selection for the Escherichia coli gpt gene, we have introduced a functionally rearranged κ light chain Ig gene into an Abelson murine leukemia virus (A-MuLV)-transformed lymphoid cell line. One transfectant was shown to make κ mRNA and polypeptide chains and to assemble the κ chain product with γ_2b heavy chains to form apparently complete IgG2b. When bacterial lipopolysaccharide was added to the growth medium, levels of κ mRNA and polypeptide increased, showing regulated expression of the introduced κ gene. We are currently introducing heavy and light chain Ig genes into a variety of lymphoid cells lines, including other A-MuLV transformants, B lymphomas and myelomas, to study their expression and regulation.

0342 STUDY OF THE JCV GENOME USING SV40 OR BKV GENE SEQUENCES AS EXPRESSION PROMOTERS. Eugene O. Major. NINCDS, NIH, Bethesda, Md. 20205

Experiments to try to understand the molecular nature of the host restricted growth of JCV, now limited to propagation in primary human fetal glial cells, have led us to develop human embryonic kidney cells containing a replication deficient SV40 genome and also to use COS-1 cells developed by Gluzman. Total JC genome transfected into these cells, however, is not replicated. We have found that the JC origin of replication will not bind SV40 T protein sufficiently for the initiation of replication. Since this type of genetic recombination is not effective, we have begun constructing recombinant hybrid viral genome molecules between JC and BK DNAs taking advantage of the permissive nature of human embryonic kidney cells for BK gene sequences. Plasmid-BK vectors containing the BK origin and regulatory sequences both to the late and early region of its genome have been constructed and are being used as vectors to insert segments of the JC genome. Since there is no well defined genetic map of the JC genome, transfecting these vectors into conventional laboratory cell cultures such as HEK, has provided a better system for the study of JC genome expression. In order to determine whether the JC genome was expressed, a monoclonal antibody directed against the SV40 T protein, Cl 108, was found to cross react with the JC T protein and used as a probe in immunoprecipitation tests of DNA transfected cells.

0343 MUTATIONAL ANALYSIS OF AAV REPLICATION AND GENE EXPRESSION USING AAV-PLASMID CLONES. J.D. Tratschin, P. Senapathy, M.H.P. West, and B.J. Carter, N.I.H., Bethesda, Md.

Adeno-associated virus DNA is 4.7 kb long. We are using recombinant plasmids (pAV) containing the entire AAV2 genome for analysis of AAV replication and to evaluate AAV as a eukaryotic cloning vector. The right half of AAV DNA codes for the virus capsid protein and the left half contains open reading frames coding for putative rep proteins. Transfection of human 293 cells with helper adenovirus particles and pAV DNA results replication of infectious AAV. Deletion of the terminal repetition from both AAV termini (pAV rep$^-$) results in a cis-dominant replication defect. However, pAV rep$^-$ can complement other replication defective AAV clones. Deletion of AAV DNA between map positions 41/91 yields AAV plasmids (pAVΔ 41/91) which accumulate only a very low level of RF DNA. pAVΔ 41/91 can be complemented and replicated efficiently in mixed infections with pAV. Under these conditions pAVΔ 41/91 inhibits replication of pAV and mimics, the properties of AAV DI genomes. Other AAV deletions are being tested. In another construction, pAVgD, 2.3 kb of AAV DNA (map positions 40/91) was replaced with a 3.2 kb fragment of herpes simplex virus type 1 DNA containing the gene for glycoprotein D. pAVgD is completely replication defective but is complemented for RF replication in mixed transfections with pAV or pAVrep$^-$. In either case, 5.5 kb pAVgD duplex RF molecules accumulate but no pAVgD single strands (ss) are seen even though in the tranfections together with pAV, pAV ss are seen. This supports previous suggestions that a) strands longer than AAV genome length (4.7 kb) are not packaged and b) in the absence of packaging single strands do not accumulate even if capsids are present.

0344 REGULATION OF TRANSCRIPTION OF MINUS-STRAND RNA SYNTHESIS IN ALPHAVIRUS INFECTED CELLS. Dorothea Sawicki and Stanley Sawicki, Medical College of Ohio, Toledo, Ohio.

The alphavirus infectious cycle in vertebrate cells can be divided into three phases: an immediate early phase lasting about one hour during which the synthesis of viral encoded macromolecules is difficult to detect, an early phase occurring between one and four hours post-infection during which the rate of viral RNA synthesis increases exponentially and a late phase which is characterized by a relatively constant rate of viral RNA synthesis and the shedding of infectious virus. We have hypothesized (J. Virol. 34:108,1980) that the rate of alphavirus RNA synthesis is governed by the accumulation of minus-strand RNA templates. Minus-strand RNA synthesis ceases normally at about 3-4 hours post-infection, ceases prematurally if protein synthesis is inhibited before 3-4 hours post-infection, ceases selectively when Sindbis virus ts11 infected cells are shifted to the non-permissive temperature, and ceases even under conditions when nonstructural proteins continue to be synthesized at a high rate. We have found (Virology 115:161,1981) that the cessation of minus-strand RNA synthesis appears to be regulated by a viral encoded function that is temperature sensitive in Sindbis virus ts24. If the accumulation of a viral encoded regulatory protein (which would be temperature sensitive in Sindbis virus ts24) were to inactivate the minus-strand RNA polymerase or an activity required for the initiation of minus-strand RNA synthesis, it should be trans-acting. We have attempted to test this hypothesis by co-infection experiments. In cells co-infected with Sindbis virus ts24 and either Sindbis virus HR or Semliki Forest virus, the cessation of minus-strand synthesis appeared to involve a cis-acting function.

0345 THE EXPRESSION OF XENOPUS GLOBIN GENES FOLLOWING INJECTION INTO FERTILIZED XENOPUS EGGS, Mary M. Bendig, Hiro Mahbubani and Jeffrey G. Williams, Imperial Cancer Research Fund, London NW7 1AD.

During metamorphosis in Xenopus, there is a dramatic switch from tadpole to adult hemoglobin production. In order to begin to investigate the kinds of regulatory mechanisms operating during this transition, we have injected cloned Xenopus globin genesinto fertilized Xenopus eggs and analyzed the transcription of the injected genes during subsequent development of the eggs. Although previous experiments showed that the adult globin genes are not transcribed when injected into Xenopus oocytes, we find that both α and β adult globin genes are transcribed following injection into fertilized eggs. Detectable levels of transcripts with authentic 5' ends are present by gastrula stage and persist through hatching and early swimming tadpole stages. We are also determining the effect in vitro methylation of the adult genes prior to injection has on their expression during development. Our results indicate that the injected genes are not in the same totally repressed state as the endogenous adult genes. However the level of transcription of the injected adult genes, particularly in vitro methylated genes, during tadpole stages is very low when compared to the level of globin transcription in immature red blood cells. Experiments are now being continued to later developmental stages using an insertion-modified adult β globin gene whose transcripts can be readily distinguished from those of the endogenous gene.

0346 SV40 MUTANTS THAT DIFFERENTIATE THE LYTIC AND TRANSFORMING FUNCTIONS OF T ANTIGEN. M. Michele Manos and Yakov Gluzman, Cold Spring Harbor Lab, Cold Spring Harbor, N.Y.

Large T antigen of SV40 is a multifunctional protein important in viral DNA replication, regulation of viral transcription and cellular transformation. It possess DNA-binding and ATPase activities. Mutants that are deficient in only a subset of these activities have been useful tools in attempts to assign various activities to distinct domains of the protein.

Mutant SV40 DNA insertions have been rescued from a variety of permissive cells transformed by UV-irradiated SV40. Viral DNA was rescued by fusion of these lines with COS-1 cells which provide a functional T antigen in trans that allows mutant insertions to excise and replicate in the heterokaryons. The rescued SV40 inserts were cloned into the plasmid vector pK1, restoring a complete SV40 genome containing the early region (BglI-BamHI) from the mutant inserts. These DNAs were analyzed by marker-rescue analysis and DNA sequencing. A total of seven mutants were analyzed (C6-1, C6-2, C2, C8, C11, T22 and BSC-SV1). The mutants contain lesions in different parts of T antigen which render the protein incompetent in viral DNA replication but functional in transforming cells in vitro. Several mutants appear to have an enhanced tumorigenicity in hamsters.

The data define regions of T antigen necessary for replication, but dispensable for transforming activity. DNA-binding, ATPase and autoregulatory properties of the mutants and their coincidence with replicative or transforming activities will be described. Assays for complementation between these mutants will be discussed.

0347 VACCINIA VIRUS: A SELECTABLE EUKARYOTIC CLONING AND EXPRESSION VECTOR.
Geoffrey L. Smith, Michael Mackett and Bernard Moss. National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20205

Live vaccinia virus recombinants have been constructed that express foreign genes. Foreign DNA is inserted site-specifically into non-essential regions of the vaccinia genome by homologous recombination in cells infected with vaccinia virus and transfected with plasmids. These plasmids contain foreign DNA flanked by vaccinia sequences. To obtain expression of foreign genes, vaccinia promoters are ligated to the 5' termini of these genes. Vaccinia recombinants have been selected in two ways. Insertion of foreign DNA within the coding sequences of the endogenous vaccinia thymidine kinase gene results in TK^- recombinants selectable in BUdR. Alternatively, insertion of the herpes TK gene into a TK^- vaccinia mutant produced a TK^+ recombinant selectable in HAT medium. Independent confirmation of expression of herpes TK was obtained by a plaque autoradiography utilizing the ability of herpes TK to phosphorylate ^{125}I-deoxycytidine. Foreign genes expressed within the vaccinia TK gene include bacterial chloramphenicol acetyl transferase (CAT) and hepatitis B surface antigen (HBsAg). The large capacity of vaccinia virus for foreign DNA was demonstrated by the stable insertion of 25 kb of bacteriophage lambda DNA within the vaccinia TK gene.

0348 INTEGRATION SITES OF THE MOUSE MAMMARY TUMOR VIRUS IN MAMMARY TUMORS, R. Nusse and H.E. Varmus, Dept. of Virology, Antoni van Leeuwenhoekhuis, Amsterdam and Dept. of Microbiology, U.C., San Francisco.

A region of the mouse genome has been identified, called MMTV-*int1* , which harbors proviruses of the Mouse Mammary Tumor Virus (MMTV) in many different mammary tumors (Nusse & Varmus, Cell 31, 99-109, 1982). This region contains a fragment that is transcribed into poly A^+ RNA in mammary tumors. We have proposed that this region is active in MMTV-induced mammary carcinogenesis by means of expression of a novel cellular oncogene.

We have performed detailed restriction enzyme mapping on the proviral integration sites. The results can be summarized as follows: First, proviruses are integrated over a total distance of 20 Kb surrounding the *int* gene. Second, proviral integration sites are clustered but are not identical in different tumors. Third, proviruses at one side of *int* are all integrated in the same transcriptional orientation, and the proviruses at the other side of *int* are all integrated in the opposite orientation, with both orientations directed away from the *int* gene.

We have also analyzed the transcript from the *int* region and have found that all tumors with an MMTV provirus integrated at *int* have a 2.6 Kb RNA, except for some tumors where the provirus is integrated close to the transcriptional unit itself. In those cases a longer transcript was observed, suggesting that the integration site was affecting the length of the transcript.

These findings will be discussed in view of other cases of expression of cellular genes as a consequence of integration of mobile genetic elements.

0349 ALTERNATIVE APPROACHES TO THE STUDY OF ONCOGENE EXPRESSION IN NEOPLASTIC PROGRESSION
B.D. Crawford[1],E.H.Chang[2], J.L. Goodwin[2], C.E. Hildebrand[1],P.M. Kraemer[1], J.L. Longmire[1], and R.D. Palmiter[3]. Los Alamos Nat. Lab., Los Alamos, N.M. 87545[1], Unif. Serv. Univ. of the Hlth. Sci., Bethesda, MD. 20014[2]; & Univ. of Wash., Seattle, WA. 98195[3]

The role of oncogene expression in the multiple stages of carcinogenesis can best be studied by 1) introduction of such genes into various cells, preferably by means non-selective for <u>in vitro</u> markers of neoplasia, and 2) correlation of phenotypic characteristics with the level of oncogene expression. We have constructed chimeric plasmids designed to place the v-Ki-ras murine sarcoma virus oncogene [which encodes a 21 kd protein (p21) with transforming activity in NIH3T3 cells] under transcriptional control by the metallothionein promoter, in response to Zn^{+2}or Cd^{+2}. Subcloning of this construct in the pSV-2 Eco-gpt plasmid vector permits gene transfer by mycophenolic acid or HAT selection into wild-type or $HPRT^-$ cells, respectively. $HPRT^-$ mutants of Syrian hamster embryo (SHE) and human foreskin (HF) cells have been derived. In early passages, $HPRT^-$ SHE cells are diploid, contact-inhibited, anchorage-dependent, and non-tumorigenic. $HPRT^-$ mutants of HF cells are diploid, non-tumorigenic, and senesce <u>in vitro</u>. 1-10 ug of pSV-2 DNA added by the calcium phosphate coprecipitate method yields HAT-resistant SHE cells at frequencies between 10^{-5}-10^{-4}/treated cell; experiments with $HPRT^-$ HF cells are in progress. HAT-selected transformants are being examined for Zn^{+2}or Cd^{+2} regulated expression of v-Ki-ras mRNA and p21, <u>in vitro</u> markers of neoplastic transformation, and tumorigenicity in athymic mice. These combined approaches should permit an evaluation of the role of oncogene expression in the neoplastic progression of normal, diploid cells.

(This work supported in part by the U.S. D.O.E./O.H.E.R.)

0350 IDENTIFICATION OF A BKV ENHANCER SEQUENCE AND ISOLATION OF A HUMAN HOMOLOGUE.
Nadia Rosenthal, Michel Kress, Peter Gruss and George Khoury. National Cancer Institute, NIH, Bethesda, Maryland 20205

The structure and genetic organization of the human papovavirus BKV has been defined by complete sequencing (Seif, Khoury, and Dhar, Cell 18, 963, 1979) and resembles the monkey virus SV40, except for the region near the replication origin, where the two viruses show no homology. Within this region of the SV40 genome, a pair of 72 bp tandem repeats has been defined as essential for gene expression from the early promoter. These repeats can also enhance expression of heterologous genes. In an analogous position within the BKV genome are three 68 bp repeats. The proposed role of these repeats as an enhancer element was demonstrated by their ability to activate the expression of the chloramphenicol acetyltransferase gene in HeLa cells, as efficiently as the SV40 repeats in the same system. The sequence dissimilarity among papovaviruses enhancers suggests that viral early gene control regions may have been derived from the genome of the host species during evolution and may be recognized by species-specific regulation mechanisms in host cells. This hypothesis predicts that similar sequences may persist in human or monkey cells and that they may retain an enhancer role in the host genome. Using the BKV 68 bp repeat (without the viral replication origin sequences) as a probe to screen a human genomic library, several hybridizing clones have been isolated. In one clone, a BK-related region has been identified which represents a unique sequence in the human genome. The sequence of this region will be presented together with results of experiments designed to test its enhancer activity.

0351 ACCELERATED GROWTH OF MICE FROM EGGS MICROINJECTED WITH METALLOTHIONEIN-GROWTH HORMONE FUSION GENES,
Neal C. Birnberg,Richard D. Palmiter,Ralph L. Brinster,Robert Hammer, Myrna Trumbauer, Michael G. Rosenfeld and Ronald M. Evans,Salk Institute, SanDiego,Ca.92138, Univ. of Washington,Seattle,Wa. 98195,Univ. of Pennsylvania,Philadelphia,Pa.19104,Univ. of California,San Diego 92093

The promoter region of the mouse metallothionein I gene was fused to the coding and 3' flanking region of the rat growth hormone gene in a pBR322 vector. A restriction fragment of this plasmid, pMGH, was microinjected into the male pronucleus of fertilized mouse eggs and reimplanted into foster mothers. This fusion gene, in the presence of chronic zinc administration, was expressed at high levels in the livers of MGH-bearing offspring and correlated with elevated plasma growth hormone levels and accelerated growth relative to normal siblings. The MGH gene was, in addition, expressed in a number of other tissues including several from the male genital tract. We demonstrate here an important method for elevating the expression of specific genes in animals by ligating their coding sequences to an active promoter in the absence of normal feedback controls with different tissue specificity of expression than the endogenous gene.

0352 TRANSCRIPTIONAL REGULATION BY THE ADENOVIRUS Ela REGION

Nicholas C. Jones, Ourania Andrisani and Daniel L. Weeks,
Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

The 13s mRNA of the Adenovirus Ela region encodes a protein which regulates early viral gene expression during the infection of human cells.

We have fused the upstream, promotor sequences from early regions E2, E3, and E4 to the coding region of a number of assayable genes and shown that transcription of these chimeric genes is regulated by the Ela protein. These upstream sequences therefore contain elements necessary for this regulation. Localized mutagenesis of these sequences has been carried out in order to locate and identify these elements. The results of these studies will be discussed in relation to the mechanism of action of the Ela protein.

We have found that sequences at the 5' end of the Ela region enhance transcription of the linked herpes thymidine kinase gene. The sequences behave in a fashion similar to activator elements found in the SV40 and polyoma genomes. We are presently introducing mutations into this region to localize the DNA sequences involved in the enhancement phenomenon.

0353 LAMBDA SITE-SPECIFIC RECOMBINATION IN NOVEL CLONING VEHICLES, Bruce H. Howard, Jeff Auerbach, Sue Wickner and Max E. Gottesman, National Institutes of Health, Bethesda, MD 20205

We are interested in applying bacteriophage λ site-specific recombination reactions to cloning in E. coli and eukaryotic cells. Since both in vivo and in vitro site-specific recombination reactions are highly efficient, they should provide an excellent means to manipulate DNA fragments, independent of internal restriction endonuclease sites. Two approaches are currently under investigation:

1) Cloning in E. coli: λSV2, an 8.4 kb closed circular DNA vector, integrates into the chromosome of the specialized host lysogenic strain N6377 with an efficiency of 10^6 colonies/μg, and may be propagated in the single copy integrated state indefinitely by growth of transformants at 32° C. Results from various sources have suggested that DNA sequences carried in the E. coli chromosome are relatively stable. Accordingly, current experiments are directed towards determining whether λSV2 can be used to clone DNA segments which, because of large size or presence of tandem and/or inverted repeat sequences, are difficult to maintain in conventional plasmid or bacteriophage λ lytic vectors. Since maintenance of λSV2 vectors in the integrated state is dependent on the temperature-sensitive λ cI857 repressor, recombinants may be excised and amplified by brief growth of transformants at 42° C.

2) Rescue of integrated vectors from mammalian cells: integrated copies of λSV2 residing in E. coli chromosomal DNA may be rescued by in vitro site-specific excision with an efficiency of about 1000 colonies/μg HMW DNA. This result suggests that λSV2 copies integrated in mammalian DNA in the prophage configuration might be similarly rescued by in vitro site-specific excision.

0354 CHARACTERIZATION OF MVM TRANSCRIPTION IN VIVO AND IN VITRO. David Pintel, D.K.-Dadachanji, C.R.Astell[1] and David C. Ward, Department of Human Genetics, Yale University School of Medicine, New Haven, Ct., 06510, and [1]Department of Biochemistry, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Four virus-specific transcripts have been identified in murine cells infected with the autonomous parvovirus Minute-Virus-Of-Mice(MVM). These RNAs, 4.8, 3.3, 3.0, and 1.8 kilobases in length, designated R1 to R4 respectively, are all transcribed from the virion(-) strand of DNA and they are all polyadenylated and spliced. Berk-Sharp mapping of the three major transcripts indicates that MVM DNA encodes two overlapping transcription units with separate promoters near the left end (4 mu) and middle (39 mu) of the genome. In vitro transcription of cloned restriction fragments of MVM DNA confirm the existence of functional promoters at these map coordinates and sequence analysis of these regions of the viral DNA reveal the characteristic features of RNA polymerase II promoters. The complete MVM genome as well as isolated transcription units of MVM have been cloned into a Bovine Papillomavirus vector and transformed clones of C127 mouse cells have been isolatedin an attempt to study MVM gene expression under constitutive conditions. To further analyze MVM gene expression at the molecular level we have developed a unique approach to probe complex in vitro transcription systems. Template DNAs or nascent RNA molecules are "tagged" with modified nucleotides and these molecules are isolated under non-disruptive conditions utilizing affinity chromatography. The potential utility of this approach for isolating and identifying specific transcription factors from transcriptionally active cell extracts is currently being evaluated.

0355 REGULATION OF LATE GENE EXPRESSION IN SIMIAN VIRUS 40, Moshe Sadofsky, Janis Keller and James C. Alwine, Department of Microbiology G2, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

We have studied deletion mutants in the major leader region of the Simian virus 40 (SV40)late RNAs. Mixed infections between wild type and mutants result in trans acting effects indicated by the lowering of late transcription from both mutant and wild type genomes. This effect can be explained by the proposal that SV40 encodes a factor which positively effects late transcription. The leader deletions both abolish the ability to make the factor and the need for it; however in the mixed infection the presence of wild type factor abberantly affects late transcription from the mutant genomes. This aberrant interaction subsequently lowers the active concentration of factor thus the positive effect on wild type is lost. A variety of predictions can be made from this model and experiments will be presented which support it. Obviously the factor is not essential; however when it is present the virus can make more late transcripts from fewer templates (i.e. less genome amplification is required). Thus the factor would impart an advantage to the virus in the wild. In addition, the evidence supporting the existence of the factor indicate a heretofore uncharacterized mechanism of gene expression control.

0356 High Efficiency gene transfer with vectors using the Rous LTR. Cori Gorman, Raji Padmanhaban and Bruce Howard. Molecular Biology, NCI, Bethesda, MD 20205

Using the transient expression assay for chloramphenicol acetyltransferase (CAT) (Gorman et al., Mol. Cell Biol 2.1044-51) we have found that the Rous LTR is a strong promoter in many mammalian as well as avian cells (Gorman et al., PNAS in press). The high levels of expression of the vector pRSV cat, has allowed for optimization of gene transfer techniques into many cell types including: monkey CV1 cells, mouse NIH/3T3, chinese hamster ovary cells, Hela S3 and D98 and primary chick embryo fibroblasts. We have recently been able to follow transient expression in Hela cells in suspension as well as Friend ethryroid leukemic cells. We are currently optimizing conditions for gene transfer into other suspension cells such as lymphoid cells. Additionally the Rous LTR has been used in stable transformation experiments with high efficiency. Using the vector pRSVneo NIH/3T3 cells transform with an efficiency of 9×10^{-4} and Hela S3 at 2×10^{-3}. And using pRSVgpt we have repeatedly transformed monkey kidney CV1 cells at an efficiency of $5\text{-}8 \times 10^{-2}$. These very high efficiencies in gene transfer, into a wide variety of cells, greatly increase the ability to study expression of genes cotransferred into many different cell types.

0357 - IDENTIFICATION AND NUCLEOTIDE SEQUENCE OF THE VACCINIA VIRUS THYMIDINE KINASE GENE AND SPONTANEOUS FRAMESHIFT MUTATIONS. Jerry P. Weir and Bernard Moss, National Institutes of Health, Bethesda, Maryland 20205

A thymidine kinase (TK) gene has been mapped within the 180,000 bp vaccinia virus genome. The ability to readily distinguish TK^+ and TK^- variants makes the enzyme an ideal candidate for studies of vaccinia virus gene regulation, analysis of spontaneous or induced mutations, and as a selectable marker in the engineering of poxviruses as eukaryotic cloning and expression vectors. To facilitate such experiments, we have precisely mapped and sequenced the TK gene. The transcribed portion of the gene is approximately 570 nucleotides and contains an open reading frame that encodes a 20,000 dalton polypeptide. These values agree with those independently determined by RNA mapping and in vitro translation studies. The small size of the vaccinia TK polypeptide distinguishes it from that of prokaryotes, eukaryotes, and herpesvirus. The predicted amino acid sequence and composition of the vaccinia TK shows no similarity to that of herpesvirus TK. The region immediately preceding the transcriptional initiation site of the TK gene is extremely AT rich as are the upstream regions of other early vaccinia genes, suggesting a unique base composition specifically adapted to the viral RNA polymerase. Unexpectedly, the putative AT rich TK regulatory sequences lie within the coding region of an adjacent late gene. The 3' end of the late transcript overlaps the TK gene. Similar close packing has been observed for other vaccinia genes. Sequence analysis of three spontaneous TK^- mutant DNAs revealed in each case the addition of a single nucleotide identical to the one preceding it. Because of the frameshift, a nonsense codon was introduced downstream.

0358 TRANSCRIPTION PROMOTERS OF EPSTEIN-BARR VIRUS, Paul Farrell, Alan Bankier, Prescott Deininger, Carl Seguin, Richard Baer and Bart Barrell, MRC Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

We are studying the organization and expression of RNA polymerase II promoters in B95-8 Epstein-Barr virus. Most of the (about 170kb) genome of the virus has been sequenced and so far we have examined promoters in the Eco R1 H, C and Dhet fragments of the virus, comprising the right-hand 35kb of the conventional map. In vitro transcription in HeLa cell extracts has been used to identify at least seven RNA polymerase II promoters in this region. Cytoplasmic poly A+ RNAs starting at these points have been demonstrated by S1 mapping in B95-8 cells induced into virus production with the tumor promoter TPA. B95-8 cells not induced with TPA contain much less of these RNAs. One of the promoters is associated with the latent phase of the virus cycle whereas most of the others are involved with the lytic cycle. The upstream sequences near some of the promoters show striking sequence homologies and we are investigating the possibility that these may be involved in transcriptional control of promoter activity.

0359 INFLUENCE OF THE SV40 72 BASE PAIR REPEAT ON GLOBIN GENE EXPRESSION IN COS CELLS, Thomas C. Reynolds and Jeffrey L. Sklar, Stanford University, Stanford, Ca. 94305

Our lab is involved in studying the expression of the developmentally regulated globin genes. Mammalian expression vectors containing the various α-like or β-like genes have been constructed, followed by transfection into Cos cells. Preliminary evidence indicates that the SV40 72 base pair repeat is required for transcription of each of the β-like genes, while the α-globin gene shows no such requirement. S1 mapping indicates that transcription produces properly initiated, spliced, and terminated RNA. The 72 base pair repeat has been tested in a variety of orientations and at several distances from the β-globin gene; the data suggest that position and orientation of the repeat play an important role in the transcriptional enhancement of the β-globin gene.

0360 THE STRUCTURE OF THE POLYOMA VIRUS EARLY PROMOTER: SEQUENCES REQUIRED FOR EXPRESSION AND T-ANTIGEN BINDING, Christopher R. Mueller, Anne-Marie Mes, Betsy J. Pommerantz and John A. Hassell, Department of Microbiology and Immunology, McGill University, 3775 University Street, Montreal, Quebec H3A 2B4 Canada.

Transcription of the polyoma virus early region is effected by cellular RNA polymerase II and is controlled by a product of this region, large T-antigen. To understand the mechanism of transcriptional regulation by large T-antigen requires knowledge of the spatial organization of the T-antigen binding sites relative to the early promoter. In order to position these sequences on the viral genome we constructed deletion mutations in polyoma virus-plasmid recombinants and measured the capacity of the resulting mutants to be expressed in vivo and in vitro, and to bind large T-antigen. Our results reveal that two and perhaps three upstream sequence elements are required for maximal gene expression in vivo as measured by transformation assays. The 5' border of the farthest upstream element is located greater than 600 bp from the site where the 5' termini of the abundant early mRNAs map. A second sequence located near the region where DNA replication initiates may also be required for efficient in vivo expression. Finally, a third element (element 3) has been located that is required for accurate transcription in vitro, but which is dispensable for in vivo expression. Two T-antigen binding regions have been mapped. One of these overlaps with the transcriptional element 3, while the other lies farther upstream near the site of initiation of viral DNA replication. The location of the T-antigen binding sites relative to the early promoter suggests to us that large T-antigen impairs transcription initiation during the late phase of infection.

0361 ISOLATION OF HOST CELL EXPRESSION SEQUENCES, Mike Fried, Moira Griffiths, *Girolama Lamantia and *Luigi Lania, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, *Istituto di Biologia Generale e Genetica, Napoli 80100, Italy.

The early region of polyoma virus (Py) DNA devoid of most of its 5' sequences is greatly reduced in its transforming ability. We have attached fragments of host cell DNA 5' to a fragment of Py DNA containing an intact transforming region and viral sequences 60bp upstream from the initiation of transcription of the Py early mRNAs. The Py transforming region and its adjacent 5' host sequences were cloned from cellular DNA from a transformed cell colony induced by the ligated viral-cellular fragments. The initial clone which contained 6.1 Kb of 5' host DNA sequences, transformed rat cells with 10-40% the efficiency of the Py transforming region with all its 5' expression sequences. The sequence of the 150 bp of host DNA 5' to the viral sequences has been determined. Deletion studies indicate that the efficient expression of the Py transforming region is mediated by host sequences between 16-62 bp 5' to the Py sequences.

0362 TRANSCRIPTIONAL ENHANCING ACTIVITY IN THE IMMUNOGLOBULIN HEAVY CHAIN LOCUS, Mark Mercola and Kathryn Calame, UCLA, Los Angeles, CA 90024

We have identified a transcriptional enhancing activity in the mouse Cμ immunoglobulin gene using an in vivo expression system. This is the first example of a non-viral enhancing activity, although such a function has been postulated to be involved in the regulation of gene expression in higher organisms. The enhancing activity is located in the large intervening sequence between Jh and Cμ. This location is consistent with earlier studies showing the transcription of heavy and light chain constant regions in the non-rearranged configuration and with the appearance of a DN'ase I hypersensitive site in the corresponding region of an activated kappa light chain gene. The immunoglobulin enhancing region functions like viral enhancers in that it can act either 5' or 3' to a transcription unit and elevates transcription from the heterologous SV40 early promoter. We are currently studying further the precise location, structure and function of the immunoglobulin enhancing activity. VDJ joining in the heavy chain locus during B-cell development brings a Vh gene 5' promoter region into close proximity to the Jh-Cμ intervening sequence. Thus, a transcriptional enhancing activity in the Jh-Cμ intervening sequence could explain the activation of immunoglobulin gene transcription upon VDJ joining.

0363 EXPRESSION OF CLONED IMMUNOGLOBULIN GENES IN MOUSE L CELLS, S. Gillies and S. Tonegawa Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

Cloned immunoglobulin genes containing functionally rearranged V and C coding sequences, as well as 5' and 3' flanking sequences, were introduced into mouse L cell fibroblasts (tK^-) by co-transformation with the Herpes thymidine kinase (tK) gene. Cell clones containing integrated genes for λ_1 or κ light chain or γ_{2b} heavy chain were tested for the expression of immunoglobulin mRNA and protein. In the case of light chain genes, polyA(+) RNA was detected by Northern blot analysis which contained either λ_1 or κ sequences. In both cases, however, the predominant species of mRNA did not co-migrate on denaturing agarose gels with authentic immunoglobulin mRNA and light chain protein could not be detected by immunofluorescent staining or immunoprecipitation. Cell clones containing integrated γ_{2b} genes, on the other hand, were found to synthesize the correct size γ_{2b} mRNA and heavy chain protein. Comparison of the 5' termini of γ_{2b} mRNA from transformed L cells and the myeloma from which the gene was cloned suggests that transcription was initiated from the same site in both cases.

0364 PRODUCTION OF IMMUNOGLOBULIN MOLECULES IN ESCHERICHIA COLI. Michael A. Boss and Spencer Emtage, Celltech Ltd., 250 Bath Road, Slough, Berks SL1 4DY, England.

We are investigating the expression of immunoglobulin molecules in E.coli vectors using cDNAs from monoclonals to the hapten (4-hydroxy-3-nitrophenyl) acetyl (NP).

Using the trp promoter a full-length cDNA coding for λ1 light chain has been expressed at high level. Following separation of E.coli extracts into soluble and insoluble fractions, the λ1 protein is found exclusively in the insoluble fraction even under circumstances where only low levels of λ1 are being synthesised. We are currently purifying the λ1 protein and recombining the cloned product with immunoglobulin heavy chain molecules from myelomas directed to NP.

0365 EXPRESSION OF HUMAN DIHYDROFOLATE REDUCTASE cDNA IN B. SUBTILIS, Carlo Morandi, Marta Perego and Giorgio Mazza, Istituto di Genetica, Università di Pavia, 27100 PAVIA, Italy.

By inserting the human DHFR gene (cDNA) in the PstI site of pHV14 (a bifunctional E. coli - B. subtilis hybrid plasmid), we obtained a new hybrid plasmid pMP512 which is capable of expressing the DHFR gene (on the basis of its ability to confer trimethoprim resistance (Tmp^R) to the host strain) only in E. coli. Since the failure of expression in B. subtilis could be due to the inability of B. subtilis RNA polymerase to recognize the β-lactamase gene promoter of pHV14, we tried to insert a B. subtilis promoter upstream the DHFR gene in plasmid pMP512; a new plasmid was indeed obtained (pMP358), capable to confer Tmp^R to B. subtilis.

Restriction analysis of plasmid pMP358, demonstrated that its ability to confer Tmp^R to B. subtilis cells, is a consequence of a 2072 bp deletion, which places the DHFR gene under the control of the Chloramphenicol Acetyl Transferase promoter of pHV14. Plasmid pMP358, although unable to express chloramphenicol resistance due to the deletion, allows direct selection of B. subtilis transformants as Tmp^R colonies.

DNA sequence determination of the "spliced" region is in progress.

0366 RETROVIRAL VECTORS: CONSTRUCTION AND RESCUE OF INFECTIOUS VIRUS CONTAINING A FUNCTIONAL HUMAN HPRT GENE. A. G. Miller*, I. M. Verma*, T. Friedmann+, and D. J. Jolly+
*The Salk Institute, San Diego, Ca. 92138 and +University of California, San Diego, La Jolla, Ca. 91093

We are exploring the use of molecularly cloned retroviral DNA's as vectors to introduce genes, including the rat growth hormone gene and the herpes virus thymidine kinase gene, into eukaryotic cells. Recently we have used the full length cDNA clone for human hypoxanthine phosphoribosyl transferase (HPRT)(1) to construct retroviral vectors which express functional HPRT. The 5'-LTR and adjacent viral sequences from a molecular clone of Mo-MSV proviral DNA was linked to the 5' end of the DNA fragment containing the human HPRT cDNA clone. The 3' end of the HPRT DNA was linked to a DNA fragment which contained the 3'-LTR of cloned Mo-MLV DNA. The chimeric DNA molecule, with the structure 5'-MSV LTR-HPRT-3'MLV LTR, was cloned in the plasmid pBR322. Transfection of the hybrid plasmid into HPRT$^-$ mouse or rat cells resulted in the production of HAT resistant colonies expressing human HPRT. Replication defective virions containing the HPRT gene could be rescued upon superinfection with Mo-MLV helper virus.

The availability of infectious virus containing the HPRT gene opens the possibility of high efficiency transfer of the HPRT gene to various cell types such as stem cells, lymphoblasts, and others, where other modes of transfer have been very inefficient.

(1) D. J. Jolly et al. (1982) PNAS (in press).

0367 IN VITRO SYNTHESIS BY VESICULAR STOMATITIS VIRUS OF A DISCRETE READ-THROUGH RNA PRODUCT IN THE PRESENCE OF RIBONUCLEASE INHIBITORS AURINTRICARBOXYLIC ACID AND VANADYL RIBONUCLEOSIDE COMPLEXES, Sohel Talib and John E. Hearst, Department of Chemistry, University of California, Berkeley, CA 94720.

In the presence of aurintricarboxylic acid and vanadyl ribonucleoside complexes, we have isolated and characterized a small RNA, product of VSV in vitro transcription. This RNA is capped and lacks Poly(A) at its 3'-end. Nucleotide sequence analysis revealed that this RNA corresponds to the 5'-terminal 68 nucleotides of N-mRNA and thus a read-through product corresponding to two small RNA previously identified in vitro transcription of VSV. One of these RNA represents the first 40 bases of 5'-end of N-mRNA and the other starts at position 41 and terminates at position 68 in the N-gene. Synthesis of this 68 mer RNA in the presence of ribonuclease inhibitors indicates the first evidence of RNA cleavage or processing involved in VSV transcription.

Photoreaction of VSV with 4'-substituted psoralen fails to inhibit the synthesis of the 68 mer RNA under conditions where full length mRNA synthesis diminished by more than 90%, indicating that psoralen binding site is located beyond 68 bases in the N-gene. Since this is the only RNA synthesized at very high concentrations of venadyl ribonucleoside complexes and aurintricarboxylic acid, the possible role of this RNA in the transcription replication of VSV will be discussed.

0368 INHIBITION OF THE TRANSFORMING ACTIVITY OF MOLONEY SARCOMA VIRUS DUE TO CYTOSINE METHYLATION. M. L. McGeady, C. Jhappan, R. Ascione, G. F. Vande Woude, Laboratory of Molecular Oncology, National Cancer Institute, Bethesda, Maryland.

The transforming activity of cloned Moloney sarcoma virus proviral DNA was tested after in vitro methylation. Methylation of the DNA with HpaII methylase, HhaI methylase, or both enzymes resulted in a 70% decrease in its transformation efficiency. Cotransfection of a plasmid containing the long terminal repeat (LTR) of the virus with a plasmid containing v-mos results in efficient activation of the transforming potential of mos. Methylation of the LTR containing plasmid resulted in a 50% drop in the transforming activity. However, an 80% decrease was observed when the mos containing plasmid was methylated. In these experiments methylation of the DNA by HhaI methylase was as inhibitory as HpaII methylation. Knowing the location of the methylated sites in the LTR and mos suggests that methylation in the 5' half of mos is most important in preventing mos gene expression, although methylation at or slightly downstream from the transcriptional start site in the LTR is also inhibitory. The MSV proviral genomes in the cells transformed by methylated DNA were sensitive to digestion with HpaII suggesting that the loss of methyl groups was necessary for mos expression to occur. In order to obtain nontransformed cell lines containing fully methylated copies of MSV proviral DNA cotransfection with the selectable marker Ecogpt was utilized. Cell lines obtained by this method are currently being used to study the mechanism by which cytosine methylation prevents mos expression.

0369 DNA MEDIATED GENE TRANSFER IN HAMSTER FIBROBLAST CELLS. Ming-Ling Janet Wang and Amy S. Lee, University of Southern California, School of Medicine, Los Angeles, CA 90033.

We are interested in using DNA mediated gene transfer technique to determine functional DNA sequences involved in gene expression. To achieve this goal, we use K12, a ts mutant of Chinese hamster lung fibroblast as a model system to study the expression of a cloned gene specifically induced in this mutant. We have previously shown that the hamster K12 cells are highly competent for DNA mediated gene transfer using dominant selective markers, e.g., pSV2gpt and pNE03. The transformation efficiency is 5×10^{-3} for pSV2gpt and several fold lower for pNE03. Analysis of DNA extracted from individuals or pooled groups of several hundred transformants reveal that in our carrier-DNA free transformation experiments, the vector DNA are present as polymers integrated into the recipient genomes. The copy number of the vector sequence varies from 5 to 700. Work is now in progress to ligate the flanking genomic sequence of the inducible gene to vectors which contain dominant selective markers. The recombinant plasmids are then used to transform the ts mutant and the expression of the structural genes is studied at both the permissive and non-permissive temperature.

(Supported by NIH grant CA27607)

0370 INITIATION OF POLIOVIRUS REPLICATION IN VITRO, Margaret H. Baron and David Baltimore, Mass. Inst. Technol., Cambridge, MA 02139

Poliovirus replicase can be isolated in a form which depends on either oligo(U) or on a host cell protein for the initiation of copying of poliovirus virion (plus strand) RNA. The "host factor" has been purified several thousand-fold from an uninfected HeLa cell postmitochondrial supernatant. The product of the replicase reaction includes full length (35S) RNA molecules and contains the RNase T1-resistant oligonucleotides of the poliovirus minus strand.

Poliovirion RNA is covalently linked at its 5' terminus to a small protein (VPg). VPg was chemically synthesized, coupled to BSA carrier, and injected into rabbits. All antisera reacted with virus-specific proteins from HeLa cells infected with poliovirus. Three of these proteins have previously been implicated by others as precursors of VPg. No free cytoplasmic VPg could be detected. Anti-VPg antibodies inhibited host factor-dependent RNA synthesis by the replicase but not oligo(U)-primed synthesis, implicating VPg in the *de novo* initiation of replicase products. Complexes of VPg-related polypeptide and newly made RNA could be immunoprecipitated by anti-VPg antibody from the host factor-stimulated products of the replicase reaction.

0371 MAPPING OF TYPE I AND TYPE II DNA TOPOISOMERASE BINDING SITES ON SV40 CHROMATIN, Eric M. Nelson and Leroy F. Liu, The Johns Hopkins University Medical School, Baltimore, MD 21205

Using a P4 unknotting assay, we have purified several mammalian type II DNA topoisomerases. Type II DNA topoisomerases catalyze ATP (or dATP)-dependent strand passing of double-stranded DNA, including relaxation, knotting/unknotting and catenation/decatenation. At high enzyme concentrations, DNA topoisomerase II induces both single- and double-stranded DNA breaks, and the enzyme is covalently linked to the 5'-end of the breaks. Several antineoplastic drugs, such as ellipticine and adriamycin strongly inhibit the catalytic activity of DNA topoisomerase II while DNA topoisomerase I activity is unaffected. In addition, ellipticine stimulates the double-stranded cleavage reaction of DNA topoisomerase II on DNA.

We have observed that DNA topoisomerase II activity is associated with SV40 minichromosomes in a sucrose density gradient containing 0.1M NaCl. Purified topoisomerase II can catenate SV40 minichromosomes and the reaction is stimulated by histone H1 and HMG proteins. Double-stranded cleavage of SV40 minichromosomes by high concentrations of topoisomerase II has also been demonstrated. This reaction is stimulated by ellipticine. We are currently mapping the topoisomerase II binding sites on SV40 DNA, isolated SV40 minichromosomes and SV40 chromatin *in vivo*. DNA topoisomerase I has also been shown to be tightly associated with SV40 minichromosomes isolated from virions and infected cells. The binding sites of DNA topoisomerase I on SV40 chromatin will similarly be determined. We will discuss how topological changes of chromatin structures may influence gene expression.

0372 TUMOR VIRUS GENOMES IN DNA-TRANSFORMED F9 CELLS, Kay Huebner, The Wistar Institute of Anatomy & Biology, Philadelphia, PA 19104

In order to investigate the molecular bases for restriction of expression of papova and oncorna viruses in teratocarcinoma derived stem cells we have constructed a series of recombinant plasmids, each containing a linear oncogenic viral genome inserted into a pBR322/HSV-1 tk (Herpes Simplex virus type 1 thymidine kinase gene)recombinant, pHSV-106. These pBR/tk/oncovirus plasmids have been transfected into thymidine kinase deficient F9 cells ($F9TK^-$) and transformants isolated on the basis of tk expression. Transformants, designated F9/SV, F9/Py, F9/PyEC, and F9/Ki containing the SV40, polyoma, polyoma EC mutant, or the Kirsten murine sarcoma (KiMSV) viral genomes respectively, were characterized as to phenotype, by determining presence or absence of stem cell specific properties, and genotype, by analyzing organization of the transfecting viral genome within the stem cell genome. F9/SV transformants which have been characterized most thoroughly thus far have been shown to express SV40 specific mRNAs which by several criteria resemble authentic SV40 early mRNA. This report describes further studies of F9/SV cells and preliminary studies on characterization of F9/Py, F9/PyEC and F9/Ki cells.

0373 MICROINJECTION OF A HUMAN/RAT GROWTH HORMONE GENE INTO MOUSE EMBRYOS Richard F Selden, Thomas E. Wagner*, Edmund Tischer, and Howard M. Goodman. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02138 *Departments of Chemistry, Zoology-Microbiology, and Biomedical Sciences, Ohio University, Athens, OH 45701

A human/rat hybrid growth hormone gene was cloned into pBR327. Approximately 2000 copies of the construct were microinjected into the male pronucleus of mouse zygotes, which were then transferred to pseudopregnant or pregnant mothers. Close to 15% of the mice born contained the injected DNA as evidenced by Southern hybridization analyses. In the animals studied thus far, the construct was present in 5-15 copies/cell as an extrachromosomal element. It appears that in at least one case, several copies of the construct were joined to produce a large element that yields restriction fragments not predicted based on the physical map of the injected construct.

0374 STRUCTURE AND BIOLOGICAL ACTIVITY OF v-*raf*, A NEW ONCOGENE TRANSDUCED BY RETROVIRUS, U.R. Rapp, M. Goldsborough, G.E. Mark, T.I. Bonner, J. Groffen, and J.R. Stephenson, Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701

We have isolated from mouse (1,2) a new acutely transforming replication defective type-C virus (3611-MSV) and the acquired oncogene is being characterized. 3611-MSV encodes two polypeptides (p75 and p90) containing NH_2-terminal *gag* linked with nonstructural protein. Neither p75 nor p90 have intrinsic or associated protein kinase activity. The restriction map of the molecularly cloned viral genome closely resembles that of Mo-MuLV, except in a region between the middle of p30 and the middle of the polymerase gene. Heteroduplex analysis with Mo-MuLV DNA revealed that 2.4 kb of *gag*-pol DNA were replaced by 1.14 kb of cellular DNA. The exact locations of viral and cellular exchange were determined by DNA sequencing to be 517 nucleotides into the p30 sequence and 1930 nucleotides into the polymerase sequence. Comparison of the transforming gene from 3611-MSV, designated v-*raf*, with previously isolated retrovirus oncogenes by Southern blot hybridization and/or nucleic acid sequencing have shown it to be unique. Transfection of NIH/3T3 cells with cloned 3611 MSV DNA leads to highly efficient transformation and the recovered virus elicits tumors in mice typical of the parent virus. The cellular homolog, c-*raf*, is present in 2-4 copies in mouse and human DNA. The structure of c-*raf* from mouse and man as well as their chromosomal location and biological activity will be presented.

1. Rapp, U.R. and Todaro, G.J.: Science 201: 821-824, 1978.
2. Rapp, U.R., Reynolds, F.H., and Stephenson, J.R.: J. Virol., 1982, in press.

0375 PROTEINS ENCODED NEAR THE ADENOVIRUS LATE MESSENGER RNA LEADER SEGMENTS, Carl W. Anderson[1] and James W. Lewis[2], [1]Biology Department, Brookhaven National Laboratory, Upton, NY 11973, and [2]Fred Hutchinson Cancer Research Center, Seattle, WA 98104

Small fragments of adenovirus 2 DNA cloned into the single-stranded phage M13 were used to select adenoviral mRNAs transcribed from the R-strand between map positions 16 and 30. Cell-free translation of these mRNAs produced proteins of 13.5K, 13.6K, and 11.5K, respectively encoded between the first and second segments of the tripartite major late leader, within the "i"-leader segment, and immediately preceding the third leader segment. Partial sequence analysis of the 13.6K protein indicates that its initiation site is at nucleotide 7968 from the left end of the adenovirus genome and 27 nucleotides from the beginning of the i-leader open reading frame. Sequence analysis of the late region 1 (L1) 52,55K protein shows that at least a portion of this product begin at a site corresponding to N11040, just beyond the end of the VA II RNA gene. The 13.5K and 11.5K proteins are encoded by regions containing open reading frames. Efforts are in progress to determine the precise locations encoding these proteins and to identify all four products in infected cells.

0376 TRANSFER OF THE HUMAN GROWTH HORMONE GENE TO MICE, Peter J. Kushner and David W. Martin, Jr. University of California, San Francisco, San Francisco, California 94143.

We have introduced the human growth hormone gene into live mice by injection of cloned DNA into single cell embryos which are then transferred to the oviducts of pseudo-pregnant recipients. About 15 kb of human genomic DNA spanning the growth hormone gene along with the lambda phage vector is present intact and in multiple copies in an apparently normal male mouse, HGH-7, as shown by blot hybridization. The transferred DNA has a mosaic distribution, present in variable amounts (7-12 copies/cell) in HGH-7's tail, but absent from the ear. In addition, the germ tissue is likely mosaic since the human DNA appears in only 1/6 of the F_1. The progeny mice have approximately 30 copies of the transferred gene per cell. We are currently investigating the expression of the human gene as well as the mode of transmission to the F_2 generation.

0377 S-1 NUCLEASE MAPPING OF VIRAL RNA FROM MuSV tsl10 - A POSSIBLE TEMPERATURE-SENSITIVE SPLICING MUTANT OF MuSV, Edwin C. Murphy, Jr., Micheal A. Nash and Nicole V. Brown, Dept. Tumor Virology, UT M. D. Anderson Hosp. & Tumor Inst., Houston, TX 77030.

MuSV tsl10 is a ts mutant of MuSV which transforms cells at 33° but allows reversion to normal morphology at 39°. At 39°, infected cells contain a truncated gag gene product, $P58^{gag}$, and a 4.0 kb viral RNA. At 33°, a transforming gene product, $P85^{gag-mos}$, and a 3.5 kb viral RNA are also present. Heteroduplex analysis (Junghans et al., 1982) has shown that the 4.0 kb RNA is derived from wild-type 5.3 kb RNA by a central deletion of 1.5 kb, resulting in a fusion of the gag and mos genes, probably out of frame since only $P58^{gag}$ is translated from this RNA. The 3.5 kb RNA also has a central deletion, in this case of about 1.9 kb, extending about 0.5 kb further into the gag gene than the deletion in the 4.0 kb RNA. By S-1 nuclease mapping we find that the 5' border of the deletion in the 4.0 kb RNA is at about nucleotide 2412 in the wild-type DNA sequence (Van Beveren et al., 1981) (21 amino acids from the C-terminus of p30) and is joined to approximately nucleotide 3885, a position about 10 nucleotides downstream from the first mos gene ATG codon. The 3.5 kb RNA has a 5' deletion border of approximately nucleotide 1900 (20 amino acids from the N-terminus of p30) joined to nucleotide 3829 in the mos gene (about 50 bases downstream from the first mos ATG codon and 40 bases upstream from the second mos ATG codon). The presence of a splice acceptor sequence surrounding nucleotide 3829 and the fact that blot hybridization analysis shows that only one tsl10-related viral DNA is extant in infected cells suggests that the 3.5 kb RNA is derived by splicing of the 4.0 kb RNA.

0378 ALTERATION OF THE REVERSION FREQUENCY OF TRANSFORMED NIH/3T3 MOUSE CELLS, Robert F. Baker, Molecular Biology Division, University of Southern California, Los Angeles, CA 90089-1481

We have measured the frequency of reversion (to an anchorage-dependent phenotype) of spontaneously transformed (anchorage-independent, neoplastic) NIH/3T3 mouse cells. This reversion frequency can be increased from 5 - 10 revertants per 10^6 transformed NIH/3T3 cells (spontaneous reversion frequency) to a maximum of 70 - 80 revertants per 10^6 cells. This increase resulted after DNA-mediated transfection of a population of transformed NIH/3T3 cells, with DNA obtained in the following way: A first population of transformed NIH/3T3 cells was transfected with mouse or human whole cell DNA pieces and then immediately placed under selective pressure for reversion. (This selective pressure resulted from placing the transfected cells into media containing a low concentration of serum and then exposing the culture to cycles of 5-bromodeoxyuridine and UV light). Hirt supernatant (containing extrachromosomal DNA resulting from the transfection of the DNA pieces) from the revertants was spread into an increasingly larger number of transformed NIH/3T3 cells by cycles of transfection, of succeeding new populations of transformed cells, with Hirt supernatant DNA obtained from the non-passaged revertants selected at each previous cycle. Reversion activity in sixth cycle Hirt supernatant DNA was sensitive to EcoRI, but not to BamHI or Sal I. When the first population (cycle one) of transformed NIH/3T3 cells had been transfected with human DNA pieces (to initiate the extrachromosomal DNA in the Hirt supernatants obtained at subsequent cycles), the mouse cell revertants obtained at the sixth cycle contained chromosomally-integrated human Alu I-like sequences (Baker, R. F., Proc. Nat. Acad. Sci. USA, in press).

0379 **GROWTH RATE-DEPENDENT REGULATION OF GND EXPRESSION IN ESCHERICHIA COLI,** **R.E. Wolf, Jr., H.V. Baker, II, and M.S. Nasoff, University of Maryland Baltimore County, Catonsville, MD 21228**

Synthesis of 6-phosphogluconate dehydrogenase (6PGD), encoded by *gnd*, is proportional to the cellular growth rate, dependent on gene dosage, delayed after a nutritional shift-up, and not subject to stringent control. The DNA sequence of *gnd* has been determined and the startpoints of transcription and the structural gene identified. *gnd* mRNA has a 56 base leader that contains a strong ribosome binding site; it does not contain an attenuator like the one proposed to regulate the *E. coli* β-lactamase gene. However, there are several regions of potential secondary structure that may hinder ribosome binding and thereby make the translational yield of 6PGD dependent on ribosome concentration. The codon usage pattern of *gnd* resembles that of highly expressed genes. *gnd-lac* operon and gene fusions have been prepared with phages Mud1 and Mud2 and the fusion joints mapped genetically. Whereas the level of β-galactosidase increases with increasing growth rate in 7 of 8 gene fusions, it is invariant in the operon fusions. These results suggest that growth rate dependent regulation is carried out by a post-transcriptional mechanism. Both reverse genetic methods with the cloned gene, and conventional mutant selections in the fusion strains are being employed in the search for mutants affected in the growth rate dependent regulation.

0380 MOLECULAR GENETICS OF HEMOGLOBIN GENES AND ERYTHROPOIESIS IN FRIEND CELLS, Alfred I. Geller and Paul Berg, Dept. of Biochemistry, Stanford U Medical Center, Stanford Cal., 94305

Two central questions in development are how the expression of a regulated gene such as hemoglobin is controlled and how the expression of many such genes is coordinated to produce a differentiated cell type such as an erythrocyte. We have developed a system to study these two questions by transfecting Friend Cells with vectors containing a selectable marker (*E. coli* neo) under the control of the putative globin regulatory elements and obtained cell lines which acquire resistance to the drug G418 following induction. This developmental system is now equivalent to any inducible system in prokaryotes. The sequences required for the induction of each of the three adult mouse globin genes--the cis elements--reside within several KB of DNA 5' to the cap site. Deletion analysis, now in progress, should define the essential elements within this region and how they function. A genetic analysis of the genes which control erythropoiesis--the trans elements--is being attempted. Both [illegible] genetic selection for recessive mutants and complementation tests are possible. This system permits an analysis of the [illegible] details of the regulation of both the [illegible] globin genes and erythropoiesis, extending our understanding of development.

0381 DEVELOPMENTAL CONTROL OF THE XENOPUS 5S RNA GENE SYSTEM, Peggy J. Farnham, Jennifer Price and Laurence Jay Korn, Stanford University, Stanford, California 94305
There are two major classes of 5S RNA genes in Xenopus. One class codes for somatic-type 5S RNA and is expressed in both oocytes and somatic cells. The other class, which is 50 times more abundant and codes for oocyte-type 5S RNA, is expressed in oocytes but not in somatic cells. The developmentally inactivated oocyte-type genes can be reactivated when erythrocyte nuclei are injected into oocytes. Since the injected nuclei are induced to take on the natural activity of the host cell, the activation of oocyte-type 5S genes may represent a normal control mechanism. An opportunity to investigate this mechanism is provided by the existence of certain individual animals whose oocytes fail to activate 5S oocyte genes (non-activating females). We have injected extracts of oocytes from activating and non-activating females, together with erythrocyte nuclei, into oocytes of each kind. We have shown transcription of 5S genes is induced by extracts of activating oocytes and is not inhibited by similar extracts of non-activating oocytes. The activating factor is sensitive to protease and to heat treatment, suggesting that a protein is involved. This in vivo regulation can be maintained in vitro using somatic nuclei in the transcription reaction. This in vitro system, along with the in vivo injection system, provides an assay for extracts from activating oocytes. We plan to fractionate these extracts and add the fractions to the developmentally inactive systems in order to isolate the protein(s) involved in the reactivation of the oocyte 5S RNA genes. Eventually, we hope to reconstitute a molecular environment which can mimic the developmental regulation of 5S DNA.

0382 STUDIES OF THE HUMAN RECEPTOR FOR T CELL GROWTH FACTOR, Warren J. Leonard, Joel M. Depper, Jeri Roth, Thomas A. Waldmann, and Warner C. Greene, NIH, Bethesda, MD 20205
The binding of T cell growth factor (TCGF or interleukin-2) to its surface receptor is critical for the activation and expansion of cytotoxic, suppressor, and helper T lymphocytes. The action of these cells is required for a normal human immune response. Both the production of TCGF and its receptor are inducible events, occurring after exposure to antigen or mitogen; neither is found in resting, uninduced T cells. We have prepared a monoclonal antibody termed anti-Tac that appears to bind to the membrane receptor for TCGF. This receptor is a glycoprotein of approximately 50,000 daltons in HUT-102B2 cells (Nature, in press). In these cells, the receptor has a pI of 5.5-6.0, and by crude estimates represents 0.05% of total cellular protein synthesis. In PHA activated lymphoblasts, the receptor is slightly larger, perhaps due to differences in the extent of glycosylation. Time course studies of ^{125}I-anti-Tac binding following PHA activation of resting lymphocytes demonstrated that the putative TCGF receptors appeared on the cell surface within 4-8 hours of lectin addition, indicating the rapid induction of TCGF receptor gene expression. We have also found that the receptor is expressed in all Human T Cell Leukemia Virus (HTLV) infected cell lines studied, and thus may be involved in the growth of these cells. The antibody is being used diagnostically and will soon be used therapeutically in patients with HTLV and cutaneous T cell leukemias. We are using the antibody to purify large quantities of the receptor to obtain primary amino acid sequence data. Further, we are attempting to develop a cDNA probe recognizing the gene encoding the human TCGF receptor.

0383 CELL TYPE-SPECIFIC EXPRESSION AND PHEROMONE INDUCTION OF THE STE3 GENE OF YEAST, David C. Hagen and George F. Sprague, Jr., University of Oregon, Eugene, OR 97403
The yeast, Saccharomyces cerevisiae, displays three cell types -- the two mating types, a and α, and the product of mating, a/α. Cell type is determined by a single genetic locus, the mating type locus (MAT), which codes for regulatory proteins affecting the expression of various unlinked genes, genes whose products are responsible for the characteristic traits of the different cell types. STE3 is an example of a gene which, although present in all three cell types, is essential for mating only in α cells and has no known function in a or a/α cells. By employing molecular cloning techniques, we had shown earlier that STE3 RNA is present only in α cells and not in a or a/α cells. Extending these studies, we now have discovered a new mode of STE3 control. STE3 RNA is induced fourfold in α cells that are in the process of mating with a cells. Furthermore, a-factor, a pheromone produced by a cells, is sufficient to induce STE3 in α cells, and the induction can occur in the absence of new protein synthesis. Thus there are two distinct aspects to the regulation of STE3 expression: The control of STE3 by MAT and the induction of STE3 by a-factor. To understand the mechanisms of this dual control, we have constructed a STE3-lacZ gene fusion from which β-galactosidase is synthesized in yeast cells under STE3 control, and we have used the fusion to define the regulatory regions of the STE3 gene.

0384 EXPRESSION OF METALLOTHIONEINS IN SACCHAROMYCES CEREVISIAE (YEAST), Tauseef R. Butt, Dept. of Mol. Pharmacology, Smith Kline & French Laboratories, 1500 Spring Garden Street, Philadelphia, PA 19101

Conservation of cysteine residues of metallothioneins (Mt) throughout genetic phyla is a hallmark of these proteins. We have isolated two major species of yeast Mt; in its properties, like its eukaryotic counterpart, the yeast proteins are rich in cysteine, small Mol Wt and bind metals. Several yeast strains have been isolated which are resistant to cadmium, copper and gold compounds. A copper resistant strain (Cu^R) is of particular interest as it synthesized large amounts of Mt which represented 10% of the total cellular proteins. The presence of copper (1 mM) was required for continuous synthesis of Mt in yeast. When the Cu^R strain is grown in the presence of 1 mM $CuSO_4$, 50% of the total cellular cysteine is incorporated in Mt. Precursors of cysteine such as cystathionine or methionine enhanced the growth rate of the Cu^R strain up to 50%.

Amplification of the Mt gene has been implicated in the aquisition of copper resistance in yeast. In order to identify the DNA sequences involved in copper resistance we have constructed a Cu^R yeast DNA library on YEP 13, a yeast vector . Transformation of copper sensitive yeast with DNA library has led us to identify the plasmids which confer resistance to 1.5 mM $CuSO_4$. Further studies are underway to characterize the factors involved in regulation and amplification of Mt genes.

0385 Regulation of Cholesterol Biosynthesis in Mammalian Cells - Michael Sinensky Eleanor Roosevelt Institute for Cancer Research, Denver, Colorado

Somatic cell genetic analysis of the enzymes of mevalonate biosynthesis reveals a complex process which is in part regulated at the level of enzyme biosynthesis. Various somatic cell mutants have been isolated which permit the isolation of mRNA probes and genomic clones for these enzymes which should lead to the molecular mechanism for their regulation. Progress towards cloning one of these genes, 3-hydroxy-3-methyl glutaryl coenzyme A synthase, will be presented at these meetings in co-authorship with Ms. S. Leonard.

0386 GENE EXPRESSION IN DIFFERENTIATING DROSOPHILA WING, Nancy S. Petersen and Herschel K. Mitchell, Division of Biology, California Institute of Technology, Pasadena, CA 91125

Rapid changes in gene expression accompany differentiation in Drosophila wings. It is particularly easy to see these changes in wings because they represent a nearly homogeneous population of nondividing cells which differentiate synchronously. The program of gene expression involves changes of more than tenfold in rates of synthesis of all of the major proteins synthesized in wings. Most of the changes in protein synthesis reflect changes in mRNA concentration. The most dramatic changes in protein synthesis coincide with the most extensive changes in cell morphology, suggesting a direct relationship between the program of gene expression and the morphological changes.

The induction of developmental defects by heat can be used to study regulation of gene expression during differentiation and to see how changes in gene expression affect morphology. Heating does cause developmental abnormalities in a wide variety of organisms including mammals. In Drosophila, the defects are called phenocopies because of their resemblance to mutant defects. The defects are most likely the result of interruption of gene expression at crucial periods in development. A heat shock sufficient to induce a phenocopy shuts off both RNA and protein synthesis, and stabilizes messages which would normally decay as part of the developmental program. The resumption of development following heat shock involves resumption of message decay as well as new message synthesis. By looking in detail at the way specific phenocopies are induced and prevented, we hope to discover information about how gene expression is regulated during development.

0387 INDUCTION OF INTERLEUKIN 1 mRNA BY LIPOPOLYSACCHARIDE STIMULATION OF MACROPHAGES, Jolene J. Windle*, Hyun S. Shin, and John F. Morrow*, Howard Hughes Medical Institute*, The Johns Hopkins University School of Medicine, Baltimore, MD. 21205

Interleukin 1 (IL-1, lymphocyte activating factor) is a macrophage-derived protein which possesses a broad range of activities, including a) enhancement of T lymphocyte proliferation, b) promotion of lymphocyte helper functions and antibody production, c) stimulation of acute phase protein synthesis (i.e., serum amyloid A) by hepatocytes, d) stimulation of fibroblast proliferation, and e) induction of fever. We have obtained mRNA for this protein, translated it in oocytes, and measured biological activity in a thymocyte proliferation assay.

To induce IL-1, we incubated alveolar macrophages from BCG-stimulated rabbits or $P388D_1$ cells (a murine macrophage-derived cell line) with LPS (lipopolysaccharide). At various times after stimulation, poly A^+-RNA was purified and translated in X. laevis oocytes. The translation products were assayed for thymocyte proliferation activity. There was little or no detectable IL-1 mRNA in unstimulated cells from either cell type, but IL-1 mRNA was present by 2 hr. of LPS stimulation. Most of the activity was found in the oocyte culture medium rather than in the oocyte homogenate, suggesting that the oocytes are secreting the protein. The IL-1 produced by oocytes injected with macrophage RNA chromatographs on Sephadex G-75 identically to the IL-1 produced directly by the macrophages (M. 18,000d).

These results suggest that the secretion of IL-1 is controlled at the level of its synthesis. This assay provides a means for studying the production and abundance of IL-1 mRNA, and therefore for studying early events in immune and acute phase responses.

0388 ANALYSIS OF ECDYSTERONE-INDUCED EXPRESSION OF CLONED DROSOPHILA GENES IN LARVAL FAT BODIES, Yoshinobu Nakanishi and Alan Garen, Yale University, New Haven, Conn 06511

During the third-instar stage of larval development, ecdysterone induces expression of the P1 and LSP-2 genes, as indicated by increased level of the encoded transcript and polypeptide in the fat bodies. This effect of ecdysterone was demonstrated with the temperature-sensitive mutant ecd^1, in which the ecdysterone titer fails to increase after a temperature shift from 20° C to 29° C. When the shift occurs shortly before P1 expression would normally begin, expression does not occur unless the larvae are supplemented with exogenous ecdysterone. The same effect has been obtained with cultured fat bodies from shifted ecd^1 larvae; The level of P1 transcript does not increase unless the culture medium is supplemented with ecdysterone. The response to ecdysterone occurs within two hours and is not inhibited by cycloheximide, indicating that the hormone acts directly on the P1 gene rather than indirectly on another gene whose product induces P1 expression. Induction of both P1 and LSP-2 expression by ecdysterone appears to occur at the level of transcription, as indicated by the incorporation of ^{32}P-UMP into the P1 and LSP-2 transcripts synthesized in nuclei isolated from cultured fat bodies.

0389 A MOLECULAR MARKER FOR THE UNDIFFERENTIATED MOUSE EMBRYONAL CARCINOMA CELL LINES, Philippe Brûlet and François Jacob, Service de Génétique cellulaire du Collège de France et de l'Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15.

Cell lines and specific molecular markers are a necessary requirement to study the genetic regulations during the first cellular differentiation in the mouse embryos. It is now well established that embryonal carcinoma (EC) cell lines present strong developmental analogies with the early embryonic cells.

Using a cDNA clone we have shown that all EC lines contain an abundant high molecular weight RNA (about 6 kb) which disappears with the process of differentiation. Part of this RNA is moderately repeated in the mouse genome.

Results will be presented on the identification of this RNA species and on analogy between the repeated element and known sequences in the mouse genome.

0390 DEVELOPMENTAL REGULATION OF HEAT-SHOCK GENE EXPRESSION IN XENOPUS LAEVIS, Mariann Bienz, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, England.

Xenopus oocytes respond to high temperature (over 31°C) by the synthesis of one major 70 kdal protein (hsp 70) and by a gradual reduction in the rate of normal protein synthesis. These two switches are controlled at the translational level, probably independently: one control step involves the activation of preexisting hsp 70 mRNA, whereas the other control step presumably alters the translational machinery so that, under heat-shock conditions, it no longer translates normal mRNAs. It can be calculated that an oocyte contains 10-100 pg hsp 70 mRNA which must be synthesised during a considerable period of oogenesis at the normal temperature and which is stored in an inactive state by a "masking mechanism". In contrast, in Xenopus somatic cells (tissue-cultured fibroblasts), the synthesis of hsp 70 and an additional small heat-shock protein is induced, but the rate of normal protein synthesis is not reduced on heat-shock. These cells control heat-shock protein synthesis by transcriptional activation of the corresponding genes (heat-shock mRNA accumulates under heat-shock conditions), whereas they lack any translational control mechanism. The transition from one to the other regulatory system occurs in early development. After the midblastula stage, normal protein synthesis is no longer reduced on heat-shock and hsp 70 is first detectable, i.e. embryos after the midblastula stage presumably exhibit the somatic type of heat-shock regulation. The current hypothesis is that heat-shock genes in Xenopus are not only regulated temporarily by heat-shock, but that they also are subject to a developmental regulation: periods of constitutive or repressed expression may exist during oogenesis or early embryogenesis.

0391 GENES PROGRAMMING CELL DIFFERENTIATION, Roger Bertolotti and Georges Lutfalla, Centre de Génétique Moléculaire, CNRS, 91190 Gif-sur-Yvette, France.

Somatic cell genetics strongly suggest that the structural genes which code for differentiated functions are susceptible to extinction or activation in the absence of genomic reprogramming (1). Consequently, programming must involve specific regulatory genes such as those which mediate extinction of the differentiated functions in somatic cell hybrids. Contrary to the former structural genes, these regulatory genes seem to be stably activated or inactivated during the process of determination ; therefore, the isolation and sequencing of such a gene should provide major information on the molecular mechanisms of cell programming.
Our effort is focused on hepatic differentiation for which selective media are available (1). Our first goals have been : 1°) to determine under which conditions several well differentiated hepatoma lines could be transfected with exogenous DNA. For this purpose, we have worked with XGPRT selection using pSV-gpt and pML2 derivatised vectors (2,3) ; microinjection,calcium phosphate precipitate and protoplast fusion give good results. 2°) to construct two new families of mammalian transducing vectors. We have inserted rep sequences in pBB3 (3) : polyoma Tag segments and mouse mitochondrial DNA. From the Hirt extracts,we have isolated new recombinant plasmids ; their shuttle properties are under investigation. On the other hand,we have selected an appropriate set of hepatoma lines the differentiation of which correspond to distinct ontogenic stages. Using RNA for microinjection and cellular DNA for transfection, we are now investigating how liver specific functions can be activated or extinguished. 1) Bertolotti, (1977) Som.Cell Genet.3, 579 ; 2) & 3) Bourachot et al. (1982) EMBO J., 1, 895.

0392 ROLE OF DNA METHYLATION IN X-CHROMOSOME INACTIVATION. P. Yen, B. Marsh, T. Mohandas, P. Patel, D. Melton, and L.J. Shapiro, Division of Medical Genetics, Harbor/UCLA Medical Center, Torrance, California, and Howard Hughes Medical Institute Laboratories and Departments of Medicine and Biochemistry, Baylor College of Medicine, Houston, Texas.

DNA methylation has been shown to play an important role in the control of expression of several genes. Its involvement in X-chromosome inactivation has been suggested by the ability of 5-azacytidine, an inhibitor of DNA methylation, to reactivate genes located on the inactive X-chromosome. We have utilized a mouse cDNA clone for hypoxanthine-guanine phosphoribosyl transferase (HPRT) with extensive homology to the human HPRT sequence as a molecular probe to examine methylation patterns in Southern blots of human and mouse DNA. When either restriction endonucleases Hpa II+Eco Rl, or Hpa II+Bcl I were used, human leukocyte DNA isolated from normal females or patients with three or five X chromosomes gave one specific band which showed a gene dosage effect and which was not present in male DNA. In the case of Hpa II+Bcl I digestion, the extra fragment (2.9 kb) comigrated with the major band given by Msp I+Bcl I digestion, and thus represents site specific demethylation. Mouse DNA isolated from female liver, kidney, or brain all gave specific bands which were absent in the male DNA when Hpa II or Hha I in combination with PvuII, Eco Rl or Hind III were used. We, therefore, feel that there is a distinctive methylation pattern of the HPRT gene associated with the inactive X chromosomes. Regardless of whether these changes are primary or secondary to the inactivation event, these results provide direct support for a role of DNA methylation in X chromosome inactivation.

0393 GENE EXPRESSION IN CULTURED RAT MAMMARY TUMOR CELLS, Roger Barraclough, Karin J. Dawson, and Philip S. Rudland, Ludwig Institute for Cancer Research, Royal Marsden Hospital, Sutton, Surrey SM2 5PX, England.

A cuboidal cell line, Rama 25, derived from a DMBA-induced mammary adenocarcinoma, changes in culture to elongated cells. These elongated cells synthesize basement membrane components and resemble primative myoepithelial-like cells. The patterns of proteins synthesized by the cuboidal cells and the elongated cells show 6% variation when analysis is carried out by two-dimensional polyacrylamide gel electrophoresis. The change from cuboidal to elongated morphology is associated with the appearance of two acidic polypeptides of molecular weights 9,000 and 15,5000 daltons, and there are also changes in cytoskeletal proteins. Cell free translation of mRNA, isolated from the cuboidal and elongated cells, shows that changes in polypeptide synthesis results from changed populations of mRNA in these cells, and is not a consequence of control at translation or post-translational protein modification.

0394 DEVELOPMENTAL REGULATION OF THE MESSENGER RNA OF A NEURON SPECIFIC PHOSPHOPROTEIN, Louis J. DeGennaro, William C. Wallace*, Paul Greengard, Max-Planck-Institute for Psychiatry, 8033 Martinsried, West Germany and * Yale Univ. School of Medicine, New Haven CT 06510, USA.

Synapsin I is a neuron-specific phosphoprotein found throughout the central and peripheral nervous system. Immunohistochemistry has localized Synapsin I in the synaptic terminal region of neurons in association with the membranes of neurosecretory vesicles. We have studied the regulation of the expression of the gene coding for this neuronal marker during postnatal development of the rat brain. Polysomes were isolated from rat brains of various ages and used to program protein synthesis in a reticulocyte lysate. Newly synthesized Synapsin I was immunoprecipitated and quantitated. The relative level of synthesis of Synapsin I directed by such polysomes increased from birth (0.05% of total *in vitro* protein synthesis) to a maximum level at 14 days of age (0.33%), then decreased to the adult level (0.15%). We believe this assay measures active Synapsin I mRNA present during development. The changes in Synapsin I mRNA observed correlate well with the major period of synaptogenesis in the rat brain.

Synapsin I mRNA has been partially purified by specific immunoprecipitation of polysomes followed by oligo dT-cellulose affinity chromatography. cDNA copies of this mRNA have been cloned, and are presently being used to characterize Synapsin I gene structure and expression.

0395 MONOCLONAL ANTIBODIES IN THE ANALYSIS OF MULTIGENE FAMILIES, Lois A. Lampson, Dept. of Anatomy, Univ. of Penna. Med. Sch., Philadelphia PA 19104

As genetic analysis becomes simpler, the organization of proteins into multi-gene families is becoming increasingly apparent. Frequently, however, it is difficult to determine whether a particular sequence of interest actually codes for a protein *in vivo*. Our work with monoclonal antibodies (MoAbs) to products of the human major histocompatibility (MHC) complex illustrates an approach to this problem.

The MHC complex is a "superfamily" that includes genes coding for the families of Class I and Class II molecules. The Class I molecules, in turn, include a subfamily consisting of the HLA-A,B,C molecules. We have used different MoAbs as probes for Class I, Class II, and HLA-A,B,C. The MoAbs were used to assess MHC expression by human cells of neuronal origin.

We find that neuronal cell lines and tumor show unusually weak Class I and HLA-A,B,C expression (less than 1% of that seen in lymphoid or glial cells), and no detectable Class II expression. Yet our results show that at least some of the weak Class I activity is due to *bona fide* HLA-A,B,C molecules, rather than cross-reactive proteins.

In a similar way, MoAbs to intrafamily determinants, individual chains, domains, or exon products should be important tools for analyzing the products of other multi-gene families.

0396 CELLULAR DEFECTS IN TS EMBRYOGENESIS MUTANTS OF THE NEMATODE CAENORHABDITIS ELEGANS, Randall Cassada, Kenneth Denich, Edoardo Isnenghi & Einhard Schierenberg, Max Planck Institute for Experimental Medicine, D-3400 Göttingen, West Germany

To analyze the genetic program for embryogenesis in this simple metazoan, we have isolated temperature sensitive lethal mutants defining 30 "emb" genes, essential for embryogenesis. Ten emb genes map together on chromosome III in a possible cluster. We estimate there will be 200-500 total emb genes from complementation data for 54 such genes so far known (Cassada et al., 1981; Hirsh et al., 1977; Miwa et al., 1980; Wood et al., 1980). The maternal genome plays the dominant role in embryogenesis, since for most emb genes maternal expression can be shown to be necessary or sufficient (or both). We can order the 30 mutants according to temperature sensitive period and arrest stage.

The cellular anatomy and lineages in living embryos in the normal 12-hour, 550-cell embryogenesis are now known (Sulston et al., 1982). Before the 100-cell stage, we see some defective cell process(es) microscopically in most emb mutants, e.g., in meiosis (polar-body formation), pronuclear migration & fusion, egg shell formation, morphology and behavior of mitotic spindles or cell membranes, timing or orientation of cell divisions. Timing defects include changes in the cell cycle clock (a 3 x reduction for all cells, or alteration of the slope of the anterior-posterior gradient of division rates or division as if both poles were posterior) or alteration of the order of division relative to other developmental events, e.g. migration in gastrulation. Defects may be related to histone defects in some of the mutants previously reported here (Certa et al., 1981).

Gene Structure and Organization

0397 Ribosomal protein genes in X.laevis, I.Bozzoni, F.Amaldi, E.Beccari, P. Pierandrei-Amaldi and A.Tognoni, C.A.N. and I.B.C. - C.N.R. - Roma

Genomic clones containing genes for the r-proteins L1 and L14 have been isolated from a X.laevis genomic library and studied in some detail. The genes contain introns which are 9 for L1 and 4 for L14. Different copies of the L14 gene differ as for the length and restriction map of their introns. Sequences present in some introns and in the 3' nontranslated regions contain middle repetitive sequences. Hybridization and hybrid released experiments have shown that these sequences are present in specific poly-A mRNA, some of which have coding capacity for specific r-proteins. We have also studied the expression of the r-protein genes during embryogenesis. Specific r-protein mRNAs start to be synthesized around gastrula stage following the production of rRNA, while their translation occurs several hours later when the synthesis of rRNA has reached a plateau. The influence of rRNA on the production of r-proteins has been shown in anucleolate mutants which lack the genes for rRNA. In these mutants the mRNA for r-proteins is present but they are not translated and rapidly disappear.

0398 An X-Linked Gene Family Differentially Regulated in Lymphocytes. David I. Cohen, Stephen Hedrick, Ellen Nielsen, William Paul, and Mark M. Davis. Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

Numerous human and mouse X-linked mutations with immunological consequences indicate that genes on the X chromosome play an important role in lymphocyte development. The frequency of such mutations suggests a large target size. A gene family could provide such a large target first by providing multiple genes for potential mutation, and second by introducing the possibility of some mechanisms for gene rearrangements or mutations such as "slipped mispairing" or gene conversion. We report here the isolation of a cDNA clone which detects a family of X-linked genes that are differentially regulated in lymphocytes.

The cDNA clone was isolated from a selected cDNA library made from an antigen-specific murine T cell hybridoma, 3.3T; 95% of the cDNA clones in this library are expressed in 3.3T but not in BAL 17, a murine B cell lymphoma. The original isolate was noted to detect a family of 8-10 bands on Southern analysis of Hind III digested mouse liver DNA. Using DNA from somatic cell hybrids, provided by Peter D'Eustachio and Frank Ruddle, we established that the family is X-linked. Northern analysis reveals that the gene is expressed as a range of transcript sizes within any given cell; at least four are distinguishable. Expression occurs in a variety of functional T cell lymphomas, but not in two immature, T cell lymphomas. Among B cell lines, expression occurs in myelomas and in a mature B lymphoma line, but not in Abelson pre-B cell lines or in the B lymphomas, WEHI231 and BAL17. Thus, at least among B lymphomas, expression seems most pronounced in more mature tumors.

0399 OESTROGEN DEPENDENT METHYLATION CHANGES AT THE 5'END OF THE CHICKEN VITELLOGENIN GENE. A.F.WILKS & J-P.JOST.(Friedrich Miescher Institut, Basel,Switzerland.)

The chicken vitellogenin gene is an oestrogen dependent gene ,expressed in the liver of egg-laying hens.Previous studies have demonstrated an oestrogen dependent under-methylation of HpaII sites at the 5'end of the gene (Wilks et al,PNAS 79 (1982) 4252-4255).One such site is located 611 b.p. upstream of the cap site.The under-methylation of this site was observed to begin around 24 hr. after hormone injection, and thus follows the onset of transcription of the gene (4-6 hr. after hormone injection). The currently favoured mechanism by which methylation changes are thought to be brought about involves replication of DNA in the absence of DNA repair methylation. Implicit in this model is the presence of hemimethylated intermediates,which would be produced during the first round of replication.Single stranded clones complementary to the region of the genome around the 5'end of the gene have been prepared and have been used to demonstrate the presence of hemimethylated DNA at early times in the induction of the vitellogenin gene.

0400 A GENETIC APPROACH TO HISTONE H2B FUNCTION IN YEAST, John Wallis, Mary Rykowski, and Michael Grunstein, Molecular Biology Institute, University of California, Los Angeles, CA 90024

Yeast histone H2B is 130 amino acids in length. The conserved carboxyl terminus of H2B (amino acid residues 45-130) is part of the apolar nucleosome core. The amino terminus (amino acids 1-44) of this molecule is variable in evolution, is physically less constrained and may have a different function outside of the conserved core. Suggestions have been made that the amino terminus may be involved in mediating higher orders of chromosomal folding.

Our laboratory is pursuing the function of this portion of H2B by constructing mutations in the protein. Deletions have been made in residues 14-18 (producing a protein only 125 amino acids in length). Insertions have also been introduced at this location producing H2B proteins 135 residues long. Yeast cells containing only the altered proteins and lacking wild type H2B are viable and grow at near normal growth rates. New deletions were constructed which lack residues 13-22 and 4-22. The effect of these mutants on viability and of any histone mutations on chromatin structure remains to be determined.

0401 CONSERVATION OF BOTH THE NUCLEOTIDE SEQUENCE AND THE SECONDARY STRUCTURE IN A SEGMENT AROUND THE TRANSLATION INITIATION SITE IN THREE COLLAGEN GENES. Yoshihiko Yamada, Maria Mudryj and Benoit de Crombrugghe, National Cancer Institute, NIH, Bethesda, Maryland 20205

Because several lines of evidence suggest a possible translational control of type I and type III collagen synthesis, we compared the sequences around the translation initiation site in three collagen genes. Two cDNAs were synthesized, corresponding to the 5' terminal parts of α1 Type I and of Type III collagen mRNA, respectively. The α1 type I cDNA was made using a synthetic oligonucleotide primer, whereas the Type III cDNA was synthesized using as primer a DNA fragment isolated from a genomic clone for Type III collagen. The nucleotide sequence of these cDNAs was determined and compared with the previously published sequence of the corresponding segment of the α2 Type I collagen gene. In each mRNA the 5' untranslated region is about 130 n long and contains two AUG codons upstream of the AUG used for initiation. There is a highly conserved sequence of about 50 nucleotides surrounding the initiation codon in all three collagen mRNAs whereas the segments preceeding and following the conserved sequence diverge from each other. The conserved segment contain an inverted repeat sequence suggesting that an identical stem-loop structure could be formed in all three mRNAs. Both the second and third AUG codons are found within this stem structure and the distance between them is the same in all three mRNAs. Because this secondary structure is highly conserved in all three collagen mRNAs, it is likely that it has a regulatory role. The requirement for this secondary structure was probably the principal factor in the conservation of the nucleotide sequence.

0402 THE STRUCTURE OF FOUR FUNCTIONALLY DIFFERENT REGIONS IN DICTYOSTELIUM rDNA CHROMATIN
Cynthia A. Edwards and Richard A. Firtel, Biology, UC-San Diego, La Jolla, CA 92093
We have used DNase I and micrococcal nuclease (MNase) to examine the structure of chromatin in Dictyostelium rDNA. rRNA is encoded on a linear, 88 kb palindrome that is repeated 90 times per haploid genome. The regions we have examined are 1) the central region, thought to contain a bidirectional origin of replication, 2) a 5.7 kb noncoding region, 3) the coding region for the 36S rRNA, and 4) the terminus, which has a $(C_nT)_m$ repeat (Emery & Weiner, Cell 26:411). Southern blots were prepared with DNA from isolated nuclei and naked DNA that were treated with nuclease. These were hybridized to probes from each of the rDNA regions. Naked DNA from the central region exhibited a 165 bp repeat pattern when digested with either nuclease. Chromatin gave the same result except that MNase gave an additional pattern of nuclease sensitive "hot spots". Naked DNA in the noncoding region was randomly digested with MNase, while chromatin gave a pattern similar to the central region. Naked DNA from the coding region gave a random digestion pattern with both nucleases, as did DNase-treated chromatin. MNase gave a 180 bp repeat pattern, the same length seen for bulk chromatin. Coding region chromatin was digested more rapidly with both nucleases than the noncoding regions or bulk chromatin. The terminus showed an interesting pattern with both enzymes: regions that were insensitive in chromatin were sensitive in naked DNA and vice versa. Indirect end-labeling and sequence analysis are being used to examine each region. These data suggest the phasing of nucleosomes and that some, but not all, of the nuclease sensitive sites in chromatin are due to physical properties of the DNA and not to the arrangement of chromosomal proteins. We are currently using these techniques to examine the chromatin surrounding developmentally regulated genes.

0403 In vivo REMODELLING OF MAMMALIAN CELL CHROMATIN USING THE H2a HISTONE GENE OF DROSOPHILA, Raymond Reeves*, Cornelia M. Gorman[δ], and Bruce H. Howard[δ], *Washington State University, Pullman, Wa. 99163; [δ]National Cancer Institute, N.I.H., Bethesda, Md. 20205
Derivatives of the expression vector pSV2 have been used to introduce the Drosophila H2a histone gene into CV-1 monkey tissue culture cells in DNA-mediated transfection experiments. Studies on the expression of this gene inside mammalian cells indicate that when the gene is under control of endogenous insect DNA sequences its expression in terms of both messenger RNA synthesis and histone protein synthesis is low but detectable. Additionally, pulse-chase experiments indicate that the Drosophila histone mRNA synthesized in transfected cells has a rapid rate of turnover. However, in pSV2 vector constructs in which the insect H2a gene is placed downstream from the Rous sarcoma virus 3' long terminal repeat sequence and immediately before the SV40 small tumor antigen RNA splice site in the pSV2 vector, expression of the Drosophila gene is efficient and readily detectable levels of both insect histone mRNA and H2a protein can be detected within transfected CV-1 cells. Analysis showed that the bulk of the insect mRNA made under these conditions contained a 3' polyadenylate tail and both in vitro primer extension and S1 nuclease protection studies suggested that the start site for transcription of the histone mRNA was within the Rous LTR sequence. Furthermore, monomer nucleosomes isolated from CV-1 cells transfected with the H2a gene under promoter control of the Rous LTR sequence were found to contain authentic Drosophila H2a histone protein. This demonstrates the ability to experimentally alter the composition of mammalian cell chromatin by the introduction into cells of foreign genes coding for components of the chromosomes themselves.

0404 ASSOCIATION OF TRANSCRIBED GENES WITH THE NUCLEAR MATRIX OF DROSOPHILA CELLS BEFORE AND DURING HEAT SHOCK, Donald Small*, Barry Nelkin*, David Gilmore[+], John Lis[+], and Bert Vogelstein*, *Cell Structure and Function Laboratory, The Oncology Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, [+]Department of Cell and Molecular Biology, Cornell University, Ithaca, New York 14853
During the heat shock response in Drosophila, transcription of the heat shock genes is greatly stimulated while transcription of most other genes is greatly diminished. In this study, the representation of a number of genes in the DNA associated with nuclear matrix was analyzed both before and during heat shock in Drosophila K_c cells. All the genes examined which are known to be transcribed at 25°C were enriched in nuclear matrix DNA prepared from cells at 25°C. These genes included those coding for 70K and 23K heat shock proteins, actin, and the genes coding for ribosomal RNA. These genes were also enriched in the DNA associated with nuclear matrix prepared from cells which were heat shocked, under which conditions the transcription of actin and ribosomal DNA is greatly diminished. On the other hand, a chorion gene, which is not transcribed in these cells, was depleted in nuclear matrix DNA both before and during heat shock. Removal of greater than 99.7% of the hnRNA during preparation of the nuclear matrices did not affect those findings. It thus appears that neither active transcription nor association with hnRNA are necessary events for a gene to be located near the nuclear matrix.

0405 STRUCTURE AND DNASE I SENSITIVE DOMAIN OF THE GENE CODING FOR THE GLYCOLYTIC ENZYME GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPDH), Martin C. Alevy, Edward M. Stone, Robert J. Schwartz, Ming-Jer Tsai, and Bert W. O'Malley, Baylor College of Medicine Houston, TX 77030

It is well documented that actively transcribed genes are maintained in chromatin in a configuration such that they are relatively sensitive to degradation by DNase I. However, until now, the only genes studied in this regard have been either of viral origin or involved in differentiation. In order to further relate DNase I sensitivity to gene regulation, we set out to clone a housekeeping gene and compare its DNase I sensitive domain to that of ovalbumin, an hormonally regulated gene switched on during differentiation of the hen oviduct. We have cloned a 34 kb segment of chicken DNA containing the gene coding for GAPDH, a glycolytic enzyme, which is expressed constitutively in all cell types. The coding region has been determined to be about 4 kb. Sequencing of this area has revealed the presence of a number of introns. Using solution hybridization, we have determined the DNase I sensitivity of the GAPDH gene and its flanking sequences in the hen oviduct. Using ovalbumin as a standard, we find the GAPDH coding sequences to be in an open configuration. However the GAPDH domain closes down about 4 kb from the 3' end of the gene. This is in contrast to the ovalbumin domain which remains open for approximately 20 kb on the 3' side of the gene and then closes down gradually over a region of about 10 kb. For GAPDH, the 9 kb of 5' flanking DNA we have isolated so far is all in the open configuration.

0406 DNASE I HYPERSENSITIVE SITE AND A PUTATIVE ENHANCING ELEMENT FOR THE IMMUNOGLOBIN KAPPA LIGHT CHAIN GENE. Su-yun Chung, Virginia J. Folsom and John C. Wooley, Department of Biochemical Sciences, Princeton University, Princeton, NJ 08544

We have employed DNase I digestion and β-globin or immunoglobin (C_K and V_{K21}) probes to examine the specific chromatin structure of the mouse immunoglobin kappa light chain genes in rearranged and unrearranged chromosomes. In immunoglobin-secreting myeloma cells, the C_K gene and the rearranged V_K genes are very sensitive to digestion by DNase I, but non-rearranged (cross-hybridizing) V_K genes (in the same family) are as resistant as β-globin; all these genes are equally resistant in brain. One possible explanation is that transcriptional potential is marked on a C_K chromosomal domain during lymphocyte development, and that recombination events involve translocating V_K genes from an inactive V_K chromosomal domain to a transcriptionally active C_K domain. For rearranged kappa light chain genes, three tissue-specific DNase I hypersensitive sites have been mapped by indirect end-labeling. One is 5' to the start of transcription as found for other active genes. Two novel sites are observed at 0.7 kb and 1.7 kb upstream from the 5' end of the C_K gene, i.e., within the J_K-C_K intron region in myeloma cells. The C_K-proximal hypersensitive site coincides with a region conserved between mouse and human genes (Leder and colleagues). We note that the sequences (obtained from Max, Maizel and Leder) in this region show several stretches of homology to the 72 bp tandem repeat of SV40; recent studies in the field implicate this sequence as a genetic "enhancer" element. By analogy, the C_K-proximal site might also act in cis to effect the differential expression of the translocated V_K genes. (Supported in part by NIH GM26332.)

0407 ONCOGENE ACTIVATION BY IMMUNOGLOBULIN-MEDIATED TRANSLOCATION IN MYELOMAS Stuart Kim, Lee Hood and Barbara Wold, California Institute of Technology, Pasadena, CA 91125

Studies of nonproductively rearranged immunoglobulin genes from the myelomas M603 and M167 have shown that an immunoglobulin constant region gene, C_a, resides next to a translocational breakpoint and that the cellular homologue of the MC29 viral oncogene, V-myc, resides on the other side of the breakpoint. The c-myc gene is normally located on chromosome 15 whereas C_a resides on chromosome 12. Preliminary evidence indicates that the murine c-myc gene contains at least one intron and that the translocational breakpoint occurs within this intron. Thus, the 5' end of the c-myc gene is removed by the translocation. When c-myc expression is analyzed by Northern blots, bands at 2.3 and 1.5 kb are seen in spleen cells whereas additional, smaller bands at 2.0 and 1.8 kb are seen in myelomas. Almost all myelomas have a rearranged c-myc gene and express smaller RNAs. Our results suggest that immunoglobulin genes rearrange aberrantly in some cases and that, if the rearrangement occurs to c-myc, then the oncogene can become activated resulting in a myeloma. Presently, we believe that the mechanism of activation is to make a different RNA rather than overexpress the normal gene product. We are currently comparing the structure of the c-myc RNAs in myelomas to normal lymphocytes, studying the changes in c-myc expression in normal proliferating or non-proliferating lymphocytes and attempting to transform normal spleen cells using activated c-myc vectors.

0408 ANALYSIS OF THE GENES ENCODING Ia ANTIGENS, Michael Steinmetz and Leroy Hood, California Institute of Technology, Pasadena, CA 91125 USA

The cloning of the immune response region of the major histocompatibility complex of the BALB/c mouse with human cDNA clones and chromosomal walking steps has provided the genes for probably all four biochemically defined Ia antigens and has established the linkage relationship of these genes on chromosome 17. Correlation of the molecular map with the genetic map of the immune response region by mapping of restriction enzyme site polymorphisms has allowed us to confirm the location of the genes encoding the Ia antigens in the I-A and I-E subregions and to confine the I-B and I-J subregions to less than 3.4 kb of DNA where a recombinational hot spot has been detected (1). We have recently cloned the same chromosomal region from the AKR mouse and could show that the organization of this region in the k haplotype DNA where the I-J subregion has been defined is very similar to the d haplotype DNA of BALB/c. We are currently continuing our chromosomal walk with AKR mouse DNA and will present attempts to express cloned Ia genes by gene transfer experiments.

(1) Steinmetz et al. (1982) Nature 300, 34-42.

0409 DISSECTION OF THE HLA-D REGION AT THE MOLECULAR LEVEL: STRUCTURAL FEATURES AND GENE EXPRESSION, M. ROUX-DOSSETO, C. AUFFRAY, A.J. KORMAN, R. BONO, J. M. BOSS, K. OKADA and J. L. STROMINGER, Harvard University, Cambridge, Ma 02138. In collaboration with J.G. SEIDMAN, Harvard Medical School, Boston and R. DEMARS, U. Wisconsin, Madison.

The HLA-D region contains at least three serologically defined systems: DR, DC/MT/MB and SB. The molecules encoded at these loci are involved in antigen recognition by T lymphocytes and display the tissue distribution and the characteristic molecular structure of class II antigens. By using DNA probes from the HLA-DRα chain gene, cDNA clones encoding cross-hybridizing sequences have been isolated and one of them was identified as coding for the DCα chain. The corresponding DCα gene and a DR-distinct, DC-related α chain gene were isolated. Similarly, we screened for cross-hybridizing class II β chain sequences by using a mouse I-Aβ genomic clone. The comparaison of the amino acid sequence derived from one isolated cDNA clone with DR and DC β chains points out the existence of multiple differences in the Ig-like second extracellular domain where a strong homology was expected. Together with the finding that the intracytoplasmic region is different in size allowed us to identify the product of a third β chain gene locus. A study of the relation of the antigens encoded by these cDNAs with the primarly defined human class II antigens has been initiated using a set of γ-ray induced mutant cell lines that are DR null, DC null or SB null. Such analysis should provide of interest in further dissecting the HLA-D region. D region α and β chain genes have been isolated from several genomic libraries derived from homozygous B cell lines. Separate and joint expression of these genes in mouse L cells should allow a better understanding of the role that the class II antigens play in eliciting the immune response.

0410 AN INVERTED SEA URCHIN HISTONE GENE SEQUENCE WITH BREAKPOINTS NEAR mRNA CAP SITES, Eric S. Weinberg and Luigi Vitelli, University of Pennsylvania, Philadelphia, Pa., 19104

A sea urchin histone gene fragment containing inverted regions of the normal repeat has been cloned in pBR322. Restriction enzyme mapping and homoduplex analysis of this fragment indicate that the H1-H4 spacer of one repeat is situated alongside the inverted H2A-H1 spacer of another repeat. The site of inversion has been sequenced and compared with homologous stretches of the normal repeat. The breakpoints were found to be within 4-6 bp of the H4 mRNA cap site in one of the original sequences and within 8-10 bp of the H1 mRNA cap site in the other original sequence of the standard repeat. Both sites of crossover contain short direct repeats, (GC)CATCAT in the H4 region, and GCCGAGCCGA in the H1 region, the underlined bases being a 3 bp overlap of sequence at the breakpoint. There is some additional homology in the region near the TATA and cap sites in both original duplexes. As this is the first inversion breakpoint to be analyzed at the level of DNA sequence, we speculate whether structural features we identify are typical of such rearrangements. It is suggested that promoter regions are particularly susceptible to rearrangements and that the exact site of crossover may be determined by small stretches of localized homology and the presence of short direct repeats.

0411 THE MURINE IMMUNOGLOBULIN α GENE EXPRESSES MULTIPLE TRANSCRIPTS FROM A UNIQUE MEMBRANE EXON, Charlotte J. Word, J. Frederic Mushinski and Philip W. Tucker, University of Texas Southwestern Medical School, Dallas, TX 75235

We have sequenced the DNA spanning the coding region for the carboxy-terminal end of the α_m chain in a BALB/c genomic clone and have examined α mRNA expression in transformed and normal B cells. Several unique features were revealed. Unlike other murine heavy chain genes which have two membrane exons (M1 and M2), the α gene has only one membrane exon (αM1). This unusual exon encodes a highly conserved transmembranal peptide plus flanking amino acids singular to IgA. Also unusual is the complexity of the α gene transcription. Downstream of αM1, three AATAAA hexanucleotides are used to terminate as many as six distinct α membrane mRNAs. These appear to differ only in length and splicing pattern of their 3' untranslated regions, and thus, encode the same α_m protein. The α_s protein is ∿4000 daltons smaller than α_m as judged by in vitro translation and is coded by a single 2.0 kb α_s mRNA. We propose that the expression of α_s and the multiple α_m mRNAs is differentially controlled at the level of transcriptional termination.

0412 ANALYSIS OF A MAMMALIAN PROCESSED tRNA PSEUDO GENE, J. Garrett Reilly,* Richard Ogden,† and John Rossi*, *City of Hope, Duarte, CA 91010 and †Agaron Institute, La Jolla, CA 91010

Using a synthetic oligodeoxynucleotide probe we have directly identified a mouse genomic clone that is partially homologous to $tRNA^{Phe}$. This clone is considered a pseudo gene because the 5' half is completely absent in the 1.6 kb segment. The 3' end of $tRNA^{Phe}$ (38 nucleotides) is present in one-to-one homology. Our short synthetic probe (19 mer) is contained in this section. The point of divergence is located 3' of the anti codon, in a location where intervening sequences have been reported in yeast and xenopus. This clone is unique in that it encodes CCA at the 3' end. CCA is a post-transcriptional processing event common to all mammalian tRNAs. Of the more than 60 eukaryotic tRNA genes so far sequenced, none have an encoded CCA. This suggests that the clone was derived from a tRNA, that was copied into DNA and reinserted back into the genome. Small nuclear RNA pseudo genes have been reported to have homology to the 5' end yet are missing the 3' end. In addition they have in some cases direct flanking repeats. Our clone is markedly different in that it does not have a 5' end and is not flanked by repeats. This suggests several different molecular mechanisms that lead to the origin of processed pseudo genes.

0413 Ig V_H GENES AND THE IMMUNE RECEPTORS OF CYTOTOXIC T LYMPHOCYTES, David S. Ucker and Susumu Tonegawa, Center for Cancer Research, M.I.T., Cambridge, MA 02139.

The presence of idiotypic determinants mapping to the immunoglobulin (Ig) heavy chain locus on some thymus-derived (T) lymphocytes suggests that genes related to heavy chain Ig variable region (V_H) genes may play a role in encoding the antigen receptors of T lymphocytes. We have screened ^{H}RNA from clonal T lymphocytes with a battery of V_H probes and have found cross-hybridizing species. In particular several clonal cytotoxic ^{H}T lymphocytes (CTL) lines having specificity for H2-D^d targets bearing fluorescein on their surface exhibit a unique mRNA species hybridizing with a V_H probe derived from a nitrophenol (NP) specific myeloma. A full length cDNA copy of the mRNA^{H} species has been cloned and analyzed. It represents the spliced, polyadenylated transcript of an unrearranged V_H gene. The open reading frame extends slightly beyond the normal Ig gene rearrangement site. ^{H}We are presently assessing the function of the predicted polypeptide encoded by the mRNA species in two ways. Antibodies against synthetic peptides corresponding to the nucleotide sequence have been prepared to test for the presence of the polypeptide on the CTL surface. Further, the ability of such antibodies to block the cytotoxic action of the cells may indicate the involvement of that protein in the immune response. An independent approach tests whether the target specificity of a different CTL line not normally cytotoxic for fluoresceinated H2-D^d targets can be converted upon gene transfer of the cDNA clone. A method for efficiently introducing DNA into lymphocytes has been developed for this purpose. The expression of the V_H gene in the absence of rearrangement and the correlation with a target specificity different from the V_H associated B cell specificity are intriguing issues.

0414 STRUCTURAL AND FUNCTIONAL STUDIES OF TRANSPOSITION INTERMEDIATES OF THE DROSOPHILA ELEMENT COPIA. Andrew J. Flavell and David Ish-Horowicz, ICRF Laboratories, London, UK

Structural comparisons between the Drosophila mobile element copia and integrated retrovirus proviruses have indicated a familial relationship between these genetic elements. We have previously isolated from cultured Drosophila cells circular copia elements whose structures are strikingly similar to the unintegrated circular proviruses presumed to be intermediates in retroviral intergration. These circular molecules contain either one or two long terminal repeats (LTRs). We have examined the LTR-LTR junctions of several 2LTR copia circles to ascertain their origin. In no case have we found circle junctions similar to those seen in circular retrovirus proviruses. We observe highly heterogeneous DNA sequence in this region with insertions of up to 15 nucleotide pairs between the LTRs. The sequence of these insertions bears no relation either to the TTAA insertion usually found in provirus LTR-LTR junctions or to any analogous sequence predicted from the reverse transcription of a putative copia retrovirus. We conclude that at least the majority of these circles derive by excision of genomic elements. We have also found a 2LTR circle in which a large fragment of the copia element, bounded at one end by the former LTR-LTR junction, has been precisely inverted with the duplication of 5bp of a sequence at the other inversion break point. Such a structure almost certainly derived from self-integration of a 2LTR copia circle, suggesting strongly that such molecules are capable of integration. We conclude that copia transposition need not require an intermediate RNA transcript but can proceed via DNA exclusively.

0415 ORGANIZATION AND SEQUENCE OF IMMUNOGLOBULIN HEAVY CHAIN VARIABLE REGION GENES AND FLANKING REGIONS IN A PHYLOGENETICALLY DISTANT SPECIES, Gary W. Litman, Linda Berger, Kristin R. Hinds and Kent D. Murphy, Sloan-Kettering Institute, Rye, New York 10580

The mechanisms governing the organization, stabilization and evolutionary diversification of complex multigenic families are of considerable importance in understanding gene expression and regulation. Immunoglobulin heavy chain variable region (V_H) genes represent an extensive multigenic family and undergo somatic reorganization during lymphoid development. We previously have identified V_H gene homologs in Southern blots of restriction enzyme-digested DNA isolated from Caiman crocodylus, a phylogenetically ancient reptile, using murine V_H probes (Nuc. Acids Res. 10:3371-3380, 1982). Low stringency hybridization techniques have been employed to screen a Caiman genomic DNA library constructed in λ47.1. Linked vs. unlinked V_H homologs were identified and subclones representing coding and both 5' and 3' noncoding regions were derived. The complete nucleotide sequence of the presumptive coding region of one V_H gene exhibits ~65% homology with murine V_H genes. Both 5' and 3' flanking regions exhibit little homology to murine prototype sequences with the exception of the preserved recombination signal sequence located 3' to the V_H coding sequence. When the Caiman V_H homolog was employed under high stringency conditions as a blot hybridization probe, a remarkably large number of hybridizing components were identified in restriction enzyme-digested Caiman genomic DNA. Similarly, probes representing 5' and 3' noncoding regions exhibited complex hybridization patterns. These studies indicate the presence of extensive V_H gene families in phylogenetically distant species and establish approaches for examining their structure and organization.

0416 ANTIGENIC VARIATION IN Trypanosoma brucei, A.Y.C. Liu, A. Bernards, L.H.T.van der Ploeg, P.A.M. Michels, T. De Lange and P.Borst, Section for Medical Enzymology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, P.O.Box 60.000, 1005 GA Amsterdam, The Netherlands.

In the host's bloodstream, trypanosomes synthesize a surface coat composed of a single species of glycoprotein, the variant surface glycoprotein (VSG). There are over a hundred individual VSG genes in the genome, and many are clustered. The expression of VSG genes 117 and 118 involves a duplicative transposition whereby the gene is placed under the control of a promoter in an expression site. We infer this from the finding of a common 35-nucleotide sequence on the VSG mRNAs of two 117 and four 118 clones isolated independently. This segment is not encoded contiguously with the main body of the mRNA sequence. The transposition unit of VSG 118 has been sequenced. Its 5' border consists of five approximately 70 bp repeats. Each repeat begins with a repeating TPuPu motif; then a stretch of alternating purine-pyrimidine; and ends in a conserved block of AT sequence. Between this border and the 118 structural gene is a segment of 1.4 kb. Probes derived from this area hybridize to variant-specific transcripts. This segment does not contain the 35-nucleotide exon. The 3' border of the transposed segment ends in the untranslated region of the mRNA which also has characteristic sequence elements dominated by a conserved 14-mer before the poly(A) addition site(s). Presence of these homology blocks delimiting the transposition unit suggests that they might be involved in a recombination process that displaces one VSG gene by another from the expression site.

0417 IMMUNOGLOBULIN GENE ARRANGEMENTS IN HYBRIDOMA AND HYBRIDOMA VARIANT CELLS. Seth H.Pincus,Condie E.Carmack,and Clifford J. Stocks, Dept.of Pathology, University of Utah School of Medicine,Salt Lake City,Utah,84132

We have examined the arrangement of immunoglobulin heavy and light chain genes in a series of hybridoma and hybridoma variant cell lines by the Southern blotting technique.The original hybridomas secrete antibody to the synthetic polypeptide TGAL. These antibodies have been characterized idiotypically using both monoclonal and polyclonal anti-idiotypic antibodies. Analysis of V-region gene arrangements show that there are two patterns for both the heavy and light chain genes.Expression of an idiotype is dependent upon the arrangement used by both the heavy and the light chain.Further, the use of a different J-gene in the heavy chain alters the idiotype. Hybridoma variant cells have been selected using complement mediated cytotoxicity directed by antibody to idiotypes and heavy chain isotypes. Eight idiotypic and six isotypic variants have been selected.The isotypic variants were chosen because of their failure to secrete antibody to TGAL.Several of these variants secrete kappa chains,although none appear to secrete the original heavy chain. Southern blot analysis of these cells shows that,surprisingly, there is instability in the kappa chain genes. In contrast to findings with normal B-lymphocytes and myeloma cell lines, we find that these hybridomas have heavy chain genes in the germline arrangement. This finding has been strengthened by the use of several restriction enzymes as well as several J-region gene probes. We are currently performing karyotypic analysis to see if this is the result of fusion of more than two cells.

0418 ASSOCIATION OF TWO REPETITIVE DNA ELEMENTS NEAR IMMUNOGLOBULIN LIGHT CHAIN GENES, Ronald E. Wilson and Ursula Storb, Dept. of Microbiology and Immunology, Univ. of Washington, Seattle, WA 98195

We have sequenced repetitive DNAs located approx. 5kb 3' of the mouse C-kappa immunoglobulin light chain gene. We find the R element described by Gebhard et al. (J.Mol.Biol.(1982),157,453), but more sequencing revealed a BAM5 element (Fanning(1982),Nuc.Acid Res.,10,5003) lying adjacent to the R. Neither element was surrounded, by itself, by a direct repeat, but the R/BAM5 composite element was surrounded by a direct repeat of 15bp. Since several lines of evidence imply that direct repeats may arise during an insertion event of repetitive DNA elements, we believe that the R/BAM5 composite element integrated 3' of C-kappa as a linked unit. Two R/BAM5 composite elements are also found 3' of the C-lambda1 gene, in inverted orientation, but in all V gene clones so far examined, R elements exist alone, without an adjacent BAM5. Further studies on the interaction and cooperation of different repetitive elements may reveal their biological function.

0419 ISOLATION AND CHARACTERIZATION OF A GENE CODING FOR AN H-2DP MOLECULE. Mary J. Macchi, Johanna A. Griffin, Jerold G. Woodward, Elizabeth McLaughlin- Taylor, Minnie McMillan, Leroy Hood and Jeffrey A. Frelinger. USC School of Medicine, Los Angeles CA 90033. In order to study structure-function relationships among class I major histocompatibility (MHC) gene products, we have begun the identification and characterization of class I genes and gene products of the $H\text{-}2^p$ haplotype. Southern blot analysis of Bam H-1 cut B10.P DNA demonstrated extensive restriction fragment length polymorphism of the class I genes from the $H\text{-}2^p$ haplotype as compared with other inbred haplotypes. Thus we have the potential for isolating previously uncharacterized class I gene products. A genomic library was prepared from the sperm of a B10.P mouse in lambda-47 and screened with a mouse class I cDNA probe. Six different clones which most strongly hybridized with the cDNA probe were isolated. Following co-transformation of Ltk^- cells with a genomic clone and the herpes simplex virus thymidine kinase gene, expression of the gene products was verified by their reactivity with a panel of monoclonal antibodies made against $H\text{-}2^p$ trasnsplantation antigens. One transformant, L20a showed significant reactivity with five different monoclonals with specificities mapping to the D-end of the $H\text{-}2^p$ haplotype. Immunoprecipitation and 2-D gel analysis of the gene product expressed on the transformant demonstrated that it was in fact the $H\text{-}2D^p$ molecule. The $H\text{-}2D^p$ molecule expressed on the transformant L20a was also capable of functioning as a target antigen for $H\text{-}2D^p$ specific cytotoxic T lymphocytes. We are currently in the process of determining which viruses, if any, are recognized in the context of the $H\text{-}2D^p$ gene product by virus-specific cytotoxic T lymphocytes. Further analysis of $H\text{-}2^p$ gene products in this manner will yield useful information on the degree of functional heterogeneity of class I genes.

0420 A MAJOR REARRANGEMENT OF H-2-RELATED GENES IN MOUSE t HAPLOTYPES, John Rogers[1] and Keith Wilkison[2], [1]MRC Lab. of Molecular Biology, Hills Rd., Cambridge, and [2]Chester Beatty Res. Inst., Fulham Rd., London SW3, England.

The t haplotypes of mouse chromosome 17 are rearranged and do not normally recombine with wild-type, so that they are genetically isolated. The affected region includes the H-2 complex. We present a Southern blot analysis of specific regions of the H-2 complex in t haplotypes. Cloned probes were kindly provided by L. Hood (Steinmetz et al., Cell 28: 489, 1982).

We find that the t^{w12} and t^{w5} haplotypes both show a major rearrangement in the Qa-2,3 region. Whereas the BALB/c Qa-2,3 region contains a cluster of several H-2-related genes on large homology units, the t haplotypes have fewer homology units and an altered restriction pattern. This probably represents the loss of approximately two H-2-related genes in this region. We are currently investigating whether the loss of these genes is of functional significance, and whether it is related to the largescale rearrangement of the t-haplotype chromosome in the vicinity of the H-2 complex.

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0421 DNA RECOMBINATION IN B-CELL TUMORS. Erik Selsing, Jeffrey Voss and Ursula Storb. Brandeis University, Waltham, MA 02254 and Univ. of Washington, Seattle, WA 98112

The V-J joining event which recombines antibody gene segments has been thought to involve the deletion of the DNA between V and J segments. We have shown, however, that, in some B-cell lines, this DNA is retained in the genome in a recombined context (remnant DNA). Remnant DNAs in several cell lines exhibit directly-ligated V and J recognition sites. Thus, remnant DNAs are apparently by-products of V-J joining and may be formed by excision of the DNA separating V and J segments to form an episome in the B-cell genome. This episome, however, apparently undergoes secondary recombinations and may eventually reintegrate into a chromosome. Our studies suggest that sequential recombination can operate within a B-cell and that the mechanism which "turns-off" V-J recombination during B-cell differentiation may operate in a localized manner near the constant region exon.
We have also found, in two B-cell lines, kappa DNA recombinations which are apparently not linked to antibody gene expression. In both these B-cell lines, a DNA segment has been transposed into V-J joined genes, replacing the constant region exon. The transposing DNA that we have detected is quite often found to be recombined in B-cell tumors, but not in T-cell lines. In most cases, the DNA does not recombine into the kappa locus. The transposing DNA that we have found may be related to the chromosomal translocations that have been observed in many B-cell tumors. It is possible that this transposing DNA carries an oncogene which is brought under the influence of an active antibody gene promoter by a recombinational mechanism. Supported by grants from NCI, NIDR and NSF to U.S.

0422 BROMODEOXYURIDINE INDUCED AMPLIFICATION OF PROLACTIN GENE IN GH CELLS. D. K. Biswas; D. J. Wilson; S. D. Hanes & M. H. Pichler - Pharmacology Lab; Harvard School of Dental Medicine & Harvard Medical School Boston, MA 02115

Treatment of a 5-bromodeoxyuridine-resistant ($BrdUrd^r$), and prolactin nonproducing (PRL^-) subclone of GH cells with the drug led to amplification of prolactin (PRL) gene and induced PRL synthesis. Withdrawal of drug treatment reversed both of these processes. Dot hybridization measurement revealed that at the amplified state, the drug treated cells have 50-100 PRL gene copies per haploid genome. The increased PRL synthesis observed during late pregnancy and during lactation in normal rats, does not seem to be associated with amplification of the gene. The PRL gene amplification can not be associated with the mechanism which conferred $BrdUrd^r$ phenotype to these cells. The level of growth hormone (GH) gene sequences and expression of GH gene is unaffected by BrdUrd treatment of these cells. BrdUrd induced amplification of PRL gene can be identified with the low molecular weight, extra-chromosomal, supernatant DNA fraction isolated by Hirt's method. These results suggest that in rat pituitary gland, hormonally controlled increased PRL synthesis during normal developmental process is not caused by gene amplification. Whereas, the BrdUrd induced amplification of PRL gene in GH cell substrains seems to be linked to the mechanism of drug induced expression of the suppressed PRL gene.

0423 SELECTIVE OVERPRODUCTION OF ADENOSINE DEAMINASE IN MAMMALIAN CELLS, Cho-Yau Yeung, Diane E. Ingolia, Chefin Bobonis and Rodney E. Kellems, Baylor College of Medicine, Houston, TX 77030.

The objective of this work was to isolate cultured mouse cells with amplified adenosine deaminase (ADA) genes. Since ADA expression is not required for growth of cells in culture, the first step involved the development of selective conditions in which ADA activity is required for survival. This was accomplished by developing a new selection system, termed 11AAU, which selected simultaneously for ADA and adenosine kinase. We used the 11AAU selection in conjunction with stepwise selection for increasing resistance to deoxycoformycin, an ADA inhibitor, to obtain highly drug-resistant cells with a 4000-fold increase in ADA activity. ADA accounted for approximately 40% of the soluble protein in highly drug-resistant lines and was indistinguishable from that in the parent as judged by isoelectric focusing, electrophoretic mobility on starch gels, and by deoxycoformycin binding studies. Increased ADA was also correlated with the presence of hundreds of double-minute chromosomes, cytogenetic structures indicating the presence of amplified DNA. Growth in the absence of of selection was accompanied with the loss of double-minute chromosomes and a corresponding decline in ADA levels. Based on the stepwise selection protocol employed, the presence of double-minute chromosomes and the instability of the phenotype, we believe that the increased ADA is most likely the result of amplification of ADA genes.

0424 GENETIC ORGANIZATION OF MURINE RENIN LOCI, Kenneth W. Gross, Nina Piccini, John L. Knopf and Douglas P. Dickinson, Roswell Park Memorial Institute, Buffalo, NY 14263

The renin regulatory locus (Rnr) is a genetic element governing mouse submaxillary gland (SMG) renin levels. We have recently identified a cDNA recombinant for murine SMG renin and used it in Southern Blot analyses to probe the genetic organization of complementary DNA sequences in strains exhibiting high (Rnr^S) and low (Rnr^D) SMG renin levels. The results of these studies led us to suggest that the chromosome 1 locus is the site of a structural gene duplication event in high renin strains (Piccini et al. (1982) Cell 30, 205-213). In fact, all inbred mouse strains that we have surveyed exhibit the restriction digest patterns of the high and low SMG renin prototype strains SWR/J and C57BL/10J, respectively. We have now extended our survey to include wild M. musculus populations and closely related subspecies. These populations have been found to be polymorphic, harboring both the Rnr^D and Rnr^S alleles characteristic of inbred strains and additional genetic variants not observed within inbreds. These variant alleles, as observed by restriction digest patterns in Southern blots, appear to be of two types. Firstly, within some strains, for example M. spretus, there appears to be additional quantitative variation in the copy number of homologous sequences. Whether these additional sequences represent additional structural, or for example, pseudo genes is not yet apparent. Secondly, three populations, M. hortulanus, M. caroli, and M.d. praetextus, appear to harbor an allele with a novel restriction pattern. Interestingly, this pattern appears to be a hybrid pattern of that normally observed for the sequence found in Rnr^D strains and the second sequence found in Rnr^S strains. A new phenotype of expression for SMG renin may also be present in these strains. We are presently attempting to use in vitro methods to examine structure-function relationships for renin expression using these natural genetic variants.

0425 MOLECULAR ANATOMY AND EXPRESSION OF THE RELAXIN GENE, Marguerite Cronk*, Peter Hudson+, John Haley+, Hugh Niall+ and John Shine*, Australian National University, Canberra* and University of Melbourne, Vic.+

The peptide hormone relaxin is secreted from the ovaries into the blood stream just prior to parturition causing a marked softening of both the pubic symphysis and the cervix. Apart from facilitating the birth process, relaxin also inhibits uterine contractions and thus may influence the timing of parturition. The nucleotide sequence of both rat and porcine relaxin cDNA clones has demonstrated that relaxin is synthesised as a preprorelaxin molecule with a large peptide connecting the two chains of normal relaxin.

Using the cloned rat Rx cDNA as a hybridization probe, we have isolated the rat and the mouse relaxin genes from cloned libraries of rat and mouse genomic DNA. Using the pig Rx cDNA as a hybridization probe, we have isolated the human Rx genes from a cloned library of human genomic DNA. A comparison of the sequence and structure of these genes, and possibly related genes will be discussed.

0426 MOLECULAR CLONING OF THE GLUCOAMYLASE GENE OF ASPERGILLUS AWAMORI, Jack Nunberg, Vicki Schweikart, Frances Lawyer, Michael Innis and Jeffrey Flatgaard, CETUS Corporation, Berkeley, CA 94710

Glucoamylase is an inducible extracellular glycoprotein of Aspergillus awamori which is involved in the utilization of hexose sugar polymer substrates. The enzyme, an "exo-amylase," will progressively hydrolyze starch at α1-4 and α1-6 linkages, to yield glucose. The 74 kd enzyme is secreted into the medium upon growth on starch, glucose, or a variety of hexose sugar polymers. No secretion is observed upon growth on xylan, a pentose sugar polymer, or on glycerol. We have purified, and prepared antibody to, Aspergillus glucoamylase (GA). Antibody was used to characterize in vitro translation products of A.awamori mRNA obtained from cultures grown on starch and xylose. Results indicate that the induction of GA protein results from increased GA mRNA levels in cultures grown on starch. This result was subsequently confirmed in Northern analyses using molecularly cloned probes. A comparison of MeHgOH/agarose gel patterns of mRNA isolated from induced and non-induced cultures revealed a unique, predominant induced mRNA. This mRNA was isolated from gels and shown, by immunoprecipitation of in vitro translation products, to encode GA. This mRNA was then labeled in vitro and used to locate genomic A.awamori DNA sequences encoding the GA gene from several libraries of A.awamori DNA in phage lambda. The identity of the isolated gene was confirmed by its ability to hybridize and select mRNA which will direct the in vitro synthesis of GA protein. The structure of the A.awamori GA gene will be discussed, as will current experiments aimed at obtaining expression of this gene in S.cerevisiae.

0427 ANALYSIS OF THE HUMAN Ia ANTIGENS BY GENE CLONING, Henry Erlich, Deborah Stetler, Rosy Sheng-Dong, Jack Nunberg and Donald Pious*, CETUS Corporation, Berkeley, CA 94710 *University of Washington, Seattle, WA 98105

The human Ia antigens, encloded within the HLA-D region, are heterodimers, composed of a 34,000 dalton (α) and a 29,000 dalton (β) glycopeptide, and expressed on the surface of B cells and macrophages. We have constructed a cDNA library from mRNA derived from membrane-bound polyribosomes from a B cell line and have identified a subset of clones containing B cell-specific sequences. One of the clones in this subset, pDRα-1, which encodes the entire α chain of the HLA-DR antigen, was isolated with a synthetic oligonucleotide hybridization probe and identified by DNA sequence analysis (1). Using the technique of genomic blotting with DNA derived from a somatic cell variant (6.3.6) which contains a small deletion on chromosome 6, we have mapped genomic restriction fragments complementary to the pDRα-1 probe to the region defined by the 6.3.6 deletion. A polymorphic Bgl II site has been localized to the 3' end of the HLA-DRα gene and the population distribution of these polymorphic restriction fragments determined. These genomic blot studies indicate that the HLA-DRα gene is a single copy locus. However, we have also identified a number of cDNA clones, related to but distinct from the pDRα-1 clone, suggesting that there are at least three, and possibly several more, genes encoding DRα-related chains.

(1) Stetler et al. Proc. Natl. Acad. Sci. USA 79:5970,1982.

0428 ANALYSIS OF HLA-DR-β RELATED SEQUENCES USING pDR-β-1 TO PROBE FOR RESTRICTION SITE POLYMORPHISMS, Victoria Henson, Noel A. Maclaren, Dan Larhammer, Per A. Peterson, and Edward K. Wakeland, University of Florida, Box J-275, Gainesville, Fla., 32610 and Uppsala University, Box 562, S-751 22 Uppsala, Sweden

We have developed a panel of HLA-DR phenotyped individuals using HLA-DR alloantisera and the microcytotoxicity test. Family studies have established that several individuals in this panel are homozygous for specific HLA-DR antigens. Individuals representing homozygous phenotypes of HLA-DR1 through HLA-DR7 were selected and total cellular DNA extracts were prepared from their peripheral blood leukocytes by standard methods.

Plasmid pDR-β-1 contains a cDNA insert corresponding to the complete coding region of an HLA-DR-like β chain present in the Raji cell line (P.N.A.S. 79:1703). This plasmid was used to probe for restriction site polymorphisms in restriction endonuclease digests of the total cellular DNA preparations from our panel of HLA-DR serotyped individuals. Genomic digests with various restriction endonucleases, including BamHI, Hind III, and EcoRI, were electrophoresed through 0.9% agarose gels and transferred to nitrocellulose filters. ^{32}P-labeled pDR-β-1 hybridized with varying intensity to several bands in these digests. Although most of these bands were present in the digests of all the individuals tested, some restriction site polymorphisms were detected. We are in the process of assessing the relationship of these restriction site polymorphisms to HLA-DR and MT serotypes.

0429 ISOLATION OF HUMAN BIOTIN-DEPENDENT CARBOXYLASE cDNA CLONES. K.S.Guise, F.Quan,F.Tsui, R.A.Gravel, Hospital for Sick Children, Toronto, Canada

Four biotin-dependent carboxylases are known in man.Three are mitochondrial enzymes-propionyl CoA carboxylase(PCC),the catalyst for transfer of bicarbonate to propionyl-CoA;ß-methyl-crotonyl-CoA carboxylase(MCC),important in leucine degradation;and pyruvate carboxylase(PC), a gluconeogenic enzyme-while the fourth,acetyl-CoA carboxylase which initiates fatty acid biosynthesis,is cytosolic. Specific defects in man have been confirmed for PCC,MCC and PC. A six amino acid sequence (ala met lys met glu thr) surrounding the biotin binding site(lys residue) is conserved in carboxylases of sheep,fowl and some bacteria. Furthermore,16 amino acids are identical around the biotinyl-lysine in sheep and avian PC. Based on this sequence we designed two 17mer probes to minimize mismatching and to take advantage of mammalian codon preference and the unique codon for met. We have screened two cDNA libraries - a 1.4 x 10^6 independent clone library (from Okayama and Berg)derived from SV40 transformed human fibroblasts and engineered to increase the chances of full-length cDNA capture and a 2000 clone human adult liver library from A.Beaudet. Among the 27 selectants from these two libraries, filter selection of sheep mRNA and subsequent translation and immunoprecipitation have identified cDNA clones of the biotin binding polypeptides of PC and PCC. We are currently using these cDNA clones to re-examine genomic clones,previously selected from a λ Charon 4A Maniatis human library using the 17mer probe.

0430 A TRANSPOSABLE ELEMENT WHICH SPLITS THE PROMOTER REGION INACTIVATES A DROSOPHILA CUTICLE GENE. Michael Snyder and Norman Davidson, Divisions of Biology and Chemistry California Institute of Technology, Pasadena, California 91125

We are studying the sequence organization and expression of the larval cuticle genes of Drosophila. Five major cuticle proteins are synthesized and secreted by third instar larvae in order to provide a protective pupal coat. A 50 kb DNA segment of the Drosophila genome has been cloned and characterized. This segment contains four related cuticle genes clustered in 7.9 kb of DNA. The four genes encode four of the five major third instar larval cuticle proteins. These cuticle genes are coordinately expressed in the integument of third instar larvae, and they are not abundantly expressed at other developmental stages. A fifth cuticle-like gene lies within this gene cluster. It is judged to be a pseudogene, because several features of its structure suggest that it is nonfunctional. Sequence comparisons indicate that it arose by an unequal crossing over event involving two closely related and adjacent cuticle genes. Eleven kb away from the cuticle gene cluster lies another gene family comprised of three genes that are clustered within 8 kb of DNA. The three genes are expressed together in larval stages and adults, but show a completely different pattern of developmental expression from the third instar larval cuticle protein genes. Thus, two small gene families can lie adjacent on the chromosome and exhibit different patterns of developmental expression, even though individual genes within a clustered family are coordinately expressed.

Additionally, a mutant Drosophila strain has been studied which fails to synthesize one of the major cuticle proteins. A molecular characterization of this strain is reported, including the finding of a copia-like transposable element in the TATA box of the unexpressed gene.

0431 MOUSE URINARY PROTEIN GENES: STRUCTURE AND EXPRESSION. E. Derman and R. Stack, The Public Health Research Institute, New York

Mouse urinary protein (MUP) genes constitute a family (15-25) of tissue-specific genes, some of which are hormonally regulated. The developmental regulation of MUP genes in the liver and in the submaxillary gland was characterized. Liver MUP mRNAs are present only in post-pubescent animals. MUP mRNAs in the submaxillary gland are synthesized predominantly, but not exclusively, prior to the onset of puberty. Run-off transcription experiments have demonstrated the existence of transcriptional controls in the developmental regulation of MUP mRNAs in the liver.

We are currently studying the structure and organization of various MUP genes. We are also attempting to assign a function to the different MUPs synthesized by the liver.

0432 STRUCTURE AND FUNCTION OF THE BOVINE PARATHYROID HORMONE GENE, Byron Kemper, Christine Weaver, David F. Gordon and Martin S. Kissil, Univ. of Illinois, Urbana, IL

The initial translation product of parathyroid hormone (PTH) mRNA is preProPTH which contains 115 amino acids. The sequence of bovine PTH mRNA has been derived from sequence analysis of cloned cDNA and sequence analysis of restriction fragments hybridized near the 5' terminus of the mRNA and extended with reverse transcriptase. The extended transcripts terminated at three major sites within 8 nucloetides of each other and one minor site about 30 nucleotides 5' of the major sites. The sequence derived for the mRNA at the 5' terminus begins, 5'-XXXAUAUAUAAA. Since this TATA box sequence is about 25 nucleotides upstream from the major termination sites for reverse transcriptase, a minor aberrant initiation site for transcription may be present about 30 nucleotides upstream from the major sites. The gene for PTH mRNA has been cloned on a 6500 bp EcoRl fragment from a λ Charon 30 partial bovine library. The bovine PTH gene contains two introns, one of about 1800 bp which interrupts the 5' noncoding region and one of about 100 bp which interrupts the sequence coding for the Pro-specific sequence. The sequence of the gene near the site of transcription initiation included the TATA sequence described above, as well as a second TATA-like sequence, ATAAAA, about 30 bp upstream from the first one in the appropriate location to direct the transcription of the minor transcript described above. Fifteen bp around each of the TATA sequences are completed conserved between the human and bovine genes while the region between these two sequences has only 43% homology, suggesting that these sequences are important in the transcription of the gene.

0433 CLONING AND EXPRESSION OF THE MALOLACTIC GENE FROM LACTOBACILLUS DELBRUECKII IN ESCHERICHIA COLI AND SACCHAROMYCES CEREVISIAE, Steven A. Williams, Robert T. Hodges, Richard Snow and Ralph E. Kunkee, University of California, Davis, CA 95616.

A genomic library of Lactobacillus delbrueckii was constructed by ligating Sal I restricted DNA fragments to the plasmid pBR322 followed by transformation of E. coli strain RRl. The library was screened for expression of malolactic gene activity by assaying for enzymatic conversion of L-malate to L-lactate. An E. coli clone that converted L-malate to L-lactate was isolated and further characterized. This clone contained plasmid pBR322 with a five kilobase insert of L. delbrueckii DNA. Expression of this gram-positive bacterial gene was low in the gram-negative E. coli but increased upon transfer of the active DNA fragment to other multicopy vectors.

When transformed into the lower eukaryote Saccharomyces cerevisiae on a variety of yeast/E.coli "shuttle" vectors, expression of the malolactic gene was measurable but very weak. We are currently constructing fusions between this gene and cloned yeast promoter-active fragments in an effort to increase expression in the eukaryotic host. This research is directed towards improved understanding of the factors controlling expression of a gram-positive prokaryotic gene in a eukaryotic host. It should also lead to development of a yeast strain of considerable importance to the wine industry, because the malate to lactate conversion is often desirable in wine production. The knowledge gained from such cross-species gene transfers, and study of the factors that modulate the expression of genes in heterologous hosts, will have important applications in the genetic engineering of agriculturally important organisms. (Supported by CRGO grant 80-00021 from the USDA).

0434 STRUCTURE AND EXPRESSION OF THE HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE (HPRT) GENE, David W. Melton, John Brennand and C. Thomas Caskey, Baylor College of Medicine, Houston, TX 77030

HPRT cDNA recombinants, isolated from a mouse neuroblastoma mutant cell line (NBR4) with amplified HPRT genes, have been used to study the structure of the mouse HPRT gene. A simple map of the gene was obtained by Southern analysis of wild-type and NBR4 DNA after digestion with EcoRI, HindIII and XbaI. Most of the restriction fragments with homology to HPRT cDNA were amplified in NBR4 cells. However, a 3.4 kb EcoRI fragment, with homology only to the very 3' end of the coding sequence and the 3' non-translated region, was present in low copy number in both wild-type and NBR4 cells. This sequence, which was not present in DNA-mediated gene transferents containing a functional mouse HPRT gene in a Chinese hamster background, may represent an HPRT pseudogene. Considerable difficulty was experienced in isolating the entire gene from lambda genomic libraries due to its large size and the high frequency of repetitive sequences within it. A series of overlapping clones spanning 65 kb of DNA have been isolated and restriction mapped. The 5' and 3' ends of the gene, which extends over 35 kb of DNA have been defined and the 5' end plus flanking regions have been subcloned into pBR322 for sequence analysis. Full length HPRT cDNA from NBR4 was ligated into an expression vector adjacent to a retrovirus long terminal repeat. The vector was unable to rescue HPRT-deficient cultured cells following DNA-mediated gene transfer, probably due to the mutant nature of the NBR4 protein. The experiment will be repeated with wild-type HPRT cDNA recombinants which have recently been obtained.

0435 COORDINATE AMPLIFICATION OF METALLOTHIONEIN I AND II GENE SEQUENCES IN CADMIUM-RESISTANT CHO VARIANTS. C.E. Hildebrand, B.D. Crawford, M.D. Enger, B.B. Griffith, J.K. Griffith, J.L. Hanners, P.J. Jackson, J. Longmire, R.L. Stallings*, J. Tesmer, and R.A. Walters. Los Alamos Nat. Lab., Los Alamos, NM 87545, *Univ. of Texas, Smithville, TX 78957.

Two mammalian metallothioneins (MT) are inducible by Zn^{2+} or Cd^{2+}. We have constructed in pBR322 two cDNA clones, pCHMT1 and pCHMT2, encoding the two Chinese hamster isoMTs. Sequence analyses of pCHMT1 and pCHMT2 cDNA inserts show 80% homology in the coding region vs ~35% in the 3' untranslated regions. Cot analyses of genomic DNAs from clonal Cd^{2+}-resistant (2-200 μM) CHO variants using a 150 bp tracer from the coding region of the pCHMT2 insert revealed 5-40-fold amplification of MT-specific sequences vs. single-copy levels defined in CHO cells. Southern blots of genomic DNAs digested with EcoRI, BamHI, PstI, or HindIII and hybridized to the same 150 bp probe from pCHMT2 revealed 2 prominent hybridization bands, amplified coordinately in the Cd^{2+} variants. Probes discriminating pCHMT1 and pCHMT2, derived from nonhomologous 3' untranslated regions, hybridized differentially to the DNA fragments generated by BamHI or HindIII digestion, verifying coordinate amplification of sequences homologous to 3' noncoding and coding portions of MT1 and II. In situ hybridization of pCHMT2 DNA to chromosomes of Cd^{2+} cells revealed a single site of hybridization, suggesting linkage of MT genes. Mapping of these genes, using hamster/mouse cell hybrids and chromosomes isolated by flow sorting, is in progress. These studies suggest that the isoMTs constitute a functionally related gene cluster which amplifies coordinately in response to toxic metal stress. Confirmation of this hypothesis by analysis of cloned genomic fragments is in progress. (This work supported by USDOE).

0436 SEQUENCE ORGANIZATION OF THE SPLIT AND PROCESSED CHICKEN CALMODULIN GENES, J.P.Stein*, T.Tanaka†, E.C.Lai† 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

Two calmodulin genes have been isolated from a chicken DNA library using an eel calmodulin cDNA probe. The first (CaM 1) was originally isolated as an 11Kb EcoRI fragment that encoded CaM at the 5' end. Sequence analysis has shown that this CaM gene fragment does not contain introns, but does contain all but the first 33 nucleotides (NT) complementary to the coding region of CaM mRNA (410 NT), 481 NT of 3'-nontranslated DNA, and a polyadenylation site (AATAAA). The NTs complementary to CaM mRNA begin with amino acid (aa) 12 (phe), and continue in an open reading frame through the termination signal after aa 148. The adjacent 2Kb EcoRI fragment that should contain the 5' end of the CaM 1 gene is now being sequenced to determine the organization of the 5' end, and 5' flanking region. The second gene (CaM 2) was isolated as a 13.5Kb fragment which was cleaved by EcoRI into 7.0Kb (5') and 6.5Kb (3') fragments. Sequence analysis of these fragments demonstrated that CaM 2 contains at least 3 introns in the coding sequence, and at least one intron in the 5'-untranslated region. Whereas the aa sequence derived from CaM 2 is identical to that of eel, cow, and human CaMs, the aa sequence derived from CaM 1 differs from these sequences at 16 positions. Furthermore, two species of RNA complementary to CaM 2 are present in all seven different chick tissues examined (at 1600 and 1800 NT), but only muscle contained an RNA (at 800 NT) that hybridized to CaM 1. These results suggest that CaM 2 is the primary gene responsible for production of CaM mRNA, and that CaM 1 is a processed gene (and possibly a pseudogene) derived from CaM 2.

0437 MOLECULAR BIOLOGY OF TYROSINE HYDROXYLASE, Jacques Mallet, Annie Lamouroux, Nicole Faucon-Biguet, CNRS, Bât 409, 91405 Orsay.

The peripheral autonomic nervous system provides a useful model of neuronal development because the neurons in the sympathetic ganglia, which arise from the neural crest, become either cholinergic or adrenergic. Both *in vivo* and *in vitro* studies have shown that the neurotransmitter choice is labile during a prolonged period of life. Tyrosine hydroxylase (TH) is a specific marker for adrenergic neurons and has been widely used to study their ontogeny. The regulation of the expression of this enzyme has been shown to be influenced by the target tissue, by neural activity and by chemical or hormonal factors such as glucocorticoids and nerve growth factor. A full understanding of the regulation of this enzyme requires an analysis of the DNA encoding its gene and of its RNA transcripts. Recombinant DNA plasmids have been constructed that contain structural gene sequences for rat TH (PNAS, 79 p. 3883). The rat TH cDNA probe allowed the characterization of TH mRNA from PC12 cells and human pheochromacytoma. The use of this probe to analyse the TH gene and the transcriptional and post-transcriptional regulation of its expression during development and electrical activity of catecholaminergic neurons will be discussed.

0438 NEW MEMBERS OF NEUROENDOCRINE PEPTIDE FAMILIES: ISOLATION BY IN VIVO RECOMBINATION. Valerie M. Watt, William J. Rutter & C. James Ingles, University of California, San Francisco and University of Toronto, Toronto, Canada

We have used the method of πVX in vivo recombination (Brian Seed, personal communication) to isolate gene families related to several hormones. The recombinational probes used in screening contained parts of four pancreatic hormone cDNAs: human somatostatin, anglerfish glucagon and anglerfish and human insulin. At least five different human somatostatin-related genes, including the homologous gene, were isolated. Some of these isolates contained two linked somatostatin genes. Four different human glucagon-related genes were obtained. In addition to having recombined with anglerfish glucagon DNA, three of these genes also hybridized to a hamster glucagon cDNA probe. Insulin-related genes were isolated with B chain recombinational probes. Using the human DNA we isolated four human insulin-related genes in addition to insulin itself. None of these five phage selected with human insulin appeared to be among the more than 30 different human phage obtained with anglerfish insulin. These studies suggest that the gene families related to these hormones are much larger than anticipated from known proteins or by hybridization with nucleic acid probes.

0439 ISOLATION AND CHARACTERIZATION OF HUMAN IL2 mRNA AND IDENTIFICATION OF CLONES CONTAINING HUMAN INTERLEUKIN 2 cDNA, Hilde Cheroutre, René Devos, Geert Plaetinck, Walter Fiers, State University of Ghent, 9000 Ghent

Total RNA was isolated from PHA + TPA induced human splenocytes. Purification over an oligo (dT)-cellulose column yielded polyadenylated mRNA representing 1-3% of the total RNA. This mRNA fraction was further enriched for IL2 mRNA by sedimentation in a 5-20% sucrose gradient. The presence and amount of IL2 mRNA was monitored by translation in Xenopus laevis oocytes, followed by an assay of IL2 activity, based on (H^3) thymidine incorporation of IL2 dependent human spleen derived cells. The peak fractions of these gradients were further used for the synthesis of double stranded cDNA and cloning into the eukaryotic expression vector pSV529. Screening of the cDNA clones is performed using the same methodology (translation of hybridized selected mRNA) as used for the screening and identification of a human γIFN cDNA clone (also represented on the poster).

0440 MOLECULAR CLONING OF cDNA SEQUENCES SPECIFIC FOR HUMAN ACTIVATED T LYMPHOCYTES, Nancy T. Chang and Susan Tam, Centocor, 244 Great Valley Parkway, Malvern, PA 19355

PolyA-mRNA was isolated from human peripheral blood T lymphocytes (PBL) activated by phytohemagglutinin (PHA) and used as the template for cDNA synthesis. Recombinant bacterial plasmids have been constructed by insertion of the double-stranded cDNA sequences into plasmid vector pBR325 using a procedure which permits the recovery of plasmid recombinant with longer and possibly full-length cDNA inserts. The average size of cDNA inserts is greater than 2.5 Kb. Clones were screened by hybridization with ^{32}P-labeled-polyA-mRNA from both induced and resting PBL. Those clones only reactive with labeled RNA from activated T cells are selected for further studies. In parallel experiments, polyacrylamide gel electrophoretic analyses of in vitro translated products of polyA-mRNA from activated or resting PBL indicated that at least ten polypeptides with monomeric molecular weight ranging from 25,000 to 90,000 daltons were induced in PHA treated PBL. Studies involving positive hybridization-translation, immunoprecipitation and functional tests are planned to search for bacterial clones coding for these polypeptides.

0441 CLONING AND CHARACTERIZATION OF THE HUMAN METALLOTHIONEIN GENE FAMILY, Lap-Chee Tsui*, Martin L. Breitman*, A. Leonard Naismith† and John R. Riordan†, Departments of Genetics* and Biochemistry†, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

Since metallotionein (MT) proteins of mammalian species show extensive homology in their amino acid sequence, a MT cDNA clone derived from Chinese hamster ovary (CHO) cells was used as a probe to obtain human MT gene sequences from a genomic library contained in bacteriophage lambda. Six recombinant phages containing MT-related sequences were isolated. All of them were found to be distinct by restriction enzyme analysis, indicating the presence of a family of MT genes in the human genome. This observation is consistent with the fact that 10 EcoRI fragments are detectable in total human genomic DNA by Soutern hybridization analysis using the CHO MT cDNA probe. In addition, two of the phage clones were shown to contain at least two stretches of MT-related sequences by gel blotting analysis, suggesting clustering of human MT genes. These putative human MT genes were subcloned into an SV40-derived eukaryotic vector for DNA transfection studies and preliminary results indicate that at least 2 of these are active at the transcriptional level.

0442 POLYMORPHISM IN THE 5' FLANKING REGION OF THE HUMAN INSULIN GENE: A GENETIC MARKER FOR NON-INSULIN DEPENDENT DIABETES (NIDD), Peter Rotwein*, John Chirgwin*, William Knowler (NIH, Phoenix, AZ), Howard Goodman (Massachusetts General Hospital, Boston, MA) and Alan Permutt*, Washington University School of Medicine, St. Louis, MO 63110.

The genetics of diabetes remains an area of controversy and speculation. Using a cloned human insulin gene probe, DNA from peripheral leukocytes of American blacks, Caucasians, and Pima Indians was studied to determine whether differences in insulin gene structure may contribute to diabetes. Using a filter hybridization technique a region of DNA highly variable in length immediately flanking the 5' portion of the gene (within 0.5 kb) was identified. None was found within or 3' to the gene. We analyzed 434 alleles from 217 individuals. The common sized allele was found in 291, and polymorphic alleles in 143. Deletions (0.1-0.2 kb) were found in 15, and insertions (0.6-5.5 kb) in 128 alleles. Eighty-five percent of the insertions were 1.6 kb. The relationship between length heterogeneity and diabetes was investigated. Analysis by genotype for frequency of insertions suggested an association of inserts with NIDD compared to non-diabetics. A stronger relationship between the 1.6 kb polymorphism in NIDD ($p = .011$) than between all inserts in NIDD ($p = .084$) was found. The odds ratio for any 1.6 kb insert in NIDD compared to non-diabetics was 1.8 ($p = .03$), and for inserts in both alleles was 21 ($p = .002$), suggesting a dosage effect. Thus length polymorphism in the 5' flanking region of the gene may provide a genetic marker for NIDD. Whether this polymorphism accounts for variable insulin gene expression and insulin deficiency in certain NIDD has yet to be determined.

0443 STRUCTURE AND EXPRESSION OF RAT METALLOTHIONEIN-I GENES, Bruce W. Birren, Robert D. Andersen, Tomas Ganz, and Harvey R. Herschman, UCLA, Los Angeles, CA 90024

Transcription of the rat metallothionein genes is controlled by both heavy metal ions and glucocorticoid hormones. To study the regulation of MT expression we have used a rat MT-I cDNA probe to isolate genomic regions from a rat λ library. Two unique clones were selected for analysis based on their hybridization to the cDNA at high wash stringencies and their ability to select MT message for translation. We have shown by DNA sequencing that one of these clones contains the complete MT-I gene. A region 5' to the transcription start site contains palindromic sequences homologous to the secondary structure regions implicated in the control of metal induction of the mouse MT-I gene (Brinster et al., 1982). We are currently analyzing clones obtained by cotransfection of the rat MT-I gene with the HSV thymidine kinase gene into cultured human cells to investigate the nature of the metal and hormonal regulation. The second clone contains a MT-I processed pseudogene which lacks introns and terminates in a poly(A) tail. Sequencing of the flanking regions, in progress, may help explain the origin and insertion of this sequence.

Other λ clones showing lesser homology to the MT-I cDNA than the MT-I gene intself are being analyzed. The majority contain a moderately repeated dispersed 2 kb Eco R1 fragment which binds a probe of the MT-I coding region. In addition, a second metallothionein-I gene has been isolated. This displays a 9 bp deletion in the coding region, corresponding to an in frame deletion of 3 amino acids. No message from this gene has yet been identified by Northern analysis of rat liver RNA.

0444 SEQUENCE ANALYSIS OF HUMAN METALLOTHIONEIN GENE FAMILY, Umesh Varshney and Lashitew Gedamu, The University of Calgary, Calgary, Alberta, T2N 1N4, Canada.

Metallothioneins are low molecular weight, cysteine rich ubiquitous proteins. These proteins are induced by heavy metals and glucocorticoid hormones. Two major metallothioneins (MT-1 and MT-2) have been characterised in humans. Each of them is 61 amino acids long.

We have screened the human genomic library (T. Maniatis) by using a heterologous probe (mouse MT-1 cDNA, R. Palmiter) for the clones possessing metallothionein gene sequences. These isolated clones represent several of the bands obtained in a southern blot of EcoR1 digest of the genomic DNA. Our preliminary data on restriction mapping, southern blot analysis and sequence studies indicate that metallothioneins are a family of genes, some of which do not have introns. Moreover, some of the metallothionein genes are possibly arranged in tandem. One of the clones on which sequence studies are almost complete seems to be a variant of human MT-2. In this variant gene a single base transition (G→A) in the first base of the tenth codon results in a glycine (in MT-2) to serine (in variant) transition. The rest of the nucleotide sequence of this variant gene is similar to that of the MT-2 cDNA (Karin and Richards, 1982). The 5' flanking region of this gene is highly A,T rich. Data on sequence analysis of different human metallothionein genes will be presented.

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0445 ANALYSIS OF α1-ANTITRYPSIN DEFICIENCY BY GENOMIC MAPPING USING SPECIFIC OLIGONUCLEOTIDES, Vincent J. Kidd, R. Bruce Wallace*, Keiichi Itakura*, Earl W. Davie+ and Savio L. C. Woo, Baylor College of Medicine, Houston, TX 77030, *Division of Biology, City of Hope National Medical Center, Duarte, CA 91010, and +Department of Biochemestry, University of Washington, Seattle, WA 98105

Alpha-1-antitrypsin deficiency is a human genetic disorder which predisposes affected individuals to development of chronic obstructive pulmonary emphysema and/or infantile liver cirrhosis. 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, and is inherited by an autosomal recessive trait with a prevalence of 1 in 2,000 among Caucasians of Northern European ancestry. We have recently cloned the human α1-antitrypsin gene and determined its nucleotide sequence. Oligonucleotides homologous to both the M and Z genotypes were synthesized chemically and used to analyze genomic DNA isolated from MM, MZ and ZZ individuals by Southern mapping. Under specific conditions these oligonucleetide probes will hybridize preferentially to the DNA of the respective genotypes. This analysis can therefore be used for the prenatal diagnosis of the deficiency syndrome in conjunction with genetic counseling and amniocentesis. In addition, the normal α1-antitrypsin gene has been introduced into hepatic cells using the pSV_2-neo and pSV_2-gpt vectors. Analysis of the expression of the normal and variant α1-antitrypsin genes in heterologous cell systems should provide important information about the nature of the defect.

0446 ANALYSIS OF PHENYLKETONURIA (PKU) BY RESTRICTION POLYMORPHISM MAPPING OF THE HUMAN CHROMOSOMAL PHENYLALANINE HYDROXYLASE GENE LOCUS, Savio L.C. Woo, Alan Lidsky, T. Chandra and Kathryn J.H. Robson, Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030

Phenylketonuria (PKU) is a human genetic disorder caused by an inborn error in amino acid metabolism. The hereditary disease is characterized by absence of the hepatic enzyme phenylalanine hydroxylase which catalyzes the hydroxylation of phenylalanine to tyrosine. The lack of this enzmatic activity causes severe retardation in untreated children and the condition is autosomal recessive. In order to gain a better understanding of the molecular basis of phenylketonuria the human phenylalanine hydroxylase gene must be isolated and characterized. We have previously reported the purification of rat liver phenylalanine hydroxylase mRNA by specific polysome immunoprecipitation and the cloning of the corresponding cDNA. Using the rat phenylalanine hydroxylase cDNA clone as a probe, several human phenylalanine hydroxylase cDNA clones as well as the corresponding chromosomal DNA clones were isolated. These human phenylalanine hydroxylase gene clones were used as probes to analyse families with the hereditary disorder by gene mapping. These studies have shown that PKU is not caused by deletion of the entire phenylalanine hydroxylase gene, and polymorphic DNA patterns in the human phenylalanine hydroxylase gene locus generated by several restriction endonucleases were observed. These polymorphic patterns will be very useful in the development of an analytical method for heterozygote detection and prenatal diagnosis of the hereditary disorder by gene mapping.

0447 MOLECULAR BASIS OF INHERITED ANTITHROMBIN III DEFICIENCY, Edward V. Prochownik, Stylianos Antonarakis, Kenneth Bauer and Stuart H. Orkin, Division of Hematology and Oncology, Children's Hospital Medical Center and the Sidney Farber Cancer Institute, Boston, MA 02115 and the Genetics Unit, Department of Pediatrics, the Johns Hopkins University School of Medicine, Baltimore, MD 21205

Antithrombin III (ATIII) is a plasma serine protease inhibitor of M_r 56,000 which inhibits the conversion of prothrombin to thrombin. Familial ATIII deficiency is inherited in an autosomal dominant manner and results in a state of pathologic hypercoagulability. We have previously characterized a cDNA clone containing coding sequences for all except the 10 most N-terminal amino acids of ATIII. This has been used as a probe to examine the genomic organization of ATIII sequences and in studies of families with inherited ATIII deficiency. The entire ATIII gene has been isolated in a recombinant bacteriophage clone. The gene resides within a 16 kb DNA fragment and contains at least two intervening sequences. In digests of genomic DNAs from normal individuals a common PstI restriction polymorphism was observed and is attributable to a translationally silent A→G transition in the third codon position of Gln_{305}. The polymorphism is inherited in simple Mendelian fashion. We used this polymorphism in linkage analysis of two families with inherited ATIII deficiency. In one family, the deficient state was due to deletion of ATIII coding sequences. In a second family, gene deletion did not occur. This study demonstrates a novel use of restriction enzyme polymorphisms in dominantly inherited disorders and establishes a molecular basis for the most common form of ATIII deficiency.

0448 MOLECULAR CLONING OF RARE GENE SEQUENCES INDUCED PROMPTLY (WITHIN 1 HOUR) BY THE PLATELET-DERIVED GROWTH FACTOR. Charles D. Stiles, Sidney L. Hendrickson and Brent H. Cochran, Department of Microbiology and Molecular Genetics, Harvard Medical School and Sidney Farber Cancer Institute, Boston, MA 02115.
Platelet-derived growth factor (PDGF) is a connective tissue mitogen which has been purified to homogeneity from clinically outdated human blood platelets. Nanomolar concentrations of pure PDGF stimulate the growth of fibroblasts, smooth muscle cells and other cells of mesenchymal origin. We have isolated molecular clones of gene sequences which are induced 16-fold in 3T3 cells within 1 hour following exposure to PDGF. Pure PDGF induces these gene sequences at 10 ng/ml or less, whereas other mitogens (epidermal growth factor, insulin) have no effect. In the fully-induced state, these gene sequences constitute 0.05% to 0.5% of the poly A^+ mRNA within BALB/c-3T3 cells. Preliminary screening of 8000 clones from a cDNA library prepared from PDGF-treated 3T3 cells indicates that at least two and probably no more than six different mRNAs are induced by PDGF. Induction of these gene sequences by PDGF is unaffected by cycloheximide; this result indicates that the cloned inserts correspond to "early genes" which are not induced during the course of cell growth but rather are regulated directly by PDGF. One gene sequence is in fact "super-induced" more than 200-fold by the combination of PDGF and cycloheximide; cycloheximide alone has no specific effect. The super-induction phenomena suggests that expression of this gene sequence is subject to feedback inhibition. Preliminary screening data indicate that the PDGF-induced gene sequences are not related to any of the known viral oncogenes.

0449 THE DUNCE GENE OF DROSOPHILA MELANOGASTER, Ronald L. Davis and Norman Davidson, California Institute of Technology, Pasadena, California 91125
The normal function of the dunce$^+$ gene is required for memory, since flies carrying lesions in the gene exhibit an ephemeral memory. Indirect evidence suggests that dunce$^+$ codes for cyclic AMP phosphodiesterase, one member of a family of enzymes which hydrolyze cyclic nucleotides. We have isolated a 100 kbp segment of the X-chromosome which contains dunce$^+$, by the technique of chromosomal walking. Two methods have been employed to delimit the dunce region on the cloned DNA. First, the breakpoints associated with several chromosomal aberrations known to reside on the left or right side of the gene have been mapped by genome blotting experiments. Second, we have searched the region for restriction site polymorphisms between different strains, and have followed the segregation of detected polymorphisms and dunce mutations after meiotic recombination near or within the dunce gene. This analysis has mapped dunce to a region containing a gene which produces no less than 5 poly A^+ RNA transcripts, ranging in size from 1.3 to 9.6 kb. Analysis of RNAs produced by this region in dunce mutants, shows that three produce aberrant transcripts. We have also analyzed the RNA expression from a 50kbp region containing dunce$^+$, in heads, thoraces, and ovaries; and find that in general, all the genes in this region are expressed about an order of magnitude more abundantly in heads than in ovaries, and 4-5 times more abundantly in thoraces than in ovaries. This general expression pattern parallels that observed for cyclic AMP phosphodiesterase activity, and invites speculation that the similar expression pattern reflects some functional relationship between these genes.

0450 Characterization of the I Region Genes in the Mouse H-2 Locus, Richard Maki, Linda Clayton, Susumu Tonegawa, Center for Cancer Research, MIT, Cambridge Ma. 02139
The I region of the mouse H-2 complex codes for gene products which regulate the immune response and probably encodes at least portions of factors which are secreted by T cells. In order to identify specific genes in this area of the genome as well as isolate probes for genomic cloning we have isolated cDNA clones for I-A_α ,I-A_β , and I-E_β genes. We are currently sequencing these cDNA clones and isolating the genomic clones corresponding to these genes. Eventually we plan to use these clones to transform L cells or B cells and study their expression.

0451 ORGANIZATION AND EXPRESSION OF MOUSE VL30 GENES, Michael J. Getz, Clague P. Hodgson, Chandrakant P. Giri and Douglas N. Foster, Dept. of Cell Biology, Mayo Clinic, Rochester, MN 55905.

Mouse VL30 genes are a dispersed class of moderately repetitive sequence elements with structural and functional properties analogous to both integrated forms of retrovirus proviruses and transposable genetic elements. Two recent findings in our laboratory have raised new questions regarding the origin and physiological significance of VL30 genes. We have found that cloned VL30 genes share discrete regions of sequence homology with murine leukemia virus (MuLV) proviruses. In a VL30 long terminal repeat (LTR) this includes the almost perfect conservation of a short inverted terminal repeat sequence believed to function in facilitating integration of MuLV proviral DNA. Since VL30 RNA can be pseudotyped into MuLV virions, this conservation indicates a common integration mechanism for VL30 genes and MuLV proviruses and suggests a retrovirus-assisted mechanism for duplication and dispersal of VL30 genes. Studies on the expression of VL30 genes have shown that closely related RNA transcripts are rapidly and specifically induced following epidermal growth factor (EGF) stimulation of cultured mouse embryo cells. VL30 genes may, therefore, contain specific nucleotide sequences involved in the regulation of expression. Sequencing studies have demonstrated the presence of putative promoter and polyadenylation signals in a VL30 LTR as well as several pairs of tandem direct repeats. One of these, an approximately 40 bp repeat, is partially homologous to the 72 bp tandem repeat (enhancer sequence) of the Moloney murine sarcoma virus. Supported by NIH grant GM 25510 and by the Mayo Foundation.

0452 ISOLATION OF PROMOTERS FROM THE NIF GENE REGION OF ANABAENA VARIABILIS, Rona Hirschberg[1], Bruce Kimmel[2], Sue Samson[2], and Lyn Yarbrough[2], University of Missouri[1], Kansas City, MO 64110 and University of Kansas Medical Center[2], Kansas City, KS 66103

When ammonia and other fixed nitrogen sources are limiting, some vegetative cells of the cyanobacterium Anabaena variabilis differentiate into nitrogen-fixing cells called heterocysts. This is an interesting system for studying gene regulation since it is one of the few examples of terminal differentiation in procaryotes. During development, genes for nitrogen fixation, heterocyst envelop synthesis, and proteases (among others) are turned on while the genes for photosynthesis are turned off. In order to study the mechanisms involved in control of differentiation, we have constructed an Anabaena genetic library and screened it using a Klebsiella nif D-H probe (from W. Brill). A phage clone was isolated that appears to contain the Anabaena nif H and D genes within a 9 Kb EcoRl fragment. This fragment contains a 2.8 Kb HindIII fragment which hybridizes to the Klebsiella probe as well as five smaller (<600 bp) HindIII fragments lying on either side. We have cloned all of the HindIII fragments produced by digestion of the 9 Kb EcoRl fragment into the galactokinase expression vector pK_o-1; two of the small fragments exhibited promoter activity. One approximately 400 bp fragment has about half of the activity of the gal promoter while the other (about 300 bp) was about 15% as effective in promoting transcription. The two promoter containing fragments were subcloned into Ml3mp8 for sequencing by the dideoxy method. Preliminary data from one clone shows sequences about 25 nucleotides apart that are nearly homologous to the -10 and -35 regions of the tet promoter.

0453 CHARACTERIZATION OF THE TUBULIN GENES FROM THE AFRICAN TRYPANOSOME, TRYPANOSOMA RHODESIENSI, L. Yarbrough+, B. Kimmel+, S. Samson+, S. Samuelson+, and R. Hirschberg*, U. of Kansas Medical Center, Kansas City, KS+ and U. of Missouri, Kansas City, MO*.

Trypanosomes are protozoans which cause severe diseases in both man and animals. These organisms exhibit a variety of forms some of which contain a single flagellum composed largely of microtubules. In addition, they contain intracellular microtubules; hence, they represent a good system for study of tubulin gene structure and gene expression. Using a chick β tubulin cDNA probe provided by M. Kirschner we performed genomic Southern analysis of DNA from the human pathogen, T. rhodesiensi. A strongly hybridizing band of about 3.7 Kb was observed in DNA digested by Bam Hl, Eco Rl, or Hind III. In some cases a larger more weakly hybridizing fragment was also seen. Using the chick β tubulin cDNA as a probe we screened a Charon 4 partial Eco Rl library constructed by J. Donelson. Eight clones were purified and all contain the 3.7 Kb fragment which hybridizes to the β tubulin probe. This fragment also hybridizes to a chick α tubulin probe under stringent conditions. Based on amino acid sequence data for porcine tubulin, a minimum of 2.67 Kb is required to code for α and β tubulin. The 3.7 Kb fragment has been isolated and cloned into pBR322 for DNA sequence analysis. Preliminary sequence data show that a region coding for 36 amino acids of trypanosome β tubulin differs in only 4 positions from that of chick and porcine β tubulin. Three of these four amino acid changes could have been produced by a single base change. Comparison of the nucleotide sequence with that of chick β tubulin cDNA shows about 86% conservation. Thus, the β tubulin genes of trypanosomes, chicks, and pigs show strong sequence conservation. (Supported in part by NIH grant 24599 to LY).

0454 A COMPARISON OF VERTEBRATE INTERFERON GENE FAMILIES DETECTED BY HYBRIDIZATION WITH HUMAN INTERFERON DNA, P.G. Boseley,* P.M. Slocombe*, A. Easton* and D.C. Burke, University of Warwick, Coventry, U.K.; V. Wilson, A.J. Jeffreys, P.A. Barrie, University of Leicester, U.K.; *Present Address: G.D. Searle and Co. Ltd., High Wycombe, U.K.

Cloned human interferon complementary DNAs were used as hybridization probes to detect interferon α and β gene families in restriction endonuclease digests of total genomic DNA isolated from a wide range of vertebrates and invertebrates. A complex interferon-α multigene family was detected in all mammals examined, whereas there was little or no cross-hybridization of human interferon-α cDNA to non-mammalian vertebrates or invertebrates. In contrast, human interferon-β cDNA detected a single interferon-β gene in all mammals tested, with the exception of the cow and the blackbuck, both of which possessed a complex interferon-β multigene family which has presumably arisen by a recent series of gene duplications. Interferon-β sequences could also be detected in non-mammalian vertebrates ranging from birds to bony fish. Detailed restriction endonuclease mapping of DNA sequences neighbouring the interferon-β gene in a variety of primates indicated a strong evolutionary conservation of flanking sequences, particularly on the 3' side of the gene.

0455 THE STRUCTURAL ORGANIZATION AND REGULATORY SEQUENCES OF THE PREPROSOMATOSTATIN 1 GENE FROM ANGLERFISH. Robert J. Crawford+, Peter Hobart* and W.J. Rutter*. *Department of Biochemistry, University of California, San Francisco, CA, and +Howard Florey Institute, University of Melbourne, Australia.

Somatostatin inhibits the secretion of several peptide hormones, including growth hormone, insulin and glucagon. It may also be involved in neurotransmission. We have begun to compare the structural organisation and regulatory sequences of somatostatin genes in several vertebrates. Accordingly we constructed an anglerfish genomic library and isolated clones containing the preprosomatostatin 1 gene. Several striking features about the organization of sequences surrounding this gene are evident. The gene is bounded by small inverted repeat sequences positioned at both the 3' end, and adjacent to the CAAT sequence at the 5' region. The 5' promoter region contains three significant regions of dyad symmetry surrounding both the CAAT sequence and the TATAA region. Comparison of the promoter region of the anglerfish somatostatin 1 gene with that of human (Shen & Rutter, manuscript submitted) reveals negligible sequence homology. However, some features of dyad symmetry have been conserved, suggesting that secondary structure within the promoter region is important in regulating somatostatin gene expression.

0456 CHROMOSOMAL ASSIGNMENT OF ALCOHOL DEHYDROGENASE STRUCTURAL AND REGULATORY GENES, Moyra Smith, University of California, Irvine California 92717.

Human cathodal alcohol dehydrogenase (ADH) isozymes represent homodimers and heterodimers of 3 distinct but structurally similar polypeptides. Our previous biochemical studies indicate that these polypeptides are the products of 3 different gene loci which exhibit developmental and tissue specific regulation.

In order to define the chromosomal location of human ADH genes and to examine factors involved in the regulation of ADH gene expression, we derived somatic cell hybrids from the fusion of FU5 rat hepatoma cells and human fibroblasts. Shortly after fusion it was noted that in a number of hybrid clones production of rat liver ADH was suppressed. Following further cultivation and additional loss of human chromosomes from the hybrids, rat liver ADH was re-expressed in a number of clones. In a sub-set of these rat liver ADH expressing clones, human liver ADH was also expressed. Through analysis of the human chromosomal content of these hybrids, we have obtained information on a) the chromosomal assignment of one of the human structural ADH genes and b) the possible role of additional human chromosomes in the regulation of ADH gene expression.

To examine the ADH gene and ADH gene expression at the molecular level, we have isolated and transcribed polyA mRNA from human liver. In vitro synthesized ADH has been immuneprecipitated. These studies indicate that mRNA for ADH is an abundant message species in liver. Studies are currently in progress to derive a cDNA probe for ADH.

0457 TERRITORIAL EFFECTS ON THE OCCURRENCE OF PSEUDOGENES AND POORLY TRANSCRIBED GENES ON EUKARYOTE CHROMOSOMES, Hiroto Naora, Research School of Biological Sciences, The Australian National University, Canberra, A.C.T., Australia.

Pseudogenes have been reported in various types of multigene families in a variety of eukaryote organisms. However, their biological significance remains obscure. Recently we have pointed out that when two genes lie on the same DNA strand and have an intergenic distance shorter than a defined length, the transcriptional activities of one or both genes are, in most cases, inactivated or reduced. This is called a "territorial" effect. In this paper, the various arrangements of pseudogenes and a few poorly transcribed genes are taken from recent publications, and the relationship between the occurrence of pseudogenes and territorial effects was studied. Evidence is presented which confirms the previous suggestion that there exists a correlation between the occurrence of some (but not all) pseudogenes and territorial effects. Furthermore, our results show that the occurrence of those pseudogenes which are thought to be under territorial control is proportional to the size of the multigene family clusters, whereas another class of pseudogenes, which appears not to be under territorial control is independent of cluster size. It is also noted that pseudogenes have been discovered more frequently in the dispersed form of multigene family.

0458 *EXPRESSION OF THE MAJOR CO_2-FIXING ENZYME IN WHEAT*, *Gayle Lamppa, Richard Broglie, Gloria Coruzzi, Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, New York, New York 10021*

A homologous in vitro transcription system is being developed for wheat using the nuclear-encoded, small subunit gene for ribulose bisphosphate carboxylase (RUBISCO) as a template. At least 6 genes are present in wheat for the RUBISCO small subunit. R-looping and sequencing data for one genomic clone demonstrate the presence of a 287 base (b) intron that separates the transit from the mature polypeptide sequences. A comparison of the genomic clone with a full-length cDNA sequence indicates that major base changes have occurred in two different genes in their 5' and 3' non-translated regions. R-loops of full-length suggest that the gene we have isolated is transcribed in vivo. Transcriptionally active cell-free extracts have been prepared from wheat seedlings, using as a template a subcloned genomic fragment that includes the entire gene and 600 bases at the 5' end. S1-mapping suggests that several sites of mRNA initiation may be recognized in vitro. The S1-resistant products are dependent on the addition of the carboxylase small subunit gene template, and are also strand-specific. We are concomitantly defining the in vivo transcripts of the RUBISCO genes. The major poly A+ RNA species is 850 b; minor bands appear on gels at about 1250 and 1500 b. The 1250 b species approaches the size expected for a precursor RNA containing the intron and a poly-A tail.

0459 NUCLEOTIDE SEQUENCE OF A cDNA FOR THE COMMON α-SUBUNIT OF THE BOVINE PITUITARY GLYCOPROTEIN HORMONES: DEVELOPMENTAL EXPRESSION OF THE α-SUBUNIT GENE. J.H. Nilson‡ A.R. Thomason,+ M.T. Cserbak‡ C.L. Moncman* and R.P. Woychik‡,++ Departments of Pharmacology*, Molecular Biology and Microbiology‡,++ Case Western Reserve University, Cleveland, OH 44106; and Applied Molecular Genetics‡ Thousand Oaks, CA 91320

Synthetic oligodeoxynucleotide probes were used to isolate a cDNA clone which codes for the common α-subunit of bovine LH, FSH, and TSH. The cloned α-cDNA contains 549 bp and is almost full-length. As expected, there is considerable amino acid and nucleotide homology shared between bovine, human and murine pre-α subunits. In contrast, the sequence homology between the 3'-untranslated region of the cow and mouse is 14%, while the homology between cow and human is 56%. Moreover, there is a stretch of 93 nucleotides in the 3'-untranslated region of cow and human where the homology is 85%, suggesting that this region may be of functional significance. We have also used both the α-subunit cDNA clone, and prolactin and growth hormone cDNA clones, to quantitate the levels of these mRNAs during development. The steady-state levels of the α-subunit mRNA remain relatively constant throughout development. In contrast, prolactin mRNA levels increase over 100-fold, while growth hormone levels fall by a factor of two. The change in mRNA levels for at least one of these hormones, prolactin, is probably a result of differential gene expression.

0460 A SOLO-LTR-LIKE MULTICOPY INSERTION SEQUENCE IN THE BOVINE GENOME, R.E. STREECK, Institut Pasteur, Paris. France

Two insertion sequences present in the bovine genome at a high copy number have been analyzed (R.E. Streeck, Science 213 443-445 (1981); Nature 298, 767-769 (1982)). They are 0.6 and 1.2 kb long, respectively, highly homologous at their ends, and completely unrelated in their internal sequences. The 1.2 kb element which generates a 6 bp duplication at the site of insertion has striking similarities to a retrovirus LTR (solo-LTR). First detected in satellite DNA, the distribution of these sequences in the genome is now analyzed. Their possible role in the generation of a retrovirus will be studied.

0461 EVOLUTION AND COMPLEXITY OF THE GENES ENCODING THE KERATINS OF HUMAN EPIDERMAL CELLS, Elaine V. Fuchs, Israel Hanukoglu and Kwan Hee Kim, The University of Chicago, Chicago, IL 60637

The cytoskeletal keratins are a family of closely related proteins (MW 40-70 kd) that form 80 Å intermediate filaments in the cytoplasm of most vertebrate epithelial cells. Each epithelial cell expresses its own subset of keratins which consists of about 2-6 polypeptides. In epidermis the keratins are especially abundant, comprising 30-85% of the total protein of these cells. We have shown that in all human epithelial cells, there are multiple mRNAs for the keratins. These RNAs can be grouped into two distinct classes as judged by their ability to hybridize to one of two separate classes of cloned keratin cDNA sequences. Each of these two classes of sequences is encoded by a multigene family of about 10 genes each and these two families are coordinately conserved throughout vertebrate evolution. Recently, we have determined the DNA sequence of a cloned cDNA insert which is complementary to > 90% of the coding region for a 50 kd keratin belonging to the class of small keratins. A comparison of the predicted amino acid sequence of this cytoskeletal keratin with the partial sequences known for other intermediate filament proteins indicates that the keratins may be the most distantly related of this class of 80-100 Å filamentous proteins. This is in contrast to the actins and tubulins, which are highly conserved components of vertebrate cytoskeletons. The wide flexibility in the amino acid sequence of intermediate filament proteins suggests that these proteins have evolved to meet subtly different requirements in the cytoskeletal architecture.

0462 An mRNA FRACTION ENRICHED FOR CHICKEN MYOGLOBIN mRNA, David A. Konkel, Tushar N. Patel, Gregory Alsip, and Amanda McWatters, University of Texas Medical Brach at Galveston, TX 77550.

Total polysomal RNA was prepared from 19-day embryonic chick thigh and heart muscle by the method of Kelly et al (Biochem. Biophys. Acta. 609, 278-285, 1980). Poly A+ mRNA was incubated in a rabbit reticulocyte cell-free translation system and ^{35}S-products tested for presence of myoglobin by immunoadsorption to formalin-fixed staph A cells. SDS-polyacrylamide gel electroploresis showed myoglobin to be synthesized at about 0.4% of products made from heart mRNA, but at less than 0.02% of products from thigh muscle. The latter result is unexpected and will be investigated further at a later date.

Poly A+ mRNA was fractionated by size on an automated slab gel eletrophoresis system (1.5% agarose - 6M urea, pH 3.5; Wertz et al, Anal. Biochem. 106, 148-155, 1980). About 10-fold enrichment (by weight of RNA) was obtained from a single electrophoresis run on 1.5 mg of Poly A+ heart mRNA. Resolution was significantly decreased below that expected, due to temporary RNA sticking to the dialysis membrane, so that higher molecular weight fractions are somewhat contaiminated by "smaller" RNA species. This problem is significantly reduced by using the Bethesda Research Labs continuous elution tube gel apparatus with the same gel system, although total capacity of the gel is decreased to about one milligram. Thus, when fractionating total mRNA rather than discrete species, the continuous elution method seems superior to automated periodic elution. We are currently constructing a cloned cDNA library from the enriched fraction. The library will be screened for myoglobin-specific clones by the hybridization - elution method.

0463 PATTERN FORMATION IN DEVELOPMENT:MOLECULAR ANALYSIS OF THE engrailed LOCUS IN DROSOPHILA, Jerry M. Kuner, Mikiye Nakanishi, Barry Drees, Thomas Kornberg, and Patrick H. O'Farrell, University of California, San Francisco, CA. 94143

The engrailed locus in Drosophila functions in subdivision of the embryo into segments and smaller developmental units called compartments. Homoeotic loci, like bithorax, act to determine the developmental program to be followed by the compartments previously delineated by genes like engrailed.

We have isolated as recombinant DNA clones over 250 kb spanning the polytene band 48A, the cytological position of the engrailed locus. Southern blot analysis has been used to locate the positions of engrailed mutations caused be chromosome rearrangements; all of these have breakpoints at 48A and alter restriction fragments within the cloned region. Seven mutations are clustered in a 20 kb region. Three transcripts have been detected in a 50 kb region surrounding the breakpoints. Some of the breakpoints are located in regions where no transcripts have been detected. Current work focuses on mapping positions of open translational reading frames and producing hybrid polypeptides from the cloning vector—Drosophila DNA fusion constructs. These will provide antigens for immunocytological studies to test whether engrailed expression is positionally confined to those compartments where it functions.

0464 DNase I HYPERSENSITIVE REGIONS CORRELATE WITH A TOPOISOMERASE-LIKE ACTIVITY ON THE r-CHROMATIN OF TETRAHYMENA, Bjarne Bonven, Elmar Gocke and Ole Westergaard, University of Aarhus, DK-8000 Aarhus C, Denmark

A novel nuclease activity have been detected at three specific sites in the chromatin of the spacer region flanking the 5'-end of the ribosomal RNA gene from Tetrahymena. The endogenous nuclease does not function catalytically in vitro, but is in analogy with the DNA topoisomerases activated by strong denaturants to cleave DNA at specific sites. The endogenous cleavage sites are associated with micrococcal nuclease hypersensitive sites and DNase I hypersensitive regions. Thus, a single, well-defined micrococcal nuclease hypersensitive site is found approximately 130 bp upstream from each of the endogenous cleavages.

Exposure of the r-chromatin to strong detergents results in a cleaved DNA molecule with a polypeptide chain covalently attached to the free DNA ends created in the process. This suggests similarities between the observed nuclease activity and the topoisomerases, where these DNA-protein complexes represent trapped intermediates in the normal catalytical cycle of the enzyme.

The location of the endogenous nuclease activity with the hypersensitive sites near the 5'-end of the coding region strongly suggests that the nuclease participates in the transcriptional control of the chromatin.

0465 THE REGULATION OF EXPRESSION OF YOLK POLYPEPTIDE GENES IN DROSOPHILA MELANOGASTER, Charles J. Kunert, John H. Postlethwait, Parviz Minoo, and Paul D. Shirk, University of Oregon, Eugene, Or. 97403.

The yolk polypeptide (YP) genes of Drosophila melanogaster are under the regulation of the hormones 20-hydroxyecdysone and juvenile hormone and, in addition, are expressed in tissue- and stage-specific ways in the developing fly. They provide, as such, an interesting system for the study of genetic regulation in eucaryotic organisms. Three separate YPs are coded for by single-copy genes located on the X chromosome. The mature products of these genes form bands on SDS-PAGE gels which correspond to molecular weights of 47, 46, and 45 Kd (YP1, YP2 and YP3, respectively). Several female sterile mutants have been isolated which map to these gene loci and result in electrophoretic or quantitative variants in the amount of YP synthesized. One of these (fs(1)1163) causes a ten-fold reduction in the amount of YP1 observed. While mRNA levels are normal, a defect has been found in the secretory form of this polypeptide. Both the primary translation product and the intermediate product (formed by cleavage with pancreas microsomes) are about 300 daltons heavier than wild-type. Time course experiments in vivo indicate an accumulation of intermediates between the IYP1 and MYP1 not seen in wild-type. E.M. studies indicate peculiar vesicle formation in mutant cells which are secreting YPs. This mutant thus alters the YP sequence in such a way as to preclude normal processing and secretion. Structural studies of the gene and message indicate the changes which occur in the mutant gene which modify its cpacity to be secreted.

0466 ORGANIZATION AND EXPRESSION OF THE BOVINE GROWTH HORMONE GENE, Fritz M. Rottman and Richard P. Woychik, Case Western Reserve University, Cleveland, OH 44106
Our laboratory is interested in the genomic organization and regulated expression of bovine pituitary hormones. As part of these studies we have established a bovine genomic library and isolated a gene coding for bovine growth hormone. This gene is likely to represent the gene expressed in the pituitary since the nucleotide sequence of the coding regions of the gene was found to be identical with that of a nearly full-length growth hormone cDNA clone. The gene sequence is approximately 1800 bp in length and contains four intervening sequences. The second intervening sequence (227 nt), is considerably shorter than that found in the rat growth hormone gene and lacks the repetitive element containing potential pol III initiation sites also noted in the rat gene. A comparison of the 5'- and 3'-flanking and untranslated regions of the bovine, human and rat growth hormone genes revealed many areas of conserved sequence. Of special interest, however, was the observation that all three genes contain within their 5'-flanking regions a highly conserved sequence of 38 nucleotides. This sequence is at least 90% homologous between the three species and is located about 100 bp upstream from the transcription initiation site of each gene. Whether this sequence functions as a hormone recognition signal remains to be established. Preliminary DNA-mediated gene transfer experiments indicate that the bovine growth hormone gene is transcribed in transfected cells.

0467 REGULATION OF RIBOSOMAL PROTEIN GENES, Jonathan R. Warner, Howard M. Fried, Norbert Kaufer, Chung Kim and Nancy J. Pearson, Departments of Biochemistry and Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461.
The isolation of sixteen ribosomal protein genes from Saccharomyces cerevisiae has made it possible to study the levels of regulation which lead to the coordination of the synthesis of the ribosomal proteins with each other and with the ribosomal RNA. Many of the ribosomal protein genes appear by indirect evidence to have an intron. We have determined the sequence of the cyh2 gene and demonstrate from comparison with known protein sequences, from examination of canonical splice sites, and from direct observation, that it has a single intron of 514 nucleotides. This is the first yeast gene with a selectable phenotype shown to contain an intervening sequence.

While each of the ribosomal protein genes has an individual transcript unit, their transcription is tightly coupled, as shown by two results: (1) approximately equimolar amounts of mRNA for each ribosomal protein is transcribed; (2) after a mild temperature shift the transcription of each ribosomal protein gene is drastically but temporarily inhibited. An additional level of regulation is observed when extra copies of a ribosomal protein gene are introduced into the cell. Although there is four-fold more active mRNA for that gene present within the cell, only slightly more of the ribosomal protein is made than in the control cells. Thus Saccharomyces must use both transcriptional and translational levels of regulation to maintain an equimolar synthesis of ribosomal proteins.

0468 NUCLEAR GENES CONTROLLING PRODUCTION OF THE MITOCHONDRIAL CYTOCHROME b mRNA IN YEAST, Carol L. Dieckmann, Gregory Homison, T.J. Koerner and Alexander Tzagoloff, Columbia University, New York, NY 10027. By mutation, 130 nuclear genes have been identified that are necessary for mitochondrial respiration in Saccharomyces cerevisiae. Five of the 130 genes code for elements involved in the transcription and maturation of one mitochondrial gene transcript; cytochrome b. The cytochrome b transcript contains two intervening sequences. Northern blot analyses of the mitochondrial RNA of mutant strains show three of the nuclear genes are involved in excision of the first intron, and the fourth in excision of the second intron. The fifth gene, CBP1, is involved in the initiation of transcription of the cytochrome b gene or in transcript processing. cbp1 mutants exhibit very little cytochrome b hybridizable material. Anomolous transcripts with 5' ends 200 nucleotides downstream of the AUG codon in exon 1 are present in RNA from two cbp1 isolates, E3 and N5-26. These mutants have no transcripts with normal 5' ends -940 nucleotides upstream of AUG. The +200 site has some homology to the -940 site and no homology to any exon/intron boundary. Studies are under way to determine if -940 is the transcription start site or is a 5' processing site. cbp1 mutants have normal amounts of glu tRNA, the gene for which ends at -1085 relative to the cytochrome b AUG. Double mutants constructed between those blocked in excision of I_1 or I_2 and the cbp1 mutants, like the cbp1 mutants, have little hybridizable cytochrome b material. The CBP1 gene has been cloned, subcloned to 2.4 kb, and sequenced. The sequence reveals a reading frame of 1962 bp coding for a highly basic protein of 76,000 daltons. Three very basic domains are found; at the NH_2-terminal, at the COOH-terminal, and residue 500 to 593. The above properties are being exploited to purify the CBP1 gene product.

0469 THE TWO ADULT β-GLOBIN GENES OF THE C57BL MOUSE HAVE UNDERGONE GENE CONVERSION. Steve Weaver, Mark A. Erhart & Kimberlee S. Simons, University of Illinois at Chicago, Chicago, IL 60680.

In Mus musculus, hemoglobin β chains are synthesized from a pair of tandemly duplicated genes. The species is dimorphic with respect to the number and kind of β globins produced by these two 'adult' genes. Animals homozygous for one alternative form of the gene cluster, the so-called 'single' haplotype, have but one molecular species of β globin, β-single. Strains carrying the alternative 'diffuse' haplotype produce two distinct types, β-dmajor and β-dminor.

We have sequenced the two genes (designated β-s and β-t) of the 'single' mouse C57BL/10, and compared them to their alleles (β-dmaj and β-dmin) carried by the'diffuse' strain BALB/c. As expected, the β-s and β-t genes encode identical polypeptides. Surprisingly, the two genes are virtually identical: no silent substitutions in any of the three exons, and almost no differences in either of the two introns. This is very different from the situation with the 'diffuse' haplotype. The β-dmaj and β-dmin genes are in the process of divergence. They have accumulated replacement changes at 9/146 condons, and their large introns have become radically different. (Konkel, et. al 1979 Cell 18, 865-873)

The identity of β-s and β-t appears to be due to gene conversion. The gene conversion event(s) have occurred within defined interval, the limits of which can be discerned. The 5' boundary lies very near the ATG initiation codon and the 3' boundary lies very near the termination codon. Outside these limits, β-s and β-t are just as divergent as are β-dmaj and β-dmin, and in fact are almost identical with them.

0470 ORGANIZATION AND EXPRESSION OF A GENE FAMILY CONTAINING A REPEATING TRINUCLEOTIDE SEQUENCE IN DICTYOSTELIUM. Alan R. Kimmel, LCDB/NIADDK, NIH, Bethesda, MD 20205.

I have identified a unique organization and transcriptional pattern of the repeating trinucleotide (AAC/GTT)n in Dictyostelium. The repeat does not occur in long tandem arrays (as satellite DNA) but in short tandem blocks of 35 to 150bp interspersed with single-copy DNA. 1% of the poly(A)+ RNA from vegetative cells contain sequences which hybridize this repeat. The complementary RNA is heterogeneous in size and 90% of its mass hybridizes to single-copy DNA. Sets of cDNAs derived from poly(A)+ RNA present at various developmental stages have been isolated which are complementary to the repeat. Comparisons of various genomic and cDNA sequences indicate that (AAC/GTT)n is the common element. DNA and RNA blot hybridizations with probes from regions adjacent to this element or probes specifically deleted of (AAC/GTT)n sequences hybridize to unique restriction fragments and unique poly(A)+ RNA species. The (AAC/GTT)n is asymmetrically transcribed; only (AAC)n sequences are represented in RNA. The (AAC)n stretches are located at the 5' end of cDNAs upstream from single-copy regions. One stretch has been localized within 30bp of the TATA "promoter" of a particular gene. The AAC gene family is expressed in a unique developmental pattern. Individual members of the AAC-containing RNAs are expressed with similar developmental properties suggestive of coordinate expression. The relative rates of transcription, the relative abundancies of nuclear,polysomal and non-polysomal cytoplasmic RNA and the organization in chromatin of these genes during development are being studied. By using DNA-mediated transformation to introduce specifically mutated derivatives of this gene family into Dictyostelium it should be possible to monitor the relationship of the (AAC)n sequence to their expression.

0471 MULTIPLE "TATAA" SEQUENCES ARE ASSOCIATED WITH MULTIPLE TRANSCRIPTIONAL START SITES FOR THE RAT SVS IV GENE, W. Stephen Kistler, Jagannadha C. Kandala, Malathi K. Kistler and Robert P. Lawther, University of South Carolina, Columbia, SC 29208

The major secretory proteins of the rat seminal vesicle are under investigation as differentiated gene products whose synthesis is regulated by androgens. We have isolated a single 3.3 kb Eco Rl fragment from a genomic library that contains the entire gene region for protein SVS IV. S1 mapping coupled with restriction mapping and partial sequence analysis have produced a complete transcriptional map of the gene. It is composed of three exons measuring 98, 310, and 164 base pairs that are separated by introns of approximately 450 and 710 bp. The locations of the introns roughly divide the message into functional regions. The first intron separates the signal peptide from the sequences coding for the bulk of the mature protein, while the second intron lies entirely within the 3'-untranslated portion of the message. Accordingly, virtually the entire coding sequence for the body of the secreted form of the protein lies within a single exon. Upstream from the coding region of the gene are clustered three AT-rich regions with varying degrees of homology to the TATAA box sequence generally found 25 to 30 bp ahead of the Cap site of eucaryotic genes transcribed by RNA polymerase II. S1 mapping employing 5'-labeled restriction fragments as probes indicated that all three TATAA sequences are capable of directing transcriptional initiation in vivo. However, studies using primer extention catalyzed by reverse transcriptase indicated that the quantitatively most significant TATAA site is that closest to the coding sequence. Supported by NIH grant HD 13472.

0472 REGULATION OF AVIAN LIPOGENIC ENZYME GENE EXPRESSION, Sidney M. Morris, Jr., Larry K. Winberry, and Alan G. Goodridge, Case Western Reserve University, Cleveland, OH 44106

Fatty acid synthase (FAS) and malic enzyme are two of the set of lipogenic enzymes which are acutely regulated in liver cells by nutritional status *in vivo* and by thyroid hormone and glucagon *in vitro*. We have recently isolated cDNA clones for these two enzymes from goose and are using them to characterize regulation of their corresponding mRNAs. In liver of neonatal goslings which have been fasted and then refed for 24 hours, FAS and malic enzyme mRNA levels are increased by about 70-fold and 50-100-fold, respectively. This matches well with the corresponding changes in the enzyme synthesis rates. In chick embryo hepatocytes in culture, changes in malic enzyme mRNA levels parallel the approximate 100-fold increase in enzyme synthesis rate due to thyroid hormone treatment. Furthermore, this increase is virtually completely blocked by exposure to glucagon. These results indicate that nutritional and hormonal regulation of synthesis of these two enzymes occurs at the pre-translational level. Experiments are underway to determine the degree to which the rate and extent of response to thyroid hormone and glucagon are coordinated for the FAS and malic enzyme mRNAs.

0473 CONSERVATION AND EXPRESSION OF A FAMILY OF MODERATELY REPETITIVE MOUSE DNA SEQUENCES, Sheau-Mei Cheng, Cancer Biology Program, Frederick Cancer Research Facility, Frederick, Maryland 21701

When mouse DNA is digested to completion with restriction endonuclease EcoRI and subjected to agarose gel electrophoresis, a distinct 1.3-kb segment comprising about 0.5-3% of the total genome is observed. This DNA is not tandemly repeated in the genome and is not derived from highly repeated mouse satellite DNA. After being cloned, the 1.3-kb fragments could be categorized into two classes of repeated DNA sequences. Class I DNA hybridized predominantly to 1.3-kb genomic EcoRI fragments, whereas class II hybridized both to 1.3-kb fragments and to many other genomic EcoRI fragments. There was no cross homology between class I and class II sequences. Analysis of RNA by Northern blotting and hybridization indicated that both total mouse L cell RNA and cytoplasmic poly(A) RNA contain abundant heterodispersed transcripts of the two classes of repeats.

Repetitive sequences complementary to both classes of 1.3-kb EcoRI fragments were found in rat and CHO DNA, but not in human DNA. RNA homologous to both classes of repeated sequences was detected in rat brain tissue, but not in rat liver and muscle tissue, nor in a rat myoblast cell line. This tissue-specific expression of DNA suggests a regulatory function for the repetitive DNA sequences during evolution and differentiation.

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0474 THE MAJOR URINARY PROTEINS OF THE MOUSE: MULTI-TISSUE EXPRESSION AND DIFFERENTIAL REGULATION OF A MULTI-GENE FAMILY. W.A. Held, N.D. Hastie, P. Shaw, J. Knopf, R.E. Hill, and S. Remson. Roswell Park Memorial Institute, Department of Cell and Tumor Biology, Buffalo, New York 14263.

The major urinary proteins (MUPs) constitute a family of approximately 20 genes which are clustered at the Mup a locus of chromosome 4. Several different MUP proteins are synthesized in the liver, secreted, and rapidly excreted into the urine. A number of other tissues (submaxillary, sublingual, parotid, lacrimal, mammary glands) have been found to contain MUP mRNA sequences. The MUP genes expressed in some of these tissues appear to be quite distinct from those expressed in liver; and the liver, lacrimal, mammary, and salivary glands each have distinct developmental patterns. In some tissues (submaxillary, sublingual, and parotid gland), there is no apparent hormonal regulation. Several hormones (testosterone, thyroxine and growth hormone) regulate expression of MUP genes in the liver and different hormonal states result in different patterns of MUP gene expression. Measurements of transcription rates suggest regulation by both transcriptional and post-transcriptional mechanism. In contrast to the complex regulation of the MUPs in the liver, lacrimal MUPs appear to be regulated only by testosterone. Thus, the MUPs constitute a family of genes which are subject to a diversity of tissue specific, developmental, and hormonal controls. Possible functions of MUPs suggested by the unique tissue distribution and hormonal regulation are discussed. Further, since each tissue has a unique MUP array and hormonal regulation, it should be possible to relate this to a physical map of the genes.

0475 MOLECULAR ORGANIZATION OF THE QA-1 REGULATORY LOCUS OF NEUROSPORA CRASSA,
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The qa-1 locus of Neurospora positively regulates the inducible enzymes responsible for quinic acid catabolism. The enzymes, which are induced by quinic acid, are encoded by three genes (qa-2, qa-3 & qa-4) tightly linked to qa-1. The entire qa gene cluster has been cloned into E. coli and the individual genes localized by transformation of Neurospora. In order to further understand the regulation of this genetic system, the qa-1 locus has been characterized at the molecular level.

By transforming Neurospora qa-1 mutants with various plasmids and hybridizing these plasmids to poly(A)$^+$ mRNA, it has been shown that the locus is composed of two distinct genes. These genes correspond to the mutant types qa-1^S (uninducible, semi-dominant) and qa-1^I (uninducible, recessive), earlier interpreted to be separate domains of a single gene[1]. The entire six kb region has been sequenced and the coding regions of qa-1^S and qa-1^I determined. The implications of the results for the regulation of the qa genes will be discussed.

1. Case & Giles (1975) Proc. Natl. Acad. Sci. USA 72, 553-557.
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