MOLECULAR APPROACHES TO DEVELOPMENTAL BIOLOGY

Richard A. Firtel and Eric H. Davidson, Organizers

March 30 — April 6, 1986

Plenary Sessions

Poster Sessions

Late Additions

Oogenesis and Maturation

N0 TOTAL AND SPECIFIC RNAs IN MOUSE OOCYTES AND EGGS, Rosemary Bachvarova, Barbara V. Paynton, Victor De Leon, Gail Kaplan, and Andrew Johnson, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, N.Y. 10021.

The main classes of RNA accumulate steadily during growth of mouse oocytes. mRNA is synthesized at a rate that is rapid relative to somatic cells, but 40 fold lower than that on lampbrush chromosomes of Xenopus oocytes (1). The full-grown mouse oocyte contains about 0.5 ng of total RNA (2), of which approximately 20% is polyadenylated RNA (3). During the 12 hours of meiotic maturation, when the pattern of protein synthesis changes significantly, half the mRNA is deadenylated or degraded, and total RNA decreases by almost 20% (4). Much of the remaining mRNA is probably lost by the late 2-cell stage.

Northern blotting has been used to examine specific RNAs during maturation. Total RNA from samples of 100-1000 oocytes is run on denaturing gels, blotted, and filters hybridized with double or single stranded probes. β-actin mRNA is present in similar amounts in full-grown oocytes and in eggs, but is deadenylated during maturation, seen as a small decrease in MW (4). Northern blotting carried out on fractions collected from sucrose gradients shows that 90% of the actin mRNA is on polysomes in oocytes, and very little is polysomal in eggs, consistent with its known pattern of translation. Samples collected at several time points during in vitro maturation indicate that most of the β-actin mRNA molecules lose most of their poly(A) between 7 and 10 hours of maturation. The mRNAs for α-tubulin and for HPRT do not show any change in MW during maturation.

Blots have also been analysed with probes containing a mixture of "Alu" repetitive sequences. Homologous sequences are observed in the MW range of mRNA, but less than that in an equivalent amount of liver or brain RNA (5). The probe also reacts with a set of low MW RNAs 100-600N in length, including 4.5S RNA, a small RNA associated with mRNA. Using single stranded probes from specific Alu-related sequences, it is found that 4.5S RNA is abundant in oocytes and eggs, and its complement in mRNA-sized molecules. One strand of a B2 sequence detects a few mRNAs as well as a set of low MW species in oocytes. One or more of the Alu-containing mRNAs show small changes in MW during maturation.

1. Bachvarova, R. (1981). Devel. Biol. 86: 384.
2. Bachvarova, R. (1985). in "Developmental Biology" vol. 1 (L.W.Browder, ed.), p. 453.
3. De Leon, V. et al. (1983). Devel. Biol. 98: 400.
4. Bachvarova, R. et al. (1985). Devel. Biol. 108: 325.
5. Kaplan, G. et al. (1985). Devel. Biol. 109: 15.

N1 GENETIC COMPLEXITY OF CONTROL FOR OOGENESIS, Anthony P. Mahowald and Norbert Perrimon, Genetics & Anatomy, Case Western Reserve Univ., Cleveland, Ohio.

Molecular studies utilizing either hybridization kinetics or protein synthetic patterns have clearly indicated that the majority of the genome is active during oogenesis. Only a few genetic loci, however, are active only during oogenesis, whereas most loci also perform critical functions during other stages of the life cycle. By producing ovarian germline clones to lethal alleles we have found that a portion of late larval-early pupal lethal mutations show distinct embryonic pattern defects in embryos produced from a homozygous germline clone. We have now completed our analysis of over 610 lethal loci on the X-chromosome and have identified 10 essential loci with distinct, maternally dependent, effects on segmentation. In addition, we have found at least 25 loci showing either the "topless" or neurogenic phenotypes. Because of the extensive nature of these screens, we are able to estimate the approximate number of these pattern elements in the whole genome. Detailed description will be provided for a new "gap" mutation and a new "segment-less" mutation.

N2 PROCESSING AND TERMINATION ON THE XENOPUS RIBOSOMAL GENES, Ronald H. Reeder and Paul Labhart, Basic Sciences Division, Hutchinson Cancer Research Center, Seattle WA 98104

Three major sites of 3' end formation, designated as sites T1, T2, and T3, have been identified within the intergenic spacer of the Xenopus laevis ribosomal genes. Each site has different sequence signals and characteristics which suggests that each form 3' termini by a different mechanism. Site T1 corresponds to the unique HindIII restriction site located at the 3' end of the mature 28S rRNA (and at the 3' end of the 40S precursor). T1 is a processing site and transcription continues past T1 without attenuation for another 235 nucleotides to site T2. With appropriate probes it is possible to detect a low amount of a high molecular weight precursor that contains these extra sequences. Site T2 has no sequence homology with T1 and seems to mark the point where the transcript becomes highly unstable (half-life less than one minute). T2 is not a termination site since several types of experiments show that T2 does not cause polymerase release. Site T3 is located on the extreme right-hand end of the spacer, at position -215 relative to the 5' end of the 40S precursor. T3 shares some sequence homology with T2 but experiments suggest that, in contrast to T2, T3 can cause polymerase release. Thus, site T3 is currently the best candidate for a true termination site on the ribosomal gene repeat. The proximity of T3 to the gene promoter suggests that, even if it is a termination site, there may be some mechanism for passing the polymerase from T3 to the promoter without allowing the polymerase to enter a free pool of competing polymerases.

N3 MULTIPLE MECHANISMS REGULATE THE TRANSLATION OF MATERNAL mRNA. L. Dennis Smith, M. A. Taylor, A. D. Johnson and R. Gelfand. Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

Previous studies involving the injection of heterologous mRNAs into Xenopus laevis oocytes have demonstrated that protein synthesis in full-grown (stage 6) oocytes is limited by components of the translational machinery other than mRNA availability(1). However, recent evidence shows that message availability does limit protein synthesis in growing (stage 4) oocytes, since injected messages do not compete for endogenous mRNAs for translation. Rather, total protein synthesis in stage 4 oocytes can be increased to the level seen at stage 6 by injection of increasing amounts of mRNA(2). We suggest that the limiting component in stage 6 oocytes, present in excess at stage 4, is a protein(s) which binds to mRNA prior to initiation and enhances the probability of subsequent initiation and translation. The limited availability of mRNA in growing oocytes appears to result from two events. On the one hand, small oocytes contain proteins which can suppress translation when reconstituted with mRNA(3). As these proteins decrease during oogenesis, more mRNA would become available for translation. In addition, a large proportion of the maternal poly(A) RNA stockpile is not translatable without structural modifications(4). Recent evidence suggests that this interspersed cytoplasmic poly(A) RNA could result from deficiencies in the content of certain small nuclear RNAs. Together the evidence suggests that multiple mechanisms regulate the translation of maternal mRNA during development. In Xenopus oocytes, recruitment of message from the maternal pool is a progressive and continual process and the various mechanisms involved may be temporally separable.

1. Richter, J. D. and Smith, L.D. (1981) Cell 27, 183.
2. Taylor, M. A., Johnson, A. D. and Smith, L. D. (1985) Proc. Nat'l. Acad. Sci. 82, 6586.
3. Richter, J. D. and Smith, L. D. (1984) Nature 309, 378.
4. Richter, J. D., Anderson, D. M. Davidson, E. H. and Smith, L. D. (1984) J. Mol. Biol. 173, 227.

N4 MOLECULAR ANALYSIS OF DORSAL, A GENE ESSENTIAL FOR POLARITY IN THE DROSOPHILA EMBRYO, R. Steward, Department of Biology, Princeton University, Princeton, NJ 08544.

The establishment of polarity is one of the prime steps for subsequent pattern formation and morphogenesis. Two classes of "maternal effect" genes, which seem to be essential for establishing polarity along the two major axes, have been isolated. Lesions in one class, the bicaudal genes, disrupt the anterior-posterior axis, while lesions in the other class disrupt dorsal-ventral polarity. The dorsal locus is one of the dozen maternal effect genes involved in the establishment of dorsal-ventral polarity. Mutant alleles of these genes all have a dorsalizing effect with a similar range of phenotypes, differing however in their response to "rescue" injections of wildtype cytoplasm or wildtype poly A+ RNA. The dorsal phenotype is rescued only by wildtype cytoplasm and only locally, at the site of injection. In the wildtype embryo the dl rescuing activity becomes distributed asymmetrically during the very early stages of embryogenesis.

We have cloned the dorsal gene, identified the coding region, and determined the site and time of expression of the gene by both northern analysis and DNA-to-RNA in situ hybridization to tissue sections.

Cytoplasmic Localization

N5 LOCALIZED ACTIVATION OF DORSAL DEVELOPMENT IN XENOPUS EGGS, J. Gerhart, University of California, Berkeley, CA 94720

In the Xenopus laevis embryo, gene expression does not begin until the 4000 cell stage. Prior to this time, there are several important maternally-controlled events whereby the egg and early embryo establish systematic cytoplasmic differences needed later for region-specific gene expression. One of these events will be reviewed.

1) The unfertilized egg has two hemispheres, animal and vegetal, which differ in their contents. The sperm can enter anywhere in the animal hemisphere. The meridian through this entry point (with termini at the hemispheric poles) predicts the ventral midline of the embryo; the opposite meridian predicts the dorsal midline, including the aligned notochord and neural tube, key elements of the chordate body axis. The egg can initiate dorsal or ventral development at any meridian, and makes a unique selection based on the point of sperm entry.

2) The main process by which dorsal-ventral assignments are made in the single-celled egg is not sperm entry itself, but a global rotation of the contents of the egg in which the cortex and sub-cortical cytoplasm move 30° relative to one another, in a period 40-80 min after fertilization, in a direction dictated by the sperm centrosome. The sperm just cues the direction; it is not actually needed for the rotation. The prospective dorsal meridian is the one along which the sub-cortical cytoplasm moved farthest toward the vegetal pole of the cortex. The direction of rotation is highly predictive of the position of embryonic body axis.

3) The rotation can be inhibited by microtubule-depolymerizing agents, by cold shock, hydrostatic pressure, and UV-irradiation (only on the vegetal surface). The extent of rotation controls the anterior-posterior completeness of the eventual body axis. The less the rotation, the more posterior is the level of truncation of the axis. With no rotation, the embryo lacks an axis altogether, though it still develops as a symmetrical "invertebrate" having a gut, red blood cells, and ciliated epidermis. Thus, the rotation at the one celled stage sets the preconditions for all later patterns of gene expression.

4) The rotation can be driven artificially by holding the egg in a gravitionally unstable orientation, in which the dense subcortical material of the vegetal hemisphere slips relative to the cortex. The extent of forced slippage dictates the completeness of the body axis. Double and opposite forced rotation causes twin axes to form. These results are discussed in terms of localized activation, rather than active localization, of widespread latent determinants.

See Scharf and Gerhart, Dev.Biol.99:75 (1983). Gimlich and Gerhart, Dev.Biol.104:117 (1984). Black and Gerhart, Dev.Biol.108:310 (1985). Vincent and Gerhart, Dev. Biol., in press.

N6 MATERNAL mRNA LOCALIZATION IN XENOPUS EGGS, D. Melton, M. Rebagliati, and D. Weeks. Department of Biochemisty and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

We have identified several maternal RNAs that are localized to different regions of unfertilized Xenopus eggs. While most egg RNAs are uniformly distributed in the cytoplasm, we find that there are some maternal RNAs localized to the animal or vegetal pole. These two poles of the unfertilized egg have different prospective fates giving rise to the ectoderm and endoderm, respectively. Whether the localized RNAs are involved in specifying the fates of animal/vegetal blastomeres or have some other developmental function is currently under investigation. In addition, we are using cDNA clones of localized mRNAs to study the mechanism of RNA localization. Injection experiments using sense and anti-sense SP6 transcripts of cloned cDNAs are being used to address these issues.

N7 CYTOPLASMIC DETERMINANTS OF CELL FATE IN ASCIDIAN DEVELOPMENT, J. R. Whittaker, Laboratory of Developmental Genetics, Marine Biological Laboratory, Woods Hole, MA 02543

We have used two approaches to examining the properties of maternally preformed egg cytoplasmic determinents: obtaining of multiple and complex differentiation expressions in the same cleavage-arrested zygotes and microsurgical fragmentation of cytoplasm from one daughter blastomere to another at the time of the 8-cell stage. Complex ultrastructural features of up to four quite diverse cell lineage components were observed in regions of the common cytoplasm in cytochalasin B-arrested ascidian zygotes: epidermal (extracellular test material), muscle (myofilaments and myofibrils), notochordal (leaflet and filamentous sheath components) and the particular localized combinations of microtubules, filamentous structures and cilia characteristic of neural tissues. Almost all the embryos examined contained two such expressions, half of them had at least three, and a few expressed all four. Clear examples of muscle acetylcholinesterase and endodermal alkaline phosphatase development at essentially normal quantitative levels were also noted in association with two of these traits. The finding of so many possible features of differentiation developing within a common cytoplasm is consistent with the idea of gene "activating" factors that are autonomous and relatively non-interfering in their functions. The results of differentiation within cells containing artificially contrived combinations of cytoplasm (by microsurgical partitioning at the 8-cell stage) are also wholly consistent with this conclusion about the occurrence and properties of histodetermining egg cytoplasmic factors.

Molecular Analysis of Hemeotic Genes

N8 HOMEOTIC GENES, THE HOMEOBOX AND THE GENETIC CONTROL OF DEVELOPMENT, Walter J. Gehring, Biozentrum, University of Basel, CH-4056 Basel, Switzerland

Homeotic genes share a highly conserved DNA segment, called the homeobox, which encodes a specific domain within the homeotic proteins. The homeobox first discovered in Drosophila is not only present in arthropods and their ancestors, but also vertebrates and man (1). Using the homeobox as a probe we have isolated and characterized more than 14 Drosophila genes that are involved in the spatial organization of the embryo. The caudal gene, isolated on the basis of homeobox homology, is first expressed during oogenesis (2). Its transcripts accumulate in the nurse cells and the oocyte (germline) but they are absent from the surrounding follicle cells (soma). The transcripts are distributed uniformly throughout the mature oocyte. However, after fertilization the transcripts accumulate in the cortical cytoplasm and during the syncytial blastoderm stage an antero-posterior gradient forms with the highest concentration of transcripts at the posterior pole. After cellularization a single band of labelled cells is found near the posterior end of the blastoderm, corresponding to the last abdominal segments (A9/10) on the fate map. The mechanism by which the transcripts become localized is under investigation.

The control mechanisms of expression of the fushi tarazu (ftz) gene have been analyzed by germline transformation and construction of fusion genes between ftz and β-galactosidase (3). In comparison with the coding region of 1.9 kb, the 5' control region of ftz is very large: In transformation experiments $\sim$ 6.1 kb of 5' flanking sequences are required for complementation of ftz mutants. Fusion of these 5' flanking sequences to the lacZ gene of E.coli leads to the expression of β-galactosidase in a "zebra" pattern in transformed embryos. This pattern corresponds precisely to the pattern of ftz$^+$ gene expression. Therefore, the 5' flanking sequences contain a morphogenetic control element capable of generating a precise spatial pattern of gene expression. Deletion experiments led to the identification of an enhancer-like element, an element which prevents ftz$^+$ expression in the

anterior head segments, a neurogenic element required for expression in the nervous system, and a "zebra" element located most closely to the initiation site of transcription. In addition to these cis-acting control elements, we have identified several transacting zygotic and maternal-effect mutants involved in the control of ftz^{+} expression.

1) Gehring, W.J. (1985). Cell 40, 3-5. Minireview.
2) Mlodzik, M., Fjose, A. and Gehring, W.J. (1985). The EMBO J. 4, in press.
3) Hiromi, Y., Kuroiwa, A., and Gehring, W.J. (1985). Cell 43, in press.

N9 PARASEGMENTAL REGULATION OF HOMOEOTIC GENE EXPRESSION IN THE EMBRYO OF D. MELANOGASTER, Alfonso Martinez-Arias, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, U.K.

A conspicuous feature of most insects is their segmentation. In Drosophila, the first manifestation of this property occurs shortly after gastrulation, when the embryo becomes visibly subdivided into a series of metameric units consisting of the posterior compartment of one segment and the anterior compartment of the neighbouring segment. We have called these units: parasegments. Throughout germ band extension, a phase of cellular proliferation, the embryo is clearly subdivided into 14 parasegments, a head and a telson. Later in development, the early parasegmental organization is manifest in the nervous system and in the organization of the muscles.

Before the morphological signs of metamerism are visible, transcription of selector genes such as Ubx and Ant suggests that parasegments are set up during blastoderm formation and that they precede compartmentalization in the ectoderm. Indeed, the parasegmental organisation of the embryo is a key feature in the expression and regulation of selector genes. This observation poses, therefore, the question of segmentation in relation to selector gene expression. It might be that definition of the domains of selector genes at blastoderm not only requires maternal information, but also some of the zygotic elements responsible for the definition of parasegments. This is easily tested by monitoring the expression of selector genes in segmentation mutant backgrounds.

Gene Expression in Embryogenesis — I

N10 ANALYSIS OF GENE EXPRESSION IN CELL LINEAGES OF THE SEA URCHIN EMBRYO. Robert C. Angerer and Lynne M. Angerer, Department of Biology, University of Rochester, Rochester, NY 14627

The development of the sea urchin embryo to the pluteus larva can be considered in terms of the determination and differentiation of individual subsets of blastomeres whose fates in the normal embryo are reproducible and predictable. Our laboratory has undertaken to define and analyze these lineages at the molecular level by using in situ hybridization to determine the distribution of individual messenger RNAs. This analysis shows that a relatively small set of mRNAs is sufficient to identify the major lineages (aboral ectoderm, oral ectoderm, gut, primary mesenchyme and secondary mesenchyme) either by their unique expression of one or more mRNAs, or by expression of a unique combination. The earliest evidences of cell-type specific mRNA content to date are observed at hatching blastula stage (about 300 cells in Strongylocentrotus purpuratus), and by the criterion of specific patterns of mRNA accumulation, the major lineages are all determined by mesenchyme blastula stage. In the cases of several lineages, sequential expression of different mRNAs provides an indication of the state of differentiation achieved by these cells. These studies of normal embryos have provided a set of molecular markers that will be useful in analysis of embryos whose development is experimentally altered.

N11 EXPRESSION OF CELL ADHESION MOLECULES IN EACH OF THE THREE GERM LAYERS OF THE SEA URCHIN EMBRYO AT GASTRULATION. David R. McClay, James Coffman and Gary M. Wessel. Department of Zoology, Duke University, Durham NC, 27706.

Morphogenetic events at gastrulation have been studied in the sea urchin embryo by combining cell adhesion experiments with the monoclonal antibody technology in order to characterize the cell rearrangements at the molecular level. Monoclonal antibodies were raised against cell surface components of the gastrula and were screened for interference with adhesive function. In two kinds of screens seven antigens (of more than 120 cell surface-specific antibodies tested) were identified by blockage of cell interactions. Each of the seven antigens was localized to restricted cell lineages. This report presents data on expression of three of the antigens, one specific for the ectoderm, one for the mesoderm, and one for the endoderm.

The ectodermal antigen (Ecto V) is a glycoprotein of 116 kD and is expressed at low levels over the entire presumptive ectoderm at the mesenchyme blastula stage. At the gastrula stage Ecto V becomes confined to the ventral ectoderm and during stomodael induction,also appears on the surface of the stomodaem. Ecto V remains localized to the ventral surface throughout development. Radiolabeled immunoprecipitates suggest that the protein is first translated prior to the mesenchyme blastula stage. The same protein, as determined by Western blots, is later translated in the stomodael cell lineage.

The mesodermal antigen (Meso I)is a glycoprotein of 380 kD. Meso I appears on the surface of the primary mesenchyme cells coincident with their ingression into the blastocoel. Expression of the Meso I epitope appears to be the result of a post-translational modification and is first apparent in the trans golgi as determined by EM immuno localization. Pulse-chase radiolabeling experiments indicate further that translation of the protein portion of Meso I occurs well in advance of expression of the Meso I epitope. Antibodies to the glycoprotein specifically inhibit the migration of primary mesenchyme cells that occurs following their ingression.

The endodermal antigen is a glycoprotein of 320 kD. Endo I is first translated during archenteron invagination and its expression is restricted to the surface of cells in the posterior 2/3 of the endoderm. The boundaries of Endo I expression are very sharp even before anatomical constrictions delineate the parts of the gut. This antibody specifically inhibits the adhesion of endodermal cells without affecting adhesion of cells of other germ layers.

Together, these data show a diversity in timing, spatial localization, and regulation of expression of three germ layer-specific molecules that are relevant to the morphogenetic rearrangement of cells in the embryo.

N12 CONTROL OF X-CHROMOSOME EXPRESSION AND SEX DETERMINATION IN EMBRYOS OF C. ELEGANS, W. B. Wood, P. Meneely*, C. Trent, P. Schedin, L. Donahue, B. Quarantillo, J. Manser, and S. Burgess, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado and *Fred Hutchinson Cancer Research Center, Seattle, Wasington.

Sex is determined in C. elegans by the ratio of X chromosomes to autosomes (X/A ratio) acting through a set of at least seven autosomal genes, which have been defined, characterized, and shown to function as a regulatory pathway in which the first gene is her-1 (Doniach and Hodgkin, Dev. Biol. **106**: 223, 1985). C. elegans compensates for the difference in X dosage between hermaphrodites (XX) and males (XO). For at least some X-linked genes, this is accomplished by regulating levels of the corresponding mRNA's. The level of X expression is also determined by the X/A ratio, acting through a second set of autosomal and X-linked genes, whose interactions are not well defined. Some of the genes implicated in determination of X expression level also influence determination of sex, suggesting roles for these genes in both decisions. Mutations isolated as suppressors of a dominant her-1 allele may define additional genes involved in interpreting the X/A ratio.

Experiments are in progress to explore the questions of where and when in the embryo the sex determination decision takes place. Analyses of triploid intersexes with intermediate X/A ratios have extended earlier observations by Madl and Herman (Genetics **93**: 393, 1979) that in a single animal different cell lineages can follow different pathways of sexual development, indicating that the sex determination decision can be made independently by different cells in the early embryo, and providing some evidence on the timing of this decision. In addition, molecular cloning of the her-1 gene by transposon tagging has been undertaken in order to approach these questions more directly through analysis of her-1 expression.

Gene Expression in Embryogenesis — II

N13 THE TRANSCRIPTIONAL ACTIVATION OF MUSCLE-SPECIFIC ACTIN GENES IN EARLY XENOPUS DEVELOPMENT, J.B. Gurdon, T.J. Mohun, S. Brennan, and S. Cascio, CRC Molecular Embryology Unit, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England.

Previous work from this laboratory has established that the cardiac and skeletal muscle-specific actin genes of Xenopus are first transcribed and expressed at the mid-late gastrula stage in the presumptive muscle cells (1), and that the information for the regional activation of these genes is already localized in the subequatorial region of the 1-cell egg (2). Work from many laboratories (e.g. Nieuwkoop and colleagues (3)) has shown that some of the cells which express muscle genes do so as a result of an induction of animal by vegetal cells in the blastula. We have defined temporal and other details of this induction reaction, using nucleic acid probes and antibodies specific for muscle genes (4).

The experiments to be described aim at a cellular and molecular analysis of this induction process. We are particularly concerned with the mechanisms by which muscle genes are activated in only a minority of animal cells exposed to the vegetal inducing influence. We are analyzing this process by carrying out inductions in culture chambers of such a shape that the amount of animal or vegetal tissue can be varied without changing the number of animal and vegetal cells in contact. A molecular analysis of muscle gene activation is being undertaken by injecting genomic clones of muscle actin DNA into ooctyes and fertilized eggs.

1. Mohun, T.J., Brennan, S., Dathan, N., Fairman, S., and Gurdon, J.B. (1984). Nature 311, 716-721.
2. Gurdon, J.B., Mohun, T.J., Fairman, S., and Brennan, S. (1985). Proc. Nat. Acad. Sci. USA 82, 139-142.
3. Nieuwkoop, P.D. (1977). Curr. Top. Dev. Biol. 11, 115-132.
4. Gurdon, J.B., Fairman, S., Mohun, T.J., and Brennan, S. (1985). Cell 41, 913-922.

N14 THE SPEC GENE FAMILY IN STRONGYLOCENTROTUS PURPURATUS, William H. Klein, Susan H. Hardin and Paul E. Hardin, Department of Biochemistry and Molecular Biology, M. D. Anderson Hospital, University of Texas, Houston, TX 77030.

The Spec genes encode a family of low molecular weight calcium binding proteins belonging to the troponin C superfamily. The mRNAs and proteins of the family are expressed exclusively in the dorsal (aboral) ectoderm of sea urchin embryos and larvae and may play a role in changing the shape of the ectodermal cells during embryo and larval development. We have been investigating the structure of the Spec genes in the genome of Strongylocentrotus purpuratus and have discovered several unusual features. When comparing certain Spec genes, we find that the transcribed 5' and 3' untranslated sequences are highly conserved (over 80 percent conservation of sequence) while the protein coding regions have diverged greatly (less than 30 percent conservation of sequence). This observation suggests that the untranslated portions of the Spec messages are evolving at a much slower rate than the translated portions or that the untranslated portions are being periodically corrected by a rectification mechanism. We have also investigated a family of repeated elements roughly 200 bp in length (termed Rsp's) that are found upstream and sometimes downstream from the Spec genes. In one case an Rsp element is the 5' end of a Spec mRNA. Approximately 100bp of the Rsp's from sea urchins are strongly conserved in several other animal genomes including chicken, Drosophila melanogaster and Caenorhabditis elegans. In the case of the chicken genome an Rsp element has been found 3kb from the chicken calmodulin gene, which like the Spec genes is another member of the troponin C superfamily. D. melanogaster and C. elegans genomic libraries were screened with a sea urchin Rsp element. Two recombinant clones from each library were isolated and hybridized with a 43 base oligonucleotide that contains approximately 80 percent homology with the third calcium binding domain of any of the troponin C superfamily members. One of the Drosophila clones and one of the C. elegans clones hybridized strongly with the oligonucleotide. In both cases the calcium binding domain maps within a few kb of the Rsp element. Thus Rsp elements and genes encoding troponin C-like proteins appear to be closely linked. Given their high conservation it is possible the Rsp elements are involved in the evolution or expression of the troponin C gene superfamily.

Molecular Biology of Cellular Differentiation in Lower Eukaryotes

N15 DEVELOPMENTALLY CONTROLLED TELOMERE FORMATION AND GENOMIC REARRANGEMENT IN TETRAHYMENA, E. Blackburn, C. Greider, D. Larson, B. Spangler, G. Yu, Dept. of Molecular Biology, University of California, Berkeley, California; E. Orias, P. Yaeger, Dept. of Biological Sciences, University of California, Santa Barbara, California.

The polygenomic somatic macronucleus develops from a division product of the germline micronucleus following conjugation of Tetrahymena. Macronuclear development involves extensive genomic rearrangement, site-specific chromosome fragmentation, and telomere formation at the ends of the resulting linear macronuclear DNA molecules. These subchromosomal DNA molecules are then replicated up to multiple copies. We have analysed these processes at three different levels: analysis of micro-and macronuclear counterpart sequences (1,2) and their molecular intermediates, biochemical analysis of enzymic activities involved in new telomere formation (3) and genetic analysis.

We have been able to demonstrate a telomere elongation reaction in vitro (3), and the biochemical properties of the enzyme(s) involved in this reaction are being analysed. Because the formation of amplified rRNA genes in macronuclear development exemplifies many of the genomic rearrangement processes, we have analysed the pathway of rDNA amplification by a combination of molecular and genetic approaches. A mutant with altered macronuclear rDNA replication properties was found to have a cis-acting mutation in its rDNA which changes the sequence of an enhancer-like element near the replication origin. The replication behavior of this mutant and another genetic variant rDNA were analysed. Our findings provide evidence that macronuclear development puts macronuclear DNA replication under copy number control in addition to cell cycle control.

1. E. Howard and E. Blackburn, Mol. Cell Biol. 5, 2039-2050, 1985.
2. J.M. Cherry and E. Blackburn, Cell 43, in press, 1985.
3. C.W. Greider and E. Blackburn, Cell 43, in press, 1985.

N16 REGULATION OF GENE EXPRESSION DURING CYTODIFFRENTIATION OF DICTYOSTELIUM. Alan R. Kimmel, Charles L. Saxe and Stephen A. Saxe, Laboratory of Cellular and Developmental Biology, NIADDK, NIH, Bethesda, MD. 20892.

Dictyostelium discoideum grows as undifferentiated ameboid single cells. Development can be initiated by plating cells on filter pads in a low salt buffer in the absence of a food source. Synchronous development then proceeds at the air-water interface; gradients of cAMP are established and amebae move toward regions with high cAMP concentrations. Stimulation of amebae by extracellular cAMP results in a rise in intracellular cAMP concentrations. Cyclic AMP is then secreted, thus amplifying and propagating the original cAMP concentration wave. After relaying the cAMP signal, cells become transiently refractory to further stimulation. During this period of adaptation or desensitization, extracellular cAMP concentrations decrease and cells recover their sensitivity to extracellular stimulation. This adaptation/ deadaptation cycle repeats itself with cAMP pulses intiating from aggregation centers with a periodicity of ~6 min. The result is the assemblage of groups of ~10^5 cells into multicellular aggregates. We have been studying the regulation of gene expression during this early period of development in Dictyostelium. Our studies have focussed on a diverse set of genes.

M4-1 is expressed in undifferentiated cells but is repressed specifically by cAMP signalling. Genes expressed at maximal levels after aggregate formation and which may exhibit cell-type specific expression are stimulated by high exogenous levels of cAMP. We also describe conditions of cell culture which promote or inhibit the expression of the cell surface receptor for cAMP. Finally, we describe a set of single-copy genes associated with a common repetitive element which exhibit apparent coordinate expression. Plausible molecular mechanisms involved in developmentally regulated gene expression of these independent genes sets will be discussed.

N17 ADHESION MECHANISMS AND MULTICELLULAR CONTROL OF CELL-TYPE DIVERGENCE OF DICTYOSTELIUM, William F. Loomis, David A. Knecht, and Danny L. Fuller, Department of Biology, University of California, San Diego, La Jolla, CA 92093

Differentiating cells of Dictyostelium discoideum sequentially acquire two distinct mechanisms of cell-cell adhesion. During early aggregation (T=2 to T=8 hr.), adhesion is sensitive to 10^{-2}M EDTA while later (T=8 to T=20 hr.) adhesion is resistant to disruption by EDTA (1). Experimental evidence will be presented showing that neither mechanism is Ca^{++} dependent.

We have used monoclonal antibodies to a surface glycoprotein (gp80) to block late cell adhesion. Moreover, we have isolated mutations in a locus, modB, that result in the loss of this adhesion mechanism (2). Cells carrying these modB mutations retain the early adhesion mechanism and can form small fruiting bodies.

We have raised polyclonal antisera that block the early adhesion mechanism by injecting rabbits with membrane proteins purified from developing modB cells. The ability of these antibodies to block the early adhesion mechanism can be neutralized by a 28 Kd surface glycoprotein. Monoclonal antibodies have been raised against gp28 that will be used to screen for mutants and sequences cloned in expression vectors.

All cell adhesion in modB cells, as well as multicellular morphogenesis, is blocked by antibodies to gp28. Under such conditions, late cell-type specific genes fail to be expressed under normal developmental conditions.

1) Gerish, G. (1980) Univalent antibody fragments as tools for the analysis of cell interactions in Dictyostelium. Top. Dev. Biol. 14, 243-269.
2) Loomis, W.F., Wheeler, S.A., Springer, W. and Barondes, S. (1985) Adhesion mutants of Dictyostelium discoideum lacking the determinant recognized by two monoclonal adhesion blocking antibodies. Devel. Biol. 109, 111-117.

N18 ISOLATION AND CHARACTERIZATION OF GENES THAT REGULATE CONIDIOPHORE DEVELOPMENT IN Aspergillus nidulans, Margaret T. Boylan, Peter M. Mirabito, Catherine E. Willett, and William E. Timberlake, Department of Plant Pathology, University of California, Davis, CA 95616

In Aspergillus nidulans, three genes, called brlA, abaA and wetA, appear to be involved in regulating major developmental steps leading to the elaboration of the multicellular asexual reproductive apparatus, the conidiophore. Mutations in each of these genes cause the formation of morphologically abnormal conidiophores without affecting vegetative growth rates or patterns. For example, strains carrying the mutant allele brlA1 produce conidiophore stalks but are incapable of forming other structural components of the conidiophore. Strains carrying the mutant allele wetA6 produce spores that autolyse as they near maturity. Mutations in all three genes are recessive in diploids and autonomous in heterokaryons. The brlA1 mutation is epistatic to the abaA1 and wetA6 mutations, and the abaA1 mutation is epistatic to the wetA6 mutation. We have found that mutations in these genes reduce or prevent expression of numerous other genes that are selectively activated during conidiophore development.

We cloned the brlA, abaA and wetA genes by complementation of appropriate mutant strains with a genomic recombinant DNA library constructed for this purpose. The identities of the cloned genes were confirmed by site-directed mutagenesis and tests for allelism with previously described mutations. With each gene, we found that loss of gene function led to the mutant phenotype. We also found that the genes have complex structures in comparison to many other Aspergillus genes. For example, the brlA gene contains one small intron and one large intron, and multiple transcripts may be generated by alternative splicing patterns. The activities of all three genes are strongly regulated during conidiation. Our results are consistent with the hypothesis that brlA, abaA and wetA are regulatory genes that act in trans to induce the expression of many other conidiation-specific genes. The results of experiments to test this hypothesis further will be described.

Cell Lineages in Terminal Differentiation

N19 ACTIVATION OF RECEPTOR AND RESPONSE GENES IN DEVELOPING T LYMPHOCYTES, Ellen Rothenberg, Division of Biology, California Institute of Technology, Pasadena, CA 91125

T (thymus-derived) lymphocytes specifically recognize cells bearing foreign antigens, then respond to this recognition by proliferating, by secreting growth and differentiation hormones for other leukocytes, and in some cases by killing the foreign cells directly. These functions depend on the correctly regulated expression of a large array of genes: those encoding components of the antigen-binding receptor, which are probably expressed constitutively, and those involved in antigen-triggered responses, most of which are expressed only upon induction by the sequelae of antigen recognition. T-cell proliferation appears to be completely dependent upon the unique growth hormone interleukin-2, which is one product made by activated T cells themselves. Responsiveness to IL2 is conferred by expression of a specific IL2 receptor, which is also triggered by antigen recognition. In the absence of antigen, both IL2 and its receptor cease to be made and their mRNAs disappear. Thus, the development of T cells whose proliferation is triggered by antigen depends on establishing correct regulation of these two genes.

We have examined T-cell precursors in the thymus to determine when IL2 and the IL2 receptor are first expressed or first become inducible. A relatively large fraction of thymocytes is proliferating, yet few of the proliferating cells express any IL2 receptors. Thus, the primary mitogenic mechanism in the thymus is distinct from the IL2-dependent mechanism that dominates mature T-cell proliferation. However, the thymocytes that do express IL2 receptors are among the most primitive, suggesting that the IL2 receptor gene can be transcribed early even if its product will not be used to regulate growth until several generations later. It is not certain whether the primitive cells express IL2 receptors constitutively or in response to an unknown inductive stimulus.

A different sequence of events is found for IL2 gene expression. There is no evidence as yet for ongoing IL2 production in vivo by any population in the thymus. Primitive pre-T cells, such as those found in the fetal thymus, are not inducible for IL2 expression. However, among the phenotypically immature cells in the adult thymus are cells which can be induced to make IL2 when treated with stimuli that mimic the intracellular events following antigen binding. These cells cannot be stimulated by conventional mitogens that bind to the T-cell receptor of mature T cells. Only with the assumption of a mature phenotype do thymocytes begin to make IL2 in response to conventional T-cell mitogens. Thus, cells may achieve inducibility of the IL2 gene before they acquire the cell-surface receptor apparatus that enables them to be triggered by antigen.

N20 NORMAL AND NEOPLASTIC EARLY LYMPHOCYTE MATURATION, Irving L. Weissman, Christa Muller, George Tidmarsh, Gerald J. Spangrude, Becky Adkins, Pam Sherwood, Sophie Ezine, Craig Okada and Cheryl Whitlock, Laboratory of Experimental Oncology, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305

T and B lymphocytes apparently share a common precursor with other hematopoietic cells in the bone marrow. Using surface phenotype-defined bone marrow cells and clonogenic assays for pre-T and pre-B cells we have defined several stages of early lymphocyte maturation by bone marrow cells. We have examined the maturation of B lymphocytes in Whitlock-Witte cultures, and have defined 2 stages of early lymphocyte maturation prior to the expression of the B cell form of the lymphocyte common antigen (B220). The earliest of these two stages resides in a population of Thy-1^{lo} cells which make up only 0.1% of bone marrow cells, yet contain all known precursors for the various hematolymphoid lineages, including pluripotential stem cells. These cells undergo early maturation on certain cloned stromal elements (e.g., AC 6.21) from the Whitlock-Witte cultures, reaching the stage of expression of the B220 antigen, but prior to the expression of surface immunoglobulin. Transfer of the cells from cloned stromal cells to mixed stromal elements in the Whitlock-Witte culture system gives rise to surface Ig^+ B cells in a short time, indicating at least 2 stromal elements necessary for the proliferation and differentiation of 2 major stages of B lymphocyte maturation. Abelson leukemia virus transformed pre-B cells require stromal elements in the early phases of leukemogenesis for their growth, and the cloned stromal line (AC 6.21) responsible for the earliest B lymphocyte maturation from the Thy-1^{lo} cell is sufficient for this task. Later, at about the time the Abelson pre-B transformants become feeder-layer independent, they begin to express a tumor-associated B lineage antigen which we call $gp160^{6C3}$. The normal cells expressing $gp160^{6C3}$ are the cloned stromal elements which support normal and neoplastic pre-B cell proliferation, as described above. Thy-1^{lo} cells also contain precursors of clonogenic thymus homing pre-T cells, and we demonstrate that the progeny of any clone have the full maturation potential of T cells, at least in terms of surface phenotypes and some functions. We are currently involved in an analysis of these T cell receptor gene arrangements.

Molecular Biology of Plant Development

N21 REGULATED GENE EXPRESSION OF NUCLEAR GENES FOR CHLOROPLAST PROTEINS IN TRANSGENIC PLANTS: PHYTOCHROME-INDUCTION, LIGHT-REGULATION, TISSUE-SPECIFICITY AND POSITION EFFECTS. Robert Fluhr, Cris Kuhlemeier, Ferenc Nagy, Giorgio Morelli, and Nam-Hai Chua Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021-6399, USA.

We are interested in characterizing cis-acting elements that are important for regulated expression of nuclear genes encoding chloroplast proteins. We have isolated from peas, three members (E9, 3A and 3C) of the multigene family for the small subunit (rbcS) of ribulose-1,5-bisphosphate carboxylase. All the genes are expressed predominantly in leaves and their expression is regulated by light and phytochrome, depending on the leaf developmental stage. We have transferred these genes into petunia and tobacco and analyzed the transgenic plants. From these analyses we conclude that (1) Transgenes are expressed in a tissue-specific manner and show the expected pattern of regulation by light and phytochrome. (2) Chromosomal locations appear to modulate the quantitative level of transgene expression but have no significant effects on their tissue-specificity.[1] Deletion derivatives and site-specific mutants of some of these genes as well as chimaeric constructs have been introduced into petunia and tobacco and their expression patterns will be discussed.[2] Cis-acting components modulating light expression have been localized to a region surrounding the TATA box and also include an upstream enhancer-like region. This work was supported by a grant from Monsanto Company.

[1]Nagy, F., Morelli, G., Fraley, R.T., Rogers, S.G., and Chua, N.-H. 1985. Photoregulated expression of a pea rbcS gene in leves of transgenic plants. EMBO J. 4. (in press).

[2]Morelli, G., Nagy, F., Fraley, R.T., Rogers, S.G., and Chua, N.-H. 1985. A short conserved sequence is involved in the light inducibility of a gene encoding ribulose-1.5-bisphosphate carboxylase small subunit of pea. Nature 315. 200-204.

N22 INDUCTION OF GENE EXPRESSION BY THE PLANT HORMONE ETHYLENE, Robert L. Fischer & James E. Lincoln, Division of Molecular Plant Biology, University of California, Berkeley, CA 94720

The plant hormone ethylene, the simplest unsaturated carbon compound (C_2H_4), has a profound influence on plant physiology. Active in trace amounts, it has been shown to have an effect on fruit ripening, seed germination, seedling growth, root growth, leaf growth, many kinds of stress phenomena, and plant senescence. An excellent model system for studying the effect of ethylene on plant development is tomato fruit ripening. In tomatoes (and in many other fruits) ethylene initiates and coordinates many of the subsequent ripening processes. That is, fruit ripening is preceded by a burst of ethylene biosynthesis, fruit ripening can be induced to occur prematurely by exposing unripe fruit to exogenous ethylene, and removal of ethylene from fruits inhibits their ripening. Once begun, the changes in physiology that occur during fruit ripening are very dramatic (e.g., degradation of chlorophyll, acumulation of carotenoid pigments) and many of these morphological changes reflect changes in gene expression (e.g., the softening of the fruit results from increased expression of genes that encode cell wall degradation enzymes, cellulase and polygalacturonase).

In my laboratory we are studying the effect of ethylene on gene expression during fruit ripening. In particular, we would like to analyze the primary genetic response to the accumulation of this hormone. To this end we have cloned three genes (E8, E4, and E17) whose expression is rapidly induced when unripe fruits are exposed to exogenous ethylene. Specifically, we can detect an increase in their steady state mRNA levels within 30, 60, and 120 minutes, respectively. Furthermore, during the course of normal fruit ripening their expression is activated only when endogenous ethylene biosynthesis increases, leading to a 65, 75, and 15 fold increase in their mRNA levels, respectively. At present we are determining whether their expression is regulated at the transcriptional level, and whether they are expressed in other plant organs that produce ethylene at specific times during plant development. In addition, we are studying their genetic structure and organization. We find that E4 and E17 appear to be single copy genes, while E8 is a small multigene family

(3 genes). We have recently isolated their genomic clones in order to determine the regulatory sequences that render these genes responsive to ethylene.

N23 MOLECULAR ANALYSIS OF TRANSPOSABLE ELEMENT MEDIATED PHENOTYPES IN MAIZE, Susan R. Wessler*, George Baran*, Rita Varagona*, Paul Chomet+ and Steve Dellaporta+, *Botany Department, University of Georgia, Athens GA 30602; +Cold Spring Harbor Laboratory, New York 11724.

There are eight unstable alleles of the waxy (Wx) locus of maize caused by insertion of either the autonomous Activator (Ac) or non-autonomous Dissociation (Ds) controlling element. The Wx gene encodes an enzyme responsible for the synthesis of amylose in the endosperm of the developing kernel. Excision of Ac or Ds during endosperm development can be observed by staining kernel cross sections with KI revealing the black or pale staining amylose sectors that are diagnostic of the clonal restoration of Wx function. In this way it was determined that each Ac/Ds allele displays a unique unstable phenotype with respect to the quality, timing and frequency of Ac or Ds excision. Stable germinal derivatives can also be derived from each allele. The amount of Wx expression and the frequency of these derivatives is also characteristic of each allele.

The goal of our investigation is to determine the alterations in Wx gene expression responsible for these characteristic phenotypes. By cloning and analyzing the expression of many of these alleles and several germinal derivatives we have determined that (1) each allele results from the insertion of either Ac or Ds into a different position within the Wx gene, (2) because excision is usually imprecise, the position of the element strongly influences the phenotype; imprecise excision of one Ds element produces a gradient of Wx enzymatic activity, (3) the orientation of the element determines whether transcription will terminate within the element or readthrough into Wx sequences; if readthrough occurs most of the element can be processed from the final transcript in the absence of DNA excision events, and (4) Ac can cycle from active to inactive; this change is correlated with its level of modification in the genome.

Regulation of Gene Expression During Later Development — I

N24 THE REGULATION OF ACTIN AND MYOSIN GENES DURING MYOGENESIS IN THE MOUSE, Buckingham M., Alonso S., Barton P., Cohen A., Daubas P., Garner I., Robert B. and Weydert A., Department of Molecular Biology, Pasteur Institute, 75724 Paris Cedex 15, France.

The initial formation of skeletal muscle fibres is accompanied by the co-ordinate expression of muscle-type actin and myosin genes. This process can be followed in tissue culture. During subsequent maturation of the fibres in vivo, developmental changes in the foetal/adult isoforms of these proteins occur (see (1)). Skeletal muscle specific transcripts coding for different myosin heavy chains accumulate sequentially and a genetic analysis demonstrates that these genes are clustered (2), implicating cis acting regulatory factors in this case. The actin and myosin alkali light chain gene families demonstrate a different developmental strategy with the transient expression of an adult cardiac isoform with the corresponding skeletal protein. These genes are not linked (3). This interplay between cardiac and skeletal genes can also be re-activated in adult tissue. Under conditions of cardiac overload adult rat hearts accumulate skeletal actin mRNA, as well as transcripts from the cardiac actin gene. The presence of a mutation affecting the cardiac actin gene in some mouse lines results ,in the adult hearts, in a reduction of this mRNA and accumulation of skeletal actin mRNA.

Genes expressed in the same foetal or adult muscle phenotype are not linked (3), suggesting that their co-expression is regulated by trans-acting factors. The promoter regions of such genes in the mouse have no common features of primary structure with the exception of a polyoma B-type enhancer core sequence which has a conserved 5' flanking element, seen for actin and myosin light chain genes. Interspecies comparisons for the same gene demonstrate the presence of conserved regions of promoter sequence, e.g. for the myosin alkali light chain gene which encodes $MLC1_F$ and $MLC3_F$ (4). Re-introduction of genes into mouse muscle cell lines permits a functional definition of such regions important in myogenesis, provided that the construct mimics the expression of the endogenous gene. With the mouse cardiac actin gene promoter, in transient expression experiments, this is the case; products of the marker CAT gene accumulate in muscle fibres, but not in precursor myoblast cultures. For this and other mouse muscle genes study of the regulation of their initial co-ordinate

expression in differentiating muscle cell cultures, is therefore feasible. Transgenic mice provide the best experimental system in which to look at developmental regulation within different actin and myosin gene families during skeletal muscle maturation.

1. Buckingham M.E. (1985) Essays in Biochemistry, 20 77-109. 2. Weydert et al. (1985) Proc. Nat. Acad. Sci. USA. 82 Nov. In press. 3. Robert et al. (1985) Nature 314 181-183. 4. Daubas et al. (1985) Nucl. Acids Res. 13 4623-4643.

N25 CONTROLS OF CYTOSKELETAL ACTIN GENE EXPRESSION IN ANIMAL CELLS. Norman Davidson[1], Beverley J. Bond[2], Nevis Fregien[1], and Sandra B. Sharp[1]. Church Chemical Laboratories[1] and Division of Biology[2], California Institute of Technology, Pasadena, CA 91125

The cytoskeletal β-actin gene is highly expressed in most non-muscle animal cells. Its expression is down regulated in the myoblast to myotube differentiation step of myogenesis. We wish to explore the molecular basis of both high level expression and its down regulation.

Elements from the 5' promoter region of the chicken β-actin gene have been fused to several constructions for expression of the neomycin resistance gene or the CAT gene. Assays for transformation frequencies for *neo* resistance or for CAT expression after gene transfer into mouse cells show that a) the chicken β-actin promoter itself drives high level expression, comparable to or superior to, for example, the RSV LTR, b) elements from the β-actin promoter region can augment (enhance?) expression from a disabled *tk* or SV40 promoter after stable chromosome insertion, but not during the transient expression phase. Further studies to identify the *cis* acting sequences within the 5' region of the β-actin gene which contribute to its strong promoter activity or the enhancement of other promoters will be reported.

When introduced by gene transfer into the mouse myogenic cell line, BC3H-1, the chicken β-actin gene shows down regulation upon differentiation, whereas the chicken skeletal α-actin gene shows variable constitutive or up regulated expression. (The endogenous β and α genes are down and up regulated, respectively.) Hybrid genes were constructed by exchange at a conserved site within the respective protein coding regions. The 5'β 3'α gene behaves similarly to the intact α gene, whereas the 5'α 3'β gene is down regulated like the intact β gene. Thus, some of the information for regulated expression resides within the 3' part of one or both of these genes. Further studies of the molecular basis for these phenomena will be reported.

We find that transcription of the actin 5C gene, one of the two cytoskeletal genes of Drosophila, initiates at two alternate cap sites, separated by about 1kb, each with its own short 5' untranslated leader exon, spliced to the downstream common protein coding exon. There is no evident developmental regulation of these alternate mRNA starts. Contructions in which segments of the upstream promoter drive the CAT gene give good levels of CAT expression in transient assays with Kc cells in culture. Additional constructions will be tested to identify the *cis*-acting sequences that contribute to the high level expression of the actin 5C gene, and to determine if the alternate downstream leader exon has an independent promoter.

N26 MYOGENIC CELL LINEAGE DETERMINATION AND THE COORDINATE REGULATION OF MUSCLE GENES Charles P. Emerson, Jr. and Stephen F. Konieczny, University of Virginia, Charlottesville, VA 22901

5-Azacytidine converts multipotential C3H10T1/2 cells into stable, clonally distinct lineages of myogenic, adipogenic and chondrogenic stem cells that can be induced to differentiate and activate cell type specific genes in high density cultures (Konieczny and Emerson, Cell 38, 791, 1984). C3H10T1/2 cells also can be induced to undergo myogenic determination by transfection with genomic DNA isolated from embryonic quail myoblasts and C3H10T1/2-derived myoblast cell lineages (Konieczny, et al, UCLA Symp. Vol. 29, 1985). These findings suggest that C3H10T1/2 conversion is controlled by the expression of lineage-specific regulatory loci that mediate mesodermal stem cell determination. cDNA and cosmid genomic libraries currently are being used to facilitate the identification and isolation of these regulatory loci.

The role that C3H10T1/2 lineage determination plays in establishing a program of muscle-specific gene activation also is being investigated by transfection of cloned muscle and non-muscle genes into C3H10T1/2 derived cell lineages (Konieczny and Emerson, MCB 5, 2323, 1985). For example, cis-acting regulatory elements associated with troponin I and troponin C genes appear to respond solely to differentiation-specific signals. We conclude that myogenic determination and muscle differentiation are under the simple genetic control of a few regulatory genes that act to establish myogenic cell lineages and to activate coordinately the expression of muscle gene transcriptional regulatory signals during muscle differentiation.

N27 **MULTI-LEVEL REGULATION OF GENE EXPRESSION IN HUMAN MYOGENESIS: DISSOCIATION OF FACTORS RESPONSIBLE FOR MODULATION AND ACTIVATION AND EVIDENCE FOR NON-COORDINATE EXPRESSION.** Larry Kedes, Adrian Minty, Robert Wade, Peter Gunning, Edna Hardeman*, Helen Blau*, Patricia Benton-Vosman, Linda Boxer, Harry Erba, David Feldman, Reinhold Gahlmann, Takeshi Miwa, George Muscat, and Alan Taylor. MEDIGEN Project and Departments of Medicine and *Pharmacology, Stanford Medical School and Palo Alto Veterans Administration Medical Center, Palo Alto, CA 94305.

At least two upstream regions of the human cardiac actin gene contain sequences that interact with muscle specific factors which direct high-level transcription of this gene in differentiated muscle cells. These factors have already accumulated in dividing mouse C2C12 myoblasts before differentiation of the cells. The endogenous cardiac actin gene in the C2C12 line is expressed only at low levels in myoblasts but at a high level when these cells differentiate into multinucleate myotubes. In contrast, human cardiac actin genes stably introduced into C2C12 cells show high level expression in both myoblasts and myotubes, indicating that the endogenous cardiac actin gene is repressed in myoblasts by a mechanism which does not affect transfected genes. In a second muscle cell line (the rat L8 line) the level of transfected cardiac actin genes increases when these cells differentiate into myotubes, paralleling the expression of the endogenous sarcomeric actin genes. We propose that the level of transcriptional modulating factors is low in L8 myoblasts and increases when these cells differentiate into myotubes. Thus at least two steps are necessary for high-level cardiac actin gene expression: activation of the gene and subsequent modulation of its transcriptional activity. These two steps can be dissociated and thus the factors involved in modulation are distinct from those involved in gene activation. By examining the expression of more than 12 muscle specific genes or isoforms, we can distinguish a variety of patterns of gene expression in myogenesis that differ in time of onset and duration. These observations do not support the concept that the expression of muscle genes is coordinately regulated.

Regulation of Gene Expression During Later Development — II

N28 **MOLECULAR PROBES FOR THE DIFFERENTIATION OF NEURAL CREST DERIVATIVES.** Richard Axel, David Anderson, and Reuven Stein. Howard Hughes Medical Institute, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

The cells of the neural crest migrate from the top of the neural tube throughout the embryo to generate a diverse array of differentiated derivatives including the components of the peripheral nervous system. We have isolated cDNA clones homologous to mRNA expressed in crest-derived neurons. We have used these clones as developmental markers to follow the differentiation of a sublineage of crest cells, the sympathoadrenal lineage, which generates both sympathetic neurons and the chromaffin cells of the adrenal medulla. One such clone, SCG10, is expressed in the neurons of the sympathetic ganglia, but not in mature adrenal chromaffin cells. However, this clone can be induced to high levels upon exposure of chromaffin cells to NGF in culture. Since sympathetic neurons and adrenal chromaffin cells are thought to share a common precursor, we next asked whether this gene was expressed in precursor chromaffin cells and what factors may be responsible for the inhibition of SCG10 RNA expression in mature adrenal chromaffin cells. Using *in situ* hybridization to SCG10 mRNA and immunoperoxidase histochemistry to tyrosine hydroxylase and neurofilament, we have observed that adrenal medullary precursors transiently express neuronal markers, but this expression is extinguished as the cells migrate into the adrenal medulla. *In vitro* culture experiments with primitive adrenal chromaffin cells suggest that this transient expression may be due to the conversion of neuron-like cells to chromaffin cells under the influence of adrenal corticosteroids. Thus the plasticity of neural-specific genes in mature chromaffin cells may be explained by transient expression of these genes early in embryonic development. Neural-specific gene expression is ultimately extinguished, but the memory of this expression is retained such that the genes may be re-expressed under appropriate environmental influences.

N29 STRUCTURE AND EXPRESSION OF HUMAN GLUCOCORTICOID RECEPTOR cDNA: A TRANS-ACTING FACTOR RELATED TO THE c-erbA PROTO-ONCOGENE FAMILY.

R.M. Evans, C. Weinberger, and S. Hollenberg, Gene Expression Laboratory, The Salk Institute, San Diego, California; and M.G. Rosenfeld, School of Medicine, University of California, San Diego, California.

We are interested in developmental and inducible regulation in the neuro-endocrine system. In animals, the endocrine and nervous systems produce a diverse set of molecules which interact to exert profound effects on development, physiology and behavior. All these molecules exert their effects by binding specific membrane or intracellular receptors. Our interest in steroid receptors is that these molecules serve as a paradigm for sequence-specific DNA binding proteins that are capable of directly regulating gene transcription. To gain information about the structure of this molecule and to begin an analysis, the molecular mechanisms by which it regulates gene transcription, we have cloned receptor cDNA sequences. Analysis reveals two human glucocorticoid receptor (hGR) variants (alpha, 777 amino acids; and beta, 742 amino acids), differing at their carboxy termini. The proteins contain a cystine-lysine-arginine rich region which may define the DNA-binding domain. Expressed full-length hGR is immunoreactive and possesses intrinsic steroid binding activity characteristic of the native glucocorticoid receptor. In addition to multiple protein forms, we find receptor sequences to be localized to human chromosomes 5 and 16 and have identified at least three mRNA forms. Analysis of the receptor sequence reveals it to be distinct from other previously charcterized DNA binding proteins and suggests that it may be part of a new class of regulatory molecules. Furthermore, both alpha and beta hGR are related to the oncogene v-erbA from the Avian Erythroblasosis Virus (AEV) suggesting that steroid receptor genes and the c-erbA proto-oncogene are derived from a common primordial regulatory gene. Oncogenicity by AEV may result, in part, from the inappropriate activity of a truncated steroid receptor or related regulatory molecule encoded by the v-erbA gene. This suggests a mechanism by which trans-acting factors may facilitate or potentiate transformation. Additional characterization of the relationship of the glucocorticoid receptor to these oncogene products may provide insight into the evolution of a family of eukaryotic transcriptional regulatory factors. Finally, A portion of the receptor is also homologous to the Drosophila homeotic proteins encoded by Antennapedia and fushi tarazu.

N30 NEUROPEPTIDES: MULTIPLE REGULATORY MECHANISMS AND THEIR ROLES IN MEDIATING SIMPLE BEHAVIORS. Richard H. Scheller, Department of Biological Sciences, Stanford University, Stanford, CA 94305

The central nervous system of the marine mollusk Aplysia is a useful model system for studies of the cellular basis of simple behaviors. We have isolated several genes encoding the precursors for biologically active peptides used as intercellular messengers by identified neurons in the abdominal ganglion. Cell specific expression of the neuropeptide genes is being studied by microinjection of cloned genes into the neurons. The large size of Aplysia neurons makes it possible to analyze the expression of microinjected genes in single cells. Antibodies to synthetic peptides define the anatomical and subcellular distribution of the molecules. The peptide products are expressed in subsets of central neurons and are packaged in multiple populations of dense core vesicles. The distribution of immunoreactive processes suggests three anatomical locations of neurosecretion, the neuropile, the connective tissue sheath and specific peripheral targets. The structure of the neuropeptide precursors defined by analysis cDNA clones suggests sites of proteolytic processing which liberate mature, biologically acive peptides. HPLC in conjunction with protein microsequencing are used to define the precise proteolytic processing pathway. Once cleavage products of the precursors are defined, physiological analysis of their actions is possible. The peptides act directly as excitatory and/or inhibitory transmitters and also modulate the actions of other substances on both neuronal and muscle targets. Voltage and patch clamp analysis are being used to define the cellular mechanisms of action of the peptide products. The multiple roles of biologically active peptides in the nervous system are being defined by this multidisciplinary approach aimed at understanding the simple behaviors of Aplysia.

Regulation of Gene Expression During Later Development — III

N31 RETROVIRUSES AS GENETIC MARKERS FOR MAMMALIAN DEVELOPMENT, Philippe Soriano and Rudolf Jaenisch, Whitehead Institute for Biomedical Research and M.I.T., Nine Cambridge Center, Cambridge, MA 02142.

Chimeric mice derived from combining blastomeres of different mouse strains have been used to study fundamental processes of mammalian development such as cell lineage relationships or the origin of tissue primordia. The interpretation of data obtained with aggregation or injection chimeras are, however, complicated because blastomeres of two different genotypes are combined to generate a single animal. It has been argued that this may lead to cell selection of one genotype over the other which possibly can distort normal cell interactions in the developing embryo. Indeed, the fraction of the two genotypes contributing to a given tissue has been shown to change during the lifetime of a chimeric animal in many instances, a phenomenon designated as chimeric drift.

We have explored the potential of retroviruses to serve as cell lineage markers in mammalian development. Preimplantation mouse embryos were infected with a recombinant retrovirus at the 4-16 cell stage. Founder animals were derived from infected embryos which, as predicted from previous observations, were mosaics for individual proviruses in somatic and germ line tissues. Becasue a retroviral genome introduced into a given blastomere serves as a stable genetic marker for the mitotic progeny of the infected cell, the molarity of a given proviral band on a Southern blot reveals the fraction of cells the respective blastomere has contributed to the analyzed tissues. Careful quantitative analysis of approximately 35 individual proviruses carried in 13 founder animals indicated that the molarity of a given proviral band was equal in all different organs tested of an individual founder animal. The molarity of individual proviral genomes, however, varied between .15 and 1.0. This indicates that the somatic tissues of the animal are derived from at least 6 founder cells which contribute equally to all organs. Extensive cell mingling has to occur prior to tissue allocation. Our observations contrast with the distribution of genotypes in chimeric mice which vary considerably among different tissues.

The founder animals were bred to study the distribution of proviruses in the germ line. When compared with the uniform occurence of proviral bands in somatic tissues, considerable variation was observed in proviral bands recovered in the offspring. This suggests that cells forming the germ line are set aside prior to somatic tissue allocation.

The genetic labeling of early embryonic cells by retroviruses appears to be a reliable and sensitive lineage marker which does not disturb normal cell interactions in mouse development.

N32 GENETIC, DEVELOPMENTAL, AND MOLECULAR STUDIES OF THE DROSOPHILA ENGRAILED COMPLEX, Thomas Kornberg, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143

The engrailed locus of Drosophila encodes an essential function that participates in the processes that organize the early pre-cellular embryo and that maintain the segmental organization of the later developmental stages. After cellularization of the embryo, during the gastrulation and larval stages, engrailed locus expression is limited to the cells of the posterior developmental compartments. engrailed mutants that break the locus into two parts define a region of 70 kb that is involved in engrailed gene function. Within this region, three types of engrailed mutations have been found: embryonic lethal breakpoint mutations located in the proximal 45 kb of the locus, viable breakpoint mutations located in the distal 25 kb of the locus, and embryonic lethal apparent point mutations that have not been localized and that have a complex pattern of complementation with the lethal breakpoint mutations. The engrailed locus has a single 4 kb transcription unit that produces, predominantly, a 2.7 kb poly(A) mRNA. Antibodies directed against an E.coli - synthesized engrailed translation product detect a nuclear protein that is localized to the posterior compartment cells.

Neighboring the engrailed locus is a gene, invected, that is related both in sequence and in pattern of expression. The invected gene contains an engrailed - like homeobox and its expression is also limited to the posterior developmental compartments.

N33 CIS- AND TRANS-ACTING REGULATORS OF THE DROSOPHILA 68C GLUE GENE PUFF

Elliot M. Meyerowitz, M.Crosby, M.Garfinkel, C.Martin, P.Mathers, and K. Vijay Raghavan, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The polytene chromosome puff found in the 68C region of the third chromosome in Drosophila melanogaster salivary glands contains the genes for three coordinately expressed salivary gland secretion proteins, sgs-3, sgs-7 and sgs-8. These proteins are part of the "glue" that is secreted by third instar larvae just prior to pupariation, and that is used in affixing the puparial case to a solid surface for the duration of the pupal period. The mRNAs for the three proteins are coded in a region of less than 5,000 base pairs, and are coordinately expressed in both tissue and time. The initiation of accumulation of the three RNAs depends on at least two trans-acting signals. One is the steroid hormone ecdysterone, the other the product of an x-chromosomal genetic locus. Ecdysterone also serves, 40 hours after initiation of glue gene expression, as the signal that represses new accumulation of the glue mRNAs. One question raised by this system is that of the coordination of expression of the three RNAs: is each RNA controlled independently of the others, each with its own regulatory sequences, or is there a single control region responsible for expression of all three genes? The answer is that there are separate regulatory regions for at least two of the genes, with the third gene not yet tested. When upstream and genic regions of a few hundred base pairs from either the Sgs-8 or the Sgs-3 gene are fused to E. coli β-galactosidase coding sequences, then introduced to the Drosophila genome, each of the upstream regions independently controls the reporter gene, causing abundant production of E. coli β-galactosidase in third instar larval salivary glands. In both cases this production requires the activity of the trans-acting x-chromosomal regulatory locus.

Another question is: what is the relation between RNA expression and polytene chromosome puffing at the 68C locus? In the absence of ecdysterone or of the wild-type product of the x-chromosomal regulatory locus, a puff forms at 68C in third instar, but no RNA from the puffed region accumulates. Thus puffing may be a precursor, but not a consequence, of glue gene expression. When the Sgs-3 gene, which accounts for 60 to 80% of transcription at the 68C puff (as measured in 15 minute pulse-labeling experiments), is inserted into the fly genome alone, it does not cause a puff, even though it expresses at a normal level, tissue, and developmental stage. However, if the Sgs-7 and Sgs-8 genes are introduced along with the Sgs-3 gene, the new sites containing this construct do puff in third instar salivary glands. Thus some sequences near Sgs-7 and 8, or a combination of sequences near these genes and sequences near Sgs-3 are able to cause puffing. The location of these sequences, and the relation of puffing to gene expression in the puffed DNA are now being studied.

N34 REGULATION OF DROSOPHILA CHORION GENE AMPLIFICATION, Richard L. Kelley, Terry Orr-Weaver, Lynn Cooley, Gene Leys, Suki Parks and Allan Spradling, Department of Embryology, Carnegie Institution of Washington, 115 W. University Pky., Baltimore, MD 21210

Two 80-100 kb chromosome regions containing clustered chorion genes undergo specific amplification during Drosophila oogenesis. A series of small deletions was constructed in the 3.8 kb region of the third chromosome gene cluster essential for amplification. Analysis of amplification of the constructs in the follicle cells of transformed lines has further narrowed the essential cis acting regulatory region, the amplification control element (ACE), to a 500 bp sequence upstream of the s-18 gene. Transcription in cis from either the nearby s-15 or s-18 genes is not required for proper gene amplification, but our studies suggest that some regulatory elements may be common between amplification and s-18 transcription.

Two unlinked female sterile mutations from the Nusslein-Volhard collection, fs(3)272 and fs(3)293, which disrupt proper chorion gene amplification were examined and mapped. The fs(3)293 mutation was shown to be a hypomorphic allele of a previously described locus, l(3)K43, whose null phenotype is pupal lethality and the absence of imaginal discs. A search for specific trans-regulatory loci affecting the genes in the chorion clusters is underway using an s-18-Adh gene fusion that directs the production of alcohol dehydrogenase in follicle cells of late egg chambers.

Oogenesis and Cytoplasmic Localization and Homeotic Genes and Gene Expression in Embryogenesis

N35 ORGANIZATION OF MURINE HOMEO BOX LOCI AND THEIR SPATIALLY RESTRICTED EXPRESSION DURING DEVELOPMENT, Alexander Awgulewitsch[1], Manuel F. Utset[2], Charles P. Hart[1], Abraham Fainsod[1], William F. McGinnis[3] and Frank H. Ruddle[1,2], Departments of [1]Biology, [2]Human Genetics, and [3]Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

A number of genes controlling morphogenesis in D. melanogaster have been found to share a 180 bp DNA sequence called the homeo box. The conceptual translation of the homeo box sequence provides a protein domain with putative DNA binding function. Highly conserved copies of the homeo box have been identified in a wide variety of animal phyla, including mammals, which lead to the speculation of functional relations between the Drosophila homeo box genes and those from other species. We have studied the structure and organization of various cloned murine homeo boxes by chromosomal and restriction mapping and by limited sequence analysis. So far we have identified three different homeo box loci located on mouse chromosomes 6, 11 and 15. The mouse homeo box genes display a clustered organization, superficially similar to that found in the homeotic gene complexes in D. melanogaster. The transcriptional activity during mouse embryogenesis was tested by Northern blot analysis and by in situ hybridization to tissue sections. The results show complex transcription patterns and spatially restricted expression in different body regions of the developing mouse and in adults. Using polyclonal antibodies raised against a mouse-10 homeo box/E. coli ß-galactosidase fusion protein, homeo box gene products have been identified in specific subregions of the somites in tissue sections from 13 day mouse embryos.

N36 HOMEO DOMAIN EXPRESSION IN X. LAEVIS EMBRYOS, Andres E. Carrasco and George Malacinski, Department of Biology, Indiana University, Bloomington, IN 47405

The homeo domain (a 180 bp protein coding sequence) has been reported to be expressed in the embryos not only of Drosophila melanogaster but the amphibian Xenopus laevis (Carrasco et al., Cell 37:409-414, 1984 and Muller et al., Cell 39:157-162, 1984) and probably other vertebrates as well.

To analyze the role of the homeo domain containing genes in vertebrates, we have isolated several cDNAs expressed in early stages of embryogenesis. One of these clones corresponds to the AC_1 genomic fragment previously reported. Our work is being directed to (1) the nucleotide sequence of these cDNAs (2) the temporal pattern of expression during embryogenesis and (3) the spatial distribution of the transcripts. Northern blots of microdisected regions of X. laevis embryos probed with AC_1 cDNA show a regionally restricted distribution of transcripts and a different pattern of transcripts in the different regions.

N37 GENE EXPRESSION DURING EARLY LENS DEVELOPMENT, Timothy S. Charlebois and Robert M. Grainger, University of Virginia, Charlottesville, VA 22901

We are studying how the lens cell type is specified in embryonic ectoderm, and are particularly interested in changes in gene expression which occur during this process. In vitro culture techniques have been used to assess the developmental potential of various regions of ectoderm. In the 36 hour chicken embryo, before any overt lens differentiation has occurred, the isolated presumptive lens ectoderm can differentiate in vitro into lentoids which express delta-crystallin, the major lens-specific protein. This potential is sharply contrasted by that of a large region of dorsal ectoderm overlying the somites, which fails to exhibit lens differentiation in response to our in vitro conditions. In order to identify specific molecular correlates of cell fate, we are examining the pattern of gene expression in these ectodermal tissues which clearly have different developmental potentials. We have compared the set of abundant polypeptides synthesized in presumptive lens ectoderm with that of dorsal ectoderm, using two-dimensional polyacrylamide gel electrophoresis. In these experiments, over 500 polypeptide spots could be resolved, yet no reproducible differences could be found. More subtle differences in the pattern of gene expression are being examined using a cDNA library prepared from 36 hour embryonic tissue. This library is sufficiently large ($2-3 \times 10^6$ independent clones) to encode many rare mRNAs. This library is presently being screened to identify clones representing RNAs which are expressed in a region-specific manner within the ectoderm In this way it may be possible to determine if ectodermal gene products are activated during the period when the lens-forming bias is established.

N38 ISOLATION AND CHARACTERIZATION OF HOMEOTIC GENES IN PIG & SHEEP, Chung-Lit Choi[+], Alfred Stauder[+], Peter Hudson[+], Malcolm R. Brandon[+]. [+]Veterinary Preclinical Sciences, The University of Melbourne, Parkville, Victoria, Australia 3052. [+]CSIRO, Division of Protein Chemistry, Parkville, Victoria, Australia 3052.

The homeotic gene family in Drosophila appears to be the orchestra of the body-plan of the insect during morphogenesis. Members of this gene family have a highly conserved nucleotide sequence of 180 base-pairs, called the "homeo-box", which encodes for a basic helical protein domain. Besides Drosophila, the homeo-box sequence is also conserved in analogous developmental genes in xenopus, mouse and man.

Using cloned Drosophila homeotic genes, ubx, ftz and antp, and synthetic oligonucleotides specific to the homeo-box as DNA probes, clones that appear to be homeotic genes in pig and sheep have been isolated from Sau3A pig and sheep genomic phage libraries. Restriction and Southern hybridization analyses revealed that there may be three unique pig homeotic genes and over six unique sheep homeotic genes. Some of these clones have been further confirmed by nucleotide sequence analysis. In situ hybridization studies using probes prepared from the homeo-box specific oligonucleotides and the pig and sheep clones have illustrated the activity of the homeotic genes during embryonic development.

N39 OOCYTE-SPECIFIC GENE EXPRESSION: MOLECULAR CHARACTERIZATION OF A cDNA CODING FOR ZP-3, THE SPERM RECEPTOR OF THE MOUSE ZONA PELLUCIDA Jurrien Dean, Maurice Ringuette, Donna Sobieski and Steven Chamow, Laboratory of Cellular and Developmental Biology, NIADDK, NIH, Bethesda, MD, 20892

The mouse zona pellucida genes are expressed uniquely during oogenesis where they are developmentally regulated in the absence of cell division. Little is known about the mechanisms that control the expression of these germline specific genes which play crucial roles in early mammalian development. We have constructed a λgt11 cDNA expression library from ovarian poly(A)$^+$ mRNA and have isolated two clones coding for ZP-3, the mouse sperm receptor. To confirm the identity of the clone, the ZP-3 protein was digested with V-8 protease and a peptide was isolated by affinity chromatography using an anti-ZP-3 monoclonal antibody. A sequence of nineteen amino acids was found that corresponded to an open reading frame of the DNA sequence of the ZP-3 cDNA clone. Further studies demonstrate that the ZP-3 gene is transcribed as a 1.7 kb polyadenylated mRNA which is transcribed exclusively in ovarian tissue. This germline specific expression is reflected in the observed hypomethylation of the ZP-3 locus in ovarian but not liver or brain DNA. The ZP-3 gene is otherwise identically organized in somatic and germline DNA where it appears to be present as a low copy number or single copy gene. Even though the mouse sperm receptor has species-specific functions, the ZP-3 cDNA cross-hybridized with a variety of mammals including rat, rabbit, dog, pig, cow and human. These data suggest that the sperm receptor gene is evolutionarily conserved among mammals.

N40 DNA BINDING MAY HAVE A ROLE IN THE ACTION OF A DROSOPHILA DEVELOPMENTAL GENE. Claude Desplan, Jim Theis, Deann Wright & Patrick O'Farrell, Dept. of Biochemistry, University of California, San Francisco.

We have used molecular approaches to investigate the action of developmental genes in pattern formation. A considerable body of information suggests that some developmental genes regulate expression of other developmental genes; these interactions are likely to play important roles in forming embryonic pattern. We have focused on the role of the engrailed homeo domain in mediating presumed regulatory interactions. The engrailed gene is involved in specifying cells of the posterior compartment of each segment.

We constructed gene fusions which were expressed in E.Coli to produce the entire engrailed protein or subdomains linked to beta-galactosidase. A fusion containing the homeo domain plus 44 flanking N-terminal aminoacids and 39 C-terminal aminoacids binds specific DNA fragments from non coding sequences of the engrailed gene itself and of the fushi-tarazu (ftz) gene. This sequence specific binding could be an indication of a regulatory interaction. Perhaps sequence specific DNA binding activities are involved in proposed combinatorial and autoregulatory interactions of homeo domain containing proteins.

We are currently determining the sequence of the site recognized by the engrailed fusion protein. Also using other fusion constructions we are in the process of defining more accurately the region of the engrailed protein sequence specifying binding and are investigating the DNA binding activities and specificities of homeo domains encoded by other developmental genes.

N41 ISOLATION OF A HOMEO BOX-CONTAINING GENE EXPRESSED DURING EMBRYOGENESIS IN SEA URCHINS, Gregory J. Dolecki, Surawit Wannakrairoj and Tom Humphreys, Pacific Biomedical Research Center, University of Hawaii, Honolulu, HI 96813

A 180 nucleotide protein-coding region, the homeo box, is present in several phyla of higher metazoa. It is conserved in several genes which control development in Drosophila and in developmentally regulated genes in frogs and mice. The 60 amino acid homeo domain encoded by this region is postulated to be a DNA- or chromatin-binding polypeptide which regulates sets of developmental genes. By hybridizing Drosophila homeo box DNA probes to Southern transfers of restriction endonuclease digested genomic DNA from the Hawaiian sea urchin Tripneustes gratilla, we have shown that the sea urchin genome apparently has four or five homeo box sequences and that the DNA in the vicinity of these sequences exhibits very little polymorphism from individual to individual. We have cloned and sequenced one of the T. gratilla homeo boxes. It is 69-73% homologous to the Drosophila homeo box probe DNAs. The homeo domain which it encodes is 78-88% homologous to the Drosophila homeo domains encoded by the probe DNAs. The sea urchin gene which we have cloned is expressed during embryogenesis and produces two transcripts. One is 6.9 kb and can first be detected in Northern transfers of poly(A)$^+$ RNA from blastula stage. It increases in abundance reaching a maximum at gastrula stage before it decreases considerably by pluteus stage. The second transcript is 7.7 kb and can be detected in poly(A)$^+$ RNA only at gastrula stage. This transcription of a homeo box-containing gene in the sea urchin embryo, an organism with a non-segmented body plan, indicates that such genes cannot be solely involved in the specification of the homologous serial structures which define segmented body plans.

N42 DIFFERENCES IN THE CONTROL OF SYNTHESIS OF BASEMENT MEMBRANE COMPONENTS AT DIFFERENT STAGES OF DEVELOPMENT, John H. Fessler, Andrew G. Campbell, Bruce Blumberg and Liselotte I. Fessler, Molecular Biology Institute, UCLA, Los Angeles, CA 90024

During development a thin, defined layer of basement membrane is deposited by epithelial and certain other cells at their interface with the extracellular matrix. Special gene products make up the basement membrane, in particular procollagen IV, laminin, entactin and proteoglycans. Efficient assembly of supramolecular complexes requires coordinated synthesis of these materials in order to give a high local concentration of each. Furthermore, the total amounts made must be limited, to assure that only a thin basement membrane is formed. With specific antibodies against these components of Drosophila basement membranes we found that they are first made during the period of organogenesis. Neither early blastoderm stage Drosophila embryos nor the cells obtained from them synthesize these materials initially. However, upon culture these cells start to express basement membrane components at about the same total age as the embryos do. Therefore some blastoderm stage cells are programmed for subsequent synthesis, and later this is initiated independently of the surroundings of the cells. We found that intense synthesis of basement membrane collagen is related to periods of active growth and remodeling of a tissue such as lung. While in chick this occurs at about 66% of embryonic development, in rats it happens near birth. Such changes of biosynthetic rate are only partly under transcriptional control. While the levels of RNA coding for procollagen IV sharply dip and rise again in the rat neonatal lung, closely paralleling the corresponding changes in the synthesis of this protein, a given amount of this specific lung RNA sustains different rates of procollagen IV synthesis at birth and three weeks later. When cell division is initiated by partial hepatectomy of rats, procollagen IV synthesis follows a few hours later. However, when DNA synthesis of parietal endoderm cells is blocked with the inhibitor aphidocolin, procollagen IV synthesis continues unabated. We conclude that the rate of synthesis of basement membrane components varies during development and repair and that transcriptional control only partly accounts for these changes.

N43 **GNU: A DROSOPHILA MUTATION THAT IS DEFECTIVE FOR NUCLEAR DIVISION**
Matthew Freeman and David M Glover,Cancer Research Campaign Molecular Genetics Research Group,Biochemistry Department,Imperial College,London SW7.

THE Drosophila embryo is a syncitium for its first 13 nuclear divisions; then at cycle 14,when there are about 6000 nuclei,the embryo cellularises. We have characterised a maternal effect lethal mutation,gnu,in which embryos from an homozygous female (GNU embryos) are defective for nuclear division,but not DNA replication. Embryos with a small number of giant nuclei are thus formed; GNU embryos never cellularise. Using immunofluorescent techniques,we have found that centrosomes continue to divide in GNU embryos,in the absence of nuclear division: these centrosomes migrate to to the cortex of the syncitium and are completely disassociated from giant nuclei. Using an antibody against tubulin, we also find aster-like structures near the surface of the embryo: their striking feature is their size - up to twenty times the diameter of wild type asters. When the giant nuclei start to break down,at about 24 hours,small pieces of chromatin are released. These appear able to provide functional kinetochores since apparently normal spindles are often found to form over them. These observations suggest that centrosomes,asters, kinetochores,and spindles are all functionally normal in GNU embryos. Thus the gnu mutation appears to uncouple the process of nuclear division from many of the cytoplasmic events of the mitotic cycle. Further phenotypic characterisation,and a molecular analysis of gnu,is now in progress.

N44 Synthetic Peptides as Nuclear Compartmentalization Signals. D.S. Goldfarb, J. Gariépy, G. Schoolnik, and R.D. Kornberg. Departments of Cell Biology and Medical Microbiology, Stanford University School of Medicine, Stanford, CA 94305.

The nuclear envelope is a porous structure that presents a diffusion barrier to large proteins in transit to the nucleoplasm. SV40 large T-antigen (96 Kd) efficiently traverses the nuclear envelope and accumulates in nuclei by virtue of a short amino acid sequence, the nuclear localization signal (Kalderon et al. (1985) Cell 39, 499-509). We find that a synthetic peptide, based on the large T-antigen signal, serves as a nuclear localization signal when covalently coupled to either BSA or IgG and microinjected into Xenopus laevis oocytes. The accumulation of peptide-protein conjugates in nuclei is peptide sequence specific. We also find that initial rates of nuclear uptake are saturable, with an apparent K_m of 10^{-6} M, and that free peptide competes with the peptide-protein conjugate for uptake. These studies suggest that nuclear uptake is a high affinity, receptor-mediated transport process and also demonstrate the utility of peptide reagents as autonomous nuclear localization signals.

N45 ISOLATION AND CHARACERIZATION OF *SEVENLESS* - A GENE THAT CONTROLS PHOTORECEPTOR CELL DEVELOPMENT IN DROSOPHILA. Ernst Hafen, *Jan E. Edstroem and Gerald M. Rubin, University of California, Berkeley, CA. 94720/ *EMBL Heidelberg, FRG

The compound eye of Drosophila consists of 800 ommatidia - the functional units of phototransduction. Each ommatidium consists of eight photoreceptor cells arranged in a trapezoidal fashion which is repeated from ommatidium to ommatidium. Each photoreceptor cell has a discrete identity based on its position in the unit as well as based on its projection into the optic lobes of the brain. The mutation *sevenless* (*sev*) specifically affects the development of a single type of photoreceptor cells. Flies homozygous or hemizygous for *sev* lack the photoreceptor cell R7 in each ommatidium. Morphological studies on the development of the R7 cells in *sev* mutants by Tomlinson and Ready suggest that the progenitors of the adult R7 cells form in the early stage of eye development, but develop as cone cells - a non neuonal cell type in the eye.

We have cloned the chromosomal region encompassing the *sev* gene by a combination of microdissection cloning and chromosome walking. Deficiency braekpoints that define the extent of the *sev* locus were mapped on the cloned genomic DNA by whole genome Southern analysis as well as by *in situ* hybridization to the deficiency chomosomes. A 15 kb region was identified that contains at least part of the *sev* gene. Within this region a single transcription unit was identified that is specifically expressed in the eye imaginal disc. Northern blot analysis indicat that a single transcript of approximately 3 kb derives from this region. This transcript is only expressed in the eye imaginal disc of the third instar larva. *In situ* localization of the *sev* transcript in tissue section of third instar larvae indicat that the transcripts specifically accumulate in at least a subpopulation of developing photoreceptor cells. Presently, germ line transformation is being used to determine whether a 15 kb DNA fragment is sufficient to rescue the *sev* phenotype.

N46 CONTROL ELEMENTS OF THE DROSOPHILA SEGMENTATION GENE FUSHI TARAZU

Yasushi Hiromi, Atsushi Kuroiwa and Walter J. Gehring, Biozentrum Basel,Switzerland

Fushi tarazu (ftz) is one of the Drosophila homeo box-containing genes required to establish the segmental pattern. Homozygous ftz mutants have a pattern deletion in parts of alternating segments and die as larvae with half the number of segments of the wild type embryos. Localization of the ftz transcripts by in situ hybridization has revealed that the ftz gene is expressed in cells forming seven equally spaced bands in blastoderm embryos; these cells appear to coincide with the segment primordia deleted in the ftz mutants.

We are trying to to understand the control mechanism of ftz expression by mapping cis-acting regulatory sequences of the ftz gene and searching for genes encoding trans-acting factors. A series of fusion genes in which 5' flanking sequences of the ftz gene are fused to the E. coli lacZ gene were constructed and introduced to the germ line by P-element mediated transformation. A fusion gene containing 6.1kb of ftz 5' flanking sequences is expressed in a "zebra" pattern along the germ band, and also in certain neuroblasts of all segments. Deletion analysis revealed that the morphogenetic control element generating the "zebra" pattern is located within a 0.74kb fragment immediately upstream of the coding region. The region further upstream contains an enhancer-like element and an element necessary for expression in the nervous system. A number of mutations affecting the larval cuticle pattern also affects expression of the ftz/lacZ fusion gene.

N47 Tissue specific expression of early embryonic genes in Xenopus laevis.
Milan Jamrich, Thomas D. Sargent, and Igor B. Dawid.
Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD. 20205.

We investigated the spatial expression of genes activated for the first time at or shortly after the mid-blastula transition in Xenopus embryos. By analyzing a large number of early embryo-specific genes we found molecular markers suitable for monitoring the steps involved in the formation of tissue specific expression in Xenopus embryos. DG42 is a gene expressed during early embryogenesis, and RNA complementary to this gene is specifically accumulated in the endoderm of stage 12 embryos. However, the RNA is initially accumulated in the animal pole of the stage 9 embryo, and only later in the vegetal hemisphere from which endoderm derives. In contrast, the mRNAs complementary to embryonic cytokeratin genes are specifically expressed in epidermis of stage 14 embryos. At earlier stages, as judged from preliminary experiments, these genes are expressed in presumptive neuro-ectoderm as well and are only later specifically turned off in these cells. It appears, therefore, that early embryo-specific genes are first activated in large regions of the embryo while the final location of their expression is determined by subsequent spatial restriction of their transcription and/or accumulation.

N48 TISSUE AND DEVELOPMENTAL STAGE SPECIFIC EXPRESSION OF MYC FAMILY GENES
E. Legouy, K. Zimmerman, R. DePinho, N. Kohl, and F. Alt.
College of Phys.and Surgeons of Columbia University, New York, N.Y.
The myc family of cellular oncogenes contains three well-defined members, c-myc, N-myc, and L-myc; our recent studies suggest that this family contains multiple additional members. Previously We have demonstrated that the N-myc and c-myc genes have a similar oncogenic potential and that the two genes have a similar overall structure. Despite the apparent structural and functional similarities of myc family genes, we have demonstrated that high level expression of the N- and L-myc genes is very restricted with respect to tissue and developmental stage while that of c-myc is more generalized. The expression patterns of these genes in normal development predicts the types of tumors in which they are activated; in particular, activated N-myc expression may be a characteristic of a variety of tumors which derive embryonic tissues and have a genetic component to their etiology. Our intitial transfection experiments suggest that sequences which confer tissue, stage, or tumor-specific expression patterns of N-myc expression are located within or near the N-myc gene. In addition, we find that high level expression of N-myc appears to down-regulate (cross-regulate) expression of the c-myc gene. Together, our findings suggest that differential myc-family gene expression could play an important role in normal differentiation processes.

N49 MURINE TERATOCARCINOMA; A MODEL OF DIFFERENTIATION AND VIRAL GENE EXPRESSION, John M. Lehman, Larry Couture and Siobhan Gagen, Department of Microbiology and Immunology, Albany Medical College, Albany NY 12208.

The murine teratocarcinoma is an interesting tumor model system which differentiates into representatives of all three embryonic germ layers. This phenomenon of differentiation while not unique to tumor model systems, is of interest and may be potentially useful to study both neoplastic and normal differentiation. It has been recognized that certain viral genomes such as SV-40 and polyoma are unable to infect, transform, or replicate in the stem cell of the murine teratocarcinoma, embryonal carcinoma. However, these viruses are able to express their genomes in the differentiated cells. As a result of the availability of this system which can modulate the expression of viral genomes dependent upon the state of differentiation, studies have been undertaken to characterize the mechanism(s) that block viral expression in the stem cell but allow expression in differentiated cells. A number of mutant polyoma viruses have been isolated which replicate in embryonal carcinoma cells. Characterization of these mutants may allow a definition of why and where wild type infection is blocked. This system is of interest since the change of gene expression during differentiation modulates the expression of a well-characterized viral genome. Studies will be presented that characterize regulation of cellular and viral gene expression in this model system. (This work was supported by a grant from the National Cancer Institute, CA16030.)

N50 DOMINANT PRE-NATAL LETHALITY IN A TRANSGENIC MOUSE LINE IS ASSOCIATED WITH A CHROMOSOMAL TRANSLOCATION, Kathleen A. Mahon, Paul A. Overbeek, and Heiner Westphal, NIH, Bethesda, MD 20205

Nine independent lines of transgenic mice were generated which contain the promotor of the Rous Sarcoma Virus (RSV) fused to the coding region of chloramphenicol acetyltransferase (CAT). The founder mouse of one line fathered unusually small litters (ave. 3.5 pups instead of the normal 9.5 pups/litter) when crossed to wild-type females. Of the progeny, ~50% bore the RSV-CAT sequences and also produced small litters when mated to wild-type animals. An analysis of embryogenesis revealed that normal numbers of embryos were implanting, but that 50-60% failed to develop past day 7. Eight other independent transgenic lines containing RSV-CAT show no evidence of embryonic lethality, thus it is unlikely that the defect is due to the direct effects of RSV-CAT expression. In addition, the characteristics of this line are not easily explained by insertional activation or inactivation of cellular genes by the RSV-CAT sequences at the site of integration. We have found that affected animals bear a chromosomal translocation which can explain the dominant embryonic lethality observed. When a mouse carrying the translocation is crossed to wild-type, 25% of the embryos will be wild-type, 25% will be balanced for the translocation and viable, and 50% will inherit an unbalanced karyotype and will die. Since the spontaneous rate of translocation in mice is quite low (1/10,000), and since the translocation always segregates with RSV-CAT sequences, we propose that this rearrangement did not occur randomly and was in some way related to the introduction of foreign DNA.

N51 ALTERED SEGMENTATION PATTERN OF DROSOPHILA TAILLESS EMBRYOS AT BLASTODERM IS REVEALED BY IN SITU HYBRIDIZATION WITH FTZ AND HAIRY PROBES.
Paul A. Mahoney[1], Teresa R. Strecker[2], John R. Merriam[2], & Judith A. Lengyel[1,2], [1]Molecular Biology Institute and [2]Dept. of Biology, University of California, Los Angeles, CA 90024.

Tailless (tll) is a zygotic lethal mutation which affects the anterior and posterior ends of Drosophila embryos. Mature tll embryos are missing the eighth abdominal segment and the telson, and have shortened pharyngeal ridges in the head skeleton. At 8 hrs post-fertilization, tll embryos are observed to have fewer tracheal pits than wild type embryos. By 9 hrs, tll embryos are missing segments A8, A9, and A10. In addition, the clypeolabrum, procephalic, and optic lobes are abnormal (Strecker et. al., Dev. Biol. 113, 1986).

We have hybridized radioactive single-stranded probes in situ to whole tll and wild type embryos to examine the effects of the tll mutation on the expression of the genes ftz and hairy. Autoradiography of plastic sections and whole mounts demonstrates that the patterns of gene expression seen in wild type embryos at blastoderm are altered in tll embryos. This indicates that the tll gene acts very early in embryogenesis, prior to or concomitant with the expression of the ftz and hairy genes, and is required for the normal spatial expression of these genes. Thus, we can use cloned probes to investigate, many hours prior to the time at which segmentation becomes apparent, the role of the tll gene in segment pattern determination

N52 HORMONAL REGULATION OF EGG COAT PROTEIN SYNTHESIS IN XENOPUS, Joan Marsh and Jamshed R. Tata, National Institute for Medical Research, London NW7 1AA, U.K.

The three jelly coats (equivalent to the zona pellucida of mammalian eggs) of Xenopus and other vertebrate eggs have important functions in fertilization and early embryonic development but little is known about the site and regulation of their formation. We have approached this problem by establishing primary cultures of Xenopus oviduct and follicle cells to characterize egg coat and other proteins synthesized and secreted by these cells and their regulation by estrogen, progesterone and follicle stimulating hormone (FSH). In parallel studies, cloned cDNAs to estrogen-induced oviduct mRNAs have been used to analyze the induction of genes encoding jelly coat proteins. The accumulation of three mRNAs coding for as yet uncharacterized proteins synthesized in the oviduct and which are possibly part of the jelly coats was thus studied. The addition of estrogen and progesterone to oviduct cell cultures enhanced the accumulation of these mRNAs but with different kinetics and to different extents. Comparison of their transcription with that of estrogen-inducible genes in other tissues has also allowed us to get further insight into the important question of the tissue specificity of the hormonal regulation of gene expression.

N53 Preliminary Characterization of the maternal transcription unit of the fs(1)h locus of Drosophila. Brian A. Mozer, Susan R. Haynes, Francoise Forquignon and Igor B. Dawid. Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD. 20892

Females carrying mutations at the X-linked female sterile homeotic locus fs(1)h show missing organs as well as homeotic transformations of T3 to T2. The maternal homeotic effect is enhanced if such progeny are heterozygous for some Ubx alleles or trx alleles. In addition, individuals homo- or hemi-zygous for non-conditional fs(1)h mutations are zygotic lethals. The fs(1)h locus has been cloned by chromosome walking and four alleles have been mapped along the walk. Proximal localization of a linked mutation 1(1)mys as well as Northern analysis show that the fsh locus lies within a stretch of 20 kb of DNA. Northern analysis using polyA+ RNA isolated from ovaries or 0-4hr embryos has identified two maternal polyadenylated transcripts (7.6 and 5.9 kb) derived from the locus. A moderately repetitive element called pen was identified within several of the fsh probes. These probes hybridize to additional RNA bands which are derived from other parts of the genome. Sequence analysis of pen containing clones as well as partial length cDNA clones corresponding to the 7.6 and 5.9 mRNAs confirms the presence of the pen repeat which consists of multiple tandem copies of the sequence GGX.

N54 FACTORS CONTROLLING THE TISSUE SPECIFIC EXPRESSION OF A CELL SURFACE ANTIGEN. Carey Phillips and Rebecca Akers. Bowdoin College, Brunswick, ME and Stanford University, Palo Alto, CA. The distribution of a tissue specific antigen was studied in Xenopus laevis embryos with a monoclonal antibody. Antibodies were generated against a membrane preparation from neurala stage embryos. One tissue specific antigen, Epi 1, is first detected at early neural plate stage and distinguishes between neural and non-neural epithelium. The Epi 1 antigen is also expressed in cultures of early blastomeres explanted prior to mid-cleavage. All of the surface cells resulting from cultures of animal hemisphere blastomeres will express Epi 1. However, the vegetal cell blastomere cultures do not express the Epi 1 antigen. This indicates that the cells of the animal hemisphere possess the information necessary for expression of Epi 1 very early in development. In fact, if the uncleaved zygotes are ligated into animal and vegetal hemispheres the animal portion of the embryo will express the Epi 1 antigen after the appropriate time in culture. Two questions regarding the factors controlling the expression of this antigen are being studied. First, where in the animal hemisphere is the information which is necessary for the expression of Epi 1. Second, what is responsible for the inhibition of Epi 1 expression in a subset of the surface ectoderm, the neural epithelium?

N55 THE ENGRAILED COMPLEX CONTAINS TWO CLOSELY RELATED GENES. S. Poole, K. Coleman, M. Weir, B. Drees, and T. Kornberg
Dept. Biochemistry, UCSF, San Francisco, CA 94143

The engrailed gene is required for the maintenance of anterior/posterior compartment boundaries in Drosophila segments and is expressed only in posterior compartments (Kornberg et al, Cell 40:45). We have identified a nearby related gene, invected, ~15 kb downstream from engrailed. It encodes a 2.7 kb transcript and shares two regions of homology with engrailed, a 51 bp region ~1 kb into the open reading frame and a 267 bp region commencing with a homeo box and extending almost to the termination codon of each gene. The genes share no homology outside of these two regions. The invected gene is also expressed only in posterior compartments, but shows a different time course of developmental expression. We have fused engrailed and invected upstream regions with lac Z structural sequences to assay those regions necessary for compartment specific expression.

N56 U1 RNA BASE-PAIRED WITH POLY(A)RNA IN SEA URCHIN EGGS. S. D. Ruzdijic and T. Pederson, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Echinoderm and amphibian eggs have recently been found to contain a large amount of cytoplasmic polyadenylated RNA which, surprisingly, cannot be translated in vitro or after oocyte microinjection. This non-messenger RNA accounts for over 50% of the total egg poly(A)$^+$ RNA and, importantly, contains covalently-linked single-copy and repetitive DNA sequence transcripts, a feature reminiscent of the nuclear pre-mRNA (hnRNA) of somatic cells. We have investigated the possibility that these interspersed repeat-containing poly(A)$^+$ RNA's are complexed with a known component of the pre-mRNA splicing machinery: U1 small nuclear RNA. Eggs were homogenized and incubated in the presence or absence of aminomethyltrimethylpsoralen (AMT) followed by irradiation with 365 nm light to photocatalyze AMT cross-links in base-paired regions of RNA. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography and the presence of U1 RNA was determined by gel blot hybridization with a cloned sea urchin U1 DNA probe after crosslink reversal. These experiments revealed that U1 RNA was paired with poly(A)$^+$ RNA in AMT-treated samples but not in untreated controls. To further investigate this association, we used a monoclonal antibody, Sm, for proteins associated with U1 and related small nuclear RNA's. RNA immune-precipitated from egg homogenates by Sm antibody was found to hybridize with cloned DNA probes for interspersed single-copy and repetitive sequences represented in egg poly(A)$^+$ RNA. Similar experiments performed on nucleate vs. anucleate egg halves confirmed that the Sm antibody-precipitable, interspersed repeat RNA's are largely confined to the anucleate fraction. We speculate that the egg may contain cytoplasmic pre-mRNA's whose processing is arrested during oogenesis. This hypothesis carries substantial developmental significance, and is therefore being explored further.

N57 Regulation of Alpha-Fetoprotein Gene Expression during Embryonal Carcinoma Cell Differentiation. J. Schindler and P. Coughlin, Department of Anatomy and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267

Nulli-SCC1 murine embryonal carcinoma (EC) cells grown in monolayer culture can differentiate into several cell phenotypes in an inducer-dependent manner. Exposure of these EC cells to hexamethylene-bisacetamide (HMBA) induces differentiation into visceral endoderm with concurrent expression of alpha-fetoprotein (AFP). In experiments designed to investigate the temporal expression of AFP, we observed a bimodal response following HMBA induction. Northern blot analysis of poly A$^+$ RNA isolated from HMBA treated cells showed detectable AFP mRNA within one hour of exposure. Detectable levels of authentic AFP mRNA decreased dramatically by 6 hours and reappeared following 48 hours of exposure to HMBA. Immunoprecipitation of radiolabeled AFP demonstrated detectable intracellular levels of protein 3-6 hours after initial exposure to HMBA. Levels of secreted AFP could not be detected in the medium until 48 hours of induction. Thus, HMBA treated Nulli-SCC1 EC cells demonstrate two unique characteristics; 1) AFP specific mRNA exhibits a bimodal temporal response to HMBA, initiated by an extremely rapid burst of AFP mRNA synthesis and 2) an extremely long lag period exists between the appearance of intracellular AFP and the secretion of AFP into the medium.

N58 AN INDUCIBLE ENHANCER ELEMENT IN THE UPSTREAM REGION OF METALLOTHIONEIN GENES. Edgar Serfling (1,2) and Walter Schaffner (1)
1) Institut für Molekularbiologie der Universität Zürich, 8033 Zürich, Switzerland
2) present address: Institut für Virologie und Immunbiologie der Universität Würzburg, Versbacher Str. 7, 8700 Würzburg, FRG

We have isolated SV40 recombinant viruses which are dependent on heavy metal ions for efficient propagation. They were obtained after co-transfection of enhancerless SV40 DNA (the so-called enhancer trap) with sonicated DNA from the mouse metallothionein-I (mMT-I) or human metallothionein-IIA (hMT-IIA) upstream regions. To substitute for the SV40 enhancer, these viruses have incorporated a segment of the immediate upstream region of the metallothionein genes. Two recombinant viruses of SVMT-I type carry segments of mMT-I gene from position -73 to -187, and -39 to -194, respectively, inverted with respect to their natural configuration. The overlapping segment contains two of the four metal responsive elements involved in the induction of the mMT-I gene by heavy metal ions. The SVMT-II recombinant virus contains a segment of the hMT-IIA gene from position -39 to -366 which harbors the metal and hormone responsive elements of the hMT-IIA gene. Insertion of the mMT-I segment downstream of a rabbit ß-globin test gene enhances the number of ß-globin transcripts upon metal ion stimulation. This shows that the immediate upstream region of the mouse metallothionein-I gene, when detached from its TATA box, can act as an inducible enhancer. It may be generally true that the enhancer/promoters of inducible genes are composed of several regulatory sequence elements which are interspersed with constitutive elements.

N59 EGG ACTIVATION AS SEEN BY NMR. Mary Lee Sparling. California State University, Biology Department, Northridge, CA 91330.

31-P NMR on live (S. purpuratus) eggs and 1-H NMR of membrane extracts comparing them before (U) and after fertilization (F), and after treatment with A23187 (I), zinc (Z), ammonia (A), or lithium (L), show distinct altered metabolic patterns and membrane lipid content. (Bruker WM-500, 31-P broadband probe, 202 MHz, peak width 40, AQ = .803, SI = 16-32K, non-spinning, 1-H decoupling or not, 15°C, 2 ml packed eggs in 10 mm tubes. U and A have a peak at -3 ppm (arginine phosphate) missing in all others. F has 5% new peaks from -15 to -18 ppm. A23187 causes greatly decreased ATP; increased AMP, Pi, pyrophosphate. L has less ATP than normal and has a new peak at 8.5 ppm. Z has more bound ADP and less ATP. Little internal pH change is found. Proportions of NADP to NAD and total P differ, somewhat more for L and Z. Membrane lipids isolated from U,F,I,A were observed (1-H NMR 500 MHz probe, 5 mm sample, 32°C, PW=6, AQ=1.36, 16-32K). Four sucrose density membrane fractions prepared from each group show lipid effects following fertilization or treatments. U lacks peaks found in all others. There is considerable variation of a) protons of the double bond region, b) Phosphatidylethanolamine peak intensity at 3.16 ppm (highest in U), c) the kind of glycolipid peaks between 3.6 and 3.8 ppm, with U being most unique and d) a great difference at the acyl alpha methlylene peak at 2.5 ppm, as well, especially in F. The striking result is that each treatment, within minutes, gives such unique metabolic and lipid content changes. This work was done at the Southern Regional NMR Facility at California Institute of Technology.

N60 A HIGHLY REPETITIVE SEQUENCE ASSOCIATED WITH mRNAs FROM EMBRYOS AND ADULT TISSUES OF X.LAEVIS, Georges Spohr and Walter Reith, laboratoire d'Embryologie Moléculaire, Université de Genève, 1211 Genève 4, Switzerland

A highly repetitive sequence consisting of a tandem array of subunits of 77-79 bp has been identified by hybrid selection translation experiments in mRNA extracted from oocytes, embryos of stage 40, adult liver and adult intestin of X.laevis. A total of 35 different polypeptides that are encoded for by mRNAs which carry the same repeat have been detected. Some of these peptides have been identified exclusively in one of the tissues studied, whereas others have been found in various of them or in all. Expression of genes associated with this repetitive sequence does not seem therefore to be restricted to a defined stage of development or tissue.
To find out whether the repeat influences the transcription of neighbouring sequences, chimeric genes containing pol II promotors and the repetitive element have been constructed and assayed by injection into fertilized eggs. Preliminary data suggest that the presence of the repeat has a stimulatory effect.

N61 **CLONING AND CHARACTERIZATION OF MOUSE HOMEO BOX-CONTAINING SEQUENCES,** Naoki Takahashi and Masami Muramatsu, Department of Biochemistry, University of Tokyo Faculty of Medicine, Tokyo 113, JAPAN

We have constructed a BALB/C mouse genomic library in Charon 28. Approximately 1 x 10^6 plaques of this library were screened using fragments containing homeo box sequences of Antennapedia and fushi tarazu as probe (kindly supplied by W. Gehring). Thirty homeo box-containing recombinants contain at least six homeo box sequences. Two of them are linked each other within 12 kilobases.
Analysis of structure and expression of these sequences are in progress.

N62 REGULATION OF EXPRESSION OF THE DROSOPHILA SERENDIPITY GENE CLUSTER. Alain Vincent, Constantin Yaniscostas, François Schweisgut, Institut J. Monod, 75251 PARIS CEDEX 05 France and Michael Rosbash, Dept of Biology, Brandeis Univ. Waltham MA 02254.

The serendipity (sry) locus found in the 99 D region of the third chromosome in Drosophila contains three tightly clustered genes; beta, alpha and delta (Vincent et al.,(1985) J. Mol. Biol. 186 ...). The alpha gene lies in between beta and delta and is expressed predominantly at the blastoderm stage of embryogenesis. Additional unusual features of this locus include read-through transcription and the fact that the predicted beta and delta polypeptides show remarkable homology to the Xenopus polymerase III transcription factor TFIIIA (Miller et al. (1985) EMBO J. 4, 1609) and to Krüppel, a Drosophila segmentation gene (H. Jäckle, pers. communication). To identify cis-acting elements controlling the expression of the alpha and beta transcripts, we created transformed lines of flies containing modified versions of these genes. In lines containing derivatives of both the alpha and beta genes, the expected mRNAs are transcribed under proper developmental control. An alpha gene variant containing only 798 bp of upstream DNA is specifically transcribed at the blastoderm stage. This and other experiments show that the transcription of the beta and alpha genes is independently regulated and that beta-alpha read through transcription is not required for proper bastula-specific alpha gene expression. Experiments now in progress will determine the spatial distribution of the sry transcripts and polypeptides using sry/lacZ gene fusions.

N63 DEVELOPMENTAL EXPRESSION OF A NOVEL MURINE TESTIS-SPECIFIC TRANSCRIPT CONTAINING HOMEO BOX HOMOLOGY, Debra J. Wolgemuth and Eric Engelmyer, Columbia University College of Physicians and Surgeons, NY, NY 10032.

pHBT-1, a cDNA clone isolated from a mouse testis cDNA library, contains sequences homologous to the homeo box region of the Antennapedia gene of Drosophila. Sequences flanking the 3' end of homeo box homology are highly diverged from other murine homeo box-containing genes characterized to date. RNA blot hybridization of mouse testis poly(A)$^+$ RNA using HBT-1 revealed transcripts of ~1.4 kb in length. Within the limits of sensitivity of Northern blot analysis, no transcripts were detected in any of the adult somatic tissues examined, including other tissues that contain stem cells, namely those of the hemopoietic system. HBT-1 transcripts were limited to male germ cell-containing tissues, since poly(A)$^+$ RNA from juvenile and adult ovaries did not contain HBT-1 transcripts. A developmental regulation of the expression of HBT-1 in the testis was observed: no transcripts were detected in RNA isolated from immature testes, which contain spermatogonia but which lack cells in meiosis. HBT-1 transcripts were detected in purified populations of pachytene spermatocytes, early spermatids, and in a mixed population of cytoplasmic fragments of elongating spermatids and residual bodies. Experiments to define the precise localization of HBT-1 transcripts in spermatogenic cells by in situ hybridization are in progress. A role for the expression of the HBT-1 gene in sperm differentiation is postulated.

N64 THE XENOPUS HOMEOBOXES, Christopher Wright, Andreas Fritz and Eddy De Robertis, Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024

We have previously reported, in collaboration with W. Gehring's group, the cloning of a Xenopus homeobox-containing gene using Drosophila probes. This constituted the first identification and isolation of a vertebrate homeotic-like gene. Similar genes are probably present in all vertebrates, and the mouse and human homeoboxes have been characterized in detail by other investigators. To date we have cloned six different homeobox-containing genes from frog gastrula cDNA libraries. These were characterized by sequencing using synthetic primers complementary to the 3' end of the homeobox. In addition to the homeobox, a new region of homology (sequence Ile Tyr Pro Trp Met) has been found in 4 of these genes in the exon preceding the homeobox. A similar sequence is also found in Antennapedia, as determined by S. Schneuli and W. Gehring. All of six genes are expressed during early frog development, with 3 of them being transcribed only during very narrow periods of development.

Three of the frog genes have sequences that are very similar to human genes isolated in other laboratories. Thus it seems that all vertebrates share a small number of these genes, which have been highly conserved outside of the homeobox region.

Several lines of evidence, such as their highly regulated transcription pattern during embryogenesis, the demonstration of alternative slicing for one of these genes, their tissue distribution, homologies to yeast and Drosophila genes, and the DNA-binding properties of frog fusion proteins made in E. coli, suggest that these genes are important in early vertebrate development.

Cellular Differentiation in Lower Eukaryotes and Hematopoietic Cell Lineages

N65 REGULATION OF EMBRYONIC GLOBIN GENE EXPRESSION. Margaret H. Baron and Tom Maniatis, Harvard University, Cambridge, MA 02138.

The phenomenon of "hemoglobin switching" which occurs during the ontogeny of the red blood cell involves the sequential expression of embryonic, fetal, and adult globin genes. Globin genes of the β family have been introduced into human erythroid cell lines (e.g. K562) which express only embryonic and fetal, but not adult globin, using an episomal vector derived from Epstein-Barr virus. Both a "marked" human embryonic and a mouse embryonic globin gene are appropriately expressed in these cells, and transcription initiates from the correct start site. However, the (marked) human adult globin gene, although structurally intact, is not expressed. These findings are consistent with earlier suggestions that K562 cells either lack a positive regulatory component required for the expression of the β globin gene, or synthesize a repressor which prevents its transcription.

To identify trans-acting elements involved in the regulation of embryonic globin gene expression, human K562 cells have been fused with various mouse cells to form transient heterokaryons. Expression of the mouse embryonic globin gene is induced in these heterokaryons but not in unfused mixtures of the two cell types. The mouse adult β globin gene is not induced. These experiments demonstrate the existence of diffusible factors that control the expression of differentiation-specific genes.

N66 ISOLATION OF MUTANTS OF DICTYOSTELIUM DEFECTIVE IN THE RESPONSE OF GENES TO cAMP, Edward A. Berger, Eugene D. Dulaney, and Nicholas B. Carter, Cell Biology Group, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

We have devised a positive selection scheme for the isolation of mutants defective in the regulatory pathways by which cAMP controls gene expression during Dictyostelium development. Our strategy is based on reported observations that differentiated (starved) cells lose their differentiated characteristics when returned to growth medium, and that exogenous cAMP blocks this de-differentiation. From this we reasoned that if a population of starved cells is returned to growth medium, cAMP should block the resumption of growth of the wild type cells; however, rare mutants defective in the cAMP response mechanism(s) should escape the growth block and hence be selectively enriched. We have established conditions under which cAMP treatment has no effect on the growth of unstarved cells, but drastically inhibits resumption of growth of starved cells. When a population of unmutagenized cells was passaged through several starvation and cAMP treatment cycles, most of the survivors were found to have morphogenetic defects. These spontaneous mutants fell into five phenotypic classes: one class was completely non-aggregating, whereas the other four classes formed aggregates each with a distinct altered morphology. One clone from each class has been analyzed for developmental gene expression by RNA dot blotting, using as probes a variety of recombinant DNAs detecting mRNAs whose levels are either increased or decreased by exogenous cAMP. Preliminary results reveal several distinct patterns of altered responsiveness of specific genes to cAMP, suggesting these mutants may prove useful in characterizing the biochemical pathways by which cAMP regulates developmental gene expression.

N67 CHROMATIN STRUCTURE OF DEVELOPMENTALLY-RELATED GENES IN THE CELLULAR SLIME MOLD, DICTYOSTELIUM DISCOIDEUM, Daphne D. Blumberg and Jo Ann F. Comer, National Cancer Institute, Frederick, MD 21701.

Post-aggregation Dictyostelium cells contain 2000 to 3000 new mRNA species that are absent from growing and preaggregation stage cells. The transcription of these new mRNAs is initiated late in development. We have explored the structural organization of these late genes in chromatin. Two major conclusions emerge: 1) In nuclei of growing cells the late genes are in a fully DNase I-sensitive "active" configuration, even though they are not transcribed in these cells. The DNase I sensitivity of active and inactive genes is the same. 2) Digestion with micrococcal nuclease, however, does reveal a major structural differences in the organization of the genes which are actively transcribed in the growing cells and the transcriptionally-inactive late genes. The inactive late genes are found in a regular nucleosomal ladder with a repeat of about 168 bases. The actively transcribed genes, however, are cut by the enzyme, both between nucleosomes, and at additional sites, giving rise to an irregular ladder of bands with a spacing of 70-130 nucleotides between bands. Further analysis of the structure of these single copy genes utilizing high resolution two-dimensional hybridization mapping of mono- and oligonucleosomes reveals that the irregularly cut, transcriptionally-active genes are associated with nucleosomes which lack histone H1, while the inactive late genes are in nucleosomes which contain histone H1.

N68 DEVELOPMENTAL REGULATION OF C-MYB DURING HEMATOPOIESES

David Boettiger and Stephen Duprey, Department of Microbiology
University of Pennsylvania, Philadelphia, PA 19104

The expression of c-myb in specific hematopoietic progenitor cells of the chicken was demonstrated using cell separation techniques combined with blot hybridizations of cellular RNA and determination of protein levels by immunofluorescence and western blots. The majority of RNA transcripts and p75 c-myb were restricted to a minor population of the hematopoietic progenitor cells and expressed particularly in the macrophage progenitors (M-CFC). The level of expression was 200-400 copies of M-RNA/cell which is similar to the level of v-myb expression in cells transformed by avian myeloblastosis virus which carries the v-myb oncogene.

A model for macrophage differentiation is proposed based on analogies between c-myb and v-myb in which myb functions to depress the production of macrophage-specific products to allow expansion of the progenitors prior to terminal differentiation.

N69 EXPRESSION OF HUMAN TUMOR NECROSIS FACTOR AND ALPHA INTERFERON IN VIRUS INDUCED HUMAN PERIPHERAL BLOOD LEUKOCYTES.

S.L. Berent, R.M. Torczynski and A.P. Bollon, Wadley Institutes of Molecular Medicine, Dallas, TX.

Human peripheral blood leukocytes (PBLs) induced with Sendai virus expressed alpha interferon mRNA and surprisingly high levels of tumor necrosis factor (TNF) mRNA. The PBL RNA was utilized to make a cDNA library which generated over 10 alpha interferon clones and several tumor necrosis factor clones. The TNF and α-interferon mRNAs appear to be at similar levels and the frequency of TNF clones in the cDNA library was about 10 fold higher than that previously found with an established tumor cell line. The TNF mRNA peaked at about 1-3 hrs after induction with Sendai virus. The level of TNF activity was about 250 fold lower than that of interferon activity. Since the TNF mRNA and alpha interferon mRNA are at similar levels under these induced conditions, either the TNF has a short half-life or some form of translational regulation may exist. Utilizing a P_L expression vector we have produced up to 10^9 U/l of TNF in E. coli. The recombinant TNF has been shown to be cytotoxic against tumor cell lines and necroses tumors in mice. Clinical trials were initiated at the Wadley Institutes on September 25, 1985. Since interferons and TNF act synergistically, the induction of alpha interferon and tumor necrosis factor in peripheral blood leukocytes may represent an excellent example of regulation of gene expression during late stages of development of "normal" human cells. This work was supported by grants from Meadows Foundation and an NIH grant to APB.

N70 Isolation of the brlA locus from Aspergillus nidulans,

Margaret Boylan and William Timberlake, University of California, Davis, CA 95616.

Mutations at the bristle locus prevent sporulation in Aspergillus nidulans. The conidiophores of bristle mutants elongate to more than ten times the normal length and fail to produce any of the apical structures found in a wild-type conidial head. We have used a cosmid library to isolate the brlA locus from A. nidulans by transformation and complementation of the mutant phenotype. The complementing activity in the recovered cosmid maps to a 4.5 kb Bam H1 fragment which encodes a prominent poly(A^+) RNA transcript of 2.4 kb. This transcript is detected only in sporulating cultures and is not found in vegetative mycelia or in spores. We have constructed a plasmid which disrupts this transcription unit when transformed into a wild-type Aspergillus strain. The recovery of bristle colonies among these transformants indicates that the Bam H1 fragment contains the brlA locus itself rather than a suppressor of the bristle phenotype. Structural and functional analysis of this region of the Aspergillus genome will help elucidate the role of the bristle gene product in regulating conidiation.

N71 Anke Burmester, Johannes Wöstemeyer
Institut für Biochemie und Molekulare Biologie/Botanik
Technische Universität Berlin, Franklinst. 29, 1000 Berlin 10, F.R.G.

EXPERIMENTS TOWARDS AN IN VITRO SYSTEM FOR GENETIC MANIPULATION OF MUCORACEOUS FUNGI: CLONING OF MITOCHONDRIAL ARS ELEMENTS FROM THE ZYGOMYCETE ABSIDIA GLAUCA

N72 SV40 EXPRESSION IN MICROINJECTED MOUSE OOCYTES, L.E. Chalifour[1], D.O. Wirak, U. Hansen[2], P.M. Wassarman[3] and M.L. DePamphilis, [1]National Research Council Biotechnology, Montreal, [2]Dana-Farber Cancer Centre, Boston, [3]Roche Institute, Nutley.

What promoter elements are recognized by oocytes and are these the same as adult cells? We have used deletion mutants of Simian Virus 40 (SV40) and measured the expression of both early (T- and t- antigens) and late (V-antigens) genes after microinjection of the plasmids into the nuclei of mouse oocytes. We have found both early and late genes are expressed in the injected oocyte in contrast to the lack of late gene expression in SV40 - infected adult mouse cells. Early and late gene expression does not depend upon a functional SV40 origin. Early gene expression was also independent of its TATA box and the 72 bp repeat enhancers but was dependent on retention of three of the six GC-rich motifs located in the 21 bp repeats. Late gene synthesis also required three of the GC-rich motifs and sequences between nt 197 and 273. These parameters resemble those elements essential in monkey permissive cells at late times after SV40 infection. Thus, the elements recognized as promoters in mouse oocytes are not those found important after infection of adult cells.

N73 A RETROVIRUS VECTOR FOR *IN VITRO* ANALYSIS OF ERYTHROID SPECIFIC GENE REGULATION. O.-R. Choi and J.D. Engel, Northwestern University, Evanston, IL 60201.

AEV-ES4 IS A DEFECTIVE AVIAN RETROVIRUS HARBORING TWO TRANSDUCED CELLULAR ONCOGENES, *v-erbA* AND *v-erbB*, AND DISPLAYS A MARKED TROPISM FOR ERYTHROID PROGENITOR CELL TRANSFORMATION *in vitro*. WE HAVE CLONED ONE TEMPERATURE-SENSITIVE ALLELE OF AEV (TS167), AND HAVE DEMONSTRATED THAT A SINGLE AMINO ACID CHANGE IN THE PROTEIN KINASE DOMAIN OF *v-erbB* IS NECESSARY FOR THE THERMALLY INDUCIBLE PHENOTYPE OF TS167AEV-TRANSFORMED ERYTHROID PROGENITOR CELLS.

WE ARE ATTEMPTING TO ESTABLISH TS167AEV AS A VECTOR FOR INTRODUCTION OF CLONED ERYTHROID-SPECIFIC GENES INTO THE CHICK EARLY ERYTHROID LINEAGE. THE PROPERTIES OF SUCH A SYSTEM ARE ATTRACTIVE FOR STUDYING *cis*-ACTING REGULATORY ELEMENTS OF ERYTHROID-SPECIFIC GENES SINCE: (1) A SINGLE COPY OF THE MODIFIED GENE IS RESIDENT IN EACH CLONED ERYTHROID PRECURSOR CELL; (2) SEVERAL ERYTHROID GENES APPEAR TO BE TRANSCRIPTIONALLY ACTIVATED SUBSEQUENT TO THE DEVELOPMENTAL STAGE OF CELLS TRANSFORMED BY THE RETROVIRUS; (3) SELECTION FOR ERYTHROID PRECURSOR CELLS CONTAINING THE MUTANT GENE IS ACCOMPLISHED SIMPLY BY CLONING OF TRANSFORMED BONE MARROW COLONIES *in vitro*; (4) SUCH TRANSFORMED PRECURSOR CELLS CAN BE STIMULATED SYNCHRONOUSLY TO DIFFERENTIATE INTO MATURE ERYTHROCYTES *in vitro* BY THERMAL INDUCTION IN THE PRESENCE OF ERYTHROPOIETIN; (5) PURIFIED INTERMEDIATE STAGES LEADING TO MATURE ERYTHROCYTES CAN BE ISOLATED AND IDENTIFIED USING PERCOLL GRADIENTS AND STAGE-SPECIFIC MONOCLONAL ANTIBODIES; AND (6) THE REINTRODUCED ERYTHROID GENES (IN SEVERAL INSTANCES) ARE STABLE AS PART OF THE RETROVIRUS GENOME. WHETHER OR NOT SUCH REINTRODUCED, MODIFIED ERYTHROID-SPECIFIC GENES ARE EXPRESSED IS CURRENTLY UNDER INVESTIGATION.

N74 EVOLUTION OF HISTOCOMPATIBILITY SYSTEMS: MOLECULAR CLONING AND ANALYSES OF MHC/Ig RELATED SEQUENCES IN THE COLONIAL TUNICATE BOTRYLLUS schlosseri. J. S. Danska, N. E. Sibinga, Y. Saito, I. L. Weissman and H. O. McDevitt, Stanford University Medical School, Stanford, CA. The protochordate Botryllus, which diverged from the lineage leading to mammals about 450 X 10^6 years ago, possesses a polymorphic allorecognition locus that determines the acceptance or rejection of neighboring individuals into a parabiotic colony. Such natural transplantation reactions involve blood cell contact and affect the survival and germ line diversity of the organism. Among mammals the basis of antigen-specific recognition is controlled by a set of proteins bearing primary sequence homology and similar cell surface conformation. On the hypothesis that control of colony recognition in Botryllus may be mediated by genes ancestral to the mammalian immunoglobulin family, we have sought and cloned genomic and cDNA sequences which bear resemblance to mammalian MHC genes by using the latter as molecular probes. We have analyzed their nucleotide sequence, polymorphism and number in the genome of Monterey, Ca., Botryllus populations. The ontogenesis of MHC-related gene expression has been analyzed by northern blot analysis of the free-swimming larvae and post-metamorphic, sessile adults. Efforts to prepare antibodies directed against these MHC/Ig-related gene products by insertion of our cDNA clones into prokaryotic expression vectors are underway. Characterization of the MHC/Ig related genes, their gene products, the possible correlation between the genomic polymorphisms they identify and the allorecognition phenotype, and the pattern of their developmental expression provides a novel perspective on the primary sequence conservation, biological utility and evolution of the MHC/Ig gene family.

N75 IDENTIFICATION OF PUTATIVE CONTROL SEQUENCES INVOLVED IN REGULATING THE EXPRESSION OF GLOBIN AND NON-GLOBIN GENES DURING MURINE ERYTHROPOESIS, J. Frampton, I. Chambers, W. McBain, and P.R. Harrison, Beatson Institute, Glasgow, G61 1BD, SCOTLAND.

The presence of nuclease hypersensitive sites in chromatin and the formation of minor transcripts upstream of the major cap site are thought to be associated with transcriptional regulation. Indeed, for a globin gene these two phenomena have been shown to correlate closely.

With this in mind the identification of tissue-specific cis-acting control regions was attempted by making an analysis of DNase I hypersensitive sites and upstream transcripts in alpha 1, beta major, and beta minor adult globin genes and the non-globin gene ep19 which is expressed at high levels in red blood cells and a small subset of other cell types. Since previous work has concentrated on terminally differentiated cells the temporal activity of these regions was assessed with respect to erythropeitic differentiation from a pluripotent stem cell line through to reticulocytes (including an inducible Friend erythroleukaemia system). On the basis of this data conclusions can be drawn regarding events at the chromatin and transcriptional levels prior to and during expression from the major cap sites.

Comparison of the locations of a subset of upstream transcripts in the globin and non-globin genes has suggested two consensus sequences which might be regulatory elements. Preliminary functional assays of these consensus sequences are discussed.

N76 D. Francis and T. Hatfield, Univ. Delaware, "C-MYC-RELATED PROTEINS IN A CELLULAR SLIME MOLD."

We have probed cell extracts of Polysphondylium with an antiserum recognizing human c-myc protein, and find immunologically related proteins. Cell extracts were electrophoresed on polyacrylamide gels with SDS and dithiothreitol and blotted to nitrocellulose. Plots were probed with a rabbit polyclonal antiserum raised against purified human c-myc protein (Watt et al., 1985, Mol. Cell. Biol. 5, 448-456). In Polysphondylium pallidum, a polypeptide of 45 kd apparent molecular weight (pal 45): 1) Reacted with the c-myc antiserum but not with antiserum previously blocked by incubation with c-myc protein, and not with a control rabbit serum; this indicates that pal 45 is immunologically related to c-myc protein. 2) Was present in vegetative cells but not in cells at later stages of development. 3) Was concentrated in nuclear preparations. These results suggest that pal 45 may be similar in function to human c-myc protein.

N77 Characterization and regulation of the sporulation-specific yeast gene, SPS4. Anthony T. Garber# and Jacqueline Segall*. #Department of Medical Biochemistry, University of Calgary, Calgary, Canada and *Department of Biochemistry, University of Toronto, Toronto, Canada.

Several genes preferentially expressed during sporulation of S. cerevisiae have been isolated. The sporulation-specific gene, SPS4, and its flanking regions have now been characterized. The SPS4 ORF of 1014 nucleotides encodes a basic protein of 38,575 daltons. Hybridization selection experiments identified an appropriately sized polypeptide unique to MATa/MATα cells growing under sporulation conditions. S1 nuclease and primer extension experiments identified several transcription initiation sites 65bp upstream of the start of translation. Construction of homozygous deletion-insertion mutants revealed that SPS4 is non-essential for sporulation. A series of progressively 5'-deleted SPS4:lacZ fusion genes were constructed and transformed into diploid yeast on centromeric vectors. Expression of the chromosomal and fusion genes during vegetative growth and after transfer to sporulation conditions was monitored by S1 nuclease analyses. These experiments revealed a 108bp region, 74bp upstream of the TATAA box, containing a negative regulatory element necessary for the repression of SPS4 synthesis during vegetative growth and for its ability to be induced during sporulation. (Supported by the Medical Research Council of Canada).

N78 STUDIES ON CELL-TYPE DIFFERENTIATION IN DICTYOSTELIUM DISCOIDEUM USING ANTIBODIES TO PRODUCTS OF CLONED GENES, R.H. Gomer, S. Datta and R.A. Firtel, Dept. of Biology, University of California at San Diego, La Jolla, CA 92093

On starvation, Dictyostelium discoideum differentiates from individual vegetative cells into aggregated prestalk and prespore cells. Previous work in this laboratory led to the isolation of cell-type specific, developmentally regulated cDNA clones. We have isolated and sequenced the corresponding genomic clones of a prespore-specific and a prestalk-specific gene. The derived amino acid sequence of the prespore gene product shows little homology to known sequences, while the prestalk gene product is highly homologous to the cysteine proteinase Cathepsin H. Segments of the coding regions of these genes were ligated into an expression vector to produce, in bacteria, β-galactosidase/Dictyostelium fusion proteins. These were then used to immunize rabbits. Each antibody reacts with a single molecular-weight protein, as determined on Western blots, and is cell-type specific by indirect immunofluorescence staining of cryosections of migrating slugs. The prespore-specific antibody reacts with cells in the posterior 90% of the slug, while the prestalk antibody reacts with the anterior 10% and also with cells in the periphery of the slug. Staining of cryosections shows that the antigens are also specifically localized in both pre- and post-slug aggregates. Using immunofluorescence staining with these antibodies on low-density cells growing in microwells, we have devised a microassay for the conditioned-medium factor described by Mehdy and Firtel (Mol. Cell. Biol. 5: 705-713, 1985). The expression of both proteins in this culture system is dependent on cAMP and conditioned medium.

N79 *Analysis of telomere elongation in vitro* . Carol W. Greider and Elizabeth H. Blackburn. Department of Molecular Biology, University of California, Berkeley.

Telomeres, the extreme ends of chromosomes, are characterized in lower eukaryotes by the presence of tandemly repeated, simple GC rich sequences. Recent evidence suggests that telomere replication involves the de novo addition of telomeric repeats onto the ends of chromosomes. In addition, during macronuclear development in the ciliate Tetrahymena , the germline micronuclear chromosomes are fragmented and new telomeres are added to the ends of the resulting macronuclear molecules. We have found an activity in cell free extracts of Tetrahymena which elongates telomeric sequence oligomers by the de novo addition of $(TTGGGG)_n$ repeats, which is the Tetrahymena telomeric sequence (Greider and Blackburn Cell December 1985 in press). The elongation activity is template independent and requires only dGTP and dTTP, not dATP or dCTP. To further analyze the properties of the elongation activity we have begun fractionation of the extract and biochemical characterization of the reaction.

N80 DIFFERENTIATION OF NON-TRANSFORMED PRE-B LYMPHOCYTES IN VITRO, Johanna A. Griffin and David Spalding, University of Alabama at Birmingham, Birmingham, AL 35294

B lymphocyte differentiation is marked by a series of phenotypic changes that often reflect irreversible genotypic changes in the cells. These process have been studied in transformed cells arrested at a few specific stages in the differentiation pathway, and, therefore, the mechanisms by which these processes occur have been difficult to study. We have established cultures of non-transformed cell lines at one of the earliest stages in the differentiation pathway, pre-B cells, that have only the cytoplasmic heavy chain polypeptide of the IgM molecule. We can induce these pre-B cells to differentiate in vitro to the terminal plasma cell stage of immunoglobulin secretion under the influence of only accessory cells without exogenous antigen. The accessory cells consist of dendritic cells and mitogen-stimulated T cells from different lymphoid tissues, and the pathway of differentiation that is induced in the pre-B cells is dependent upon the lymphoid tissue source from which the dendritic cells, not the T cells, are derived. Therefore, we have established a system in which we can study the mechanisms by which the phenotypic and genotypic changes occur during B lymphocyte differentiation.

N81 CONTROL OF MORPHOGENESIS IN CANDIDA ALBICANS, Bipasha Gupta Roy and Asis Datta. Jawaharlal Nehru University, New Delhi-110067, India.

Candida albicans shows environmentally controlled dimorphism between two intercovertible morphologies- the yeast form and the mycelial form through an intermediate germ tube form. This germ tube formation can be induced by various factors, such as N-acetyl-D-glucosamine (GlcNAc), proline and glucose plus glutamine, at 37°C. Inhibitors of calmodulin eg. trifluoperazine (TFP), chloropromazine and compound 40/80 can block the yeast to germ tube conversion. Moreover, calmodulin has been shown to be present in Candida albicans. This result suggests the involvement of the calcium binding protein, calmodulin, in morphogenesis of Candida albicans. Further investigations into the molecular mechanisms involved in germination induced by GlcNAc, revealed that there is a progressive increase in the rate of protein phosphorylation and decrease in the rate of protein synthesis. However, TFP brought back the rates almost to yeast phase levels by enhancing the rate of protein synthesis and by inhibiting the rate of protein phosphorylation. An increase in protein phosphorylation was observed during germination with the other inducers and this was also inhibited by TFP. Our observations indicate that the action of calmodulin in germination is possibly exerted via calmodulin modulated protein phosphorylation.

N82 ISOLATION AND PURIFICATION OF THE RAT HEMATOPOIETIC STEM CELL. M. L. Hale, P. L. Fehnel, and K. F. McCarthy. Armed Forces Radiobiology Research Institute, Bethesda, Maryland, 20814.

The rat hematopoietic stem cell contains antigenic determinants recognized by the monoclonal antibodies Ox-7 and W3/13. The monoclonal antibody W3/13 recognizes a determinant for rat leukocyte sialoglyprotein molecules, predominantly found on T-cells and polymorphs; Ox-7 recognizes the Thy-1 determinant, found on T-cells, immature B-cells, and brain. In addition, the stem cell was found to be Ox-22 negative. The monoclonal antibody Ox-22 recognizes the high molecular weight form of the leukocyte common antigen. Dual parameter sorting of rat marrow following labeling with an Allophycocyanine-conjugated F(ab)' fragment of Ox-7 and phycoerythrin-conjugated F(ab)' fragment of Ox-22 results in greater than 200-fold enrichment of the rat CFU-s. Further analysis of rat marrow and other lymphoid tissue using various monoclonal antibodies specific for T-cell and B-cell subpopulations has led to the conclusion that commitment of rat CFU-s to the granulocyte lineage is accompanied by loss of the Thy-1 antigen, commitment to the B-cell lineage is accompanied by the loss of the W3/13 antigen, and commitment to the T-cell lineage appears to take place in the presence of both of these antigens.

N83 REGULATION OF GENE EXPRESSION IN DICTYOSTELIUM DISCOIDEUM. Bodduluri Haribabu and Robert P. Dottin. Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

In Dictyostelium discoideum cell-cell contact and cAMP regulate the expression of several genes including the UDP glucose pyrophosphorylase gene. Addition of exogenous cAMP alone bypasses the requirement for cell-cell contact and UDPGP expression is observed. To define the mechanisms by which exogenous cAMP modulates gene expression we characterized the target molecule which binds extracellular cAMP using cAMP analogues. The order of potency with which these analogues affect the expression of specific genes is consistent with the specificity of their binding to a cell-surface receptor and is distinct from their affinity for intracellular protein kinase. Both the genes which are positively regulated and the discoidin gene which is negatively regulated, respond to cAMP analogues to the same degree, suggesting that the effects are mediated through the same receptor. The specificity of this receptor is indistinguishable from that of the well-characterized cell-surface receptor.

N84 IN VITRO TRANSCRIPTION OF CLONED RDNA FRAGMENT AND ISOLATED NUCLEOLI FROM TETRAHYMENA PYRIFORMIS, Toru Higashinakagawa and Tadashi Matsuura, Tokyo Metropolitan University, Tokyo 158, Japan

In an attempt to know the role of chromatin structure in the process of transcription, we have been studying on rDNA of Tetrahymena pyriformis in multiple aspects. The trascription initiation site was determined on the cloned rDNA fragment using in vitro capping of 35S pre-rRNA. Transcription in vitro of the cloned rDNA gave rise to faithful transcript, the initiation point of which coincided exactly with the in vivo point on a nucleotide level. In vitro transcription of the nucleoli was carried out with isolated extrachromosomal nucleoli. Endogenous nucleolar pre-rRNA was digested with RNase T1. Since the pre-rRNA from T.thermophila does not protect the T.pyriformis rDNA probe, the RNase digested nucleoli were incubated with crude extract from T.thermophila. S1 nuclease protection mapping of the in vitro transcript gave the signal of expected size. Addition of four nucleotides only gave no signal, indicating that the observed protection signal is coming from the transcription initiation, but not from the elongation of the preinitiated RNA chain. Nucleoli provides more efficient transcription than naked cloned rDNA fragmnent on a template molar basis.

N85 INTACT EGGS AND LYSATES OF THE SEA URCHIN, L. PICTUS, HAVE LITTLE SPARE TRANSLATIONAL CAPACITY Merrill B. Hille and Anita M. Colin, Dept. Zoology, Univ. Wash., Seattle, WA

The free translational capacity of Lytechinus pictus eggs was tested in vivo and in vitro by the addition of rabbit reticulocyte poly(A)mRNA. Injection of 0.3 and 0.5 ng mRNA into L. pictus eggs increased by less than 10% the total incorporation of [^{3}H]amino acids into peptides as compared to buffer-injected eggs. Incorporation into the putative globin band was 33-39% of the total. Similar results were obtained with L. pictus lysates. The increase in total protein synthesis was less than 30% and the incorporation of [^{3}H]valine into globin was 58 and 67% when 0.14 to 0.28 mg/ml globin mRNA was added to lysates. Both results show that L. pictus eggs have a limited translational capacity as added mRNA competes with endogenous mRNAs for some limited components. Lysates of L. pictus zygotes gave similar results. Added mRNA competed with endogenous mRNAs to a greater extent than it increased the total amount of peptide synthesis. Contrarily, when globin polyribosomes are added to egg and zygote lysates, endogenous protein synthesis does not decrease, and total protein synthesis is stimulated 3- and 2-fold, respectively. Although the recruitment of mRNAs into polyribosomes in intact eggs can be made as great as that in zygotes by making egg cytoplasm alkaline, this does not happen in lysates. Egg lysates are 7- to 10-fold less active that equivalent zygote lysates at any pH value. Egg lysates, however, can be stimulated to levels equal to that of zygote lysates by adding globin polyribosomes which reinitiate. These results show that lysates preserve some of the translational regulation of intact egg and zygotes and will be useful for determining the mechanism of translational control. Supported by NSF grant DCB-8408386.

N86 STUDY OF THE PROTO-ONCOGENE c-myb AND ITS ROLE IN THE DEVELOPMENT OF DROSOPHILA MELANOGASTER: Alisa L. Katzen[1,2], Gary Ramsay[2], Barry Drees[1], Thomas Kornberg[1], and J. Michael Bishop[2], [1]Department of Biochemistry and Biophysics,and [2]G.W. Hooper Foundation, University of California, San Francisco, California 94143.

Proto-oncogenes are thought to have roles in growth control and differentiation of the cell and in development of the organism. Vertebrates contain a proto-oncogene c-myb which is expressed predominantly in hemopoietic cells and encodes a nuclear protein. Its functions are not well understood. We have identified the presence of a single myb-related gene in the genome of Drosophila melanogaster, providing us with a suitable developmental and genetic system in which to study how a c-myb gene serves the normal organism.

Sequence analysis revealed that the Drosophila c-myb gene shares a conserved domain with the chicken c-myb gene of at least 125 amino acids of which 73% are identical. Further characterization is in progress. The Drosophila c-myb gene maps to location 13F on the X-chromosome. Based upon this information, we have obtained strains suitable for the generation of Drosophila myb mutants. The mutagenesis strategy will be discussed. We have determined that the myb gene is transcribed throughout Drosophila development and we are currently undertaking experiments to determine whether it is expressed in a tissue-specific manner. We are generating anti-peptide antisera in order to characterize the c-myb gene product.

These studies should provide further information about how closely the Drosophila c-myb gene resembles that of vertebrates and will hopefully give some clue as to the gene's role during Drosophila development.

N87 GENETIC AND ENVIRONMENTAL REGULATION OF DETERMINATION AND DIFFERENTIATION IN *VOLVOX*
David L. Kirk and Marilyn M. Kirk, Dept. of Biology, Washington University, St. Louis, MO 63130

Volvox carteri is one of the simplest multicellular organisms exhibiting a complete division of labor between two fully differentiated cell types (germinal and somatic cells), and it is amenable to genetic analysis. Therefore, it constitutes a powerful model for examining the genome-by-environment interactions that are responsible for regulating cellular specification and differentiation. In asexual wild-type individuals the first step in establishing the germ-soma dichotomy is a series of asymmetric cleavage divisions in which 16 embryonic cells in specific locations undergo sequential unequal divisions to generate 16 large cells and a number of smaller sister cells; the larger cells then normally develop as asexual reproductive cells, or gonidia, while the small cells develop as terminally differentiated somatic cells. Mutational analysis leads to the hypothesis that three control genes are centrally important in establishing this dichotomy: one locus (*gls*) that controls the asymmetric divisions that generate the two cell lineages, one (*lag*) that is responsible for suppressing somatic differentiation in gonidial initials, and one (*regA*) that is responsible for suppressing reproductive development in somatic cells. However, the actions of these genes, or their products, can be perturbed by environmental insults applied at critical stages of development: When heat shock is adminstered to embryos just after the asymmetric divisions have been completed, presumptive somatic cells are switched into the reproductive pathway and develop as gonidia. In contrast, heat shock near the end of embryogenesis has the opposite effect and causes the presumptive gonidia to develop as terminally differentiated somatic cells. At about this same stage a variety of insults (including heat shock) selectively induce stable mutations at the *regA* locus. All these results can be fitted together into a working hypothesis about when and how the control genes listed above are turned off and on in the two cell types. Attempts to clone the relevant loci and test this hypothesis at the molecular level are underway.

N88 ONCOGENE EXPRESSION IN PERIPHERAL BLOOD CELLS OF PATIENTS WITH AUTOIMMUNE DISEASES AND IN ACTIVATED NORMAL LYMPHOCYTES
D.M. Klinman, J.F. Mushinski and A.D. Steinberg NIH, Bethesda, Md. 20892

Proto-oncogene expression in normal and autoimmune human peripheral blood cells was assessed by Northern blot analysis of poly $(A)^+$ RNAs. RNA from 18 patients with systemic lupus erythematosus (SLE) and from 7 patients with other autoimmune diseases were compared with those from 7 normal volunteers. The cells from patients with SLE had significantly more c-myc RNA than normal, and those from patients with all forms of active autoimmune disease expressed significantly elevated levels of N-ras RNA. Cells from individuals with SLE, but not from those with other autoimmune illnesses, showed significantly decreased levels of the c-myb and c-fos proto-oncogenes.

To evaluate these results, RNA was prepared from T and B lymphocytes purified from the peripheral blood of normal volunteers and then stimulated with concanavalin A (Con A) or lipopolysaccharide (LPS) respectively. C-fos RNA levels were initially abundant in both T and B cells, but fell dramatically within 1.5 hours after mitogen activation. Treatment of T cells with Con A led to a sequential increase in c-myc and then c-myb RNA. Levels of both peaked at 5 hours, but remained elevated for at least 18 hours. LPS stimulated B cells, on the other hand, displayed an elevated level of c-myc but not c-myb. Thus, the pattern of proto-oncogene expression in patients with SLE closely resembled that of mitogen activated normal B cells. Our findings suggest that proto-oncogene expression can be used to characterize autoimmune states, and that sequential changes in proto-oncogene expression are associated with the activation of normal human lymphocytes.

N89 DEVELOPMENTAL ELIMINATION OF DNA SEQUENCES BY NUCLEIC ACID SPLICING IN THE CILIATED PROTOZOAN OXYTRICHA NOVA,
L.A. Klobutcher, R.M. Ribas-Aparicio, and J.J. Sparkowski, University of Connecticut Health Center, Farmington, CT 06032

During its life cycle, Oxytricha nova transforms a copy of its chromosomal micronucleus into a transcriptionally active macronucleus containing exclusively short, linear, gene-sized DNA molecules. This process of macronuclear development involves a complex series of genomic changes including the fragmentation of the micronuclear chromosomes, elimination of more than 90% of the micronuclear genome, and the addition of telomeric sequences to the resulting gene-sized DNA molecules. By comparing recombinant clones of three macronuclear genes with the corresponding regions of the micronuclear genome that give rise to them during development we have found that nucleic acid splicing is also involved in generating a macronucleus. The micronuclear copy of each gene contains within it multiple, short blocks of sequences (ranging in size from 32-53 base pairs) that are not present in the mature macronuclear gene and, thus, must be removed during development. The DNA sequences of six of these internal eliminated sequences (IESs) have been determined. All six IESs differ in their primary DNA sequences, but are structurally similar to transposable elements in that each is bounded by a 2-4 base pair direct repeat and an adjacent short inverted repeat. To date, no micronuclear copy of a macronuclear gene has been found to lack IESs and, extrapolating from our results, it is likely that greater than 50,000 splicing events occur during macronuclear development in O. nova. Additional analyses indicate that IESs are not repetitive in the micronuclear genome and represent a portion of the unique sequence DNA eliminated during development.

N90 T-CELL RECEPTOR β-CHAIN GENE REARRANGEMENTS IN HUMAN LEUKAEMIAS, Sharad Kumar, M.F. Lavin and P.J. Smith* Department of Biochemistry, University of Queensland and *Queensland Institute of Medical Research, Brisbane, Australia.

Human leukaemias originate mainly from B- and T-cells. The monoclonality and lineage of B-cell leukaemias is determined by phenotypic typing of leukaemic cells and analysis of immunoglobin heavy chain (IgH) gene rearrangements. Recent studies have shown that T-cell receptor (TCR) genes are very similar in structure to IgH genes and undergo similar type of somatic rearrangements in functional T-cells. This finding led to the use of TCR gene rearrangement studies in detecting a monoclonal leukaemic population of T-cells. In the present study, we have investigated the rearrangement of TCR β chain gene in a number of T-cell acute lymphoblastic leukaemias (T-ALL), adult T-cell leukaemias (ATL) and some B-cell leukaemias. Hind III cut DNA hybridized to a cDNA probe for $c\beta_2$ gene showed a rearrangement of TCR β gene in one case of B-cell leukaemia, all 5 cases of ATL and at least 8 out of 12 cases of T-ALL. The other 4 cases of T-ALL had a Hind III pattern which appeared similar to the germ line TCR β gene, however Bam Hl pattern revealed rearrangements of one or both alleles of TCR β in the 3 cases. One case of T-ALL did not show any rearrangement with either Hind III, Eco Rl or Bam Hl and did not reveal any mRNA for TCR β chain. None of the T-cell leukaemias showed the rearrangement of IgH gene indicating their non B-cell lineage. All 5 B-cell leukaemias studied, including the one which shows a rearranged TCR β gene, showed rearrangements of IgH genes confirming their B-cell lineage. The potential of these techniques as a tool for diagnosing human leukaemias will be discussed.

N91 Shut-off of immunoglobulin genes in myeloma x fibroblast somatic cell hybrids. A.Greenberg*, Z.Kra-Oz+, R.Ber+ and R.Laskov*. Hebrew University Medical School, Jerusalem*. Rappaport Research Institute, Haifa, Israel+.

Fusion of fibroblasts with a variety of differentiated cells usually results in extinction of the differentiated phenotype of the non-fibroblast parent. To explore the genetic basis of this phenomenon, we have prepared and characterized a series of somatic cell hybrids between Balb/c IgG or κ producing mouse myeloma cells and C_3H fibroblasts. Many adherent colonies were grown and subcloned. Cell morphology, chromosomal number and H-2 typing indicated that the adherent clones were true hybrids. None of the 20 clones analysed secreted Ig chains as measured by double diffusion in agar. Similarly Ig production and secretion could not be detected by a sensitive biosynthesis assay. In order to see if the rearranged active Ig genes were present in the hybrids, Southern blot analyses were performed using probes which detect J_H and J_κ gene segments. Almost all of the myeloma x fibroblast cell hybrids were found to contain the actively rearranged immunoglobulin genes of myeloma parental cells. Immunoglobulin heavy and light chain specific RNA transcripts were not detected in Northern blots of the hybrid-cell RNA. We conclude that the "shut-off" of immunoglobulin production occurs at the transcriptional level, and simultanuously affects light and heavy chain immunoglobulin genes. These results indicate that fibroblast cells contain a trans-acting factor(s) which directly or indirectly extinguishes the transcription of immunoglobulin genes.

N92 HUMAN B LYMPHOCYTES EXPRESS A CLASS II MHC GENE WHICH IS NOT REGULATED LIKE THE KNOWN HLA-DP, -DQ AND -DR CLASS II GENES, Eric O. Long, Laboratory of Immunogenetics, NIAID, Bethesda, MD 20892

Class II antigens of the major histocompatibility complex (MHC) are required for the recognition of foreign antigens by certain T lymphocytes. Three class II MHC antigens consisting of distinct alpha and beta chains exist in man, called HLA-DP, -DQ and -DR. The DP, DQ and DR genes are expressed constitutively in B cells and antigen-presenting cells. They can be induced in a variety of cells by treatment with gamma-interferon and in T cells upon mitogenic or antigenic stimulation. We have isolated a full-length cDNA clone encoding a new class II beta chain, designated DO. Unlike other class II genes DObeta is not induced by gamma-interferon in human fibroblast lines and is not expressed in activated T cells. Furthermore DObeta expression remains in mutant B-cell lines which have lost expression of other class II genes due to a defect in a trans-acting factor. Thus DObeta is not subject to the same regulatory mechanisms as are other class II genes. DObeta expression appears later in B cell development. The independent expression of DObeta suggests that it may be part of a functionally distinct class II molecule.

N93 REGULATED EXPRESSION OF A TRANSFECTED TOXIN GENE, Ian Maxwell, Francoise Maxwell and L. Michael Glode, University of Colorado Health Sciences Center, Denver, CO 80262

We are investigating the possibility of selectively killing cells of specific types by expression of a transfected diphtheria toxin A chain gene (DT-A), linked with tissue-specific transcriptional regulatory sequences. We have obtained indirect evidence for toxicity in transfected cells using a stable transformation assay and a transient expression assay based on the inhibition of gene expression from a co-transfected plasmid (pSV2cat). Stimulation of DT-A expression (from a truncated metallothionein promoter) in response to putative trans-activating factors was observed (a) in 293 cells, which express adenovirus E1A, and (b) in B-lymphoblastoid cells when an immunoglobulin (Ig) enhancer was included in the DT-A construct. We shall report on attempts to increase B-cell specificity by expressing DT-A from Ig promoters, and on minimizing toxicity in non-target cells using an attenuated DT-A mutant. Initial results with additional trans-activation systems will be presented. Controlled toxin gene expression should provide a useful tool for isolating trans-activator mutants and for eliminating specific cell populations in developmental studies. The concept could also eventually be applicable as a novel means of cancer therapy.

N94 MODULATION OF MURINE ERYTHROLEUKEMIA CELLS INDUCED DIFFERENTIATION BY INHIBITORS OF POLYAMINES BIOSYNTHESIS. E. MEILHOC, M.J. MOUTIN, H.B. OSBORNE, Laboratoire de Biophysique Moléculaire et Cellulaire (U.A. CNRS 520), D.R.F., C.E.N.G., 85X, 38041, Grenoble cedex, France.

Murine erythroleukemia (MEL) cells (Friend cells) can be induced to differentiate by a variety of chemical compounds (e.g. hexamethylene bis acetamide-HMBA) according to a program similar to normal erythropoiesis. We have shown that changes in the amounts of intracellular polyamines (a fall in putrescine and spermidine) are associated with the differentiation induced in MEL cells by HMBA. The role of polyamines in this process has been investigated by using two inhibitors of polyamine biosynthesis : α difluoro methyl ornithine (DFMO) and methyl glyoxal bis guanyl hydrazone (MGBG). DFMO and MGBG act at two different points in the polyamine biosynthetic pathway. Inhibition (respectively of ornithine and S adenosyl methionine decarboxylases). Thus, in many cellular systems these two inhibitors produce different profiles of the intracellular polyamines : DFMO : a fall in putrescine and spermidine ; spermine is little or not affected ; MGBG : a massive accumulation of putrescine ; a fall in spermidine and spermine. We show that DFMO and MGBG have opposing effects on the induced differentiation : DFMO stimulates the differentiation ; MGBG inhibits differentiation even if this drug is present only during the first three hours of culture with the inducer. By using a novel experimental protocol, the inhibitory effect of MGBG on the differentiation was dissociated from a pleiotropic effect on cell growth. Intracellular polyamine analysis demonstrated that DFMO increased the rapidity and the amplitude of the changes in intracellular polyamines associated with the induced differentiation. The presence of MGBG during the first 3 hours with the inducer was sufficient to produce opposing changes in the intracellular polyamines. These results imply that either intracellular polyamines or the activities of polyamines biosynthetic enzymes play a regulation role in the differentiation process of MEL cells induced by HMBA. The polyamine dependent step necessary for the subsequent commitment of the cells to erythrodifferentiation is probably an early event in the differentiation process.

N95 STRUCTURE AND EXPRESSION OF THE LYT-2 T CELL DIFFERENTIATION ANTIGEN GENE, Jane R. Parnes, Rose Zamoyska, Chen Liaw, Amy Vollmer and Kurt Sizer, Stanford University School of Medicine, Stanford, CA 94305.

The Lyt-2,3 antigen is a surface glycoprotein expressed on most mouse thymocytes but restricted to the cytotoxic/suppressor subset of mature T lymphocytes. Expression of Lyt-2,3 correlates best with recognition by T cells of class I major histocompatibility complex proteins. Monoclonal antibodies specific for Lyt-2,3 inhibit T cell function at the recognition step. The Lyt-2,3 molecule consists of heterodimers of either of two related polypeptide chains bearing the Lyt-2 determinant and a distinct polypeptide bearing the Lyt-3 determinant. We have isolated cDNA and genome clones encoding the Lyt-2 polypeptide chains. Analysis of the sequence shows that the Lyt-2 protein contains an N-terminal external domain which is homologous to immunoglobulin variable regions. The organization of the Lyt-2 gene further confirms that it is a member of the immunoglobulin gene superfamily. We have shown that a single gene encodes Lyt-2, and this gene can result in expression of both Lyt-2 polypeptide chains after transfection into L cells. The Lyt-2 chains can be expressed in the absence of Lyt-3. We have further shown that the difference between the two Lyt-2 chains is in the length of their cytoplasmic tails and that the mechanism responsible for these two forms is alternative patterns of mRNA splicing. We have demonstrated that both forms of mRNA are expressed in normal thymus, lymph node and spleen as well as in transfected L cells. The functional significance of the two protein forms and their developmental regulation are currently under study. In collaboration with Drs. John Chamberlain and Sherman Weissman (Yale University School of Medicine), we have recently put the 2.1 allele of the Lyt-2 gene into Lyt-2.2 transgenic mice. The regulation of expression of this gene during thymic development, the sequences responsible for tissue specificity, and the function of the protein are being studied in this system.

N96 MACROMOLECULAR SYNTHESIS DURING GERMINATION OF PHYTOPHTHORA (Oomycetes) ZOOSPORES, Christopher J. Penington, Bruce R. Grant and Kenwyn R. Gayler, University of Melbourne, Parkville 3052, AUSTRALIA

Zoospores of the plant pathogenic fungus Phytophthora palmivora undergo a simple developmental sequence when appropriate stimuli are applied. The pyriform, biflagellate cells become immobile and secrete cell walls to form cysts. Subsequently some 60 to 80% of the cysts germinate, producing a germ tube which can grow into a new mycelium. We have used a number of approaches to establish the timing of, and requirement for, major biosynthetic events during this sequence.

Actinomycin D and Cycloheximide, inhibitors of transcription and translation respectively, blocked germination, but not encystment, indicating that germination requires de novo synthesis of both RNA and protein, while encystment does not.

Direct isolation of mRNA was used to confirm this requirement for transcription. Populations of polyadenylated RNA were extracted from cells at different stages of development and characterized by cell free translation. The level of translatable poly A^+ RNA increased dramatically during development, and the proportion of RNA which bound to cDNA prepared against polyadenylated RNA from actively growing germlings also increased markedly. These increases began immediately following application of the primary stimulus.

We conclude that synthesis of new mRNA is necessary for germination and is one of the earliest responses of the cell to the primary stimulus.

N97 CONTROL OF THE FETAL GLOBIN SWITCH IN VITRO, S.P. Perrine, R.A. Cohen, M.F. Greene, B.A. Miller and D.V. Faller, Stanford University, Palo Alto, CA 94304 and Harvard University, Boston, MA 02115. The fetal globin swtich, from synthesis of predominantly gamma globin (fetal) to predominantly beta globin (adult), occurs in humans at approximately 32 weeks gestation and has been studied as a model system of developmental gene regulation. We have previously identified a group of subjects in which the fetal globin switch does not occur on schedule and have used this observation to investigate the physiological factors controlling this developmental process. In vitro globin chain synthetic studies from such subjects demonstrate that regulation of the switch lies at the transcriptional level, rather than via an effect on mRNA stability. Furthermore, the gamma globin genes in these subjects remain in a fully unmethylated state, suggesting a developmental delay at the genomic level. Late erythroid progenitors derived from such subjects synthesize predominantly gamma globin (60-90% of total non-alpha globin) similar to what the subjects' reticulocytes are synthesizing. More primitive erythroid progenitors from these subjects, however, synthesize predominantly beta globin (gamma chain representing only 20-40% of total); they appear to be developmentally uncommitted and to switch in culture as a result of having been removed from the fetal environment. Using this in vivo switching as an assay, we have identified a physiologic factor which prevents the fetal globin switch in culture. This fatty acid allows primitive erythroid progenitors to continue synthesis of gamma globin in culture, with final accumulation of fetal hemoglobin in amounts up to twice as great as in its absence. The ability to control human globin switching in culture provides a model system to molecularly dissect this developmental regulation.

N98 ALTERNATIVE FATES OF MEMBRANE IMMUNOGLOBULIN IN PRE-B and B CELLS: Ig MYRISTYLATION AND NUCLEAR LOCALIZATION. Shiv Pillai and David Baltimore, Whitehead Institute for Biomedical Research and the Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142

Immunoglobulin (Ig) heavy (H) chains are made in two forms, one that binds Ig to membranes (H_m) and another that allows Ig to be secreted (H_s). The fates of these two chains depend on the differentiation state of the cells. In pre-B cells where no light chain is present, H_m slowly disappears from the soluble phase of the cell and H_s remains. H_m is not put on the cell surface but appears to migrate to the nucleus from which it is only extractable by harsh treatment. The nuclear H_m may have a role in regulating gene rearrangement. In B-cells, H_m is myristylated and is converted to a detergent-soluble form. It is then, as part of a complex with light chain, placed onto the cell surface. There is apparently an intermediate stage in which heavy/light chain Ig complexes are formed but do not migrate to the cell surface, possibly because of a lack of myristylation. Plasma cells that secret H_s in Ig may also have H_m in the nucleus.

N99 REQUIREMENTS FOR TELOMERE FORMATION IN YEAST, A.F. Pluta and V.A. Zakian, Hutchinson Cancer Research Center and University of Washington, Seattle, Washington, 98102.

Terminal Bam Hl or Hind III restriction fragments from total Oxytricha macronuclear DNA can support telomere formation on linear ARS plasmids in yeast cells. Although termini from different Oxytricha DNA molecules can promote telomere formation, there is a clear preference for terminal restriction fragments derived from the 7.4 kb macronuclear DNA molecule coding for 18S and 28S ribosomal RNAs. The preference for rDNA is greater than that predicted based on the relative abundance of rDNA in total macronuclear DNA and on the estimated density of Bam Hl and Hind III sites. Moreover, one terminus of the rDNA molecule is favored over the other as a substrate for telomere formation by a factor of 10 to 1, a preference which appears to be inherent to this terminus itself and not strictly a reflection of fragment size. These results suggest that some property of the Oxytricha rDNA molecule facilitates its use as a telomere in yeast. We have tested the possibility that Oxytricha rDNA contains sequences that function as ARS elements in yeast. We detect no ARS activity for three overlapping subclones representing the entire rDNA molecule. Therefore, a terminal ARS is neither required for telomere formation in yeast nor can it account for the preferential use of the rDNA in telomere formation. We are currently testing the relative contributions of small changes in terminal sequence organization and structure on substrate requirements for telomere formation in yeast.

N100 REGULATION OF PHOSPHODIESTERASE TRANSCRIPTION DURING DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM, Gregory J. Podgorski, Jacob Franke, and Richard H. Kessin, Columbia University, New York, NY 10032

We have isolated cDNAand genomic clones encoding the cyclic nucleotide phosphodiesterase of Dictyostelium discoideum. The phosphodiesterase functions to maintain responsiveness to the chemoattractant cAMP which is emitted by cells in the initial stages of development, and serves to direct cells into a common center. The nucleotide and deduced amino acid sequences of the coding region have been determined, as well as the nucleotide sequence of the 5' flanking region presumed to play a role in the regulation of this gene. The phosphodiesterase transcript is 2.6 kb in length and contains more than 1 kb of untranslated sequence. Transcription is regulated during development in a biphasic manner. In cells shaken in buffer phosphodiesterase RNA is first detected 1 hour after starvation. The phosphodiesterase transcript becomes maximally abundant by 2 hours before a sharp decline in RNA levels. Beginning at 4 hours after the initiation of development the levels of transcript increase once again to reach a maximu by 8 hours before declining at later times. We present data on nucleotide sequences which may be important in the regulation of this gene, and the results of experiments testing the effects of cAMP and cell-cell contact on the modulation of phosphodiesterase transcription.

N101 FUNCTION OF IMMUNOGLOBULIN GENE ELEMENTS AT DIFFERENT STAGES OF LYMPHOID CELL DEVELOPMENT, Cary Queen, John Foster and Jeannine Stafford, National Cancer Institute, Bethesda, MD, 20892

To test the ability of immunoglobulin regulatory elements to function in various cell types, we constructed a series of plasmids in which a kappa light chain promoter was linked with an enhancer from either polyomavirus, Moloney murine sarcoma virus, or the kappa gene itself. The plasmids were transfected into a set of cell lines, and RNA extracted after 48 h was analyzed by an S1 nuclease assay for the presence of transcripts from the kappa promoter, with transcripts from another gene on the plasmids serving as an internal standard. We have previously shown that the kappa promoter functions only at a very low level in non-lymphoid cells, even when stimulated by the viral enhancers (Nature 315, 423-425). Now we have transfected the plasmids into cell lines representing the pre-B, B, T, and plasma cell stages of lymphoid development. We found that both the kappa promoter and enhancer function in the pre-B and B cells, but at a level 3-5 fold lower than in more differentiated myeloma cells. The kappa promoter and enhancer also function at readily detectable levels in the T cells, although not as well as in the B-lymphoid cells. Hence, it appears that either T cells contain small amounts of the same proteins that interact with these regulatory elements in B cells, or T cells contain homologous proteins to activate their own genes that cross-react with the immunoglobulin gene elements.

N102 GENE REGULATION DURING MYELOID DIFFERENTIATION APPEARS TO BE PREDOMINANTLY TRANSLATIONAL RATHER THAN TRANSCRIPTIONAL. W. Salser, R. Davis, C.-C. Chou, and S. Teraoka. UCLA, Los Angeles, CA 90024.

We have characterized more than twenty cDNA clones for genes whose mRNA levels change drastically during the differentiation of HL-60 cells to form macrophages or neutrophils. In the course of this work it became clear that the cDNA clones which correspond to mRNAs which are transcriptionally regulated constitute only a small fraction of the cDNA library, substantially less than 10%. This was surprising in view of our 2-dimensional gel electrophoresis studies of protein synthesis, which showed dramatic changes in synthesis rates for over half of the major protein species. These results also ruled out protein modifications and suggested that translational regulation was a major feature of myeloid differentiation. In accordance with this model, we found that genes with changing mRNA levels are also controlled at the translational level. For example, we have shown that changes in alpha-tubulin mRNA levels and translational regulation are both major factors in the control of alpha-tubulin biosynthesis in these cells but that the translational effects are much stronger. Also we have isolated the first human ferritin light subunit cDNA clones and shown that changes in the H/L subunit ratio can be explained by changes in mRNA ratios, however translational control has previously been shown to be a major mode of regulation for ferritin synthesis - ferritin mRNA accumulates and is translated only when iron is present. The alpha-tubulin and ferritin light chain mRNA 5'-untranslated sequences suggest a detailed mechanism for this translational regulation. Comparison of 2-D protein gels and Northern blots show that there can be more than 100-fold translational activation during differentiation of these cells so that protein synthesis rates can dramatically increase even when the mRNA level is decreasing 10-fold.

N103 DIFFERENTIAL EFFECTS OF cAMP ON GENE EXPRESSION IN DICTYOSTELIUM DISCOIDEUM, Charles L. Saxe III and Alan R. Kimmel, LCDB, NIADDK, NIH, Bethesda, MD 20892.

3',5'-cyclic AMP plays a major role in the regulation of differential gene expression in Dictyuostelium discoideum. Using several different approaches we have identified apparently different roles for intracellular and extracellular cAMP. Mutant studies and studies using a drug which blocks intracellular cAMP accumulation indicate that a gene normally regulated during the first hours of development is sensitive to changes in intracellular cAMP levels. Regulation of the gene is refractory to changes in extracellular cAMP concentrations. The expression of a series of prespore and prestalk-specific genes is stimulated by exogenous [extracellular] cAMP. In conjunction with these experiments we have monitored the appearance of cAMP receptors on the surface of the cells. Addition to cells of high levels of exogenous cAMP, early in development, blocks the normal accumulation of cAMP receptors. This loss of receptors, and presumably a loss of normal cAMP signal relay, results in a loss of regulation of the early gene. Prespore and prestalk-specific gene expression is much less affected. The relationship between expression of the cAMP relay system and differential gene expression will be discussed.

N104 ISOLATION OF A CLONE FOR THE REGULATORY SUBUNIT OF CAMP-DEPENDENT PROTEIN KINASE IN D. DISCOIDEUM, Kristin L. Schaller*, Ben H. Leichtling and H. V. Rickenberg*, National Jewish Center for Immunology and Respiratory Medicine and *Univ. of Colorado School of Medicine, Denver, CO 80206.

We have isolated a clone for the regulatory (R) subunit of the cAMP-dependent protein kinase (cA-dPK) in Dictyostelium by screening a λgt11 cDNA library with antibodies directed against the R subunit. The cAd-PK has been shown to be a developmentally regulated enzyme which accumulates preferentially in prespore cells. We are interested in studying the mechanism(s) by which the accumulation of the cAd-PK is controlled.

Extracts of E. coli cells infected with the recombinant phage contain high molecular weight β-galactosidase-fusion protein bands on SDS-polyacrylamide gels. The fusion proteins produced by these clones can be immunoprecipitated with antibodies against the R subunit. In addition they can be photoaffinity-labeled with $8N_3$-cAMP. The fusion proteins are larger by approximately 10 to 15 Kd than β-galactosidase.

Experiments are now in progress to characterize these clones.

N105 HOUSEKEEPING GENES AND THEIR EXPRESSION IN THE GENERATION CYCLE OF VOLVOX CARTERI, Rüdiger Schmitt, Kurt Müller, Helmut Rausch and Michael Salbaum, University of Regensburg, D-8400 Regensburg, Germany

The colonial aga Volvox carteri serves as a "simple" model system to study cell differentiation and development. Volvox consists of two types of cells: ~4000 somatic cells located at the periphery and 16 reproductive cells (gonidiae) located inside a spheroid. Each reproductive cell gives rise to a new colony. An unequal cleavage during embryogenesis defines the central differentiation event; spheroid inversion and embryo release are developmental steps.

The Volvox genome consists of 1.2×10^8 base pairs. A genomic library was constructed using the lambda vector EMBL3. Clones containing the Volvox ß-actin, histone H4, rDNA and α-tubulin genes were "fished" with heterologous probes. These housekeeping genes are currently being used to monitor gene expression during the generation cycle in synchronized cultures. Further, the DNA sequences of ß-actin and histone H4 genes will be compared to their known counterparts from other eukaryotes to disect (i) conserved and non-conserved portions and (ii) signals for gene expression.

N106 PHORBOL-12-MYRISTATE-ACETATE(PMA) INDUCTION OF DIFFERENTIATION IN CLONAL MOUSE TUMORS CELL-LINES WITH ALTERED MYB LOCUS, Grace L.C. Shen-Ong*, and Herbert C. Morse+, *Lab. of Genetics, NCI; +Lab. of Immunopathol., NIAID; NIH, Bethesda, MD20892

The myb oncogene transduced by the avian leukemia virus appears to confer tumorigenicity specifically to cells in the myeloid lineage. The myb locus in a small group(6) of BALB/c tumors arose subsequent to abelson virus infection has been shown to be disrupted by viral insertion of M-MuLV, the helper component of the abelson virus complex. We report here that these tumors contain hematopoeitic cells of myeloid origin. The main parameters used in the characterization of these cells include morphological appearance, FACS of cell surface antigens, and biochemical analysis for the presence of enzymes such as lysozyme and nonspecific esterases. It has been proposed that leukemic cells represent frozen stages of hematopoeitic cells. PMA has been used extensively in cell differentiation studies. Since c-myb expression appears to be restricted to immature hematopoeitc cells, it will be of interest to study if these mouse myeloid tumors with their expression of the myb gene under the control of viral promoter can be induced to differentiate by PMA. Our data indicated that different clonal cell-lines respond to PMA to varying extents. The changes of expression of c-myb and various other genes in these lines ± PMA were investigated.

N107 Gene Regulation During Early Development of Dictyostelium discoideum, Charles Singleton, Vanderbilt University, Nashville, TN 37235

We have isolated several cDNA containing plasmids that hybridize to specific mRNAs which show a decrease in amount during the preaggragation or initial segment of development in Dictyostelium. The level of change varies from three to four fold upward to more than 50 fold during the first eight hours of development. The kinetics of decrease depend upon the individual mRNA: some show a constant decline with an apparent half life ranging from two to six hours while others show a slow initial decline followed by a rapid drop after three to four hours. Given the types of regulatory processes thought to occur early in D. discoideum development, we are examining the transcriptional rates of the corresponding genes and whether the mRNAs show any changes in their polysome-nonpolysome distribution. We have also begun characterizing the expression of the genes in various early aggregation deficient mutants. The decrease in mRNA levels for most appear to occur normally or at reduced rates in the mutants; however, some of the mRNAs show misregulation reflected in a retention of the vegetative level during development. In the wild type strain, the mRNA for one clone, pLM-V4, is highly abundant in vegetative amoebae, but by eight hours has dropped by over 50 fold. In contrast, this mRNA is surprisingly absent from both vegetative and developing amoebae in a strain mutated in a developmentally essential, early acting, pleiotropic gene. Whether the lack of expression in vegetative cells of the gene corresponding to this mRNA contributes to the inability of this strain to initiate development is being addressed.

N108 Nuclear Differentiation in Tetrahymena thermophila
Elizabeth A. Spangler and Elizabeth H. Blackburn. Department of Molecular Biology, University of California, Berkeley.

The ciliated protozoan Tetrahymena thermophila contains two nuclei: a diploid micronucleus and a polyploid macronucleus which is derived from the micronucleus during conjugation. Formation of the macronucleus involves fragmentation of the micronuclear chromosomes, the elimination of 15% of the micronuclear genome, and amplification of the genes encoding the ribosomal RNAs. An essential aspect of this process is the generation of new telomeres at the ends of macronuclear DNA molecules.

In order to study the process of differentiation of a micronucleus into a macronucleus we have obtained clones of four macronuclear telomeric sequences. Evidence from genomic blots indicates that these cloned sequences are non-telomeric in the micronucleus. Thus, they must be sequences which are adjacent to a micronuclear fragmentation site. Sequence analysis shows no significant homology between these macronuclear telomere-associated sequences. Any signals for chromosome fragmentation must be present at the other side of the fragmentation site in the micronuclear genome. We are in the process of characterizing micronuclear genomic clones which correspond to the macronuclear telomeres already examined in order to determine what features are conserved at chromosome fragmentation sites.

N109 Transcription of Germ Line-Specific DNA in Tetrahymena. Susan Stein-Gavens, John M. Wells and Kathleen M. Karrer, Brandeis University, Waltham, MA 02254

In the ciliated protozoan T. thermophila 10-20% of the DNA sequences are micronucleus (germ line) specific. They are eliminated from the developing macronucleus during sexual reproduction (conjugation). Most of the micronucleus-specific sequences are members of repeated families. We cloned a 0.7 kb Hind III - Cla I fragment that is a member of such a family. Using this cloned sequence as a probe, we detected a 1.5 kb poly A^+ transcript on northern blots. The transcript was not seen in RNA prepared from vegetatively grown cells, but was detected in RNA from starved cells (a condition necessary to bring about conjugation). The abundance of the transcript decreased as the cells entered conjugation. The northern signal can be washed off under moderately stringent conditions, suggesting that a different member of the family is the template for the transcript. Two other members of this repeated family were isolated from a micronuclear genomic bank. Restriction analysis showed the clones to be different from each other as well as from the original 0.7 kb fragment. When the new clones were hybridized to northern filters, they detected a transcript with the same size and inducibility as seen with the original clone, consistent with the hypothesis that a member of the micronucleus-specific family is transcribed. Attempts to isolate the transcribed sequence are in progress. These data represent the first molecular evidence for transcription of a germ line-specific DNA sequence.

N110 GENE REPRESSION DURING MYELOID DIFFERENTIATION: A NOVEL SYSTEM WHICH PERMITS THE STUDY OF COMMITMENT IN HUMAN CELLS. S. Teraoka, S. Kania, P. Concannon*, C. Snyder, R. Nelson, S. Chada and W. Salser, UCLA, Los Angeles, CA 90024. *Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We have developed a system for using site-directed mutagenesis to study gene regulation in HL-60 cells, the only established human cell line with a well-characterized ability to undergo commitment decisions. We have shown that the levels of many different mRNAs change dramatically at various points along the pathway of differentiation to macrophages or neutrophils. The non-coordinate, complex patterns of regulation seem to indicate several independent regulatory events during myeloid differentiation. For our first detailed analysis of cis-acting signals regulating expression in these cells we are studying chromosomal clones of gene pHL1231 whose mRNA decreases about 16-fold during these differentiation steps. Tagged chromosomal clones show high level expression and appropriate regulation when transfected into HL-60 cells. We have shown that the DNA sequences required for expression and regulation during differentiation are contained within a 2.9 kb DNA fragment. We have also shown that the 3' terminus of the gene is not important for proper regulation by constructing chimeras in which the 3' terminus and flanking sequences are replaced by a portion of the corresponding cDNA clone joined to a pi-plasmid tag sequence. Continued analysis is being carried out to characterize the regulatory sequences within a 2 kb region 5' to the candidate TATAA box. We feel this gene serves as an excellent model for the analysis of gene repression during development.

N111 INVOLVEMENT OF CIS AND TRANS ACTING ELEMENTS IN TRANSLATIONAL REGULATION,
G. Thireos, D. Alexandraki, and D. Tzamarias, Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece

Translational regulation has an important role in the events of early embryonic development. Messages that are very abundant are essentially untranslated until some time after fertilization, and this translational repression-derepression is poorly understood at the molecular level. In yeast, a well defined system of translational regulation exists, and our efforts are focused on unraveling the molecular interactions involved, which might establish a paradigm for similar regulation systems. Expression of the yeast GCN4 gene is increased postranscriptionally when amino acid limitation is imposed on the cells. Under such conditions, the translational efficiency of GCN4 mRNA is increased at least 5 fold. In rich media, the low level of translation of the GCN4 mRNA is due to a region within the 5' untranslated sequence. This region includes four initiation codons (AUG), each of which is shortly followed by an in frame terminator. In this study we identify elements which are necessary for the regulated increase in translational efficiency. When any of the GCN1, GCN2, or GCN3 genes is mutated, translational derepression of the GCN4 mRNA cannot be accomplished, and thus these genes define trans acting positive regulatory elements. Deletion of sequences from the 5' untranslated region of GCN4 mRNA which include the 5' most proximal AUG results in the reduction of basal translation levels and the loss of positive regulation. Deletion of sequences that include two of the 5' most distal AUGs results in an increase of basal translation levels in rich media, with no further increase under amino acid limitation conditions. Finally, increasing the distance of the "real" AUG from the upstream ones by mutagenesis also results in the loss of translational regulation. Based on these results, we favor the idea that positive regulation of GCN4 mRNA translation employs the upstream "false" AUGs in combination with trans effectors to increase the likelihood of initiation at the "real" AUG.

N112 FUNCTIONAL ANALYSIS OF SPORE SPECIFIC GENES FROM ASPERGILLUS.

Rosa Varona, John E. Hamer, Bruce Miller and William E. Timberlake. Dept. of Plant Pathology. University of California. Davis campus. Davis, California.

Conidiation in Aspergillus is characterized by the accumulation of numerous stage and cell-specific poly(A)+RNAs. Approximately 200 poly(A)+RNAs, corresponding to 1.4% of the Aspergillus genome, are found only in differentiating or mature spores. Many of these genes are frequently organized into chromosomal clusters. In contrast, about 20% of the spore specific genes appear to reside in relative isolation from others. We have identified two of these non-clustered genes designated spo 23 and spo 28 and subjected them to structural analysis.

The positions of the genes were determined by RNA blot hybridization with subcloned restriction fragments and by DNA blot hybridization with c-DNA synthesized from spore poly(A)+RNA. The position of 5' and 3' ends and their transcriptional polarity were determined by S1 nuclease protection experiments.

A sequence comparison of the 5' flanking regions of spo 23, spo 28 and spoC1-C (an spore specific cluster gene) revealled several highly conserved sequences.

A functional analysis of these genes has been initiated. Novel alleles created by in vitro mutagenesis have been reintegrated into the genome and an examination of the effects of these mutation upon gene expression is in progress.

N113 INDUCTION OF T-CELL RECEPTOR (TCR) mRNAs IN RESPONSE TO PHORBOL ESTER OR CYCLOHEXIMIDE
Miles Wilkinson and Carol L. MacLeod
University of California, San Diego Cancer Center, La Jolla, CA. 92093

A model system for studying the induction of TCR-T3 genes utilizes closely related murine T-lymphoma clones which lack detectable expression of TCR-alpha. The SL12 clones can be induced to express TCR-alpha mRNA in response to phorbol myristate acetate (PMA). The calcium ionophore, A23187, also induces TCR-alpha expression; synergistic levels are induced by PMA together with A23187 implying that protein kinase C and elevated Ca++ both contribute to the induction of this transcript. A labile protein may be responsible for the repression of TCR-alpha mRNA expression in the non-expressing cells since treatment of the cells with cycloheximide, a protein synthesis inhibitor, also strongly induces the expression of TCR-alpha mRNA. Induction of TCR-alpha mRNA is rapid (< 1hr) and reversible; strongly suggesting that the activation of expression is not due to TCR-alpha gene rearrangements. Induction of TCR-beta, T3-delta and Lyt2 mRNA is also observed in the SL12 clones. Of particular interest is the induction of both TCR-alpha and full length 1.3kb TCR-beta mRNA in response to PMA in cells which constitutively express T3-delta and T3-epsilon. Experiments are in progress to determine whether PMA will stimulate the assembly and expression of a complete TCR-T3 receptor complex on the cell surface. The SL12 clones provide a unique model system for studying the developmental regulation of mRNAs encoding proteins involved in T-cell recognition and function.
Supported by NIH CA 37778.

N114 ISOLATION OF GENES COMPLEMENTING DEVELOPMENTAL MUTATIONS IN ASPERGILLUS NIDULANS, Catherine E. Willett, Margaret M. Boylan, Peter M. Mirabito and William E. Timberlake, University of California, Davis, CA 95616

Cloning methods recently developed for the filamentous fungus Aspergillus nidulans have allowed the isolation of genes complementing several developmental mutants. A cosmid library of A. nidulans chromosomal DNA was constructed and used to isolate the sporulation-specific genes bristle (brlA1), abacus (abaA1) and wet (wetA1) by complementation in the mutant strains. The complementing cosmids were recovered and restricted with several enzymes to localize the complementing activity. The brlA1-complementing activity has been localized to a 4.5 kb BamHI fragment that contains one developmentally regulated 2.4 kb transcript. AbaA1 has been localized to a 6.0 kb SalI fragment encoding two transcripts, a 1.2 kb unregulated and a 3.0 kb developentally regulated transcript. The wetA1-complementing activity is contained on a 8.0 kb EcoRI fragment coding for two spore-specific transcripts.

N115 The Control of Gene Expression in Dictyostelium discoideum by Differentiation Inducing Factor J.G. Williams, K.A. Jermyn, M. Berks* and R.R. Kay* Imperial Cancer Research Fund, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts. EN6 3LD. UK

Differentiation Inducing Factor (DIF) is a low molecular weight hydrophobic compound which has the properties of a cellular morphogen. It is synthesized late during cellular aggregation and it will induce isolated amoebae to form stalk cells. We have isolated cDNA clones derived from three different mRNA sequences which accumulate in response to DIF. All three sequences are expressed only in the prestalk-stalk cell pathway of differentiation but they differ in their kinetics of induction by DIF and in their temporal and spatial pattern of accumulation during normal development. Two of the three mRNA sequences accumulate only after several hours of exposure to DIF. They may, therefore, be responding to a secondary inductive signal, but they provide very useful markers of terminal stalk cell differentiation. The third mRNA sequence is very rapidly induced in response to DIF and is selectively expressed in prestalk cells of the migrating slug. It therefore has the properties expected of a transcript directly responsive to DIF induction. These results provide evidence for a direct role of DIF in controlling gene expression in the prestalk-stalk cell pathway of differentiation.

*MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 3QH

N116 ACTIN GENES OF ASCARIS SUUM. Michael A. Winrow, Pamela E. Martin, and Ann Sodja, Department of Biological Sciences, Wayne State University, Detroit, MI. 48202

Pre- and post-diminutive high molecular weight DNAs were isolated from parasitic nematode, Ascaris suum. The DNAs were digested with several restriction endonucleases. The resulting fragments were separated electrophoretically and then transferred to nitrocellulose filters by Southern technique. Radioactive heterologous actin probes from Drosophila melanogaster and Caenorhabditis elegans were hybridized to the DNA on the filters. The hybridization patterns obtained from both probes were identical and no differences were detected between the pre- and post-diminutive DNAs. This result suggests that no actin genes are lost during diminutive event. Furthermore, the several strong bands of hybridization indicate that in A. suum actin is encoded by a multigene family in which the number of members exceeds the four found in C. elegans. To study these genes in greater detail, recombinant A. suum genomic library was constructed and several actin containing recombinants isolated. Both structural and transcriptional characterization of these clones is under way. Comparison of these clones with those of C. elegans will also be made.
(Supported by NIH Grant No. RR-08167)

N117 Anke Burmester, Johannes Wöstemeyer
Institut für Biochemie und Molekulare Biologie/Botanik
Technische Universität Berlin, Franklinst. 29, 1000 Berlin 10, F.R.G.

EXPERIMENTS TOWARDS AN IN VITRO SYSTEM FOR GENETIC MANIPULATION OF MUCORACEOUS FUNGI: CLONING OF MITOCHONDRIAL ARS ELEMENTS FROM THE ZYGOMYCETE ABSIDIA GLAUCA

In order to study the regulation of differentiation towards development of zygospores we have set out to establish an in vitro system for the transfer of genes between both mating types of Absidia glauca. One possible strategy towards the construction of an autonomously replicating plasmid implicits the isolation of putative origin-of-replication elements by selection for autonomously replicating sequences in Saccharomyces cerevisiae.

Fragments from mitochondrial and chromosomal DNA of A. glauca have been cloned in S. cerevisiae using the ARS selection vector YIp5. No ARS elements could be selected from chromosomal DNA, but we succeeded in isolating fragments of mitochondrial origin that support autonomous replication in bakers' yeast. Structural properties of these plasmids as well as data concerning stability and replication behavior in E. coli and S. cerevisiae respectively will be presented.

N118 PRE-COMMITMENT DUE TO HYDROXYUREA DURING HL-60 MYELOID DIFFERENTIATION. A. Yen, L.A. Freeman, and J. Fishbaugh. Dept. Int. Med., Univ. of Iowa, Iowa City, IA.

We have proposed that an S-phase specific signal initiates a program of HL-60 myeloid differentiation. This signal induced a pre-commitment state before any overt modulation of proliferation or differentiation. Pre-commitment cells retain a memory of the previous exposure to inducer thus require only an abbreviated re-exposure to inducer for onset of GO growth arrest and phenotypic differentiation. The present results show that a pulse exposure to hydroxyurea (HU) induced a pre-commitment state associated with over-replication of a small chromosome. Exponentially proliferating HL-60 cells (doubling time of 21 h.) were exposed to 0.3 mM HU for 20 h. The cells were washed free of the HU, recultured in drug free medium for 6 h., and then exposed to 10^{6}M retinoic acid (RA). Onset of G1/0 specific growth arrest and phenotypic myeloid differentiation occurred within a period corresponding to one division cycle. In contrast, cells not treated with HU required a period corresponding to two division cycles. The HU was sub-cytotoxic. The HU treated cells had an enriched number of a group of small chromosomes detected by flow cytometric karyotyping. Use of synchronized cells confirmed these results. The HU treatment and release regimen employed here is strikingly similar to the one known to induce dihydrofolate reductase gene amplification during selection of methotrexate resistant cells. The results suggest a model where HU induced pre-commitment during HL-60 terminal myeloid differentiation is due to amplification of gene(s) carried on over-replicated small chromosomes. Such a gene(s) may serve as a master control of other genes whose expression results in the terminally differentiated phenotype.

Plant and Animal Development — I

N119 ANALYSIS OF GENE EXPRESSION DURING PRECOMMITMENT STAGES OF EMBRYONAL CARCINOMA CELL DIFFERENTIATION, Patricio Abarzúa, Mary I. Harper and Michael I. Sherman, Roche Institute of Molecular Biology, Nutley, NJ 07110. Because of their pluripotent nature and other similarities to early embryonic cells, embryonal carcinoma (EC) cells provide a model system for studying gene expression during the earliest stages of differentiation. EC cells differentiate *in vitro* in response to various chemical stimuli, e.g., retinoic acid (RA) and hexamethylenebisacetamide (HMBA). Although many resultant changes in gene expression have been characterized, most are detectable only several hours or days after addition of inducer. To learn about earlier molecular events in the differentiation pathway, we have constructed large cDNA libraries representing the mRNA populations of untreated Nulli-SCC1 EC cells and cells treated for 24 hr with RA, a time at which many cells are not yet committed to differentiate. We have isolated by differential colony hybridization six cDNA clones reflecting quantitative changes in RNA expression. Clone pNRA-4, which has been studied in most detail, hybridizes by Northern analysis to polyA^{+} RNA which is heterogeneous in size (350 to 500 nucleotides). The level of this RNA increases as early as 1 hr after addition of RA, reaches a maximum at 3-6 hr and decreases to uninduced levels within 72 to 96 hr. Early in the induction period, a discrete RNA band is notable at about 500 nucleotides. A very similar pattern of hybridization is observed after treatment of cells with HMBA, a chemically (and perhaps mechanistically) unrelated differentiation inducer. We are currently sequencing the pNRA-4 insert and attempting to learn more about its relevance to differentiation by analysis of mutant EC lines which fail to differentiate in response to RA and/or HMBA.

N120 DEVELOPMENTAL EXPRESSION OF SUNFLOWER STORAGE PROTEIN GENES, Randy Allen, Elizabeth Cohen, Carl Adams, Ray Vonder Haar, Craig Nessler and Terry Thomas, Texas A&M University, College Station, TX 77843

Two major storage protein classes are synthesized exclusively in embryonic tissues during sunflower seed development. By 5 days after anthesis(DAA), 2S albumins have accumulated to substantial levels in the sunflower embryo. 11S storage protein species, collectively designated helianthinin, are detectable at 7 DAA. cDNA recombinants representing both classes of storage proteins have been isolated and characterized. The albumin storage proteins appear to be encoded by a single copy gene which gives rise to a mRNA of 1.1 Kb. Helianthinin storage proteins are encoded by a gene family of approximately 10 members; at least 3 members of this family are transcribed into 1.9 Kb mRNAs during sunflower embryogenesis. Helianthinin and albumin mRNAs reach their maximum prevalence at about 12 DAA corresponding to the period of maximum storage protein accumulation. Levels of storage protein mRNA decrease after 12 DAA; these transcripts are essentially undetectable in RNA of mature seeds or germinating seedlings. Storage proteins continue to accumulate until seed maturation and dessication. DNA sequence analysis of both classes of storage protein genes indicate some notable differences from other genes encoding plant storage proteins.

N121 THE ACTIN MULTIGENE FAMILY IN PETUNIA: CHARACTERIZATION AND DIFFERENTIAL EXPRESSION OF ITS MEMBERS. Wm. Vance Baird and Richard B. Meagher, Genetics Dept., University of Georgia, Athens, GA 30602

The genes encoding actin, a ubiquitous protein in all eukaryotic cells, provide an excellent model system for studying the structure and tissue specific expression of a multigene family. In Petunia hybrida 'Mitchell', actin proteins are encoded by a large and complex multigene family. Southern blot analyses of genomic DNA indicates that although the members share various degrees of homology (cross-hybridization) certain members are present in one or two copies per haploid genome while others are present at 20 or more copies. Also it appears that most if not all of the 'Mitchell' actin genes are derived from the P. axillaris parent. Eight distinct clones were isolated from a phage lambda genomic library and characterized by restriction endonuclease digestion and filter hybridization to known heterologous actin clones (Dictyostelium, Drosophila, rat and soybean). Transcripts from the genomic clones were detected in the poly $(A)^+$ RNA fraction isolated from leaves, roots, stems and flowers by Northern analysis and by probing the restricted lambda clones with labeled RNA. Genes or gene pairs were characteristically expressed at different levels in each organ and one clone was not detected in any organ type. We are currently sequencing the clone most highly expressed in leaf tissues as well as constructing chimeric genes containing a reporter function linked to putative promoter and enhancer regions. Their regulation will be further characterized in transgenic Petunia plants.

N122 EFFICIENT INTEGRATION OF RETROVIRUSES INTO THE MOUSE GERMLINE AND THEIR POTENTIAL FOR INSERTIONAL MUTAGENESIS, Victoria Bautch and Sachiko Toda, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

We are investigating the potential of retroviral integration into the mouse genome to cause insertional mutations. Specific genetic crosses were tested for proviral integration into the embryonic genome and one breeding scheme (Jenkins and Copeland, submitted) was found to generate new integrations at frequencies of up to 0.3 (#integrations/#mice tested). This phenomenon was dependent on the presence in the maternal genome of two genetically linked proviral loci (Emv-16 and Emv-17) contributed by the RF/J strain of mice on the SWR/J inbred background. The results indicate that both genetic and epigenetic events contribute to the probability of an integration event and are consistent with the hypothesis that virus from the mother is infecting the oocyte or early embryo (Bautch et al., manuscript in preparation). Most of these integrations were transmitted through the germline at frequencies of 6%-35%, indicating that founder mice were mosaic for the integrations. We have established 40 lines of mice containing new proviral loci. No dominant mutations were detected in the generation of these heterozygotes. We are currently producing mice homozygous at each of the new loci to check for recessive phenotypes and recessive lethal mutations.

N123 REGULATION OF THE BIOSYNTHESIS OF THE NA,K-ATPASE DURING DEVELOPMENT OF THE BRINE SHRIMP Lee Ann Baxter-Lowe, James A. Fisher and Lowell E. Hokin, University of Wisconsin, Madison, Wisconsin 53706

The developing brine shrimp provides an excellent model system for studying the synthesis and regulation of the sodium- and potassium-activated adenosine triphosphatase (EC 3.6.1.3) (Na,K-ATPase). Previous studies have shown that the activity of the Na,K-ATPase is first detectable after 8 h of development and the level of activity continues to increase until 32 h of development. In this study, radioimmunoassay showed that the subunits of the Na,K-ATPase (α_1, α_2, and β) were present in cryptobiotic cysts. The levels of the subunits increased dramatically between 6 and 24 h, after which the levels of the subunits remained constant. The quantities of $mRNA_\alpha$ and $mRNA_\beta$ were estimated by determining the amounts of α and β subunits present in the cell free translation products produced from mRNA isolated at several times during development. It was possible to detect low levels of the mRNAs coding for the three subunits at the beginning of development. The levels of $mRNA_\alpha$ began to increase after 4 h of development and continued to increase until 24 h yielding a 9-fold change in the levels of $mRNA_\alpha$. This resulted from an increase in the proportion of mRNA present as $mRNA_\alpha$, as well as an increase in the total RNA/g embryo. The $mRNA_\beta$ demonstrated a 3-fold increase during the same time interval, but these increases resulted from changes in total RNA levels and the proportion of mRNA present as $mRNA_\beta$ remained unchanged. Although the relative proportion of the α_1 and α_2 subunits changes during development, the relative proportion of $mRNA_{\alpha 1}$ and $mRNA_{\alpha 2}$ remained constant during the first 40 h of development.

N124 MECHANISTIC SEPARATION OF AN EARLY AND LATE EVENT IN PREADIPOCYTE DIFFERENTIATION. David A. Bernlohr*, Thomas J. Kelly+ and M. Daniel Lane# from the Depts. of Biochemistry, Univ. of Minnesota; +Molecular Biology and Genetics, #Biological Chemistry, Johns Hopkins Univ. School of Medicine.

Morphological and biochemical evidence indicates that adipocytes in vivo are derived from differentiation of a fibroblastic precursor. 3T3-L1 cells undergo differentiation into adipocytes in culture and are used as a model to study adipose conversion. Differentiation of 3T3-L1 cells is initiated by the addition of dexamethasone, methylisobutylxanthine, and insulin (M,D,I). Following MDI addition the cells undergo 1 to 2 rounds of cell division, an event required for expression of the adipocyte phenotype. We have isolated two cDNA clones representing mRNAs whose kinetic profile of expression define them as early (prior to cell division) and late (following mitosis) messages. The early message, which encodes an unidentified 46 kDa protein, increases in abundance 20-fold within 4 hr following MDI addition. The message expression was not accompanied by an increase in runoff transcription from isolated nuclei. The late mRNA encodes an adipocyte-specific 14.6 kDa protein believed to be a fatty acid carrier. This mRNA increases in abundance 20-fold and is accompanied by a 15-fold increase in nuclear runoff transcription. Taken together these results suggest that early and late events in preadipocyte differentiation appear to be separable by both their temporal and mechanistic deportment. Early message expression appears to be regulated by nontranscriptional events involving control of mRNA stability whereas late message expression is correlated with transcriptional control of adipocyte-specific genes.

N125 THE STRUCTURE AND REGULATION OF THE ACTIN 5C GENE OF DROSOPHILA, Beverley J. Bond and Norman Davidson, California Institute of Technology, Pasadena, CA 91125

At least six developmentally regulated mRNAs are made from the Drosophila actin 5C gene. We have examined the structure of these RNAs in detail and have determined that they are heterogeneous at both their 5' and 3' ends. At the 5' end there are two nonhomologous leader exons which are alternately spliced to the remainder of the gene. At the 3' end of the gene there are three different sites of polyadenylation. This 3' variation is the principal cause of the length diversity observed in the transcripts. Our data indicate that in whole animal RNA the two leader exons are used with approximately equal probability in all stages of development and with all three polyadenylation sites. There is some developmental variability in the use of the three polyadenylation sites. We have begun to study the sequences necessary for the initiation of transcription from each start site. We have made fusions between putative actin 5C promoter sequences and the bacterial CAT gene and tested these by transient transformation experiments in the Kc line of Drosophila cultured cells. We have found that there is a promoter upstream of the first leader exon and have begun to narrow down the sequences necessary for efficient expression from this promoter. Our current experiments are aimed at determining whether the two start sites each have independent promoters or whether they share common regulatory sequences.

N126 DNA ELEMENTS AND CHROMATIN FEATURES INVOLVED IN THE CONTROL OF THE HORMONALLY REGULATED GLUE GENES IN DROSOPHILA.

Marc Bourouis, L.G.M.E. du CNRS, U.184 de l'INSERM, 11 rue Humann 67085 Strasbourg-Cédex - France.

The salivary gland "glue" gene sgs3 requires several distinct regulatory sequences for its normal transcriptional activity. A refine deletion analysis using transformation has revealed that a DNA sequence 5', previously observed as showing homology to a key regulatory site of sgs4, does not behave as an important sequence for sgs3. However, deletion of a further removed sequence abolishes its (transcriptional) activity. This mapping correlates with the presence in the gland cells of a cluster of DNaseI hypersensitive sites, one of which disappears in vivo when the title of ecdysone rises (1). A conserved nucleotide motif found at equivalent sites 5' of several glue genes suggests that they play analogous function, perhaps the binding of a specific transcriptional factor, the displacement of which by hormone switches transcription off. Additional complexity within the control regions of these genes, is predicted from analysis of fused gene constructs.

(1) M. Bellard, M. Bourouis, Ph. Ramain, G. Richards, G. Dretzen, A Sobkowiak, in preparation.

N127 RESTRICTED EXPRESSION OF METALLOTHIONEIN AND OTHER GENES IN ECHINOID INTERSPECIES HYBRID EMBRYOS, Bruce P. Brandhorst and Ronald C. Conlon, Biology Dept., McGill University, Montreal, P.Q. Canada H3A 1B1

The synthesis of many paternal species-specific proteins is undetectible in sea urchin interspecies hybrid embryos of all stages, due to a corresponding reduction of paternal mRNA transcripts (Tufaro and Brandhorst [1982], Devel. Biol. 92: 209-220). We have selected cDNA clones corresponding to mRNAs of Lytechinus pictus which are reduced in embryos derived from a cross of Strongylocentrotus purpuratus eggs x L. pictus sperm. Several of the corresponding mRNA's accumulate extensively during embryogenesis of L. pictus, indicating that the restricted expression of the paternal genes in hybrids is not due to a persistence of stable maternal mRNA's. Based on a run-on assay in isolated nuclei, one such gene, which encodes a metallothionein, is as actively transcribed in hybrids as in homospecific embryos, but the mRNA accumulates to only 5-10% of its normal level. A working hypothesis, which is being tested, is that the accumulation of this and/or other paternal mRNA's in hybrid embryos is autogenously regulated by a cotranslationally mediated stabilization of mRNA via interaction of nascent proteins with related structural proteins. Funded by grants from NSERC and NIH.

N128 DIFFERENTIAL REGULATION OF THE HEAT SHOCK GENES UNDER HEAT AND TERATOGEN TREATMENT. Carolyn H. Buzin, Cheryl J. Clark, Nancy S. Demcak, and John J. Rossi, City of Hope Medical Center, Duarte, CA 91010.

In Drosophila primary embryonic cell cultures, a number of teratogens inhibit muscle and neuron differentiation in vitro and also induce two of the heat shock proteins, hsp 22 (2 isoforms) and hsp 23. A different response occurs when these same cells are given a heat shock: the full set of seven major heat shock proteins are induced. The heat induction occurs very rapidly, beginning within a few minutes and reaching a maximum by about an hour. We have examined the induction of the heat shock proteins hsp 22 and 23 during treatment with the teratogen diphenylhydantoin (10^{-4}M). Synthesis of these proteins begins to increase at 8-10 hr of continuous treatment and reaches a maximum at 12-14 hr. To determine whether the differential expression of the heat shock proteins is due to transcriptional regulation, we have carried out dot blot experiments using total RNA from control, teratogen, or heat treated cells. We have shown that mRNAs from hsp 22 and 23 genes are present after treatment with heat and the teratogens diphenylhydantoin, coumarin, and pentobarbital, but not after treatment with the non-teratogen sulfanilamide. mRNAs for hsp 26 and 27 are present in the heat-treated, but not in any of the drug-treated cells. We have confirmed these results by Northern gel analysis. Transcription of the hsp 22 and 23 genes is induced by diphenylhydantoin as well as by heat shock. No mRNAs for hsp 26, 27 or 70 are present in diphenylhydantoin-treated cells, but they are induced by heat shock. Thus an apparent selective transcription of certain heat shock genes occurs in the presence of diphenylhydantoin.

N129 TISSUE SPECIFIC EXPRESSION OF THE HUMAN TYPE II COLLAGEN GENE IN MICE, K. Cheah[1], R. Lovell-Badge[2], A. Bygrave[3], A. Bradley[3], E. Robertson[3], R. Tilley[4]. [1]Biochemistry Dept., Hong Kong University; [2]MRC Mammalian Development Unit, University College, London; [3]Genetics Dept., Cambridge University, Cambridge; [4]Imperial Cancer Research Fund, London.

Type II collagen is the major extracellular component of cartilage. It is synthesized in large amounts during the differentiation of mesenchymal cells to chondrocytes but synthesis is very low in the adult. Correct expression of this protein is also important in bone formation. Because of its fundamental role in the development of form we have established a system to analyse the factors controlling the expression of type II collagen. The human type II collagen gene clone, cosHcol.1 (1), containing 4.5 kb 5' and 2.2 kb 3' flanking sequence was introduced into mouse embryonic stem cells by the calcium phosphate precipitation method. Stably transformed sublines carrying intact copies of the human gene were then used to derive chimeric mice after injection into blastocysts. Human type II collagen mRNA could be detected only in developing cartilage and bone of chimeras, demonstrating tissue specific expression. Antibody staining of chimeric mouse tissue sections in situ show that human type II collagen was present only in the matrix surrounding chondrocytes, suggesting cell-type specific expression. But quantitatively, expression was not equivalent to the endogenous mouse level. The human gene was also appropriately turned off in adult chimeras because no mRNA transcripts could be detected. The evidence suggest that the DNA sequences within 4.5 kb 5' and 2.2 kb 3' to the human type II collagen gene are able to mediate the tissue specific expression of the gene correctly from mRNA to the final protein product.

(1) Cheah KSE, et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 2555.

N130 ISOLATION OF FUNCTIONAL HEAVY AND LIGHT SUBUNIT FERRITIN GENES AND THEIR REINTRODUCTION INTO A HUMAN CELL LINE THAT CAN DIFFERENTIATE IN VITRO. C.-C. Chou, S. Chada, S. Teraoka, P. Concannon*, R. Nelson, M. Fuller, R. Davis and W. Salser, UCLA, Los Angeles, CA 90024. *Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.

We are using the HL-60 promyelocytic leukemia cell line as a model for cellular differentiation and commitment. Among a large number of cDNA clones whose expression is regulated during myeloid differentiation we have identified clones for both the heavy and light subunits of human ferritin. These were used to select chromosomal clones for further study. Ferritins are a family of ubiquitous eukaryotic proteins which function to sequester and detoxify intracellular iron. The ferritins in different tissues show remarkable variations in the ratios of the heavy (H) and light (L) subunits present in the 24-subunit ferritin molecule. Although translational regulation (in response to iron challenge) was the only mode of regulation previously demonstrated, we have used Northern blot analysis to show that the mRNA levels are strongly regulated (up to 40-fold) during HL-60 differentiation and that the change in the H/L protein subunit ratio can be attributed to a 16-fold change in H/L mRNA ratio.

Chromosomal clones for both the H and L subunit genes have been tagged and successfully transfected into HL-60 cells. One of the six independent H subunit genomic clones shows the same pattern of regulation seen for the endogenous gene. When a tagged ferritin L subunit genomic clone is transfected into HL-60 cells we observe that the expression of the tagged gene increases during neutrophil differentiation more dramatically than that of the endogenous gene.

N131 MOLECULAR ANALYSIS OF THE RAT MHC, A.L. Cortese Hassett, K.S. Stranick, J. Locker, H.W. Kunz and T.J. Gill III, University of Pittsburgh, Pittsburgh, PA 15261

Southern blot analysis with liver DNA from a unique series of recombinant (R10,R11,R16,R18, R21 and R22), congenic and inbred rats has been performed to examine the restriction fragment length polymorphisms of Class I genes. Digested genomic DNA was hybridized with murine H-2 cDNA probes. Eighteen to twenty-five bands of varying intensities could be clearly resolved in any given strain. Analysis of these hybridization patterns detected restriction fragment length polymorphisms that permitted the assignment of seventeen specific fragments to regions within the MHC: RT1.A, RT1.B/D, and the RT1.E-grc-T1a region. Fragments have been identified that are specific for grc, grc+ and RT1.E and that mark the junction sites between these loci. In addition, several markers identify the sites of recombination in some strains. The hybridization pattern of the R18 had a unique band that specified a point of recombination within the grc. The grc (growth and reproduction complex) are recessive genes affecting growth and development linked to the major histocompatibility complex of the rat (RT1). A similar region has been identified in the mouse (T/t complex). Rats carrying the grc genes are sterile because of a uniform arrest at the early pachytene stage of the primary spermatocytes, and they are small (ca. 70% of normal weight). The grc has been further divided into loci influencing small body size (dw-3) and fertility (ft) based on recombination (1). The presence of genes affecting development and linked to the MHC in the mouse and the rat suggests that this pattern of organization may be a general phenomenon in mammals. Whether these genes are mutants or whether they play a role in normal genetic structure of the animal is not known. 1. Gill,T.J.III, Siew,S. and Kunz,H.W. J.Exp.Zool. 228:325-345, 1983.

N132 ISOLATION AND CHARACTERIZATION OF GENES REGULATED DURING MYELOID DIFFERENTIATION. R. Davis, A. Thomason*, M. Fuller, C.-C. Chou, S. Chada, R. Gatti, J. Slovin and W. Salser. UCLA, Los Angeles, CA 90024. *Present address: AMgen Inc., Thousand Oaks, CA 91320.

We have constructed cDNA clone banks using mRNAs from differentiated and undifferentiated HL-60 promyelocytic leukemia cells and have isolated a large number of clones corresponding to genes which are regulated during this differentiation. Regulation of the corresponding mRNAs in HL-60 cells during both monocytic and neutrophilic differentiation was measured for twenty-one of these clones. The levels of mRNAs corresponding to some of these clones appear to change by more than 100-fold during differentiation. Individual genes are turned on and off at a variety of different times during the two differentiation pathways. This is unlike erythropoiesis or myogenesis, in which the synthesis of a few new proteins in the differentiated cells is synchronously regulated. Our results suggest that several distinct regulatory events are controlling different classes of mRNAs at different times. This correlates well with the very complex set of biological activities shown by macrophages and granulocytes and suggests that they should provide excellent model systems for studying a variety of molecular mechanisms of gene regulation during commitment and terminal differentiation. About half of the clones scored as regulated in these tests contain repeat sequences, including both Alu and novel repeat families. The great majority of these regulated genes are members of extensive gene families.

N133 ISOLATION OF A cDNA CLONE ENCODING ACTH-KINASE ACTIVITY USING A NOVEL TECHNIQUE, Lakshmi Devi[1], Jim Eberwine, Jack Barchas and Chris Evan, [1]Addiction Research Foundation, 701 Welch Road, Palo Alto, CA and Nancy Pritzker Laboratory of Behavioral Neurochemistry, Department of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305.

Kinases have been shown to play a regulatory role in neuronal cell functions during development. We are studying the role of phosphorylation of adrenocorticotropic hormone (ACTH) that is shown to be endogenously phosphorylated. To this end we have used a modification of enzyme immuno-detection assay (EIDA) developed by Eberwine and Evans (manuscript submitted) to rapidly obtain clones encoding functional kinases. The method involves using an immobilized substrate to detect the presence of a phosphorylating activity in a recombinant phage from a λgt-11 expression library. Using this method we have isolated an ACTH phosphorylating activity from an anterior pituitary expression library. Characterization of the clone and of the enzyme-fusion protein will be presented.

This technique is unique in that unlike using antibodies against protein of interest to screen expression libraries, we are able to use the expression of a functional enzyme to screen the cDNA libraries.

N134 CELL-TYPE SPECIFIC EXPRESSION OF THE RAT THYROGLOBULIN PROMOTER: INTERACTION WITH THYROID SPECIFIC NUCLEAR PROTEINS. Roberto Di Lauro, & Anna M. Musti, C.E.O.S., Napoli, Italy & Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

We have fused to the structural gene for the bacterial enzyme Chloroamphenichol Acetyl Transferase (CAT) a segment of DNA containing 1000 base pairs upstream from the transcription initiation site of the rat thyroglobulin gene. The fusion gene is able to promote CAT synthesis only in a cell line (FRTL-5) which actively expresses the endogenous thyroglobulin gene while it remains inactive if introduced in several control cell lines which do not express the endogenous gene. We have shown by deletion analysis that 170 base pairs upstream from the transcription initiation site are necessary and sufficient for cell type specific expression. Using both DNAase I and Exonuclease III footprints we have mapped three regions, within the 170 base pairs promoter, which interact with proteins present in nuclear extracts. The protein components responsible for two of the three protections are present only in the nuclear extracts derived from FRTL-5 cells.

N135 TRANSIENT EXPRESSION OF RABBIT β-GLOBIN GENES IN MURINE ERYTHROLEUKEMIA CELLS, Peter M. Dierks and Cheong-Hee Chang, University of Virginia, Charlottesville, Virginia, 22901

Cotransformation of undifferentiated and differentiated murine erythroleukemia cells (MELC) with two unlinked and genetically distinguishable adult rabbit β-globin genes has been used to examine the transient expression of β-globin genes in erythroid cells. One of the β-globin genes serves as an internal reference against which the expression of the other (test) gene can be compared by quantitative nuclease S1 mapping. Preliminary work has focussed on the effects of varying the amount of 5' and 3' flanking DNA sequences on gene expression, as well as the effects of linking the β-globin gene to the polyoma virus transcriptional enhancer element. In the absence of the polyoma enhancer all of the DNA segments tested support low levels of correct transcription in uninduced MELC. The level of β-globin transcripts accumulating per cell was not significantly different when the genes were introduced into differentiated MELC. The presence of the polyoma enhancer had little effect on the level of transient β-globin gene expression in undifferentiated MELC. However, when the enhancer was linked to a 4.7 kb Kpn I restriction fragment containing the rabbit β-globin gene the level of β-globin transcripts observed in differentiated MELC was increased about 50-fold. This effect was not observed when the enhancer was linked to β-globin genes having shorter 5' and 3' flanking chromosomal DNA segments. We conclude that enhancement of rabbit β-globin gene expression by the polyoma enhancer is developmentally controlled and may require rabbit DNA sequences that are located, at least in part, outside of the known promoter and coding regions of the gene.

N136 STRUCTURE AND HORMONAL REGULATION OF GLYCEROPHOSPHATE DEHYDROGENASE MRNA DURING ADIPOCYTE DIFFERENTIATION, Deborah E. Dobson, Douglas L. Groves and Bruce M. Spiegelman, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA02115

Glycerophosphate dehydrogenase (GPD) is a key lipogenic enzyme which is induced 2000-fold during mouse adipocyte differentiation. We have isolated and sequenced the coding region of cDNAs corresponding to this mRNA from an adipocyte cDNA library, and characterized the 5'end of the mRNA by primer extension. The GPD mRNA has a 15 bp 5' untranslated region, a 1047 bp coding region whose predicted amino acid sequence has 90% homology with rabbit GPD protein, and a 2.3 kb 3' untranslated region. The amount of GPD mRNA in adipocytes can be modulated by several hormones; northern blot analysis reveals that GPD mRNA is decreased by more than 80% in adipocytes treated with the lipolysis-inducing agents dibutyrylcyclic AMP and theophylline (0.5 mM, 1.5 hours) or with the synthetic glucocorticoid dexamethasone (10^{-6}M, 24 hours). We have isolated and characterized a 20 kb fragment of DNA encoding GPD from a mouse adipocyte genomic library. Sequence analysis of the region upstream of the transcription start site reveals a putative regulatory element shared by several adipocyte-specific genes. Experiments are in progress to identify the sequence elements necessary for adipocyte-specific regulation of GPD expression.

N137 EXPRESSION OF SEED STORAGE PROTEINS IN XENOPUS OOCYTES, D. Donaldson, L. Hoffman, R. Drong, R. Bookland, Agrigenetics Corporation, Advanced Research Division, 5649 East Buckeye Rd., Madison, WI 53716

Maize seed storage proteins, known as zeins, are a complex family of lysine deficient polypeptides with molecular weights of 22, 19, 15, and 10 kD. The 19 and 22 kD zeins are the products of a multigene family. The 15 kD zein is encoded by only one or two genes and is therefore a suitable candidate for genetic engineering to improve the nutritional quality of maize seed storage protein. A chimeric, high lysine zein gene has been constructed by replacing a segment of the 15 kD zein gene with a fragment of Phaseolus vulgaris DNA which encodes a lysine rich region of the bean storage protein phaseolin. Technologies for maize transformation and regeneration are not available, so alternative means of zein expression were utilized. Xenopus oocytes have been shown to translate total maize endosperm poly A+ RNA and package zein polypeptides into protein bodies similar to those found in maize endosperm. The wild type and modified 15 kD zein genes have therefore been subcloned into the RNA expression plasmid pSP64 and a synthetic oligonucleotide encoding 30 A residues inserted near the polyadenylation site of each gene. Use of SP6 polymerase in the presence of cap analog allows transcription of microgram quantities of capped 15 kD zein RNAs with or without poly A residues depending upon the restriction enzyme used to linearize the template plasmid. After injection into oocytes, these RNAs are translated in sufficient quantity to examine the nature and intracellular localization of their protein products.

N138 MOLECULAR CLONING, STRUCTURE, AND MAPPING OF CREATINE KINASE. Robert Dottin,[1] Janice Nigro,[1] Bernhard Haene,[1] Cherukuri Varalakshmi,[1] Clifford Schweinfest,[1] Robert Kwiatkowski,[1] Peter Rae,[2] and Mike Kamarck.[2] [1]Dept. of Biology, The Johns Hopkins University, Baltimore, MD 21218 and [2]Molecular Diagnostics, West Haven, CT 06516.

The creatine kinase gene family presents a simple system to study the molecular mechanisms controlling developmentally regulated, tissue specific isoenzyme switching in normal and diseased states of muscle. To investigate these problems, we have isolated and sequenced a full length cDNA clone of the chicken creatine kinase M subunit as well as a CK-B clone. Restriction digests of chicken genomic DNA were probed with the CK-M clone, and a restriction map of the CK-M gene deduced. The gene is more than 40 kp.p. in length and contains a minimum of 5 exons. A human heart cDNA bank was screened with the coding region of the chicken clone, and at least one large CK-M clone was isolated. The pattern of restriction sites between chicken, rat, rabbit, and human sequence is highly conserved. The human CK-M cDNA clone was used to map the CK-M gene to human chromosomes. Northern transfer experiments indicate that the accumulation of creatine kinase on RNA parallels the accumulation of the polypeptide. Therefore isoenzyme switching is controlled by mRNA synthesis and/or stability.

N139 REGULATION AND GENE EXPRESSION IN ORGANOGENESIS AND SEXUAL DIFFERENTIATION IN MERCURIALIS ANNUA (2n=16): Francis Sagliocco, Jean P. Louis, Guy Kahlem, Michel Delaigue, Saïd Hamdi and Bernard Durand (University of Orléans, F. 45067)

In this dioecious plant, a series of successively acting genes control the consecutive nodal structures. Two buds normally arise at each leaf axil, the first developing one is reproductive, the second vegetative. Two dominant genes transform the reproductive bud into vegetative at each node of male and female plants. The wild recessive alleles are necessary for the determination of floral structures. A second series of genes act later in the flower initia: the 3 sex determination genes act at the second main binary choice between stamen or carpel. Finally, 3 other genes and a maternal factor for stamen *fertility* or *sterility* determine the normal formation of sporogenous tissue. This series of genes form a hierarchy of regulatory genes switching the successive major determination steps of the node pattern and evoke regulatory successive genes of Drosophila pattern.

How these regulator genes assume the developmental regulation of these structures and particularly the normal sexual organogenesis ? Probably through cytokinins-auxins balances. Experimental variations of these hormones in vivo change the determination of every initium (floral-vegetative, stamen-carpel, fertile-sterile stamen). Hormone measurements (GC/MS) in apices of strains selected for various allelic combinations of sex and sterility genes also furnish evidences for this assumption: a clear correlation exists between certain combinations and certain active metabolites. In turn, these metabolites control mRNAs populations characteristic for sex organs: translation in vitro of mRNAs and homologous or heterologous cDNAs-mRNAs hybridization kinetics of normal or hormonal induced organs show that hormones control specific gene expression in each.

N140 A SINGLE NERVE GROWTH FACTOR GENE PRODUCES MULTIPLE TRANSCRIPTS BY DIFFERENTIAL RNA SPLICING AND AT LEAST THREE PROMOTER ELEMENTS. RH Edwards, MJ Selby, WJ Rutter, UCSF

Mature NGF is a polypeptide of 12,500 daltons and derives from processing of a larger precursor protein. Cloning of the NGF cDNA from mouse submaxillary gland originally predicted a precursor of 34 kd, if the first in-frame AUG is used to initiate translation. The only hydrophobic sequence that might act as a signal peptide to direct secretion, is located in the middle of this precursor. Our study of the NGF gene has shown that several small exons reside at the 5' end, with one large exon encoding mature NGF at the 3' end. We investigated the possibility of differential RNA splicing involving these 5' exons by S1 analysis, primer extension and further cDNA cloning. We have identified a major alternative form of NGF transcript that has no second exon, the exon which contains the first AUG. Translation of this mRNA would therefore have to begin at the second AUG, which immediately precedes the hydrophobic sequence. This sequence may then serve as a signal peptide for secretion of the NGF precursor. This transcript is in fact the predominant form in most tissues and species outside the mouse submaxillary gland, but both transcripts are highly conserved (mouse to man). We have also found several clones entirely divergent from the original NGF sequence 5' to the same intron-exon junction involved in the differential RNA splicing. We infer the presence of at least three distinct promoter elements. Differential RNA splicing and these promoter elements may alter the cellular fate and biological properties of the NGF precursor.

N141 BIDIRECTIONAL ACTIVITY OF THE RAT INSULIN II PROMOTER REGION IN TRANSGENIC MICE. S. Efrat, S. Alpert, M. Lacy and D. Hanahan, Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, N.Y. 11724.

Transgenic mice, harboring hybrid genes composed of the upstream region of the rat insulin II gene linked in either orientation to the SV40 early region, express T antigen (Tag) specifically in the β-cells of the endocrine pancreas. The phenotype of mice with the reversed promoter is undistinguishable from that of mice in which the insulin promoter is aligned to transcribe the Tag gene. In each case, the transgenic mice become hypoglycemic as a consequence of proliferation of the pancreatic β-cells, which effect hyperplasia of the islets of Langerhans and the subsequent development of solid tumors from a few of the ~ 100 islets. In one lineage - RIP1-Tag#2 - the hybrid insulin promoted Tag gene is turned on during embryogenesis in a similar fashion to the endogenous insulin genes. Developmental analysis of the other lineages - both insulin promoted and reversed - is in progress and will be presented.

Tumor RNA shows comparable levels of Tag mRNAs, with both insulin promoted and reversed orientations. The reversed promoter generates mRNAs about 300 bases longer than the insulin promoted version. This transcript is initiated at a specific site, mapping within the transcriptional enhancer region. Bidirectional transcription is observed with both hybrid gene orientations; in each case, one transcript represents the Tag gene, the other, the opposite strand extending off 5' to the Tag gene.

These results demonstrate the existance of two promoters in the region extending to -540 upstream of the insulin cap site, operating on the two strands in opposite directions. Both obey correct cell-specific regulation in the transgenic mice. The presence of a gene associated with the reversed promoter of the endogenous insulin gene is under investigation.

N142 KERATIN GENE EXPRESSION:A MARKER FOR EPIDERMAL DIFFERENTIATION, Tami Ellison and Leo Miller, University of Illinois, Chicago, IL 60680.

Studies on molecular aspects of cellular differentiation are often limited by the lack of defined ontological cell lineages by which to monitor sequential differentiative events. Further limitations have been encountered by either the inaccesibility of cells in their early states of differentiation or an insufficient amount of material which can be obtained from organ primordia. We have chosen Xenopus as a model system to study epidermal differentiation. Developmental reference points for commitment and primary differentiation of the epidermis from its ectodermal derivatives and subsequent changes in the maturational status of the skin have been well documented. These transitions have provided a framework around which progressive morphological differentiation has been correlated with biochemical differentiation of the skin, using keratin expression as the parameter (Ellison et al, in press). Utilizing the observed dependence of amphibian development on thyroid hormone, the temporal expression of the 63kd keratins during development and following precocious induction with T3 (in vivo and in vitro) has been examined using 3'-specific cDNA probes for S1 analysis of the transcripts. The three 63kd keratins show approximately 80% sequence identity at the cDNA level thus providing an interesting system in which to examine the events of gene commitment and potentiation prerequisite to the tissue-specific and developmentally-regulated response of this small multigene family to T3.

N143 THE NORMAL PROGRAM OF GENE EXPRESSION DURING SPORE GERMINATION IN *DICTYOSTELIUM DISCOIDEUM* IS DERANGED IN A GERMINATION-DEFECTIVE MUTANT, H.L. Ennis, D.R. Shaw and H. Richter, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

The synthesis of mRNA and protein during spore germination in the cellular slime mold *Dictyostelium discoideum* is developmentally regulated. After activation, wild-type spores swell and then release single amoebae in a highly synchronous manner. A mutant which swells normally but does not release amoebae was used to investigate whether this defect in spore germination affected the orderly progression of appearance and disappearance of mRNAs developmentally regulated during germination. Three previously characterized cDNA clones representing *D. discoideum* sequences that are modulated during spore germination, and are not present in growing cells, were used as probes. In the wild-type, the levels of the respective mRNAs reach a peak early during spore germination (1-1.5 h) but fall precipitously at later times, indicating that their synthesis has stopped and they are rapidly degraded. In contrast, in the mutant, after reaching their maximum level (also at 1-1.5 h), the mRNA levels remain very high, indicating that either synthesis has not been turned off, and/or degradation does not occur. It is concluded that the time of the onset of synthesis of the mRNAs and the time when their maximum levels is reached is normal in the mutant. However, the later events, the level of mRNA attained and the subsequent disappearance of these mRNAs is abnormal. This means that the emergence of amoebae, or the events leading to it, are required for the normal pattern of mRNA accumulation and therefore that the morphogenetic and molecular states of the cell are interdependent.

N144 SV40 TRANSGENIC MICE THAT DEVELOP TUMORS ARE IMMUNOLOGICALLY TOLERANT TO SV40 T-ANTIGEN, Susan J. Faas, Sueihua Pan, Carl A. Pinkert, Ralph L. Brinster and Barbara B. Knowles. The Wistar Institute and Univ. of Penn. School of Veterinary Medicine, Phila., Pa. 19104.

A number of transgenic mouse lines have been derived by the zygotic injection of various SV40 early region gene constructs, and mice from many of these lines develop tumors at specific sites. Since the ability to mount a cellular immune response to epitopes of SV40 T-antigen has been implicated in the control of SV40 tumor cell growth, such animals provide an ideal model for examining the consequences of specific immune tolerance or responsiveness to a single gene product expressed during normal development. We examined mice from two SV40 transgenic mouse lines to determine if such mice are immunologically tolerant or responsive to subsequent challenge with SV40, as compared to normal mice which mount both a humoral and cytotoxic T-cell response to the SV40 early region gene product (T-antigen). Our studies demonstrate functional immune tolerance to SV40 T-antigen in an SV40 transgenic line that succumbs to tumors of the choroid plexus at approximately 5 months of age. In contrast, mice from a second transgenic line that bear an identical plasmid construct but remain tumor-free generally mount both a humoral and vigorous cytotoxic T cell response to SV40 T-antigen. These data suggest that specific immune tolerance to the product of an integrated viral oncogene may be induced, and is likely a reflection of the time in development at which the gene product first appears. Immune tolerance or responsiveness to the gene product may in turn play a role in the tumorigenic potential of such genes.

N145 DELETION ANALYSIS OF THE STRONG PROMOTER FROM THE CHICKEN CYTOSKELETAL BETA-ACTIN GENE, Nevis Fregien and Norman Davidson, California Institute of Technology, Pasadena, CA 91125

The cytoskeletal beta-actin gene is expressed at high levels in most cells. We have found that the promoter of the beta-actin gene is very strong. We have tested the beta-actin gene for enhancer sequences. Transient and stable transformation experiments indicate that sequences in the region of the beta-actin promoter can stimulate transcription from other promoters after stable chromosomal integration but not during the transient phase of expression. To further understand the mechanisms for the strong promoter activity of the beta-actin promoter we have done deletion analysis. The promoter fragment tested consists of 1.6 kb of 5' flanking DNA including the CAAT and TATA boxes, the first (untranslated) exon, the first intron, and 6bp of the second exon up to the (now deleted) ATG for the initiation of translation of the beta-actin protein. Deletions in the 5' flanking DNA indicate that much of the DNA 5' of the CAAT box can be deleted with decreasing the level of expression. Deletions in the first intron show that removal of the 5'splice site (and/or nearby sequences) resulted in the decrease of the level of CAT expression.

N146 **The Significance and Basis for Differential Expression of Keratin Genes in Human Epithelia.** **E.Fuchs, D.Marchuk, A.Tyner, A.RayChaudhury, M.Lindhurst, C.Glass and K.H.Kim. Dept. Mol. Gen. and Cell Biol., Univ. of Chgo., Chgo. IL 60637.** The keratins are a group of 20 polypeptides (MW 40-70kd) that form 8 nm cytoplasmic filaments in vertebrate epithelial cells. The family can be subdivided into two distinct classes, type I and type II, based on the ability of their mRNAs to crosshybridize with one of two different cloned epidermal keratin cDNAs. The two keratin types are coordinately expressed in pairs, and different pairs are expressed in different tissues and at different stages of differentiation and development. Both types of keratins play an important role in filament assembly. Sequence analyses have revealed that the basic subunit structure of the filament is a heterotetramer comprised of two coiled-coil dimers of keratins. Whereas the central α-helical domains of the tetramer are similar for all keratin pairs, the nonhelical termini of different pairs are variable in size and sequence. Since these terminal sequences seem to play a role in end-to-end and in lateral interactions, the functional basis of the differential expression of these protein pairs may reside in the formation of 8 nm filaments with different properties, e.g. solubility, tensile strength, flexibility, and different interactions with cellular proteins and organelles. Our laboratory has now focused on elucidating the mechanism underlying the regulation of the differential expression of keratin gene pairs. We have isolated and characterized a number of the genes encoding keratins expressed in human epidermal and mesothelial cells. At least some genes that are coordinately expressed are tightly linked chromosomally. Sequence analyses have revealed a striking homology within the 5' untranslated regions of a coordinately expressed set of keratin genes, with no such homology for genes that are differentially expressed. We are currently investigating the role of these and other domains of conserved sequences in regulating keratin gene expression.

N147 THY-1 GENE EXPRESSION, Frank Grosveld, Vincent Giguere, Ken Ichi Isobe, Ann Britt Kolsto, Hans Prydz, Eugenia Spanopoulou and George Kollias, MRC, Mill Hill,UK.

Thy-1 is a cell surface glycoprotein, which belongs to the immunoglobulin super family of genes. It is temporally expressed on a number of tissues during development. In the adult, high expression is limited to the brain in all species and to T cells of many but not all species. To study this unusual pattern of expression at the molecular level we have cloned and analyzed the murine and human genes. We will present data on the chromatin structure of the gene, the regulatory elements of the promoter and the expression of the gene in transgenic mice.

N148 ISOLATION AND CHARACTERIZATION OF PERINATAL AND ADULT-SPECIFIC RAT MYOSIN HEAVY CHAIN cDNA AND GENOMIC CLONES. S.H. Grund, M. Periasamy, D. Bois, and B. Nadal-Ginard, Dept. of Cardiology, Children's Hospital, Dept. of Pediatrics, Harvard Medical School, Boston, MA. 02115.

To study the molecular mechanism(s) responsible for developmental and tissue-specific expression of the myosin heavy chain (MHC) multigene family, we have isolated cDNA and genomic clones corresponding to at least seven different MHC isoforms. Here we report the isolation and characterization of cDNA and genomic sequences corresponding to perinatal and adult-specific isoforms. Two adult-specific cDNAs have been mapped and completely sequenced. S_1-nuclease mapping experiments demonstrate that the respective mRNAs code for adult fast type IIA (oxidative; pMHC 40) and adult fast type IIB (glycolytic; pMHC 62) MHCs. The nucleotide homology between these two cDNAs is 85% and the amino acid sequence homology is 95%. Overlapping genomic clones covering the entire type IIA gene as well as part of the type IIB gene have been isolated. Comparison of sequences from a cDNA specific for a perinatal MHC isoform with sequences from genomic clones demonstrates that we have isolated a genomic clone which specifies a perinatal MHC. In addition, we have identified another genomic clone that is located approximately four kilobases 5' to the gene encoding adult type IIA MHC. We are currently investigating whether this represents a new, previously uncharacterized MHC isoform or a MHC pseudogene. Comparison of 5'-flanking and noncoding regions among the different MHC genes, in conjunction with expression assays, will provide information on sequences and factors which confer tissue and/or developmental regulation of MHC genes.

N149 PLASTID STARCH GRAINS REGARDED AS RESTING SPORES OF BACTERIA-ENDOSYMBIONTS, Miklos Gyenes, Dept. Biophysics, Biology Faculty, Moscow State University, Moscow, 119899, U.S.S.R.

Electron- and fluorescence microscopic staining for DNA during plastid development in Nitella translucens showed that starch was deposited within the nucleoid area. Starch deposition in dividing nucleoids resulted starch grains as if they had been stopped also at the corresponding stages of division. The number of starch grains corresponded to the number of nucleoids in a given plastid. The mature starch grains having lost nuclear genetic control after isolation formed hyphae on a moisture glass surface reminding some Actinomyces. Within the isolated starch grains just before hyphae formation the brightly fluorescing nucleoids of 0,3-0,4 μm became visible. It is supposed that starch deposition may be regarded as spore formation within the plastids which are generally believed to be bacteria-endosymbionts.

Supported by the Hungarian Academy of Sciences.

N150 ALTERNATIVE SPLICING OF THE DROSOPHILA TROPOMYOSIN II GENE GENERATES AT LEAST FOUR TROPOMYOSINS OR TROPOMYOSIN RELATED PROTEINS, Paul D. Hanke and Robert V. Storti, University of Illinois Health Science Center, Chicago, IL 60612

Drosophila has two tropomyosin genes. The tropomyosin I gene has been shown to produce two classes of developmentally regulated alternatively spliced mRNAs. The alternative splicing occurs between amino acids 257 and 258 producing two muscle tropomyosins isoforms specific to different muscle types (Basi et al. (1984) Mol. Cell. Biol. 4:2828). Northern blot analysis and DNA sequencing of genomic DNA indicated that there was also alternative splicing of the adjacent tropomyosin II gene. This has been confirmed by isolating and sequencing cDNA clones encoding a cytoplasmic tropomyosin (cTm) isoform, a muscle tropomyosin (mTmII) isoform and two tropomyosin related proteins. The results show that the four tropomyosin proteins are produced by alternative splicing of four tandemly arranged 3' terminal exons in the tropomyosin II gene at amino acid position 257. This is the same position that the alternate splicing occurs in the tropomyosin I gene. The cTm mRNA has additional alternately spliced internal exons as well. The cTm mRNA and protein is expressed in non-muscle cells, the mTmII transcript is expressed in most or all muscle, and the expression of the tropomyosin related proteins is restricted to indirect flight muscle. Thus, the tropomyosin II gene shows a novel developmentally regulated choice of four alternatively spliced exons encoding the C-terminal ends of the molecules.

N151 POTENTIAL CIS-REGULATORY ELEMENTS IN THREE ANDROGEN RESPONSIVE SEMINAL VESICLE GENES OF THE RAT, Stephen E. Harris, Aria Schifman and Marie A. Harris, W. Alton Jones Cell Science Center, Inc., Lake Placid, New York, 12946.

Two androgen responsive rat seminal vesicle genes, SVS IV and SVS V' have been isolated and sequenced and compared with another SVS V gene. By sequence homology studies, it was suggested that the SVS V' gene arose by a gene conversion event occurring in this gene family. Two highly conserved regions were noted in the 5'-flanking region of all three genes (one at $^{-92}$ CATTTACCGATAAGTAAGGT (A / T) $^{-73}$ and one at $^{-35}$ GGAAATATATAAA $^{-23}$). The SVS IV and SVS V' genes were placed in pSV2-neo and then used to transfect the androgen responsive hamster tumor cell line DDT_1-MF2. Several clones were isolated in which expression of SVS IV and SVS V' gene was observed but little increase occurred by addition of androgen to the culture media. Therefore, the 5'-flanking region of the SVS IV and SVS V' genes (-400 to -3) have been cloned into pSV2-cat and pSV0-cat. A primary culture system has been established for rat seminal vesicle epithelial cells from normal and castrate animals. This system will be tested with the above cat constructions and with the cat construction where the $^{-92}$ and $^{-73}$ region has been deleted by site-directed mutagenesis.

N152 EXPRESSION AT DEVELOPMENTALLY PROGRAMMED LOCI IS CONSERVED DURING THE CELL CYCLE BUT MODULATED BY CELL CONTACT, Raymond J. Ivatt, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

Early embryonic cells and embryonal carcinomas express a family of unusually large and complex glycans. These glycans have been implicated in cellular adhesion and are lost in a programmed manner during differentiation. In addition to these characteristic glycans, embryonal carcinomas express a variety of glycans in common with teratocarcinoma-derived endodermal cells. We have found that these two classes of glycan are regulated independently. Developmentally regulated glycans may be involved in intercellular functions; they are localized at cell contacts and their expression is conserved during the cell cycle but modulated by cellular contacts. In contrast, the glycans common to both stem and endodermal cells may be associated with household functions; they have a diffuse distribution across the cell surface, and their expression shows large, periodic changes during the cell cycle but is not altered by changes in cell contact.

Supported by March of Dimes Basic Research Grant 1-972, and Grant CA42650 from the National Cancer Institute.

N153 ARGININE-MEDIATED REGULATION OF AN ARGININOSUCCINATE SYNTHETASE MINIGENE IN HUMAN CELLS. Marian J. Jackson, William E. O'Brien and Arthur L. Beaudet, Baylor College of Medicine, Houston, Texas 77030.

Argininosuccinate synthetase (AS) activity is increased 5-10 fold in the human cell line RPMI-2650 when grown in medium containing citrulline rather than arginine. Canavanine-resistant variants (Can^r1) overproduce AS several hundred fold and the level of expression is not altered by growth in arginine or citrulline medium. The molecular bases of these regulatory events was investigated using a minigene which linked 3kb of 5'flanking sequence and the first 9kb of the structural gene for AS (including the first 4 exons) to the chloramphenicol acetyltransferase (CAT) gene. Unlike the endogenous AS gene, expression of the CAT minigene was not elevated in Can^r1 cells compared to RPMI-2650 cells. Expression of the CAT minigene in both stable and transient analyses was 4-5 fold higher in RPMI-2650 cells grown in citrulline compared to cells grown in arginine, but unchanged in Can^r1 cells. A protocol was designed to deplete the intracellular arginine pools of Can^r1 cells by transient starvation for arginine and citrulline. Expression of the CAT minigene was increased 10-22 fold in Can^r1 cells subjected to this starvation protocol for 36 to 72 hours during which time the endogenous AS activity remained unchanged. These results indicate that promotor activity and *cis* sequences involved in arginine-mediated regulation of AS are present in the AS genomic sequences in the minigene. The cellular components required in *trans* for metabolite regulation are present in both RPMI-2650 and Can^r1 cells although the endogenous AS gene is not responsive to this regulation in Can^r1 cells. These data help to distinguish various hypotheses for the mechanism leading to enzyme overproduction in Can^r1 cells.

N154 **RIBOSOMAL-PROTEIN GENES OF DROSOPHILA: SELECTIVE REGULATION IN DEVELOPMENT AND STRUCTURAL STUDIES.** M. Jacobs-Lorena, M. Kay, S. Qian, H. Tamate, A. Riedl, & J.-Y. Zhang. Dept. of Develop. Genet. & Anat., Case Western Reserve Univ., Cleveland, OH 44106.

In Drosophila, most of the abundant mRNAs are translated continuously during oogenesis and embryogenesis. Previously we have identified an exceptional mRNA (A1 mRNA) that is translated during oogenesis, **excluded from polysomes in early embryos**, and again associated with polysomes in late embryos. By comigration on 2D-gels and peptide mapping, we have demonstrated that A1 mRNA codes for an acidic ribosomal protein (rp). Further experiments performed both in vitro and in vivo, showed that **most rp-mRNAs**, but not other abundant mRNAs, follow the same regulatory pattern. The observed temporal coincidence between rp-mRNA translation and rRNA synthesis led us to investigate the expression of rp-genes in ovaries of wild type and **bobbed mutants** (flies deficient in rRNA genes). Unexpectedly, neither the rp-mRNA abundance nor its translation was altered in these mutants. The expression of rp-genes in **transgenic flies** carrying extra copies of the rp49 gene is being investigated in collaboration with M. Rosbash's lab. Initial experiments suggest that in these transgenic organisms **rp49 transcipts are overrepresented** (but not in proportion to gene dosage). We are presently investigating if the increased abundance of rp49 mRNA in transgenic organisms is compensated by decreased translational efficiency of this mRNA (**translational regulation**). To further understanding of the mechanisms that selectively regulate rp-gene expression, **several genes** coding for Drosophila r-proteins have been cloned in our laboratory and their **structure** is currently being **analyzed in detail.**

N155 CLONING AND DEVELOPMENTAL EXPRESSION OF NEUROFILAMENT GENES, Jean-Pierre Julien[1], Dies Meyer[2] and Frank Grosveld[2], [1]Institut du Cancer de Montréal, Centre Hospitalier Notre-Dame, Montréal, Canada, H2L 4M1, [2]National Institute for Medical Research, Mill Hill, London NW7.

Mammalian neurofilaments (NFs) are made-up of three proteins with apparent molecular weights of 68,000 (NF-L), 145,000 (NF-M) and 200,000 (NF-H) on SDS-gel electrophoresis. We have isolated DNA clones encoding the mouse NF proteins by cross-hybridization with a previously described NF-L cDNA probe from the rat. Screening of a cDNA library in λgt10 prepared from brain RNA led to the cloning of a NF-L cDNA that spans the entire coding region of 541 amino acids and of a partial NF-M cDNA that covers 219 amino acids from the internal α-helical region and the carboxyterminal domains of the protein. We did not detect any NF-H cDNA clones in our cDNA library by cross-hybridization but screening of a mouse genomic library at reduced stringency led to the isolation of NF-H cosmid clones. Northern blot analyses with specific NF probes show that the expression of NF genes is an early event in brain differentiation as the NF-L mRNAs of 2.5 and 3.5 Kb, and the NF-M mRNA of 3.0 Kb are detected in the early embryonal brain of the mouse at 11 days of gestation. There is a progressive increase in the levels of NF-L and NF-M mRNAs during embryonal and postnatal neuronal development, reaching a maximum at postnatal day 15. In contrast to NF-L and NF-M mRNAs the 4.5 Kb NF-H mRNA accumulates at a significant extent only in the postnatal brain.

N156 SV40 T antigen (Tag) expression directed by the α-crystallin promotor causes tumors in the lenses of transgenic mice. Jaspal S. Khillan, Kathleen A. Mahon, Paul A. Overbeek, Ana B. Chapelinsky*, Joram Piatigorsky* and Heiner Westphal. Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD. 20892 and *Laboratory of Molecular and Developmental Biology, NEI, NIH, Bethesda, MD. 20892

Naturally occurring tumors of the lens are very rare. By targeting the expression of a known oncogene to the lens we have induced lens tumors in transgenic mice. Previously, we have shown that sequences ~ 400 bp 5' to the α-A crystallin gene are sufficient for accurate developmental and tissue specific expression of linked genes in the lens of transgenic mice. By fusing these sequences to the coding region of SV40 Tag and injecting this construct into mouse embryos, we generated seven independent transgenic animals which contain this DNA stably integrated into the germ-line. The lenses of these animals are yellow and opaque - a phenotype which is detectable as soon as their eyes open. Histological analysis of a 3 1/2 month old animal has shown that the organization of the lens is distorted and that normal differentiation of the lens is not achieved. On the histological level, the abnormal lens has all the characteristics of a rapidly proliferating tumor. Immunostaining has shown that the lens cells contain α-crystallin protein. Cells from the lens grown in culture express both α-crystallin and Tag and exhibit properties of transformed cells.

N157 DIFFERENTIAL ORDER OF REPLICATION OF XENOPUS 5S RNA GENES, Laurence Jay Korn, Diane R. Guinta and David Gilbert, Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

In Xenopus laevis there are two multigene families of 5S RNA genes, the oocyte-type 5S genes which are expressed only in oocytes and the somatic-type 5S genes which are expressed throughout development. The replication-expression model (Gottesfeld and Bloomer, 1982; Wormington et al., 1983) of Xenopus 5S expression predicts that the somatic-type 5S genes replicate earlier in the cell cycle than the oocyte-type 5S genes. Hence, the somatic-type 5S genes have a competitive advantage in binding limiting amounts of the transcription factor TFIIIA in somatic cells, and are thereby expressed to the exclusion of the oocyte-type genes. To test the replication-expression model, we determined the order of replication of the oocyte and somatic-type 5S RNA genes. Exponentially growing populations of Xenopus tissue culture cells were labeled with bromodeoxyuridine (BUdR) to increase the density of newly replicated DNA. Cells were then stained for DNA content, which reflects their phase in the cell cycle, and sorted into fractions using a fluorescence activated cell sorter. DNA from cells of each fraction was isolated, and the newly replicated DNA containing BUdR was separated from the lighter, unreplicated DNA by equilibrium centrifugation. The unreplicated and newly replicated DNAs from each fraction of S-phase were hybridized with DNA probes specific for the oocyte and somatic-type 5S genes, in order to determine the time in S-phase when each class of 5S RNA genes was represented most strongly in replicating DNA. In this way we found that the somatic-type 5S genes replicate early in S-phase, while the oocyte-type 5S genes replicate late in S-phase, demonstrating a key aspect of the replication-expression model.

N158 Structure and Regulation of Genes Encoding Embryo Specific Cytokeratins in Xenopus laevis. Alan Krasner, Erzsebet Jonas, Seiji Miyatani, Thomas Sargent, Jeffrey Winkles, and Igor Dawid. Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892

A group of mRNAs coding for cytokeratins appears for the first time during gastrulation in the Xenopus laevis embryo. Three distinct type I and one type II cytokeratin genes have been characterized. There are at least two temporal patterns of keratin gene expression, however all of these genes are expressed in a tissue specific fashion, being found exclusively in ectoderm during gastrulation and in epidermis during subsequent premetamorphic development. The cytokeratin mRNAs studied could not be detected in frogs after metamorphosis. Furthermore, these keratin genes are expressed independently of inductive intercellular interactions suggesting the existence of an inherited cytoplasmic factor controlling their activation. Identification of regulatory elements shared by coordinately expressed keratin genes may aid in the isolation of such putative cytoplasmic factors. In order to identify regulatory elements, we have cloned genomic sequences and are comparing 5' regions of these genes in preparation for studying their expression after microinjection into Xenopus embryos.

N159 GENES INDUCED EARLY DURING F9 TERATOCARCINOMA STEM CELL DIFFERENTIATION, Gregory J. LaRosa and Lorraine J. Gudas, Harvard Med. School and Dana Farber Cancer Institute, Boston, MA 02115

The cultured mouse teratocarcinoma stem cell line, F9, can be induced to differentiate to extra-embryonic endoderm by exposure to low concentrations of retinoic acid (RA). This differentiation is rapid (2-3 days), synchronous and irreversible. Dibutyryl cyclic AMP and theophylline (DBcAMP) enhance the RA induced differentiation, leading to the formation of definitive parietal endoderm cells.

We are isolating cDNA clones for genes whose expression is altered early during the response to RA+DBcAMP. The identification and characterization of such early genes will facilitate the study of the mechanism(s) by which RA+DBcAMP influence gene expression leading to differentiation. Two libraries of ~$2x10^6$ recombinants have been constructed in λgt10 using double stranded cDNA synthesized from poly A^+ RNA of cells treated with; i) RA+DBcAMP + cycloheximide for 4 hours; ii) RA+DBcAMP for 8 hours. ~20,000 recombinants from each have been screened by differential plaque hybridization.

One clone containing a 480bp insert hybridizes to a ~500nt RNA which is induced ~4 fold by 8 hours after RA+DBcAMP addition. The RNA is induced by DBcAMP either alone or in the presence of cycloheximide. This clone is being further characterized. Other clones which exhibit either increased or reduced hybridization after 8 hours of RA+DBcAMP are currently being further characterized. Additionally, the libraries are being screened using a probe enriched in RA+DBcAMP 8 hour specific sequences. This should allow the detection of more clones, possibly of low abundance, induced by RA alone.

N160 MOLECULAR MARKERS FOR THE INDUCTION OF THE NERVOUS SYSTEM IN RANA PIPIENS. Brian T. Livingston and Edward L. Triplett, University of California, Santa Barbara, CA 93106

The involution of chordamesoderm at gastrulation results in the differentiation of the indifferent ectoderm which lies above it into neural ectoderm. This process involves the export of inducer molecules by the chordamesoderm, and a change in the gene expression of the ectoderm. In order to investigate the mechanisms involved, we have isolated a set of cDNAs which are induced during gastrulation in neural ectoderm. A cDNA library was constructed from adult skin, a tissue enriched in derivatives of neural crest cells. This was screened with labeled cDNA made from poly-A^+ RNA isolated from neural tubes dissected from stage 16 embryos. Positives were used to make two identical dot blots. One of these was hybridized to cDNA made from RNA isolated from embryos just beginning gastrulation. The other was hybridized to cDNA made from RNA isolated from neural tube stage embryos. Five clones that were obviously enriched in the later stage were picked and rescreened. The two clones that showed the greatest enrichment were picked for further study. The tissue specificity of these clones is being confirmed by Northern blot analysis of RNA isolated from a variety of adult tissues, and their precise regulation over development is being studied using RNA isolated from different stages of development. Results of these experiments will be discussed at the meeting.

N161 TEMPORAL RELATIONSHIP BETWEEN METHYLATION OF THE MOUSE HPRT GENE AND X CHROMOSOME INACTIVATION IN TERATOCARCINOMA CELLS AND POST-IMPLANTATION MOUSE EMBRYOS, Leslie F. Lock and Gail R. Martin*, NCI-Frederick Cancer Research Facility, LBI-Basic Research Program, Frederick, MD 21701 and *University of California, San Francisco, CA 94143

One of the two X chromosomes in each cell of female mammals is inactivated early in embryonic development resulting in equivalent doses of X linked gene products in female (XX) and male (XY). There is some evidence that suggests that X linked genes are differentially methylated when carried on the active, as opposed to inactive, X chromosome and that DNA methylation is involved in the mechanism of X inactivation. Our studies of the mouse Hprt gene have resulted in identification of restriction sites that map in the 5' region which are always unmethylated when carried on the active X and extensively methylated when carried on the inactive X. The role of such methylation differences in the mechanism of X inactivation was investigated by determination of the temporal relationship between methylation of these sites and X inactivation. It was shown that 1) in a female teratocarcinoma stem cell line containing two active X chromosomes these sites are not methylated prior to X inactivation, however, methylation of these sites occurs when the cells are induced to differentiate and undergo X inactivation in vitro and 2) extensive methylation of these sites in post-implantation mouse embryos is delayed relative to the biochemical and cytogenetic changes that are associated with X inactivation. These observations suggest that DNA methylation might play a secondary role in the maintenance of repression of genes on the inactive X. Sponsored by the NCI-Frederick Cancer Research Facility under contract NO1-CO-23909 with Litton Bionetics, Inc.

Plant and Animal Development — II

N162 CO-ORDINATE REGULATION OF EARLY GENES BY cAMP IN DICTYOSTELIUM Sandra K. O. Mann and Richard A. Firtel, University of California, San Diego; La Jolla, CA 92093

A set of genes expressed early in the developmental cycle of Dictyostelium discoideum appear to be co-ordinately regulated by cAMP. Transcripts of these genes are not detectable in vegetative cells grown on bacteria, but are present at a high level in axenically grown cells. During normal development on filter pads, cells previously grown on bacteria have a detectable level of complementary RNA beginning at about 4 hours, peaking around 6 hours, and decreasing gradually thereafter. Expression of these genes upon starvation in shaking culture is stimulated by pulsing the cells with low levels of cAMP, a condition that mimics the in vivo pulsing during normal aggregation. Expression is inhibited by high continuous levels of cAMP, a condition found later in development when in vivo expression of these genes decreases.

We have examined the structure of these genes and analyzed both the nucleic acid sequence and the derived amino acid sequence. Two of the genes, the M3 tandem duplication, are almost continuously homologous in the protein-coding region and have regions of homology in the 5' flanking sequence. Computer analysis has indicated that a 42 aa region of both these genes shares significant homology with part of the catalytic domain of a mammalian cGMP-dependent protein kinase. In light of the observed cAMP stimulation, this is interesting because there is evidence that cAMP works during aggregation by transiently increasing intracellular levels of cGMP. Another gene, D2, shows extremely significant homology over its entire known length to an acetyl choline esterase. The 5' flanking region of D2 shows some homology to the same regions in the M3 genes.

We are attempting to determine what proteins these genes code for and what their function is during development. We are also using DNA-mediated transformation to indicate which nucleic acid sequences are involved in proper regulation.

N163 A RECESSIVE PRENATAL LETHAL MUTATION IN A TRANSGENIC MOUSE LINE, W. Mark, K. Signorelli & E. Lacy, Slaon-Kettering Inst., 1275 York Ave., NY, NY 10021.

A recessive prenatal lethal mutation was identified in a transgenic mouse line that was generated by the insertion of a bacteriophage λ recombinant DNA into the germ line. This transgenic line contains 4-8 copies of the foreign DNA as a tandem array on chromosome 3. Although animals heterozygous for the integrated DNA appear normal, homozygous progeny are never observed. An analysis of postimplantation embryos derived from heterozygous intercrosses indicates that homozygous animals die in utero before day 7½ of gestation. Examination of preimplantation embryos revealed that the homozygotes are viable and morphologically normal up to the blastocyst stage and that they can trigger a decidual response. However, by day 7½ they are already resorbed, leaving only empty decidua. When embryos obtained from heterozygous intercrosses were cultured in vitro, about 25% of the embryos failed to develop normally. The presumed homozygous embryos arrest at the late blastocyst stage and do not hatch from the zona pellucida. Thus, it appears that the mutation exerts its effect at the time of implantation (day 4-5 of gestation). Cellular sequences flanking the foreign DNA were examined by a Southern blot analysis of restriction digests of the transgenic mouse DNA using the foreign DNA as a probe. A junction fragment was identified and the position of the breakpoint in the foreign DNA was mapped. By using a specific probe derived from this region of the exogenous DNA, a recombinant clone containing 4 Kb of mouse sequence adjacent to one side of the integrated foreign DNA was obtained. A subclone of the mouse DNA is now being used as a probe to isolate the wild type locus prior to the integration event and to identify transcription units present in this region of the mouse genome.

N164 Differential Effect of a Gastrula Nuclear Extract on Expression of Sea Urchin Early and Late H2b Genes Following Injection into Xenopus Oocytes. R. Maxson*, M. Ito*, S. Balcells*, L. Etkin+. *Dept. of Biochemistry, Norris Cancer Hospital and Research Institute, Univ. of South. Cal. Med. School, 2025 Zonal Ave., Los Angeles, CA 90033. +Dept. of Genetics, University of Texas M.D. Anderson Hospital, 6723 Bertner, Houston, TX 77030.

We used Xenopus oocytes as an assay system for factors involved in the differential expression of sea urchin early and late histone gene families during development. After microinjection into Xenopus oocyte nuclei, sea urchin early and late H2b histone genes were transcribed accurately and their cognate RNAs accumulated at nearly equal rates. Coinjection of a salt extract of gastrula-stage sea urchin embryo chromatin stimulated accumulation of early H2b transcripts 2-fold and late H2b transcripts 8-fold. The degree of stimulation of late H2b gene transcription was reduced when an excess of early H2b gene was coinjected, indicating that both early and late H2b genes were affected by the same stimulatory component(s) in the extract. Various fragments of the late H2b gene were tested for their ability to compete with the intact late H2b gene for transcription stimulatory factors. The most strongly-competing fragment comprised the H2b mRNA leader sequence and some adjacent 5' to 3' sequences. In vitro binding studies showed that this fragment contained a high-affinity factor binding site.

N165 IDENTIFICATION OF GENES SPECIFICALLY EXPRESSED IN REPRODUCTIVE TISSUES OF TOMATO. Sheila McCormick, Charles Gasser, and Alan Smith, Monsanto Co., St. Louis, MO 63198

Flower development is an excellent system for studying the tissue specificity and developmental expression of genes. We have prepared cDNA libraries from different developmental stages of tomato anthers and have used differential screening to identify clones expressed only in anthers. Progress on the characterization of these genes will be presented.

N166 INDUCTION OF CHALCONE ISOMERASE GENE EXPRESSION IN PLANT CELLS IN RESPONSE TO FUNGAL CELL WALL COMPONENTS, Mona C. Mehdy and Chris J. Lamb, Salk Institute, San Diego, CA 92138

Plants respond to environmental stress such as UV light by producing protective anthocyanin pigments and to pathogen infection by producing phytoalexin antibiotics. Anthocyanin synthesis is also developmentally regulated and tissue specific in leaf and flower organs. Chalcone isomerase (CHI) catalyzes the formation of an intermediate common to both pigment and phytoalexin biosynthetic pathways and elevated CHI enzyme activity is correlated with increased levels of these compounds in many plants. To assess the genetic basis of enzyme induction, we constructed a λgt 11 cDNA library prepared from mRNA from Phaseolus vulgaris cell cultures treated with a fungal cell wall preparation (elicitor). Two positive clones were obtained by screening 10^5 recombinants with an antiserum raised against purified CHI. The identity of the cloned genes was confirmed by showing that they encode proteins which are immunoprecipitated by the antiserum. Northern blots of RNA from elicitor treated and control cell cultures probed with the CHI clones show substantial accumulation of a 1.1 kb RNA within 3-4 hr after elicitor treatment. The kinetics of CHI mRNA accumulation are similar to those of phenylalanine ammonia-lyase and chalcone synthase mRNAs, which encode other enzymes common to both pathways. The accumulation of CHI mRNA is being further studied by run-off transcription experiments. The CHI cDNA clones hybridize to multiple fragments on genomic DNA blots suggesting the existence of several CHI genes. The structure and organization of the genes are being investigated in order to elucidate the regulatory sequences conferring developmental and environmental inducibility.

N167 STRUCTURE AND REGULATION OF SHEEP METALLOTHIONEIN GENES. M. Gregory Peterson and Julian F.B. Mercer, Birth Defects Research Institute, Royal Children's Hospital, Parkville, Australia.

Metallothionein's (MTs) are a family of low molecular weight, heavy metal binding proteins, unique in their high cysteine content. These proteins are inducible in experimental animals and cultured cells both by exposure to heavy metal ions and glucocorticoid hormones, making them an attractive model system to study regulation of gene expression. Sheep are an interesting species in which to study MT gene regulation because of their unusual copper metabolism in which large amounts of MT-bound copper accumulate in the liver following periods of copper loading.

We have isolated overlapping cosmid clones spanning five sheep MT genes, which represent about half of the MT genes in this species as indicated by Southern blotting. Three of these genes have been identified as MT-I like and one as MT-II like by DNA sequence analysis. Their promoter regions contain multiple copies of a heavy metal responsive elements (HMEs) and a copy of a putative glucocorticoid receptor binding site. The fifth gene has been partially sequenced and shows greater homology with the MT-I genes than the MT-II gene, but its promoter differs markedly from those of the other MT genes. It contains only a single copy of an MRE and has no putative glucocorticoid binding element or 'TATA' box. It has, however, five copies of the hexanucleotide GGGCGG, characteristic of SP1 transcription factor binding sites.

The regulation of this cluster of genes by heavy metals and the synthetic glucocorticoid, dexamethasone in cultured sheep fibroblasts, along with evidence for tissue specific expression of the individual genes will be presented.

N168 CLONING OF DEVELOPMENTALLY REGULATED RAT BRAIN mRNAS, Freda D. Miller, Cary Lai and Robert J. Milner, Research Institute of Scripps Clinic, La Jolla, CA 92037

The development of the mammalian nervous system must involve the temporal and spatial expression of particular sets of genes. In order to understand brain development at the molecular level and analyse the underlying mechanisms of gene regulation, we are attempting to isolate molecular probes for developmentally regulated brain genes. A cDNA library was cloned in pUC18 using poly (A)+ RNA isolated from the brains of embryonic day 16 (E16) rats. Approximately 10,000 colonies from this library were screened with an E16 brain cDNA probe that had been hybridized ("subtracted") with a excess of adult brain mRNA. Positive clones were further screened by differential colony hybridization with E16 vs adult cDNA probes. Using this procedure we have isolated 20 cDNA clones of embryonic mRNAs that are expressed at significantly higher levels in E16 than adult brain. Two classes of developmentally regulated mRNAs can be distinguished: those that are embryo-specific and those that are embryo brain-specific. The developmental expression of these mRNAs is currently being characterized by Northern blot analysis and the cellular localization of several of the mRNAs will be determined by in situ hybridization. Supported in part by NIH grants NS 20728 and NS 21815, NIAAA Alcohol Research Center grant AA 06420, and McNeil Laboratories. F.M. is supported by a Fellowship from the Canadian Medical Research Council.

N169 EARLY CHICK EMBRYONIC CELLS AS A 'MODEL' FOR TERATOCARCINOMA. Mitrani E, Finer M, Boedtker H, Doty P. Biochemistry, Harvard University, Cambridge, Mass 02138.

Embryonic carcinoma (EC) cells have provided a model to study differentiation during early development in higher vertebrates, mainly because of the difficulty of growing normal early embryonic cells in vitro. We have recently been succesful in growing early chick embryonic cells in culture. This has allowed us: to examine differentiation of various isolated cell populations in vitro, to study their differentiation capacities under well defined conditions resembling those of a normal embryo (Mitrani, Nature 288,800 1981), and to establish which extracellular matrix components can be synthesized by each population. We have also recently shown, that the cells can be grown as colonies of single cells as they display anchorage independent growth properties when cultured in agar (Mitrani, Exp. Cell Res. 152,148 1984). We are now using our system to study regulation of retroviral enhancers by normal embryonic cells. Retroviral genomes are not expressed when introduced into preimplantation mouse embryos or into EC cells (Jahner et al, Nature 298,623 1984, Linney et al, Nature 308,470 1985) . The promoter in the LTR of MSV does not function in undifferentiated EC cells. Replacement of the SV40 enhancer by that of MSV results in inactivation of the SV40 early promoter (Gorman et al, Cell 42,519 1985). In an effort to examine if a similar mechanism does occur in normal embryonic cells, we have succesfully transfected our cells with a replication deffective SV40 and are now in the process of replacing the enhancer by that of RSV.

N170 EXPRESSION OF A SPECIFIC GENE IS RELATED TO CONFLUENCE IN AN ADIPOGENIC CELL LINE. Marc Navre and Gordon M. Ringold, Dept. of Pharmacology, Stanford University Medical Center, Stanford, CA. 94305.

TA1 is an adipogenic cell line derived from 10T1/2 mouse embryo fibroblasts. After reaching confluence, TA1 cells express a functional adipose phenotype, and cDNAs corresponding to mRNAs induced during this differentiation have been isolated and characterized. One of these, clone 5, is unusual in that its levels increase and plateau during the pre-confluent phase of growth rather than post confluence, as is typical for other genes induced during adipogenesis. Nevertheless, clone 5 RNA expression appears to be related to the adipogenic program in that: 1) Agents such as dexamethasone and indomethacin that accelerate the adipogenic differentiation and increase the levels of adipogenesis induced RNAs also increase the levels of clone 5 RNA. 2) Clone 5 RNA is found primarily in lung and adipose tissue. This pattern of distribution is similar to that of other adipogenesis induced genes that we have studied. We do, however, see expression of clone 5 RNA in 10T1/2 cells under certain conditions. Preliminary evidence suggests that the levels of clone 5 RNA may be affected by cell growth and/or feeding and could be associated with early steps in commitment to adipogenesis.

N171 STUDIES ON THE MECHANISM OF SUP-3 SUPPRESSION IN CAENORHABDITIS ELEGANS, Anthony J. Otsuka, Department of Genetics, University of California, Berkeley, California 94720.

Suppression of paramyosin mutations by the non-informational suppressor, sup-3, has been shown to result from the formation of thick filaments of normal appearance distributed in regions of partially organized muscle lattice structure. Independently of mutations affecting muscle structure, sup-3 results in the elevation of one (MHCA) of the two body-wall myosin heavy chain isoforms. The correlation of sup-3 mutations with MHCA increase suggests that sup-3 may affect the overall expression of the MHCA structural gene and that the increased level of MHCA may result in improved muscle structure. These results are consistent with the finding by other groups that sup-3 is associated with the amplification of the MHCA structural gene.

N172 THE EXPRESSION OF CHICKEN ACTIN GENES IN MOUSE MYOGENIC CELLS DERIVED FROM EMBRYONIC AND ADULT MUSCLE. Bruce M. Paterson, Anne Seiler-Tuyns, Juanita D. Eldridge and Wolfgang Quitschke, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

We have previously shown that the beta actin, cardiac actin, and the alpha skeletal actin genes of the chicken are expressed in the mouse myogenic satellite cell line, C2C12. In this cell background the transfected chicken beta actin gene regulates in parallel with the endogenous mouse gene; however, the two chicken alpha genes are constitutively expressed at high levels even though the endogenous gene is appropriately regulated during myogenesis. The appropriate pattern of alpha gene regulation is restored when the chicken alpha genes are stably transfected into a mouse myogenic cell line of embryonic origin. These different cell backgrounds have been used to study the regulation of expression of the different actin genes with emphasis on identifying regulatory regions and proteins interacting with these regions.

N173 A MUTANT RNA POLYMERASE II AFFECTS SPECIFICALLY THE TRANSCRIPTION OF MYOGENIC GENES E. Arpala, T. Smith, M. L. Pearson

In rat L6 myogenic cells, α-amanitin selects for at least three classes of amanitin-resistant mutants all carrying lesions in their RPOII structural genes. These mutants frequently exhibit an altered differentiation phenotype as well, and are designated as Myo^+, Myo^- or conditional Myo (the latter referring to the conditional loss of the myogenic differentiation phenotype in the presence of α-amanitin). In Ama27 cells, a representative of the conditional Myo class of Ama^R mutant, the mutant RPOII affects selectively the transcription of the myogenic marker genes encoding α- and β-actin, creatine kinase, and myosin heavy chain, without altering the transcription of constitutive housekeeping genes, glyceraldehyde dehydrogenase, α-tubulin and vimentin. We have constructed a complete genomic library of Ama27 DNA in λEMBL3. This library is being screened for the mutant ama27 gene carrying the α-amanitin resistant RPOII large subunit allele in order to demonstrate by transfection that this gene alone is responsible for the conditional Myo phenotype. If this proves to be the case, this mutant gene should be useful in analyzing the molecular interactions responsible for the selective expression of muscle-specific genes during development.

N174 CHARACTERIZATION AND EXPRESSION OF THE MOUSE PROTAMINE-1 GENE:

Jacques J. Peschon and Richard D. Palmiter
University of Washington, Seattle, Washington.

During mammalian spermiogenesis, histones are replaced by small basic proteins known as protamines. Extensive disulfide crosslinking of protamines results in the formation of a compact chromatin structure devoid of transcriptional activity. In the mouse, two species of protamine exist, protamine-1 and protamine-2. Protamine mRNA is first detected in post meiotic round spermatids, and translated in elongating spermatids.

In an effort to understand the regulation of haploid gene expression, we have cloned and characterized the mouse protamine-1 gene using synthetic oligonucleotides based on the published cDNA sequence(Kleene et al., Biochemistry 24, 719-722, 1985). Sequence analysis reveals the presence of a 90bp. intron, as well as several repeated sequence elements upstream of the TATAA box. We are currently using the protamine-1 promoter to study haploid gene expression in transgenic mice. In addition, we are analyzing the consequences of protamine expression in somatic cells.

N175 REGULATION OF RIBOSOMAL PROTEIN GENES DURING XENOPUS DEVELOPMENT: POSTTRANSCRIPTIONAL AND TRANSLATIONAL CONTROL, Paola Pierandrei-Amaldi, Elena Beccari, Irene Bozzoni and Francesco Amaldi, Istituto di Biologia Cellulare CNR, Centro Acidi Nucleici CNR and II Università, Tor Vergata, Roma, Italy.

The expression of ribosomal protein genes has been studied during the development of normal and anucleolate Xenopus embryos. We have examined the regulation of these genes at the transcriptional, posttranscriptional and translational levels by measuring the synthesis and accumulation of the corresponding mRNAs, the distribution on polysomes and mRNP particles and the synthesis of r-proteins. Cloned genes for some r-proteins have been microinjected in fertilized eggs in order to study how the characteristic developmental pattern of expression of r-protein genes is modified by the alteration of gene dosage.

Moreover a translational control of r-protein gene expression has been also found and studied in oocyte.

N176 EVIDENCE FOR TWO TYPES OF CONTROL OF MYOGENESIS IN DIFFERENT SUBCLONES OF THE MOUSE C2 CELL LINE, Christian Pinset, Janet Ajioka, Christine Laurent, Paul Barton and Robert G. Whalen, Institut Pasteur, 75724 Paris Cedex, France.

We have isolated two types of clones from the mouse myogenic cell line C2. Permissive clones fuse when grown in DME plus fetal calf serum. Inducible clones are unable to differentiate in this medium, however they can be induced to fuse in DME plus insulin and transferrin. Non-permissive medium conditions can also be found, and either permissive or inducible clones grown in MCDB 202 plus serum will attain confluence but stop in the G0/G1 phase of the cell cycle. This growth arrest is not followed by the synthesis of the muscle proteins. Thus, arrest in the G0/G1 phase under these conditions is not sufficient to trigger the myogenic program. Beginning with cells arrested in G0/G1, either type of clone can be induced to undergo myogenic differentiation in serum-free medium. The first detectable synthesis of muscle proteins appears 18 hours after induction in permissive clones whereas in inducible clones synthesis begins only after 48 hours. The isoforms of the various contractile proteins synthesized by the two clones are identical. Using an inhibitor of DNA synthesis araC, we can show directly that DNA synthesis is not required for induction of differentiation in undifferentiated quiescent C2 cells, as in the case of rat L6 cells (Dev. Biol. (1985) 108:284). By refeeding quiescent cells with medium plus serum, both permissive and inducible clones can be made to divide again. The kinetics of reentry into S phase is more rapid in permissive cells (5 hours to onset of DNA synthesis) than in inducible cells (10 hours to onset). The different modes of control of myogenesis in these two cell types may make them useful models for the study of regulatory proteins.

N177 MATERNAL INHIBITION OF HEPATITIS B SURFACE ANTIGEN (HBsAg) PRODUCTION IN TRANSGENIC MICE IS MEDIATED BY DE NOVO METHYLATION. Christine POURCEL, Hend FARZA Michelle Hadchouel, Pierre TIOLLAIS. Unité de Recombinaison et Expression Génétique (INSERM U.163, CNRS UA 271) INSTITUT PASTEUR, PARIS (France).

We have previously described the production of two independant transgenic mice expressing Hepatitis B virus gene specifically in the liver (Babinet et al., 1985, Science, in press). In both mice, the expression of HBsAg was regulated by steroid hormones.
In one of the mouse strain a peculiar transmission of the viral gene expression was observed. Although HBV DNA sequences are transmitted in a mendelian fashion without any rearrangement, HBsAg synthesis is never observed when the sequences are from maternal origin. We have found that de novo methylation of the integrated plasmid and the flanking sequences occured (possibly in the maternal germ line) thus totally preventing the transcription of the viral genes. This methylation is maintained in the next generation. It is not clear at present whether this is induced by the chromosomal sequences where it is integrated or by the presence of the HBV DNA itself.

N178 POSITIVE AND NEGATIVE REGULATORY ELEMENTS OF THE CHICKEN LYSOZYME GENE
R.Renkawitz,J.Altschmied,A.Baniahmad,J.Hell,R.Miksicek*,G.Schütz*, MPI-Gen, D-8033 Martinsried, FRG,*German Cancer Res.Center, D-6900 Heidelberg, FRG

The chicken lysozyme gene is expressed constitutively in macrophages and under steroid hormone control in the oviduct. To study the mechanisms responsible for the specificity we have introduced recombinants of this gene into homologous and heterologous cells.

Fusions of several lysozyme 5'-deletions with the coding region of the bacterial CAT-gene were transfected into different cell types. Transient expression of the hybrids was measured as enzymatic CAT-activity in cell extracts. Cells transfected with a plasmid containing the HSV-TK-promoter in front of the CAT-gene were used as reference.

In every case expression was low compared to TK-CAT. Exceptions were lysozyme deletion mutants retaining approximately 200 bp immediately upstream of the start site: Their expression was high in oviduct cells only after steroid application and in MCF-7 cells (mammary gland; endogenous gene activity not yet determined), in which expression was not affected by hormone treatment.

Two different regulatory elements seem to be present in addition to the steroid control regions:

a) an inhibitory sequence upstream of -200 bp

b) an activator sequence downstream of -200 bp, for which a tissue specificity was shown.

N179 EVOLUTIONARY CONSERVATION OF SEQUENCES EXPRESSED IN SEA URCHIN EGGS AND EARLY EMBRYOS, John W. Roberts, Steven A. Johnson, Terrence J. Hall, Eric H. Davidson, and Roy J. Britten, California Institute of Technology, Corona Del Mar, CA 92625
The evolutionary conservation of several classes of DNA sequences expressed in sea urchin eggs and early embryos was determined by S1 nuclease-thermal denaturation analysis and compared to that for total single copy DNA among three common West Coast species of sea urchins. Between S. purpuratus and S. franciscanus, the measured divergences for cDNAs made from gastrula cytoplasmin poly(A)+ RNA and from unfertilized egg poly(A)+ RNA were half that measured for total single copy DNA. In contrast, sequences expressed in gastrula nuclear RNA were approximately as divergent as total single copy DNA.

N180 HIGHLY UNSTABLE GENOMIC CLONES OF BARLEY DNA CARRYING A DISPERSED REPETITIVE SEQUENCE ELEMENT AND ALEURONE-SPECIFIC GENES. John C. Rogers and Bushra Khursheed, Washington University, St. Louis, MO 63110. We want to understand the mechanism(s) governing developmental and hormonal regulation of gene expression in barley aleurone cells. These cells surround the starchy endosperm of the grain and, during germination, are activated by the plant hormone, GA, to produce very large quantities of a limited number of hydrolases. As part of this work we constructed a barley genomic library in λMG14 and screened the recombinant phage directly without amplification. Multiple, plaque-purified clones hybridizing to either of two different cDNAs representing abundant, aleurone-specific mRNAs (for amylase and PAPI) were obtained. We show that recombinant phage carrying either of these genes are highly unstable, even in E. coli FS575 (RecB21 RecC22 sbcB15 RecAΔ306). Instability is associated with a 6 kb repetitive sequence element; unstable clones rapidly deleted either the amylase or PAPI sequences leaving behind the repetitive element. More rarely, the repetitive elements were lost, leaving the desired gene. "Intact" recombinants could be obtained by avoiding liquid culture and isolating DNA from near-confluent plate lysates, probably by preventing more rapidly replicating phage with deletions from overgrowing the culture. The repetitive sequences are dispersed, representing maybe 100 copies/genome, and are transcribed in shoot but not root or aleurone tissue. The significance of these elements, if any, to tissue-specific expression of the amylase and PAPI genes remains to be elucidated.

N181 ANALYSIS OF CHORION-ADH PROMOTER FUSION CONSTRUCTS IN DROSOPHILA, Charles P. Romano, Brian D. Mariani and Fotis C. Kafatos, Harvard University, Cambridge,MA.
The chorion genes of Drosophila display strict temporal, tissue, and sex specificity in both their amplification and expression. Although genetic screens of female sterile mutants having abnormal egg morphology have uncovered several trans-acting mutants which affect amplification, no trans-acting mutants which affect expression have been found. To directly select for expression mutants, we have fused the upstream control region and promoter of the S15 chorion gene to the structural gene of alcohol dehydrogenase (ADH) and inserted the construct into the germ line of an ADH null strain by P element transformation. S1 nuclease analysis has shown that the transformed lines produce low levels of properly spliced ADH mRNA with the correct tissue and sex specificity. However, all of the transformed lines tested have less than 10% of wild type ADH activity levels and are ethanol sensitive. If expression of the fusion gene in these lines is temporally regulated, they may prove useful in selecting dominant gain of function mutants. Experiments are in progress to determine if expression of the fusion gene is temporally regulated and if other transformed lines carrying the same construct express levels of ADH sufficient to confer ethanol resistance.

N182 DEVELOPMENTAL AND HORMONAL REGULATION OF MMTV. S. Ross, Y. Choi, and A. Siebold, Dept. of Biochemistry, U. of Illinois Medical Center, Chicago, IL 60612

We are studying the regulation of MMTV transcription by subcloning regions of the viral genome and linking them to a variety of different cloned genes. These constructs are then introduced into tissue culture cells by standard transfection procedures to test for the induction of transcription by dexamethasone, or introduced into the mouse germ line by microinjection into embryos to look for tissue-specific enhancers encoded in the viral genome. Thus far, we have shown that at least two of the glucocorticoid receptor binding sites found in the coding region of the viral genome, as well as that found in the long terminal repeat, are capable of supporting hormone regulated transcription from a heterologous promoter. In addition, transgenic mice containing a construct consisting of the LTR linked to the HSV-1 thymidine kinase gene, show appropriate expression in lactating mammary gland and inappropriate transcription in testes. We are presently constructing transgenic mice containing the LTR linked to several other genes to extend these observations. The results of these experiments will be presented.

N183 REGULATION OF INTACT AND HYBRID β AND α ACTIN GENES INSERTED INTO MYOGENIC CELLS. S. B. Sharp, Caltech, Pasadena, CA 91125; T. A. Kost, Norden Labs, Lincoln, NB 68501; S. H. Hughes, Frederick Cancer Research Facility, Frederick, MD 21701; C. P. Ordahl, UCSF, San Francisco, CA 94143; N. Davidson, Caltech, Pasadena, CA 91125.
In order to determine which regions of cytoplasmic and skeletal muscle chicken actin genes contain cis-acting sequences important in regulating their developmentally timed expression, we have transferred intact and hybrid genes into myogenic BC3H-1 cells, made pools of stable transformants, and measured changes in the steady state levels of transcripts from each of the genes over eight day time courses of differentiation. The intact β and α genes were differentially regulated. Chick β mRNA was present in undifferentiated cells, and like endogenous β mRNA, showed substantial reduction during myogenesis. In contrast, chick α message, while not always appropriately upregulated, did not show the overall pattern of down-regulation typical of β. The amount of transcript from an α5'β3' hybrid decreased in a manner similar to intact β transcript. In pools with the converse β5'α3' gene, the amount of transcript first decreased and then reaccumulated to near initial levels. However, the majority of clones thus far isolated from this pool showed only a decrease, with no reaccumulation. One interpretation consistent with these results is that the 3' half of the β gene contains sequences which confer down-regulation on transcripts from the α promoter, but which are not required for down-regulation of transcripts from the β promoter. Experiments are underway to determine whether the observed regulation is transcriptional, and to further describe the regulation of expression of the β5'α3' gene during the later stages of differentiation.

N184 FIBER-TYPE DISTRIBUTION AND DEVELOPMENTAL REGULATION OF MULTIPLE SKELETAL MUSCLE MYOSIN ISOZYMES, Laura Silberstein and Helen M. Blau, Stanford University School of Medicine, Stanford, Ca. 94305.

Vertebrate skeletal muscle development progresses through a series of phases distinguished by the expression of particular sarcomeric protein isoforms, including at least three myosin heavy chain types. Genomic analysis may reveal only the minimum number of potential gene products that could result from differential mRNA splicing. Monoclonal antibodies specific for different myosin heavy chain isoforms were isolated in order to determine the number and spatial distribution of these epitopes at different developmental stages. The results indicate that several distinct stage-specific and fiber-type specific myosins are expressed at each phase of mouse and human skeletal muscle development examined. The relationship between the immunologically defined myosin heavy chain isoforms and specific cDNA clones thought to represent unique fetal, neonatal and adult myosin heavy chain genes will be the subject of future studies.

N185 GLUCOCORTICOID REGULATORY ELEMENT IN THE FIRST INTRON OF THE HUMAN GROWTH HORMONE GENE, Emily P. Slater, Peter A. Cattini, Thomas R. Anderson, Michael Karin, Miguel Beato and John D. Baxter, University of California, San Francisco, CA. 94143

DNA binding and gene transfer studies were performed to examine a potential glucocorticoid regulatory element (GRE) in the human growth hormone gene. The 5' flanking sequences and a fragment containing the first intron were retained preferentially by purified glucocorticoid hormone-receptor complexes in a nitrocellulose filter binding assay. The intron binding site from position +86 to +115 was protected from exonuclease III by the hormone receptor complex. Within this region is a 16 nucleotide sequence that matches the 16 nucleotide consensus sequence for GREs. A fragment containing this sequence was fused to human metallothionein-IIA-thymidine kinase gene fusion that has 170 base pairs of metallothionein 5' flanking DNA deleted of the GRE. Northern blot analysis of RNA from transfectants containing this construction demonstrated that this intron fragment could confer glucocorticoid regulation to this deleted metallothionein promoter. The ability of this intron fragment to regulate the intact human growth hormone gene was analyzed in rat pituitary tumor (GC) cells. The intact gene and a thymidine kinase 5' flanking DNA-human growth hormone structural gene fusion were regulated by glucocorticoids after gene transfer. The growth hormone depleted of the first intron and the 5' flanking DNA-human cDNA gene fusion were not regulated by glucocorticoids in GC cells after gene transfer. The same blots were hybridized with actin to control for RNA loading and with rat growth hormone cDNA to document the response of the rat gene to glucocorticoids. In conclusion, the glucocorticoid receptor binding site in the first intron of human growth hormone is the GRE of this gene.

N186 RELATIONSHIP OF THE AMELOBLAST BIOCHEMICAL PHENOTYPE TO MORPHO- AND CYTO-DIFFERENTIATION. M.L. Snead, E. Lau, M. Zeichner-David, P. Bringas, C. Bessem and H.C. Slavkin. USC, Lab. For Devel. Biol., Los Angeles, Calif. 90089-0191.

The developing mammalian tooth organ requires instructive-, reciprocal-interactions between dissimilar germ layers to determine its morphological, cytological and biochemical phenotype. Migratory neurocrest cells are responsible for instructing competent epithelia to become secretory ameloblasts, through the activation, transcription and translation of a family of genes termed amelogenins and enamelin, which comprise the enamel extracellular matrix. A number of investigators have examined the acquisition of morpho- and cyto-differentiation, however, only recently have specific antibody and cDNA-probes been engineered with which to access the expression of enamel genes. To attempt to align these studies, we have examined the in vivo development of mouse first mandibular molars from 16 days in utero to two days postnatal, using light microscopic techniques in concert with molecular probes for transcription (Northern) and translation (Western) analysis. The results of our experimental protocol indicates that expression of amelogenin gene products is initiated at the new born stage of development, at least two days after the acquisition of molariform shape, or the cytological hallmarks for the ameloblast phenotype. These results indicate that morpho- and cyto-differentiation are not simultaneous with enamel gene expression, and that separate instructive signal(s) may mediate these temporally resolvable events. Moreover, the analysis of unconventional heterotypic recombinants may require evaluation using molecular probes to ensure that the interaction has resulted in enamel gene expression. Supported by USPHS, NIH Grant DE-06988.

N187 CLONING AND PROPER DEVELOPMENTAL EXPRESSION OF AN ADIPOCYTE MYELIN P-2 GENE, Bruce M. Spiegelman, Clayton R. Hunt and Jason H.-S. Ro, Dana-Farber Cancer Institute and the Department of Pharmacology, Harvard Medical School, Boston MA 02115
Adipocyte differentiation involves the expression of many new gene products. We have previously cloned several adipocyte-specific sequences from a mouse 3T3-adipose cDNA library. One such differentiation-specific sequence is 650 bases in size and is a homologue of the immunogenic myelin protein P2 (MP2). RNA for this protein is increased approximately 100-fold during adipocyte differentiation. Southern blotting indicates that this sequence is present in species from Drosophila to human. Transcription of the MP2 gene is greatly increased during fat cell development, as evidenced by increased transcription detectable from isolated nuclei. We have isolated and characterized the gene from a mouse genomic library. It is approximately 4100 bases and contains 3 introns of 2300, 500 and 800 bases. Expression of the cloned gene has been examined by insertion of an oligonucleotide linker into a unique restriction site in the 3rd exon, creating a new site for S1 sensitivity in the transcribed RNA. This cloned gene is activated in stable cell lines undergoing adipocyte differentiation, whereas non-adipose cells do not express this gene. Current studies compare putative regulatory sequences shared by several adipose-specific genes.

N188 REGULATION OF THE MATERNAL-FETAL IMMUNOLOGICAL INTERACTION, K.S. Stranick, J.Locker, H.W. Kunz and T.J. Gill III, University of Pittsburgh, Pittsburgh, PA 15261.
Selective regulation of cell surface antigens is a postulated mechanism for the maternal acceptance of the fetal allograft. Only Class I MHC antigens are expressed on the placenta and the nature of these antigens is crucial in determining the maternal immunologic response to the fetus. In the rat, a nondestructive immune response is elicited by a unique, monomorphic Class I MHC (RT1) antigen, the Pa antigen, which is encoded by a locus which maps between RT1.A and RT1.B. Hybridomas secreting monoclonal antibodies to this antigen have been made from sensitized lymphocytes from the maternal spleen even in the absence of antibody in the maternal serum. Immunohistochemical studies using the avidin-biotin complex method localize this antigen to the basal trophoblast. Blot hybridizations with Class I cDNA probes have detected mRNA specific for Class I antigens in 18 day placentae and maternal liver. Although placenta has much lower levels of Class I transcripts than liver, a 1.5 Kb placental transcript can be distinguished from the prominent hepatic Class I transcripts of 1.3 and 3.1 Kb. These findings support our model that placental expression of Class I antigens with private specificities is suppressed while expression of antigens with public specificities contributes to the unique immunologic privilege of the placenta.

N189 A RAPID AND EFFICIENT BINARY cDNA CLONING AND SCREENING STRATEGY, Terry Thomas, Texas A&M University, College Station, TX 77843

A binary cDNA cloning strategy was devised that allows efficient and rapid construction and screening of large cDNA libraries in the vector λgt10. cDNA representing eukaryotic Poly A RNA populations is ligated into intermediate plasmid molecules containing SP6 and T7 polymerase promoters; recombinant DNA molecules are isolated and cloned into the Eco RI site of λgt10. These libraries can be screened by standard plaque hybridization methods. Subsequent analysis of recombinants is facilitated by the ability to synthesize hybridization probes directly from recombinant bacteriophage templates using the T7 and SP6 promoters and the corresponding phage RNA polymerase. This strategy maximizes the flexibility of the powerful bacteriophage λgt10 system so that different intermediate vectors can be utilized for special applications such as expression of cloned genetic information as a polypeptide. The configuration described here is especially adapted to the rapid isolation and analysis of large numbers of developmentally regulated genes.

N190 THE ROLE OF UPSTREAM SEQUENCE ELEMENTS IN THE TRANSCRIPTION OF XENOPUS HISTONE GENES. Gerald H. Thomsen, Michael Perry, and Robert G. Roeder. The Rockefeller University. New York, New York, 10021.

DNA sequence comparisons of the 5' regions of the Xenopus laevis histone genes of two distinctly organized clusters, Xlh1 and Xlh3, have revealed the existence of unique, gene-specific, upstream sequence elements (USEs) in addition to CCAAT and TATAA sequences. These elements appear to be specific for a given class of histone genes and, with the exception of two elements, appear to be species-specific. An examination of the expression of these genes during oogenesis, early development, and in tissue culture revealed no qualitative changes: the genes of each cluster were expressed at all stages, however minor quantitative differences were seen. This indicates that the developing embryo must alter its histone gene transcription from that of a meiotic (non-S phase, DNA replication-independent) oocyte mode, to that of a cell cycle-coupled (presumably S phase, DNA replication-dependent) somatic cell mode. We are in the process of analyzing the role of the conserved USEs in the transcription of an H4 and an H1B gene (of Xlh1) by deletion mutagenesis and Xenopus oocyte microinjection assays. The role of these elements in transcriptional control, particularly during oogenesis, will be discussed.

N191 EXPRESSION OF ANTI-SENSE ENDO B RNA IN EMBRYONAL CARCINOMA AND PARIETAL ENDODERMAL CELL TYPES, Katrina Trevor and Robert G. Oshima, La Jolla Cancer Research Foundation, La Jolla, CA 92037

Endo B is a developmentally-regulated type I cytokeratin expressed in trophectoderm and extraembryonic endoderm of early mouse embryos and in simple epithelial tissues. Embryonal carcinoma (EC) cells, which resemble early embryonic stem cells, express very low levels of the protein and its mRNA. Treatment of the F9 EC cell line with retinoic acid (RA) results in the expression of Endo B as well as other characteristics of extraembryonic endoderm. To analyze the cellular function of Endo B during embryogenesis and differentiation, we have attempted to specifically block the expression of the protein product using anti-sense Endo B RNA. The anti-sense plasmid contains the first 500 bp of Endo B cDNA opposite to its normal orientation and down-stream of the Py F101 polyoma enhancer and HSV TK promoter. Cells were cotransfected with a 1:10 molar ratio of pSV2-neo and antisense plasmids, and colonies were selected in G418. Transfected endodermal cell clones possessed up to 1,000 copies/cell of integrated plasmid but demonstrated levels of anti-sense RNA only comparable to endogenous Endo B mRNA. There was no obvious change in the level of Endo B protein. In contrast, F9 EC clones integrated 1-10 copies/cell of the plasmid and expressed much lower levels of anti-sense RNA. Retinoic acid-induced differentiation resulted in the expression of Endo B protein with no apparent effect on its regulation. Presently we are employing retroviral vectors, attempting to accumulate significant levels of anti-sense RNA.

N192 The CAAT box transcription factor is necessary for the upstream sequence dependent transcription of the ovalbumin gene. Ming-Jer Tsai, Ikuko Sagami, Heng Wang, Martine Pastorcic, Sophia Y. Tsai and Bert W. O'Malley. Baylor College of Medicine, Houston, Texas 77030.

Using deletion mutants and a cell-free HeLa cell transcription system we have demonstrated that a distal promoter containing the CAAT box is required for efficient transcription of the chicken ovalbumin gene. Competition assays utilizing DNA fragments containing the CAAT box region indicate the presence of a trans-acting factor in the HeLa cell extracts. DNase I footprinting analysis identifies a protein factor in HeLa cell extracts which binds specifically to the CAAT box region between positions -65 and -92 of the ovalbumin gene. We have partially purified this CAAT box binding transcription factor using DEAE-Sephadex, phosphocellulose, Sephacryl -300 and heparin Sepharose column chromatography. This CAAT box binding protein indeed confers upstream sequence dependent transcription of the ovalbumin gene in our in vitro system. In addition, this factor is also necessary for the efficient transcription of the β-globin gene which contains the CAAT box, but not required for the transcription of the SV40 early gene which lacks the CAAT box. Taken together, our studies indicate that the requirement of distal promoter for the expression of ovalbumin gene is due to the binding of the CAAT box transcription factor to the CAAT box region. Furthermore the CAAT box transcription factor is specific for a class of genes contain CAAT box in their promoter.

N193 THE XENOPUS LAEVIS TFIIIA GENE, J. Tso and Laurence Jay Korn, Department of Genetics, Stanford University School of Medicine, Stanford, California 94305

There are two types of 5S genes in Xenopus - the oocyte-type genes, which are expressed only in oocytes, and the somatic-type genes, which are expressed throughout development. The transcription factor TFIIIA, a protein that is specifically required for transcription of 5S genes, has been implicated in the developmental control of these genes. The level of this protein factor is high in oocytes, but is insufficient for binding all 5S genes in somatic cells. It has been postulated that the limiting level of TFIIIA, preferential binding of the factor to somatic-type 5S genes and the differential replication timing of the two classes of 5S genes, leads to the almost exclusive expression of the somatic-type 5S genes in somtic cells.

Since TFIIIA appears to play a critical role in regulating the expression of the 5S genes, we wish to study how its expression is in turn controlled. To facilitate these studies, we have isolated and sequenced the gene for TFIIIA. The TFIIIA gene is about 11 kb in length and contains 9 exons. The exon/intron structure correlates well with the repeating DNA binding structure predicted from the amino acid sequence of the protein (Miller et al., EMBO J. 4, 903, 1985), suggesting that the TFIIIA gene has evolved from a primordial DNA binding unit by multiple gene duplications.

N194 RESTING MAMMARY EPITHELIAL CELL-SPECIFIC "SILENCER" ELEMENT IN SOME MMTV PROVIRAL LTR DNA, A.B. Vaidya, R.W. Connors, and P.R. Anné, Hahnemann Univ., Philadelphia, PA

At every cycle of pregnancy and lactation mammary epithelial cells undergo proliferation and terminal differentiation with most of their biosynthetic capability devoted to the production of milk proteins. Between pregnancies the committed epithelial cells are not engaged in the milk protein synthesis and transcription from genes encoding such proteins is turned off. In some mouse strains, the endogenous MMTV proviral gene transcription is regulated as if these genes were encoding a milk protein, thus providing a system to study mammary epithelial specific gene expression. We have used an epithelial cell line (C57MG) established from the mammary glands of a retired C57BL breeder female for stable transfections with clones of MMTV and chimeric genes containing MMTV LTR. The C57MG cells appear to be of resting mammary epithelial origin. Transfected endogenous MMTV proviruses were transcribed in the fibroblastic L cells, but no MMTV transcripts could be detected in similarly transfected C57MG cells. A chimeric gene containing MMTV(C3H) LTR joined to the mouse DHFR gene was not expressed when introduced into the C57MG cells. Chimeric genes containing HSV TK gene joined to MMTV(C3H) LTRs with increasing deletions of their U3 region were then used to localize the silencer element. A deletion of the LTR sequence upstream from the -80 position released the TK gene from the suppression of transcription. We have previously observed that the C57MG cells are capable of supporting the replication of MMTV(RIII) and that the C57BL mice are susceptible to tumorigenesis by MMTV(RIII) but not by MMTV(C3H). Our present results suggest that the resting mammary epithelial cells from the C57BL mice are able to "silence" the expression from the endogenous MMTV and MMTV(C3H) LTR but not from the MMTV(RIII) LTR.

N195 REGULATION OF TRANSCRIPTION OF THE CYTOKERATIN ENDO A GENE IN THE EARLY MOUSE EMBRYO AND IN TERATOCARCINOMA CELLS. M. Vasseur, P. Duprey and F. Jacob, Unité de Génétique cellulaire du Collège de France et de l'Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15, France.

The expression of the cytokeratin Endo A has been analyzed during mouse blastocyst formation and embryonal carcinoma cell differentiation. In order to study the regulation of Endo A expression, S1 mapping experiments have been performed on RNA extracted from 2-cell to 7.5 day embryos. Low levels of Endo A mRNA begin to be detectable in 8-cell embryos. The amount of this mRNA increases at the blastocyst stage, suggesting that Endo A expression is regulated at the mRNA level during blastocyst formation. At this stage, in situ hybridization studies show that Endo A mRNA is present in the trophectoderm but not in the inner cell mass. In 7.5 day embryos, Endo A mRNAs are also detectable in the endoderm layer and in the amnion. To study the promoter of the Endo A gene, and to analyze its efficiency in different cell types, we used the Chloramphenicol Acetylase (CAT) system. We have designed constructions i) to test the efficiency of Endo A promoter and ii) to investigate the enhancing effect of sequences located upstream or downstream the mRNA cap site and to analyze their potential role in the tissue specific expression of the Endo A mRNA. The possible role of a B2 repetitive sequence in the regulation of Endo A gene transcription will be discussed.

N196 SEQUENCES CONTROLLING CELL-SPECIFIC EXPRESSION OF THE RAT INSULIN 1 GENE. Michael D. Walker*, Olof Karlsson+, Thomas Edlund+, Jennifer Barnett* & William J. Rutter*
*Hormone Research Institute, Univ of California, San Francisco, CA 94143 and +Dept of Applied Cell & Molecular Biology, Univ of Umea, Umea, Sweden

Cell-specific expression of the rat insulin 1 gene is attributable to two distinct DNA elements located in the 5' flanking DNA. The first element is an enhancer whose activity is restricted to insulin-producing cell types. It is located between nucleotides -103 and -333. Enhancer replacement experiments have revealed the presence of an additional cell-specific element located between nucleotides -113 and -1.

We have now carried out internal deletion and substitution mutagenesis experiments. Deletion of sequences from -113 to -198 has little effect on the transcriptional activity of the intact flanking DNA. On the other hand, a relatively small deletion (-104 to -112) leads to a 10-fold reduction in transcription. This result has been confirmed by construction of a substitution mutation of nucleotides -104/-112. The effect of this mutation is reflected in a corresponding loss of ability to enhance a heterologous promoter (thymidine kinase). We will report the activities of further block replacement mutants to identify precisely other sequences in this region involved in mediating cell specificity.

N197 EXPRESSION OF NEUROFILAMENT POLYPEPTIDES AND NEUROPEPTIDES IN DIFFERENTIATING F9 CARCINOMA CELLS. J. Wartiovaara[1], M. Tyynelä[1] and L. Rechardt[2]. Depts of Electron Microscopy and Medical Biology[1], Univ. of Helsinki, 00280 Helsinki, and Dept. of Biomedical Sciences[2], Univ. of Tampere, 33101 Tampere, Finland.

Mouse F9 line embryonal carcinoma cells in vitro differentiate into neural direction in presence of retinoic acid and dibutyryl cyclic AMP (Liesi, P., Rechardt, L., and Wartiovaara, J.: Nature 306,265,1983). Specific acetylcholinesterase but no markers of glial differentiation including GFA-protein, myelin basic protein or galactocerebroside are found in the appearing bi- and multipolar cells. In long term cultures (18 days) strong immunofluorescence staining with antibodies against neurofilament triplet proteins is seen but expression of 200 kD neurofilament antigen has been undetectable. Nerve growth factor (NGF) combined with the other two drugs greatly enhances differentiation and induces expression of immunoreactivity for tyrosine hydroxylase, Leu-encephalin-like peptides and serotonin. NGF strengthenes staining with anti-neurofilament triplet proteins but staining with anti-200 kD filament protein remains negative. The present results suggest that NGF has an enhancing effect on the neuronal differentiation of F9 embryonal carcinoma cells but long cultivation times are needed for expression of neurofilament peptides.

N198 ANALYSIS OF PROTEINS SPECIFIC FOR FIBROBLAST-LIKE CELLS FROM EMBRYONIC MUSCLE. Zipora Yablonka-Reuveni, Steven C. Braddy and Mark Nameroff, Department of Biological Structure, University of Washington, Seattle, WA 98195

Cell Suspensions prepared from embryonic skeletal muscle contain, in addition to myogenic precursor cells, non-myogenic cells which, in tissue culture, exhibit the morphology of fibroblasts. In order to further characterize these so-called muscle fibroblasts, we have analyzed the proteins of these cells by one- and two- dimensional polyacrylamide gel electrophoresis. Muscle fibroblasts were prepared directly from the breast muscle of embryonic chicken employing Percoll density centrifugation. Soluble and particulate cellular fractions were then prepared from the isolated cells and analyzed. When the results of these analyzes were compared with parallel fractions from myogenic cells, at least five distinct proteins specific for the fibroblast-like cells were detected. Most of these specific proteins reside in the particulate fraction. The proteins studied are of cells prepared directly from the embryo, thus reflecting the in vivo status. This is in contrast to many studies on fibroblast-like cells (including muscle fibroblasts) where cells have been prepared by sequential passaging. In the case of the muscle system, the latter approach is problematic as myogenic precursor cells may give rise to cells which morphologically resemble fibroblasts but are not nescessarily identical to those isolated directly from the embryo. (Supported by grants from the American Heart Association Washington Affiliate to Z.Y.R and from the National Institutes of Health to M.N).

N199 TISSUE SPECIFIC EFFECT OF DNA METHYLATION, Joel Yisraeli and Howard Cedar, Hadassah Medical School, Hebrew University, Jerusalem, Israel

The activation during development of a large number of mammalian genes is accompanied by specific demethylations. In order to understand what role methylation may play in the regulation of gene expression during development, we have introduced methylated and unmethylated α actin constructs into the inducible rat myoblast line L8. Although methylation significantly inhibits the expression of these constructs in fibroblasts, the myoblasts are able to overcome this inhibition. The expression of the methylated and unmethylated exogenous α actin constructs can be induced to the same extent upon serum reduction, which causes cell fusion in this line. Analysis of the methylation pattern of the introduced methylated genes reveals that very specific demethylations have occurred in approximately 25-40% of the transfected molecules. In one particular case, a site which is six nucleotides away from a site of demethylation remains fully methylated. These specific demethylations found in the exogenous genes precisely mimic the methylation pattern found in vivo in the myoblasts. To the best of our knowledge, these results represent the first demonstration of a tissue-specific effect of DNA methylation on gene expression. We have also shown that myoblasts are capable of tissue specific demethylations of transfected DNA. We are currently exploring the mechanism of these tissue-specific effects.

N200 Developmental regulation of suppressor-of-white-apricot transcription. Z. Zachar, T.-B. Chou and P.M. Bingham. SUNY, Stony Brook NY 11794

Retrotransposons are developmentally programmed transcription units apparently responding to developmental cues produced by their cellular hosts. In Drosophila and yeast allele-specific suppressor loci are known that act (formally, genetically) on specific retro-transposon insertion mutations in a way suggesting that these loci produce the cues involved in developmental regulation of retrotransposon transcription. We have cloned and analyzed one of these loci from Drosophila - the suppressor-of-white-apricot [su(w^a)] locus - that acts on the copia retrotransposon insertion allele at white, w^a. We find that su(w^a) transcription (transcript levels) is positively regulated by the su(w^a) gene product itself (autoregulated) and is independently regulated (repressed) by the product of a second allele-specific suppressor locus, suppressor-of-forked. Our results further suggest that transcription of su(w^a) may be initially activated in development by maternally deposited su(w^a) message - su(w^a) may be its own maternal determinant. We lastly find that the su(w^a) locus protein product (conceptual translation product) has an exotic amino acid composition similar to several previously characterized eukaryotic DNA binding proteins.

Late Additions

N201 TRANSVECTION AND THE CIS-REGULATION OF THE BITHORAX GENE COMPLEX IN DROSOPHILA, E. B. Lewis and Rollin H. Baker, California Institute of Technology, Pasadena, CA 91125

We have devised a global rearrangement (GR) screen that can detect virtually any rearrangement having a breakage point within the bithorax gene complex (BX-C). The screen depends upon an early finding that Cbx Ubx/+ flies have spread wings whereas flies of this genotype that are also structurally heterozygous for transvection-suppressing rearrangements (TSRs) [namely, those having one breakage point within, or proximal to, the BX-C and the other(s) elsewhere in the genome] have wild-type wings. Using the GR screen we have detected breakage points in the distal half of the BX-C as follows: two in iab-2; one in iab-3; two in iab-4; three in iab-5 and two in either iab-7 or iab-8. Such breakage points tend to show strong CIN effects (cis-inactivation of one or more genes distal to the breakage point). Certain breakage points falling in regions distal to iab-2 show, in addition, COE effects (cis-overexpression of one or more genes lying proximal to the breakage point). These results extend to the distal half of the BX-C phenomena first detected in the proximal half. To explain the CIN and COE phenomena a cis-acting regulatory entity (E) is invoked. E is assumed to track the chromosome in a direction opposite to that of transcription and to be required for efficient transcription of the BX-C. Transvection would on this model result from greater effective transport of E from one chromosome to its homologue when the chromosomes are somatically paired than when such pairing has been disrupted by a TSR. (Work supported by USPHS grant HD06331).

N202 REGULATED GENE EXPRESSION IN TRANSGENIC PLANTS, J. Schell, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, and Laboratorium voor Genetika, Rijksuniversiteit Gent, B-9000 Gent

Gene transfer methods were used to study the role played by regulatory sequences on the expression of chimeric genes in plants. These experiments were designed to test whether sequences located in the 5' upstream region of a number of regulated plant genes, could be used to control the expression of chimeric genes in transformed plants. The best studied examples thus far are nuclear genes coding for proteins transported into chloroplasts such as rbcS and LHCP (light harvesting complex). Relatively short DNA sequences in the 5' upstream region of these genes were shown to have both "Enhancer" and "Silencer" functions (M.P. Timko, A.P. Kausch, C. Castresana, J. Fassler, L. Herrera-Estrella, G. Van den Broeck, M. Van Montagu, J. Schell and A.R. Cashmore (1985) Nature (Lond.) 318, 579-582; J. Simpson, M.P. Timko, A.R. Cashmore, J. Schell, M. Van Montagu and L. Herrera-Estrella (1985) EMBO J. vol.4, n°11, 2723-2729). In the case of the chalcone synthase gene of Antirrhinum majus (H. Kaulen, J. Schell and F. Kreuzaler (1986) EMBO J., in press) 5' upstream sequences were shown to direct the expression of a NPTII reporter protein in tobacco. This expression was found to be regulated by UV light and probably also by cytokinins. Different sequence-motives were found to be responsible for enhancerment and for induction by UV light. In the case of the leghaemoglobine of soybean (J. Stougaard, K. Marcker, L. Otten and J. Schell, in preparation) it was found that 5' upstream sequences were able to direct the nodule specific expression of a reporter chloramphenicol transacetylase (CAT). Expression was regulated at the transcriptional level and followed the correct developmental timing of the original leghaemoglobine gene.
Finally 5' upstream sequences derived from the hsp70 heat-shock gene of Drosophila were shown to be able to convey heat-inducibility to a NPTII reporter enzyme in tobacco (A. Spena, R. Hain, U. Ziervogel, H. Saedler and J. Schell (1985) EMBO J. vol.4, n°11, 2739-2743). All the examples studied thus far have therefore indicated that regulatory sequences located in 5' upstream regions of regulated genes are sufficient to determine the specifically regulated expression of chimeric genes in transgenic plants. Position effects do however occur and can influence expression in a quantitative but not (thus far) in a qualitative way. Promoter sequences can now be put together with regulatory elements derived from different genes. Such chimeric promoter sequences can be used to predetermine the expression of foreign genes in transgenic plants.

N203 PLASTICITY OF DIFFERENTIATED CELLS, Helen M. Blau, Grace K. Pavlath, Edna C. Hardeman, David Engelberg, Laura Silberstein, Steven G. Webster, Steven C. Miller, Kimber Lee Poffenberger, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.

Stable heterokaryons in which cell and nuclear division do not occur provide a model system in which to examine how different tissue-specific phenotypes arise and are maintained. Following fusion of muscle cells with nonmuscle cells, muscle gene expression is activated in the nonmuscle cell type. The muscle gene products of nine different genes were detected when gene activation was studied either at a single cell level with monoclonal antibodies or in mass cultures at a biochemical and molecular level. In all of the nonmuscle cell types tested, including representatives of different embryonic lineages, phenotypes, and developmental stages, muscle gene expression was induced. However, the regulatory mechanisms responsible for gene activation are likely to differ among cell types, since the kinetics, frequency, and gene dosage requirements for gene expression differ. These results show that the expression of genes in the nuclei of differentiated cells is plastic and can be altered by exposure to a foreign cytoplasm. Since the nuclei of the two cell types remain separate, gene activation is presumably mediated by tissue-specific trans-acting regulators. The cell types activated in heterokaryons provide useful test-systems for the identification of these regulatory molecules.