DEVELOPMENTAL BIOLOGY

Organizers: Eric Davidson, Joan Ruderman and James Posakony

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Poster Sessions

Homeotic Genes

J 001 SECONDARY STRUCTURE AND FUNCTION OF THE HOMEODOMAIN: Identification of helix-turn-helix motif and sequence specific DNA binding

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Homeotic genes and several other genes controlling development share a characteristic DNA segment, the ***homeobox***. This DNA sequence of ~180 bp was first discovered in *Drosophila*, but was subsequently found in many other metazoa including vertebrates and also in man. It encodes a section of the homeotic proteins which is called the ***homeodomain***, and comprises a sequence of 62 amino acids that has been highly conserved throughout evolution (1). Computer searches through protein sequence data banks have revealed a small but significant degree of homology (22-26% identity) to the yeast mating type proteins MAT a1 and alpha 2 of *Saccharomyces cerevisiae* and P1 of *Schizosaccharomyces pombe*. Since the MAT genes of yeast encode gene regulatory proteins and determine cell type specificity, this observation, along with genetic considerations and some sequence homologies, suggested a gene regulatory function of hoemotic proteins. On the basis of structural considerations and some sequence homologies, it had been proposed earlier that the MAT a1 protein may contain a helix-turn-helix motif as is found in various prokaryotic gene regulatory proteins that bind to specific DNA sequences. Limited sequence homology between the homeodomain and the prokaryotic DNA binding proteins has also been found. This suggested that the homeodomain represents the DNA binding domain of the homeotic proteins and that it contains a helix-turn-helix motif. In order to test these hypotheses the homeodomain encoded by the *Antennapedia (Antp)* gene of *Drosophila* was overproduced in a T7 expression vector in *E.coli*. The corresponding polypeptide of 68 amino acids was purified to homogeneity. The homeodomain was assayed for DNA binding (2) and analyzed in solution by NMR spectroscopy (3). The secondary structure determination reveals a helix-turn-helix motif as prediced on the basis of DNA sequence comparisons. An additional alpha-helix region is located towards the amino terminus of the homeodomain. DNA binding studies indicate that the isolated homeodomain binds to DNA *in vitro*. It selectively binds to the same sites as a longer *Antp* polypeptide and a full-length *fushi-tarazu (ftz)* protein. Therefore, the homeodomain represents the DNA binding domain of the homeotic proteins and forms a helix-turn-helix structure.

Reference

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2) Müller, M., Affolter, M., Leupin, W., Otting, G., Wüthrich, K. and Gehring, W.J. (1988). EMBO J. **7**, No. 13
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DNA-Protein Interactions in Developmental Gene Regulation

J 002 REGULATION OF GLOBIN GENE EXPRESSION DURING ERYTHROID DEVELOPMENT,

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The globin gene family has provided a popular model system for the study of developmental regulation of gene expression. We have carried out investigations of the organization of promoters and enhancers that control the globin genes of chicken, and have identified a variety of factors, some ubiquitous and some erythroid - specific, that bind selectively to the DNA of these regulatory regions. One of the factors, which we have named Eryf1, is found only in erythroid cells and has at least one binding site in either the promoter or enhancer of every member of the α- and β-globin families. Eryf1 is likely to function as a general positive regulatory factor for genes expressed in erythroid cells. The α-globin genes α^D and α^A have Eryf1 sites in their promoter and enhancer respectively, but are otherwise rather uncomplicated. We speculate that this may reflect the simple regulatory program of these genes: α^D and α^A do not require modulation, since they are expressed in both primitive and definitive erythroid cells. In contrast, β^A, which is expressed only in definitive cells, has an enhancer and promoter with binding sites for a large number of factors. We show that one of these, the Pal factor, is probably involved in shutdown of β^A-globin synthesis at maturation. These results suggest that there are two classes of factors, those with erythroid-specific effects on all globin genes and those that serve to modulate the expression of individual members of the family.

Cell Biology of Egg Activation

J 003 Reprogramming Protein Synthesis for Rapid Cell Cycles: A Model to Explain Major Patterns of Gene Expression during Early Development, Matthew Winkler and Leslie Kelso Winemiller, Center for Developmental Biology, Department of Zoology, University of Texas, Austin, TX 78712.

Early development is characterized by a complex set of changes in the amounts and kinds of proteins synthesized. We used 2D-PAGE to study these changes and found several distinct patterns of expression (1). Actin, tubulin and other abundant cellular proteins are major synthetic products in the oocyte and reappear at the blastula and later stages. During the rapid cleavage stage another distinct set of proteins are transiently synthesized. Several of these proteins are known to play important roles in the cell cycle. A hypothesis to explain many of the observed changes is that the embryo shuts off synthesis of "housekeeping" proteins to accommodate the high level synthesis of proteins required for rapid cell cycles. We have used cloned cDNA probes, isolated from a cleavage stage library, to study changes in expression of cleavage stage mRNAs. The behavior of these mRNAs appear to fall into 2 distinct classes. The first are the more abundant mRNAs which are present at high levels during the rapid cleavage stage and then decrease sharply by the blastula stage. These mRNAs move out of polysomes and temporarily accumulate as free mRNP during the period when their levels are decreasing. This contrasts with members of the second class, such as tubulin mRNA, which is present at low levels in the egg and increase as development proceeds. These mRNAs move into polysomes at the same time that cleavage stage mRNAs are coming out of polysomes. For example, at 18 hours when 99% of the tubulin mRNA is in polysomes, only 36% of a cleavage specific mRNA is in polysomes. In addition, at fertilization, tubulin mRNA moves into polysomes significantly slower than cleavage stage mRNAs. These results suggest that there are translational regulatory mechanisms which differentially regulate the entry and exit of specific mRNAs from polysomes.

1. Grainger et al.(1986) Dev. Biol. 114, 403-415.

Gene Control of Developmental Events in Plants

J 004 SEQUENCES FOR CELL-SPECIFIC EXPRESSION IN TRANSGENIC PLANTS, Philip N. Benfey and Nam-Hai Chua, Laboratory of Plant Molecular Biology, Rockefeller University, New York, NY 10021. Analysis of the 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase gene from petunia revealed that sequences 5' of -800 can confer expression specific to floral tissue. Expression is also developmentally regulated, increasing as the flower matures. Using histochemical localization the cell specificity of expression in floral tissues has been defined. This expression is contrasted to that of the 35S promoter of Cauliflower Mosaic Virus, a nominally constitutive promoter which shows a definite specificity of expression in floral tissues. (Supported by a grant from Monsanto).

J 005 A RELATIONSHIP BETWEEN DEVELOPMENTAL AGE AND COMPETENCE DURING THE ACQUISITION OF CELL FATE IN THE MAIZE LEAF. Michael Freeling and laboratory, Department of Genetics, University of California, Berkeley, CA, 94720.-- The maize leaf has been nurtured as a system to the point where questions of plant development may actually get answered. Because plant cells do not rotate or migrate, it has been possible to distinguish a cell's age from its position. My laboratory is describing several leaf mutants that specify organ transformations that may disconnect age from position; and different maize lines differ greatly in generation-time (i.e., the meaning of a developmental minute). The maize leaf as a developmental system has been reviewed (1).-- The maize leaf is a gradient of developmental age from the tip of the blade to the base of the sheath. Between younger sheath and older blade is the auricle-ligule. Histology, mutant descriptions, and the analysis of genetic mosaics for liguleless mutant tissue implies that a specific "make auricle and ligule" signal is generated at a specific time at the midrib and is somehow propagated toward the margin. The product of the Lg1 wildtype allele is to encode a product necessary to both make ligule and to propagate the signal. This signal overrides the default cell division programs that we have also been describing for leaf growth.-- Our continuing studies of the Knotted-1 (Kn1) mutants lead to a working hypothesis predicting that the relatively undifferentiated tissue that surrounds the lateral veins of Knotted leaves is actually "young for its age" and is, thus, out-of-place sheath. SEM examination of epidermal cuticle supports this prediction. I will present evidence that cells in the primordial leaf undergo "default" cell division and maturation programs through which they acquire developmental age, and that various ages along the way correspond to particular competences to "hear" signals that pass over the leaf at various moments. Leaf developmental mutants have phenotypes that fit this notion; these are being characterized, transposon-tagged, and will eventually be used to identify meaningful molecules.

(1) Freeling, M., Pierce, D.K.B., Haarberd M., Lane, B., Hake, S., 1988. In: Temporal and Spatial Regulation of Plant Genes. Verma, D.-P.S. and Goldberg, R.B., eds. Springer-Verlag, NY pp. 41-62.

J 006 GENETIC AND MOLECULAR ANALYSIS OF FLOWER DEVELOPMENT IN *ARABIDOPSIS,* Elliot M. Meyerowitz, John Bowman, Hong Ma, David Smyth and Martin Yanofsky, Division of Biology, California Institute of Technology, Pasadena, California 91125 U.S.A.

In *Arabidopsis*, each flower consists of four sepals, four petals, six stamens and an ovary of two carpels, with all of these organs appearing in the same arrangement and number on the hundreds of flowers of each plant. These flowers develop from groups of undifferentiated cells produced by the shoot apical meristem. The mechanisms by which these cells determine their position in the flower primordium, and consequently differentiate into the cells of the appropriate organ, are unknown. As a means of discovering some of these mechanisms we have studied a series of mutations which cause homeotic transformations of organs in *Arabidopsis* flowers.

One example of such a mutation is *agamous*, a recessive mutation on the fourth chromosome. When homozygous, *agamous* plants have flowers that contain large numbers of sepals and petals, with no stamens or carpels. Observations of developing *agamous* flowers show that the primordia of sepals, petals and stamens form normally, but the cells that in wild type plants would form the ovary differentiate into a new flower, with a new set of sepal, petal and stamen primordia. This process repeats itself several times. In addition, the primordia in the position of normal stamen primordia develop into petals. Another recessive, single-gene mutation on chromosome four is *apetala2*, which causes the sepals to develop as leaves, and petals to develop as stamens. The mature mutant flower thus has a whorl of leaves surrounding ten stamens, themselves around a normal ovary.

We have taken three approaches to understanding the wild type function of the genes giving such homeotic phenotypes. One is a detailed phenotypic analysis of the development of the mutant flowers, including analysis of the temperature-sensitive period of two mutations which are fully expressed only at high temperatures. This analysis has given us an idea of what processes are affected in the mutants, and in the case of *apetala2*, one of the temperature-sensitive mutations, has shown that the wild type gene product acts very early in flower development. A second approach has been genetic, including the making of double mutant plants to study the epistatic relations of the flower genes, and mutagenesis to obtain new alleles of the original mutations. The third approach is molecular: we have used two methods in attempts to clone some of the genes. An insertional mutagenesis with *Agrobacterium* T-DNA (in collaboration with Dr. Ken Feldmann of E.I. Dupont Corporation) has allowed cloning of *agamous*. Other genes are being sought by chromosome walking. We have made an RFLP map of *Arabidopsis* so dense that 50% of the genome is within 270 kb of the DNA markers on the map. By mapping *apetala2* relative to the molecular markers, DNA less than 2 cM from the gene has been obtained. Since 2 cM represents, on average, less than 300 kb of DNA in *Arabidopsis*, we are now successively isolating overlapping clones as a means of cloning the *apetala2* gene.

J 007 GENES ASSOCIATED WITH FLORAL INDUCTION, Jim Peacock, Jill Wahleithner, Liz Dennis, Alan Neal, Marianne Lund and Ry Meeks-Wagner*, Division of Plant Industry, CSIRO, Canberra ACT 2601, Australia and *Institute of Molecular Biology, University of Oregon, Eugene, OR, 97403, U.S.A.

Since the transition from vegetative to floral development in plants is likely to be influenced by gene expression in several plant organs we have used an *in vitro* system, the tobacco 'thin cell layer' (TCL) system, as a model for investigating gene expression associated with the initiation of flowering in higher plants. cDNA cloning has been used to identify mRNAs abundant during TCL floral initiation. These genes are expressed in TCL explants initiating floral meristems but not in TCL explants initiating vegetative shoot meristems or possessing roots. Two of these genes are transcriptionally expressed in incipient floral apices during normal plant development and there are differential levels of expression of the genes in floral branches and buds. Analyses have shown that four of the genes (KS-1, -2, -3 and -5) are expresssed at low levels throughout plant development in most organs. KS-1, -2 and -5 are most highly expressed in the roots of tobacco plants that have 9-10 leaves greater than 3 cm. The involvement of roots in flowering has been demonstrated using grafting experiments. We have now identified KS-1, -2, -3 and -5, respectively, as the genes for chitinase, a gene for a product related to pathogenesis protein S (PR-S), extensin and B-1,3 glucanase. All these are pathogenesis-related (PR) proteins but previous studies have shown that healthy tobacco plants accumulate PR-proteins during floral induction, as well as during viral infection. Tobacco species which require specific daylengths to flower are being used to determine whether there is a correlation between the expression of these 4 PR-proteins and flowering.

Hormonal Regulation of Maturation and Development

J 008 **MESODERM INDUCTION BY BASIC FIBROBLAST GROWTH FACTOR**

David Kimelman and Marc W. Kirschner, Department of Biochemistry, University of California, San Francisco, CA 94143

We have previously demonstrated that bovine basic fibroblast growth factor (bFGF) and transforming growth factor-β1 can function synergistically to induce mesoderm in explants of early Xenopus embryos (Kimelman and Kirschner, 1987). Furthermore we have shown that a 1.0 kb message for bFGF is present in Xenopus oocytes and after the midblastula transition. Our cDNA clones from this message contained only the third exon of bFGF, suggesting a cloning artefact.

We have now found that a 4.0 kb message is present in both the oocyte and late-stage embryos which encodes a protein that is 84% identical to human and bovine bFGF. When the Xenopus bFGF is produced in E. coli and added to Xenopus animal hemisphere explants, it functions as an effective mesodermal inducing agent. Using anti-peptide antibodies to human bFGF we find that the FGF protein is present in early oocytes and throughout embryogenesis, strongly suggesting that FGF is indeed a true mesodermal inducer.

We have pursued our analysis of the 1.0 kb bFGF message described previously. This message is deadenylated at maturation and readenylated near the time of the midblastula transition. Most surprisingly, this message is transcribed in the opposite direction of the transcripts encoding the full-length bFGF protein. As an activity has been described that separates RNA duplexes during the early cleavage stage of the embryo, the anti-sense transcript may play a role in the regulation of the bFGF sense message during oogenesis.

Nervous System Differentiation

J 009 CELL PATTERNING AND AXON GUIDANCE IN THE DEVELOPING VERTEBRATE CENTRAL NERVOUS SYSTEM, Thomas M. Jessell, Paola Bovolenta, Marysia Placzek, Domna Karagogeos, Marc Tessier-Lavigne and Jane Dodd, Center for Neurobiology, Columbia University College of Physicians and Surgeons, New York, NY 10032

Little is known about the cellular and molecular mechanisms that determine neural cell identity and the patterning of neuronal connections in the vertebrate central nervous system. We will discuss evidence that indicates that certain aspects of neuronal differentiation and axon guidance are regulated by specialized epithelial cells that occupy the medial region of the neural plate and, later the ventral midline of the spinal cord. This cell group, termed the notoplate/floor plate appears to constitute a distinct compartment within the neural plate that is more closely related in lineage and perhaps also in function to axial mesodermal cells of the underlying notochord than to other neural plate cells. Cells of the notoplate exhibit specialized mechanical and adhesive properties that may contribute to neurulation. At later stages of development the floor plate appears to guide developing axons in the embryonic spinal cord by releasing a diffusible chemoattractant factor and by virtue of its specialized cell surface properties. We will describe results that suggest that the floor plate may also play a role in the determination of cell identity and patterning at earlier stages of neural tube development.

Developmental Immunology

J 010 EXPRESSION OF MHC MOLECULES DURING THE ONTOGENY OF XENOPUS, *Martin Flajnik and Louis Du* Pasquier, Department of Microbiology and Immunology, PO Box 016960 (R-138), Miami, FL 33101; Basel Institute for Immunology, Basel, Switzerland. The amphibian Xenopus is the most primitive vertebrate known, both functionally and structurally, to possess a major histocompatibility complex (MHC). Class II molecules are expressed by tadpoles and adults, but the tissue distribution changes dramatically at metamorphosis. Class II is expressed on all lymphocytes (T and B) and the thymic epithelium during adult life. In contrast, the tadpole shows expression of class II only on B lymphocytes and thymic epithelium as in mammals, but also on the skin, gills, and digestive tract. Class I molecules are not expressed by any peripheral tissues of the tadpole on the surfaces of cells, but is expressed ubiquitously, as in mammals, during adult life. The only organ in which high levels of class I molecules are found during tadpole life is the thymic epithelium. At the time directly preceding metamorphosis, low levels of class I molecules are detected on the tadpole tissues. We are now determining whether this differential expression of the MHC during ontogeny is somehow involved in the restructuring of tissues at metamorphosis, or whether tadpoles simply dedicate all their T cell function to recognition of antigen int he context of class II molecules. It is of interest that class II molecules are found on those tissues which are most rapidly destroyed at metamorphosis. In addition, the class I molecules have been used as markers to detect and purify cells of the erythroid lineage which are newly arising in the adult at metamorphosis. The new adult erythrocyte series is biochemically distinct from the adult lineage, not only in terms of expression of the MHC, but of most other membrane proteins as well.

J 011 ONTOGENY OF FUNCTIONAL RESPONSES IN T LYMPHOCYTES, Ellen V. Rothenberg, Paul D. Boyer, Rochelle A. Diamond, Mariam Dohadwala, Karen Pepper and Julia A. Yang, Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125

T lymphocytes are diverse not only in the target antigens they recognize, but also in the transient, transcriptional responses they make to the stimulus of antigen binding. The specific battery of genes that can be activated in a T cell upon stimulation defines its functional subtype. It is now clear that multiple distinct classes of hormone-secreting "helper" T cells exist, as well as separate classes of "killer" T cells and the less well-understood "suppressor" T cells. All these cells with distinct programs of inducible gene expression are derived from a common pool of precursors that expand and differentiate in the thymus. We have been attempting to define the mechanisms whereby these developing cells are allocated to different fates.

To dissect the process of functional maturation we have traced the changes in expression and inducibility of diverse "response" genes. The IL-2 receptor α chain gene is activatable in all mature T cells, IL-2 itself in only the type 1 helpers, and cytotoxic cell serine esterases ("granzymes") and perforin are associated specifically with killer function. Competence to express these transcripts has been correlated with the timing of initial expression of the T-cell receptor complex and of the auxiliary recognition molecules, CD4 and/or CD8. The results indicate that components of functional response pathways are acquired non-coordinately, with a loss of certain early functions in many cells. This loss of function precedes and may foreshadow the commitment of these cells to intrathymic death. Other components of function are acquired later, with the onset of T-cell receptor expression. Commitment to an exclusive functional subclass, however, may also be established by selective loss of function, rather than by selective gain of function. Finally, developmental regulation of functional response specificity appears to occur at the levels both of gene expression *per se* and of the cytoplasmic signal transduction apparatus.

Spatial Regulation of Differential Gene Expression in the Embryo-I

J 012 MATERNAL AND ZYGOTIC DNA BINDING PROTEINS REQUIRED FOR SPATIAL ACTIVATION IN THE SEA URCHIN EMBRYO, E.H. Davidson, Division of Biology, California Institute of Technology, Pasadena, CA 91125

This presentation will concern the molecular basis of the process by which differential patterns of regional gene activity are instituted in the embryo. The cytoskeletal actin gene CyIIIa provides an excellent molecular marker for the zygotic program of gene expression characteristic of the embryonic aboral ectoderm. According to our recent lineage tracer studies the aboral ectoderm derives clonally from six specific cleavage stage founder cells. An additional marker gene considered is the SM50 spicule matrix gene, expressed exclusively in skeletogenic mesenchyme cells, the clonal 5th cleavage descendants of the micromeres. Regulatory sequences of both genes have now been shown to accurately direct spatial and temporal expression of associated reporter genes, after injection of the appropriate fusion gene constructs into sea urchin eggs. By using this method of gene transfer the *cis* regulatory domain of the CyIIIa gene has been delimited. Coinjection of excess quantities of DNA fragments containing subregions of the regulatory domain results in *in vivo* competition, and thus it is possible to analyze the functional significance of individual regulatory elements. DNA-protein binding studies *in vitro* demonstrate at least ten sites where highly specific interactions occur within this domain (10^4-10^6 fold preference for CyIIIa site vs. random DNA sequence). Some of the factors that bind to these sites are probably zygotic gene products, since their concentration increases as development proceeds and they cannot be detected in extracts of unfertilized eggs. However, others are clearly maternal, and are stored in unfertilized egg cytoplasm. Analysis of the origin, the cytological distribution and possibly the activation of certain of these CyIIIa regulatory factors in the egg and early embryo, considered in conjunction with the lineage and location of the aboral ectoderm precursors, should, we believe, provide a molecular interpretation of how the CyIIIa gene becomes differentially expressed as the aboral ectoderm is formed. The same factors are likely to regulate other aboral ectoderm genes as well. This is indicated by *in vitro* intergenic competitions, carried out with regulatory regions of other genes expressed specifically in the same cells. An interesting result follows from introduction of the CyIIIa regulatory sequences into eggs of a different sea urchin species. Though the exogenous fusion construct is regulated temporally in a correct manner, spatial regulation is wholly deranged, thus providing an opportunity to identify these *cis-trans* interactions required normally for spatial control. Several of the embryonic mRNAs coding for specific regulatory factors have been purified and cloned. Theoretical interpretation of sea urchin embryogenesis integrating classical and molecular evidence will be presented.

J 013 REGULATION OF THE GENE ENCODING EMBRYONIC EPIDERMAL KERATIN XK81A1, Thomas D. Sargent, Erzsebet Jonas and Alison M. Snape, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892. XK81A1 is a type I epidermal keratin expressed in early developmental stages of Xenopus (Jonas et al., 1985, P.N.A.S. 82, 5413-5417). This and other embryonic epidermal keratin genes are activated prior to gastrulation as part of the cell-autonomous developmental program for epidermal differentiation. Regions of the epidermis can be diverted to other fates, such as central nervous system and mesoderm, by inductive interactions, and when this occurs keratin gene activity ceases. Therefore, the expression of embryonic epidermal keratin genes represents a system in which reverse genetics approaches may be used to gain insights concerning the mechanisms of both inductive and cell-autonomous processes in early development.

Cloned copies of the XK81A1 gene were not correctly transcribed when injected into Xenopus oocyte nuclei, but following injection into fertilized eggs were transcribed with high efficiency from the correct initiation site. Transcripts accumulated exclusively in the appropriate tissue, epidermis. Fusion of this promoter to a human beta globin gene lead to highly mosaic yet fully epidermis-specific accumulation of human globin mRNA and protein, indicating that the primary mode for regulating tissue specific expression of this gene is at the level of RNA synthesis, and that sequences in the 5' flanking region of the keratin gene are sufficient for this control. These regulatory sequences were further mapped by exonuclease deletion analysis to a region comprising approximately 500 base pairs, from -487 to +26. Site-directed point mutations and small deletions are being performed to precisely localize control elements, and mobility shift and footprint analyses are being carried out to detect interactions of these sequences with embryonic nuclear proteins.

J 014 **PATTERN FORMATION DURING *C. elegans* VULVAL INDUCTION,**
Paul Sternberg, Gregg Jongeward, Raffi Aroian, Russell Hill, Jane Mendel, Min Han, Andrea Holboke, Linda Huang, and Steven Clark, Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125. ¶Induction of the *C. elegans* vulva is a simple example of pattern formation in which two types of cell interactions -- induction and lateral inhibition -- can be studied among a set of seven cells. The anchor cell [AC] of the gonad induces three of six multipotent epidermal cells, the vulval precursor cells [VPCs], to generate the adult vulva. The position of each VPC with respect to the AC specifies which of three types of cell each becomes. Type **1** and type **2** VPCs generate the cells of the vulva; type **3** VPCs remain in the 'ground state' and generate non-specialized epidermis. The precise pattern of VPC fates [**3-3-2-1-2-3**] is established by two cell interactions: a graded inductive signal from the AC to the VPCs, and a lateral inhibitory signal [LIS] between neighboring VPCs. ¶To understand how intercellular signals ultimately specify the VPC types, we are studying, by genetic analysis and molecular cloning, three genes whose products are involved in the production of, or response to, these signals. Two genes necessary for induction, *let-23* and *lin-3*, are defined by mutations that cause all VPCs to be type **3** and hence prevent formation of a vulva, the "Vulvaless" phenotype. The *lin-3* locus is necessary either for the production of the inductive signal or for an early step in the response. The *let-23* locus is necessary for a response to the inductive signal. *lin-15* mutations cause VPCs to become type **1** or **2** even in the absence of inductive signal, resulting in the formation of extra vulval tissue, the "Multivulva" phenotype. The wild-type *lin-15* gene promotes the type **3** fate, and acts antagonistically to *let-23*. ¶We are pursuing several strategies to clone *lin-3*, *let-23* and *lin-15*, including RFLP mapping, chromosomal walking, transposon tagging and complementation of mutant phenotypes by microinjection of cosmid DNA. ¶Extragenic suppressors of *lin-15* or *let-23* mutations define new loci involved in VPC determination. ¶Genetic experiments indicate that *let-23* is necessary for both specification of type **1** and **2** VPCs and activation of the LIS, but that such activation requires a higher level of *let-23* activity than is required for specification of type **1** or **2**. ¶Analysis of the interactions between mutations in *let-23* and *lin-15* with those at another locus, *lin-12*, indicates that *lin-12* has two roles: *lin-12* is necessary for the action of the LIS, and is necessary to specify type **2** VPCs. These experiments also indicate that *lin-12* acts after *lin-15* and *let-23* in the pathway of response to the inductive signal.

Spatial Regulation of Differential Gene Expression in the Embryo-II

J 015 MOLECULAR GENETICS OF SEGMENTATION IN DROSOPHILA: THE ROLE OF THE PAIR-RULE GENE RUNT, J. Peter Gergen, Barbara Butler, Andrea Bonner, Mary Kania, Clare Bolduc and Joseph Duffy, Department of Biochemistry and Molecular Biology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77030. The Drosophila segmentation genes are a set of genes that act to establish the normal segmented body pattern in the developing embryo. These genes were originally grouped into three classes--gap, pair-rule, and segment-polarity--based on the size and spacing of the pattern defects observed in mutant embryos. Molecular probes have been used to demonstrate a strong correlation between the genetic requirements, as inferred from mutant phenotypes, and the spatial patterns of segmentation gene expression during embryogenesis. These probes have also been used to reveal a hierarchy of regulatory interactions among these genes. In the emerging framework, each gap gene is expressed in a unique domain spanning several presumptive segments. These localized patterns of expression are then somehow involved in generating the periodic patterns of expression of the pair-rule genes. These genes, in turn, act to generate the segmentally repeated patterns of expression of several of the segment-polarity genes.

The pair-rule gene runt plays a key regulatory role in the transition from the gap to pair-rule patterns of gene expression (1). All of the pair-rule genes so far examined require runt activity for their normal expression. Elimination of runt has reciprocal effects on the pair-rule genes ftz and eve, which are normally expressed in complementary frames. In runt mutant embryos the ftz transcript fails to accumulate normally in cells that should express runt and these cells instead accumulate eve transcripts. Mutations in another regulatory pair-rule gene, h, which is normally expressed in an overlapping but complementary frame to runt have the opposite effects on ftz and eve. We find that runt and h also act antagonistically upon each other. These results suggest that runt and h play complementary and mutually antagonistic roles in regulating the expression of other genes. We are currently extending these genetic analyses in order to determine which of the observed interactions are direct.

We have used germ-line transformation to identify the DNA sequences of the runt locus itself that are required for its normal expression. The results indicate that vital regulatory elements lie further than 8 kb upstream of the 5' end of the transcription unit (2). However, this amount of upstream DNA is sufficient for generating a qualitatively normal pattern of expression in the blastoderm embryo. We are currently further defining important regulatory elements within this region.

We have sequenced the runt gene in order to address the biochemical function of the gene product. The sequence reveals that the protein contains neither the DNA binding homeodomain found in several pair-rule genes, nor the "zinc-finger" DNA binding motifs present in the gap genes. Instead, the protein has an apparent N-terminal signal sequence and 10 acceptor sites for N-linked glycosylation. This suggests the protein is not localized to the nucleus but rather enters the secretory pathway and is glycosylated. We are currently using both in vitro and in vivo approaches to address the significance of these observations and elucidate the potentially novel role this gene product plays in regulating segmentation gene expression in the Drosophila embryo.

1. Ingham, P.W. and Gergen, J.P. (1988). Development (in press).
2. Gergen, J.P. and Butler, B.A. (1988). Genes and Development 2:1179-1193.

J 016 INT-1 AND INT-2; PROTO-ONCOGENES WITH A NORMAL ROLE IN MOUSE DEVELOPMENT, Andrew McMahon, Jeff Mann, Kevin Griffin, and Jill McMahon, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110. The development of vertebrate embryos requires cell-cell communication for the formation of many tissues and organs. For example, inductive interactions are essential for the generation of both mesodermal and neural tissue in *Xenopus*. We are interested in molecules that may be involved in cell-cell communication during mouse development. Recently we have focused our attention on the normal role of two unrelated proto-oncogenes, int-1 and int-2. int-1 is the mammalian homologue of the *Drosophila* segment polarity gene, *wingless*, and int-2 is related to fibroblast growth factors. I will discuss the characterization and properties of these and related genes, and their relevance to development.

Cytoplasmic Localization

J 017 THE DEVELOPMENTAL FATE OF FISSION YEAST CELLS IS DETERMINED BY THE PATTERN OF INHERITANCE OF PARENTAL AND GRANDPARENTAL DNA STRANDS, Amar J.S. Klar, NCI-Frederick Cancer Research Facility, BRI program, Frederick, MD 21701.

The key feature for development consists of producing daughter cells which differ in their potential for cellular differentiation. Following two cell divisions, a haploid Schizosaccharomyces pombe cell produces one cell in four "granddaughters" with a changed mating cell type, implying nonequivalence in sister cells. By these rules a "stem cell" lineage is produced. I have tested whether sisters differ because of unequal distribution of cytoplasmic and/or nuclear components to them or due to inheriting a specific parental DNA chain at the mating type locus. The DNA chain inheritance model predicts that those cells engineered to contain an inverted tandem duplication of the mating type locus should produce equivalent sisters. Consequently, two "cousins" in four related granddaughter cells should switch. The results verified the prediction. These results extended our earlier observations (Nature 1987, 326:466-470) which demonstrated that twice as many chromosomes are cleaved *in vivo* at the *mat*1 locus in strains containing the inverted duplication when compared to standard strains.

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J 018 MATERNAL PATTERN INFORMATION AND CYTOPLASMIC LOCALIZATION IN *DROSOPHILA*, Howard D. Lipshitz, Dali Ding, Teresa R. Strecker, Susan R. Halsell and Wiliam W. Fisher, Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125.

We are taking complementary molecular and genetic approaches to address the role of cytoplasmic localization in determining the anterior posterior axis of the *Drosophila* embryo. The molecular approach involves the purification of anterior and posterior polar RNA from eggs or early embryos and the construction of directionally cloned "anterior" and "posterior" cDNA libraries in vectors that allow the *in vitro* synthesis of large amounts of sense or antisense RNA. Sensitive sorting methods, including the use of subtractive hybridization, enable us to search for cDNAs representing rare RNAs localized to either the anterior or posterior pole of the egg. Subsequent molecular and genetic analysis focuses on the role of these RNAs in pattern formation, as well as on the mechanism of RNA localization. The complementary, genetic, approach involves the analysis of two classes of maternal effect genes involved in the specification either of the asegmental embryonic termini ("terminal" class) or of the abdominal region and germline ("posterior" class). Hyper- and hypoactivity mutations in the terminal gene, *torso*, result in reciprocal pattern defects and enable us to define both a positive role for the *torso* product in promoting terminal development, and a negative role in repressing central development. Cytoplasmic leakage and transplantation, and temperature-shift experiments, suggest that the *torso* RNA is unlikely to be localized in the egg, but that the timing of its function and/or differential spatial activation may be key to its role in specifying embryonic pattern. Mutations in the *tailless* gene act as dominant maternal suppressors of *torso* hyperactivity mutations, indicating that the *tailless* product acts together with, or downstream of, the *torso* product to mediate both its positive and negative functions.

J 019 **MICROTUBULE MEDIATED CORTICAL ROTATION AND DORSOVENTRAL AXIS SPECIFICATION IN THE XENOPUS EGG** Brian A. Rowning and John C. Gerhart
Dept. of Molecular Biology, University of California, Berkeley CA 94720.

The bilaterally symmetric organization of the *Xenopus* embryo is established in part by a cortical rotation in the first cell cycle. The internal cytoplasm and the entire thin cortex are displaced relative to each other by 30° and 350 μm, at the rate of 7 μm/min. The meridian along which equatorial cytoplasm is most displaced toward the vegetal pole defines the future dorsal midline. Using immunocytochemistry we observe a massive array of parallel bundled microtubules (MT) in the vegetal hemisphere at a depth of 1-3μm from the surface, at the shear zone of the rotation. These MTs appear abruptly at the onset of rotation (40 min after fertilization) and disappear at rotation's end (at 80 min). MT alignment coincides with the path of the rotation. These MTs probably act as tracks along which rotation occurs. Normally the tracks align in a direction related to the single random point of sperm entry. However, small displacements of the egg's contents by gravity in the period before rotation cause MTs to align in the direction of displacement, irrespective of the sperm's entry point. We will discuss a "bootstrap model" in which MT polymerization and rotation reinforce each other in establishing a unique orientation to the array. In order to define the rotation, we are currently investigating array MT polarity and whether the MTs are fixed to the cortex or subcortical cytoplasm.

Treatments that destabilize MTs (nocodazole, cold, hydrostatic pressure, UV irradiation) inhibit the rotation. With diminishing rotation, future embryonic development is progressively limited to posterior-ventral fates at the expense of dorsal-anterior structures. Tipping eggs off their animal-vegetal axis rescues their dorsal-anterior development. Since this gravity driven rearrangement mimics the rotation in its developmental effects without restoring microtubules, we conclude that MTs are not directly involved in dorsal development except as needed for the normal displacement of layers.

Treatment with heavy water (D_2O) enhances MT stability but paradoxically has an inhibitory effect on rotation. MTs polymerize precociously and in a disorganized manner, so the reduced rotation we see with D_2O may be a consequence of poorly aligned MT tracks. However with less rotation these eggs overproduce dorsal-anterior structures at the expense of ventral-posterior ones. Since some rotation is required for D_2O hyperdorsalization, it is unlikely that this treatment activates dorsal determinants independently of an effect on MTs. We will discuss the basis of the effect of D_2O on axis specification.

J 020 CYTOPLASMIC DETERMINANTS FOR MUSCLE CELL DIFFERENTIATION IN ASCIDIAN EMBRYOS, Noriyuki Satoh, Kazuhiro W. Makabe and Takahito Nishikata
Department of Zoology, Faculty of Science, Kyoto University, Sakyo-ku, Kyoto 606, Japan

A tadpole larva of the ascidian Ciona intestinalis contains 18 muscle cells on each side of the tail. Recent cell lineage analyses have shown that among them 14 muscle cells are derived from the B4.1 blastomere of the 8-cell embryo (primary lineage), whereas 2 muscle cells originate from the A4.1 and 2 muscle cells from the b4.2 (secondary lineage). Various descriptive and experimental studies since the turn of the century have documented the presence of cytoplasmic information or determinants responsible for differentiation of the primary muscle cells in ascidian embryos. The determinant is confined to the myoplasm, and eventually found within the muscle lineage cells of developing embryos. Although the determinant is speculated to be some regulatory factor of specific gene expression, there is still little known about the molecular identity, the pattern of spatial distribution during embryogenesis, or the mode of action at the molecular level.

Recently Jeffery (1) has isolated the myoplasmic crescent from Styela eggs. He has shown that the myoplasmic crescent fraction contains 15 polypeptides which are undetectable in the other cytoplasm fraction. The myoplasmic crescent fraction, however, does not contain specific mRNAs. In this study, we first re-examined Jeffery's investigation in Ciona eggs and confirmed his results. The Ciona myoplasm fraction contains 16 specific polypeptides but not specific mRNA. Second, we produced monoclonal antibodies against the isolated myoplasm. Twelve monoclonal antibodies, identified by indirect immunofluorescence, stained the myoplasmic region. Among them, one monoclonal antibody called IIG6B2 recognized some component which may have some roles in the process of muscle cell differentiation (2). The IIG6B2 antibody recognized a single polypeptide with a relative molecular mass of about 40,000 and pI 5.0. When unfertilized eggs were centrifuged, stratifying their mitochondria and some other cytoplasmic components, the polypeptide identified by the IIg6B2 remained at the peripheral cytoplasm of the egg. The antigen was detected in small oocytes, suggesting that the antigen is produced by oocyte itself during early stages of oogenesis. When the IIG6B2 antibody was microinjected into fertilized eggs, the antibody blocked the development of muscle-specific enzyme development.

(1) Jeffery, W. R. (1985). J. Embryol. Exp. Morph. 77, 275-287.
(2) Nishikata, T. et al. (1987). Development 100, 577-586.

Cell-Cell Interaction in Pattern Formation

J 021 EMBRYONIC INDUCTION AND GENE ACTIVATION, J.B. Gurdon, T.J. Mohun, M.V. Taylor, and C. Sharpe, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB3 3EJ, England.

Many major principles of embryonic induction are exemplified by the first induction in amphibian development, the formation of mesoderm in animal cells by vegetal cells of a blastula. To analyze this induction at a molecular level, we have cloned and fully sequenced some muscle-specific actin genes which are activated by induction [as well as some cytoskeletal actin genes which are activated at the same time in development (gastrula) but not by induction]. This induction seems to depend on locally acting substances released by inducing cells, which must be close to, but not necessarily in contact with, the responding cells. The earliest response (muscle actin gene transcription) depends on protein synthesis soon after induction. We have identified some of the upstream regions involved, using a rapid gene transfer assay. Muscle cell differentiation is helped by a community effect in which adjacent cells enhance each others' gene activation during the later part of the induction response.

J 022 DETERMINATION AND CELLULAR INTERACTIONS IN THE SEA URCHIN EMBRYO, Brian T. Livingston and Fred H. Wilt, Department of Zoology, University of California, Berkeley, Ca. 94720 The availability of a detailed lineage map of the early blastomeres of sea urchin embryos, combined with the availability of lineage specific molecular markers, has allowed us to examine in a more detailed manner the determination of blastomeres isolated from early embryos. To permit the study of the developmental capacities of animal and vegetal blastomeres, an efficacious method of isolating blastomere pairs from animal or vegetal halves of 16-cell embryos was developed. The vegetal blastomeres isolated in this manner (macromere/micromeres) formed gut, skeleton and pigment, structures characteristic to this lineage, as well as showing normal expression of a gut specific antigen, Endo I, and a gut specific enzyme, alkaline phosphatase, a primary mesenchyme specific RNA, SM50, and an oral ectoderm specific antigen, Ecto V. Animal blastomeres (mesomeres) formed hollow, ciliated spheres that did not express vegetal specific markers, and expressed Ecto V in an aberrant pattern. Treatment of mesomeres with LiCl caused them to form gut and skeleton, and evoked the expression of the vegetal specific markers Endo I, alkaline phosphatase, and SM50. Pigment was not formed, and the pattern of Ecto V expression was improved, but not completely normal. When mesomeres are co-cultured with vegetally derived micromeres, mesomeres formed guts that expressed alkaline phosphatase and Endo I. Skeleton was formed in the co-cultures, but was derived solely from micromeres. Pigment was found only rarely, and Ecto V expression resembled that of LiCl treated mesomeres. Co-culture of micromeres and LiCl treated mesomeres resulted in the formation by the mesomeres of guts expressing Endo I and alkaline phosphatase, pigment cells, and in many cases, normal expression of Ecto V. Experiments are now under way to determine the biochemical site of action of LiCl in this system.

Homeotic Genes; DNA-Protein Interactions

J 100 ROLE OF AN S1 NUCLEASE SENSITIVE POLYPURINE-POLYPYRIMIDINE REPEAT IN THE REGULATION OF MYOSIN LIGHT CHAIN 2, Rebecca Aft and Cathy Wojcicki, Department of Pediatrics, St. Louis University School of Medicine, St. Louis MO 63110
During the differentiation of rat skeletal muscle myoblasts to myotubes numerous genes associated with the assembly of the contractile apparatus are induced to be expressed. In our effort to understand the regulation of one of these genes, myosin light chain 2 (MLC2), we found that a 22 bp polypurine-polypyrimidine repeat located at approximately -150bp from the start site enhanced expression of the CAT gene when ligated in front of a neutral promoter and transfected into rat skeletal muscle myoblasts. This enhancement was non-tissue-specific, but inclusion of an additional 100bp downstream of the sequence in the construct displayed tissue-specific enhancement of expression. In vitro, the polypurine-polypyrimidine sequence displays S1 nuclease hypersensitivity which is pH and supercoil dependent. In addition, DNaseI footprinting with myotube nuclear extracts suggest that this DNA is in a non-B conformation. From this data, we speculate that the polypurine-polypyrimidine induced altered structure serves as a recognition sequence for the assembly of transcriptional regulatory factors.

J 101 IDENTIFICATION OF NUCLEAR FACTORS WHICH BIND TO THE PROMOTER REGION OF THE IL-6 GENE. S. Akira, T. Nakajima, O. Tanabe, H. Ishiki, T. Goto, T. Hirano and T. Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, Japan

IL-6 is a cytokine with many biological activities on a wide variety of tissues, such as stimulation of Ig synthesis, enhancement of myeloma and hybridoma growth, modulation of acute phase proteins by hepatocytes, and differentiation of nerve cells. IL-6 is synthesized by a variety of cells including fibroblasts, macrophages, lymphocytes, and keratinocytes. The expression of the IL-6 gene is enhanced transiently by a variety of agents including IL-1, TNF, PDGF, TPA, and cycloheximide. The dysregulation of the expression of IL-6 leads to various diseases including myeloma and autoimmune disease. We have studied the induction of IL-6 mRNA by IL-1 in a glioblastoma cell line. 5' deletion analysis indicated that the -180 to -122 bp region of the IL-6 promoter is involved in the IL-1 inducibility. This region contains homologies to c-fos SRE. We employed a gel retardation assay to detect sequence-specific nuclear factors binding to this region. A specific retarded band was identified in the untreated nuclear extract while a new slower-migration band was detected in the nuclear extract treated with IL-1 in addition to this band. Work is in progress to purify and clone these factors.

J 102 THE REGULATORY CIRCUITRY UNDERLYING THE TRANSCRIPTIONAL INACTIVATION OF SEA URCHIN EARLY H2B GENES LATE IN DEVELOPMENT, Jeffrey R. Bell, Masamichi Ito, and Rob Maxson, Department of Biochemistry, University of Southern California, Los Angeles, CA 90033. The sea urchin histone gene family includes two subfamilies that are differentially expressed during development. "Early" histone genes are maximally active between fertilization and the blastula stage, "late" histone genes from the blastula stage onwards. Our ultimate goal is to understand the regulation of the early H2B gene in terms of specific DNA-protein interactions. We have used both mutational analysis *in vivo* and protein-DNA binding studies *in vitro* to delineate the elements responsible for the inactivation of the early H2B gene late in development. We microinjected various mutants of the early H2B gene into sea urchin zygotes and measured their expression in different developmental stages with an RNAse protection assay. Preliminary mutational analysis has shown that at least two regions of DNA contribute to the inactivation of early H2B genes, one upstream of the TATA motif and the other downstream. The downstream element binds to at least two different proteins from sea urchin nuclear extracts. One of the proteins binds to a sequence near the TATA motif and it is most abundant early in development, suggesting that it has a positive effect on early H2B transcription. The second protein binds to a site on the DNA which codes for the mRNA leader. The concentration of this protein increases coincident with the inactivation of early H2B genes, suggesting that occupancy of this site exerts a negative effect on early H2B transcription. Thus, the regulation of the early H2B gene is probably the result of complex interactions between the DNA involving both positive and negative acting regulatory proteins.

J 103 **ANALYSIS OF THE MOLECULAR BASIS OF SPLICING REGULATION**, Paul M. Bingham, Inka Mims, Hao Li, Tze-Bin Chou and Zuzana Zachar, Department of Biochemistry, State University of New York, Stony Brook, NY 11794.

We are analyzing developmental regulation of the suppressor-of-white-apricot [su(w^a)] gene in Drosophila at the level of splicing. The complex developmental pattern of expression of this gene apparently results from the su(w^a) protein repressing a splicing event necessary to produce its own messenger RNA [see Trends in Genetics (1988) 4, 134, for a review]. Thus, the su(w^a) protein autorepresses its own production at the level of splicing. An implication of the autogeny of this circuit is that both the substrate RNA and the trans-acting protein involved in this regulated event are available. An implication of this, in turn, is that this circuit represents one of the very few currently available opportunities to effectively study the detailed biochemistry of metazoan splicing regulation.

To date we have investigated various aspects of this circuit including the su(w^a) primary RNA transcript sequences necessary to respond to repression of splicing by the su(w^a) protein and some aspects of the functional organization of the su(w^a) protein as a splicing regulator. Results of these and related studies will be reported.

J 104 GENOMIC AND TRANSCRIPTIONAL ORGANIZATION OF HOMEOBOX SEQUENCES BELONGING TO THE HUMAN HOX3 LOCUS, Edoardo Boncinelli, Antonio Simeone, Maria Pannese, Dario Acampora, Maurizio D'Esposito, Anna Stornaiuolo and Mauro Cafiero, International Institute of Genetics & Biophysics, CNR, 80125 Naples, Italy.

We have studied the chromosomal localization of 20 human homeoboxes and the predicted primary sequence of the encoded homeodomains. These homeoboxes are clustered in four HOX loci on chromosomes 2, 7, 12 and 17. The homeoboxes of one HOX locus can be put in a one-to-one correspondence with the homologous sequences in the other HOX loci suggesting that these had originated by virtue of large-scale duplications of a single complex locus and subsequent dispersion in different chromosomes. We studied the expression of six homeoboxes of the HOX3 locus on chromosome 12 in embryonic and adult tissues. Analysis of cDNA clones and polyadenylated RNAs from the locus suggests that at least in certain tissues transcription starts from a major promoter upstream from the homeobox-containing exons. Several polyadenylation signals are recognized giving rise to a number of polyadenylated RNAs, in turn spliced to generate several mature messenger RNAs containing different HOX3 homeoboxes. Moreover, multiple mRNAs exist in different tissues encoding different proteins containing the same homeodomain. We report in particular on the expression of homeobox sequences c8, cp11 and cp19.

J 105 SEQUENCES INVOLVED IN THE EMBRYONIC ACTIVATION OF A XENOPUS LAEVIS CYTOSKELETAL ACTIN GENE, Sean Brennan and Eric Rocher, Program in Developmental Biology and Department of Anatomy, University of Connecticut Health Center, Farmington, CT 06032. The Xenopus laevis cytoskeletal actin gene is subject to positive temporal regulation during early embryonic development, being transcriptionally activated at the gastrula stage. We are attempting to identify the sequences which mediate this temporal activation by injecting cloned copies of the cytoskeletal actin gene into embryos. The gene used for injection has been molecularly marked with a small insertion within the first exon so that its transcripts can be distinguished from those of the endogenous gene; other than this, the entire transcribed region is present in the constructs used for injection. RNA from injected embryos is analyzed at different times after fertilization: transcription of the injected gene is measured and compared to that of the endogenous gene. We find that 300 nucleotides of 5'-flanking sequence suffice to direct correct temporal activation of an injected actin gene. Additional deletions in the 5'-flanking region are currently being analyzed. In addition, we have obtained evidence that sequences within the first intron are involved in specifying the rate of transcription of the actin gene at the time of its initial activation at gastrulation.

J 106 A DNA-BINDING FACTOR REGULATING FUSHI TARAZU GENE EXPRESSION IN THE STRIPES, J. Lesley Brown, Hitoshi Ueda and Carl Wu, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Footprinting and gel mobility shift assays reveal multiple DNA binding factors that interact specifically with sequences within several hundred base pairs upstream of the Drosophila segmentation gene fushi tarazu (ftz). One factor, purified to homogeneity from Drosophila embryos, binds to a sequence repeated at least four times along the fushi tarazu gene region (consensus PyCAAGGPyCPuCCPu). The DNA-binding activity shows interesting developmental regulation, appearing in early embryos and again in late embryos with different electrophoretic mobility. P-element mediated transformation of flies with the zebra or stripes element (wild type or factor binding site mutant) fused to beta-galactosidase sequences indicate that this factor is involved in activation of some but not all of the stripes (collaboration with Sandra Sonoda and Matthew Scott).

J 107 A NEW HOMEOBOX GENE, *EMPTY SPIRACLES*, HAS DUAL PATTERNING FUNCTIONS DURING *DROSOPHILA* DEVELOPMENT, Dyana Dalton *, Robin Chadwick+ and William McGinnis+, Departments of Human Genetics* and Molecular Biophysics and Biochemistry+, Yale University, New Haven, CT

The *empty spiracles* gene has been isolated from *Drosophila* by homeobox homology. The predicted gene product is a 492 amino acid protein with a very large proline-rich domain spanning the first 4/5 of the protein. The homeodomain, near the carboxy-terminus, has a 50% amino acid homology to the *even-skipped* homeodomain. At the carboxy-terminus there is an acidic domain that may function as a transcriptional activator sequence. The *empty spiracles* gene product has a dual expression pattern and function in *Drosophila* embryogenesis. Early in development *empty spiracles* is expressed in a single anterior band of cells of the cellular blastoderm. This correlates with its function as an anterior segmental selector gene which is required for the development of the antennal sense organ and parts of the head skeleton. Later in development, *empty spiracles* is expressed in the lateral region of each segment. In segments T2-A9 the tracheal pits form in the center of the patch of lateral cells that express the *empty spiracles* protein. This expression correlates with the tracheal tree defects in the *empty spiracles* mutant embryo.

J 108 **The Need for Enhancers Is Acquired Upon Formation of a Diploid Nucleus During Early Mouse Development**, Encarnación Martínez-Salas and Melvin L. DePamphilis, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

We are attempting to identify cis-acting signals and trans-acting factors that regulate gene expression and DNA replication in mouse oocytes, fertilized eggs and developing 2-cell embryos. The activity of the polyoma virus origin of DNA replication and the expression of firefly luciferase from the herpes virus thymidine kinase promoter were used as sensitive assays for enhancer function in DNA replication and gene expression. Mouse ova were injected with plasmid DNA containing different enhancer-origin or enhancer-promoter configurations, and then allowed to continue development *in vitro*. The level of promoter activity in oocytes was independent of enhancers. However, both DNA replication and gene expression required an enhancer in developing 2-cell embryos. Competition experiments suggested that enhancers bound specific proteins that were required for replication and transcription. In contrast, DNA injected into 1-cell embryos did not need an enhancer for replication or gene expression, and no competition for replication factors was observed. In fact, the amount of DNA replication and gene expression was at least 10-fold greater in 1-cell embryos than in developing 2-cell embryos. Since 1-cell embryos that replicated injected DNA retained their pronuclei and remained 1-cell embryos, enhancers are not needed in mammalian development until a diploid nucleus is formed.

J 109 **TRANSCRIPTIONAL FUNCTIONS OF THE HOMEODOMAIN AND ZINC FINGER DEVELOPMENTAL GENE PRODUCTS.** **Yoshiaki Ohkuma, Jessica Treisman, Claude Desplan.**
Hward Hughes Medical Institute, The Rockefeller University, New York, N.Y., 10021. (212) 570 7965.

We are interested in the functions of the common motifs present in many Drosophila developmental genes: the homeodomain (HD), a highly conserved 60 aminoacid domain, is known to be a sequence specific DNA binding domain. Zinc finger motifs, also thought to be responsible for DNA binding, are found in the Gap gene class. It is our belief that, through transcriptional control, the HD and the Zinc finger containing gene products interact combinatorially in a network to establish their patterned expression which leads to the formation of body pattern.

We are addressing the question of the transcriptional role of these proteins by using an *in vitro* transcription system and our knowledge of their DNA binding properties. The goal is not to dissect a specific promoter, but to analyse the role of cis-acting sites recognized by HD proteins alone or in combination with other HD or Zinc finger proteins. We have already purified *engrailed* and *ftz* proteins to homogeneity from E. coli cells overexpressing them and we are now adding these proteins to transcription extracts from mammalian cells. While developing a *Drosophila* embryo transcription system, we are using mammalian extract as an heterologous system. This is justified by the observation that heterologous transcription machineries (such as yeast and mammalian) show a large degree of cross-species activity.

Both the general transcription functions carried out by the HD proteins and the particular properties of each gene product can be investigated in this way. Moreover, this will rapidly enlarge to an analysis of the functions of these genes in combination and will give some insight into the control mechanisms of the coordinate expression of the developmental genes. But, since these proteins are believed to act in concert with the other members of the network, a final understanding of the network will require an investigation of the spatiotemporal patterns of expression of the target genes in the developing animal, and not simply the level of expression in a cell line, which would be missing most of the members of the network. The *in vitro* information about the interactions among the developmental genes in Drosophila will provide an important base upon which to design models to be tested.

J 110 HOMEO BOX SEQUENCES HOMOLOGOUS TO THE *GUTLESS* HOMEO BOX IN *DROSOPHILA*. Scott Dessain and William McGinnis. Departments of Molecular Biophysics and Biochemistry, and Biology, Yale University, New Haven, CT 06510. Homeo box containing genes in *Drosophila* are necessary for the proper organization and identity of specific cell populations during development. Recently, a tissue-specific homeo-box containing gene, *gutless*, has been characterized which is expressed only in the musculature of the embryonic gut. It's homeo box is highly diverged from other known homeo box sequences in *Drosophila*, and low stringency Southern blotting of genomic *Drosophila* DNA indicated the presence of restriction fragments which may contain novel homeo box sequences. Screening of a genomic library allowed the isolation of five complete and two partial homeo box sequences which have been analyzed by sequence analysis, *in situ* hybridation, and Northern blotting. *in situ* hybridation indicates that three may be involved in brain development, and one may determine anterior and posterior midgut endodermal tissues.

J 111 FINGER MOTIF PROTEINS IN EARLY *XENOPUS* DEVELOPMENT, Tarek El-Baradi, Walter Knöchel[1)] and Tomas Pieler, Max-Planck-Institut fuer Molekulare Genetik, Ihnestrasse 73, D-1000 Berlin 33 and [1)]Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33.
Xenopus ovary and gastrula cDNA banks were screened with a mixed oligonucleotide probe representing the "H/C link", a sequence which joins adjacent fingers in many different proteins that contain Zn-finger domains. More than 100 different positive cDNAs were identified and sequenced. Together these sequences narrowly define a subclass of finger proteins of the type C2H2, a class which also includes some proteins from *Drosophila* and mouse. Within this subclass some of the *Xenopus* cDNAs can be further subdivided into a group of their own. One such group is characterized by the presence of two different types of alternating fingers. A finger protein of this type has recently been implicated as being a human sex determining factor. Members of another group of cDNAs share long stretches of strongly conserved elements at the amino-terminal end of their predicted protein sequence. The temporal and spatial characteristics of the expression profiles during embryogenesis of a number of the corresponding mRNAs will be discussed.

J 112 THE *C. elegans* GENE *unc-86* IS HIGHLY SIMILAR TO MAMMALIAN TRANSCRIPTION FACTORS, Michael Finney and Gary Ruvkun, Dept. of Genetics, Harvard Medical School and Dept. of Molecular Biology, Massachusetts General Hospital, Boston MA 02114

We have molecularly cloned *unc-86*, a gene that controls certain cell lineages and cell differentiations in the *C. elegans* nervous system, and found that it contains a large region of sequence similarity to three mammalian transcription factors: Pit-1, a rat pituitary transcription factor, Oct-1, a ubiquitously-expressed human transcription factor, and Oct-2, a human B-cell-specific transcription factor. The region of similarity, referred to as the pou (pit oct unc, pronounced "pow") domain, consists of three parts: two 33 amino acid regions, A and B, followed by a 60 amino acid homeodomain. The four sequences are identical at 17 of 33 positions in the A region, 18 of 33 positions in the B region, and 21 of 60 positions in the homeodomain. The homeodomains of the four pou proteins are more similar to each other than they are to other known homeodomains, including similar sequences in the "helix 3" region that may confer DNA binding specificity. The A and B regions of the pou domain are even more highly conserved among the four proteins than is the homeodomain.

unc-86 was identified genetically, based on its effects on cell fate and cell differentiation. Pit-1, Oct-1, and Oct-2 were identified biochemically as transcription factors. The fact that they have a large region of similarity not only suggests that *unc-86* encodes a transcription factor, but also that proteins first identified as transcription factors may be involved in the specification of cell type.

J 113 THE EXPRESSION OF XHOX HOMEOBOX GENES IN XENOPUS DEVELOPMENT
Richard Harvey, Molecular Biology Unit, The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia.

Vertebrate homeobox genes are postulated to participate in developmental decisions which determine the differentiation or patterning fate of cells in the early embryo. Most data on the expression of homoeobox genes in mice and frogs support this hypothesis. The Xhox-1 genes of Xenopus are expressed in a spatially restricted manner in early embryos beginning at the mid-gastrulation stage, a stage when the embryo is undergoing many morphogenetic and determinative changes. If the Xhox-1A protein is indiscriminantly expressed during early embryogenesis by injecting synthetic Xhox mRNA into developing eggs, most early events occur normally, except that somites form abnormally. They differentiate as expected into muscle tissue, but pattern abnormally so the segmental units are lost. These experiments show that somitic mesoderm is susceptible to over- or ectopic expression of Xhox-1A and may indicate that the Xhox-1A gene has a role in segmentation of mesoderm in normal embrys. To study this phenomenon further, we have made antisera against Xhox-1A fusion proteins and are in the process of documenting the cellular detail of Xhox-1A protein expression. We will be searching in particular for evidence that the pattern of expression is temporally regulated in concert with cellular changes occurring during segmentation of the axial mesoderm.

J 114 THE *Antennapedia* PROTEIN OF *Drosophila melanogaster* IS A PHOSPHORYLATED SEQUENCE-SPECIFIC DNA BINDING PROTEIN. Shigeo Hayashi, Gary Winslow and Matthew Scott, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado, 80309-0347. The homeotic gene *Antennapedia* (*Antp*) of *Drosophila melanogaster* controls insect development by specifying the identities of thoracic segments. To study the function of its protein product, we synthesized *Antp* protein in *Drosophila* Schneider line 2 cells by expressing *Antp* cDNA and studied its properties *in vitro*. The *Antp* protein produced in this system co-migrates with authentic *Antp* protein from embryos in SDS-polyacrylamide gel electrophoresis but the protein produced in *E. coli* did not. This difference in electrophoretic mobility is partly due to multiple phosphorylation of serine residues. Immunoprecipitation and gel retardation assays have established that *Antp* is a sequence-specific DNA binding protein. The *Antp* binding sites were mapped on the *Antp* P1(6kb) and *Ultrabithorax* (3.5kb) promoters. Each promoter has 3 binding sites in the upstream region and one in the untranslated leader. The binding sites located in leader sequences showed strong *Antp*-dependent enhancer activity when tested in a transient co-transfection assay. The binding of *Antennapedia* to these sites has been characterized by DNase I footprinting. An A-T rich sequence that extend over 50 bp was protected by the *Antennapedia* protein.

J 115 CLONING OF C-FOS cDNA AND ITS EXPRESSION DURING EARLY OOGENESIS OF XENOPUS LAEVIS Y. Katoh, T. Noce, Y. Fujiwara, N. Makita and T. Higashinakagawa, Department of Developmental Biology, Mitsubishi Kasei Institute of Life Sciences, Machida, Tokyo 194

A cDNA clone representing the Xenopus c-fos proto-oncogene was obtained by screening the λgt10 ovarian cDNA library with cloned v-fos DNA fragment as a probe. The nucleotide and the deduced amino acid sequence of Xenopus c-fos possess a striking homology to those of chicken, mouse and human c-fos. The cDNA encodes 370 amino acids. Near the center of the molecule 5 leucine residues are repeated with an interval of 6 amino acids, possibly allowing the formation of leucine zipper (Landschulz et al., 1988).

By Northern blotting, 2.3 kb c-fos mRNA was detected abundantly in young oocytes but scarcely in early embryogenesis. Among adult tissues examined, c-fos mRNA was detected in heart, skin, intestine, lung and testis but not in muscle, kidney and liver. Thus, the expression must have been reactivated during differentiation of certain cell lineages, but it is neither germ layer related nor cell proliferation dependent. Using anti-mouse c-fos antiserum, the c-fos protein of Xenopus oocytes was exclusively localized in the germinal vesicle.

These data suggest some positive role of c-fos gene expression in the process of Xenopus oogenesis.

J 116 EXPERIMENTAL MANIPULATION OF N-MYC ACTIVITY IN OTF9 EMBRYONAL CARCINOMA CELLS. Kazuto Katoh and Hisato Kondoh, Department of Molecular Biology, Nagoya University, Nagoya 464, JAPAN.

N-myc gene is a member of the myc gene family and encodes a nuclear protein. Its expression is extremely high during early embryonic stages and the gene is considered to play an important role in that period.

In order to gain insight into the function of N-myc gene, we examined its expression during differentiation of murine embryonal carcinoma cell line OTF9. N-myc expression is very high in the undifferentiated cells and the level rapidly decreases when the cells are induced to differentiate by retinoic acid. This reduction of expression is accompanied with the activation of Hox1.1 homeobox gene.

Next we sought agents to block N-myc activity in order to infer from the effect the possible function of N-myc. We assessed N-myc-antagonizing activity utilizing the ability of N-myc to transform rat embryonal fibroblast in cooperation with activated H-ras. We found that both N-myc antisense RNA and N-myc:β-galactosidase fusion protein can block N-myc activity. We introduced these N-myc-antagonizing genes into OTF9 cells. The effect of the blockage of N-myc activity on cell differentiation state and expression of other genes such as Hox1.1 will be reported and the possible function of N-myc gene will be discussed.

J 117 CHARACTERIZATION OF POSITIVELY AND NEGATIVELY ACTING CIS ELEMENTS IN THE TFIIIA GENE PROMOTER AND IDENTIFICATION OF TRANS-ACTING FACTORS, Hildegard Kaulen, Kathleen W. Scotto and Robert G. Roeder, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY, 10021.

Expression of the 5S gene specific transcription factor TFIIIA is regulated during Xenopus laevis development, with highest levels occuring during oogenesis. In order to investigate the molecular mechanism of TFIIIA gene transcription and its developmental control, we injected 5' deletion mutants, linker deletion mutants and point mutants into stage V and VI oocytes. These analyses revealed at least three cis acting elements closely spaced between -299 and -234 that act positively and negatively on the expression of the TFIIIA gene. Using gel retardation assays and whole ovary extracts, two DNA binding proteins interacting within the positively acting cis element could be identified. As competition experiments demonstrated, one of the factors is related to the human transcription factor USF; furthermore, a polyclonal antiserum against human USF crossreacts with the Xenopus protein. We have analysed the modulation of these factors during oogenesis and will discuss how they might be involved in TFIIIA developmental control.

J 118 STRUCTURE AND EXPRESSION OF THE MOUSE SPERM RECEPTOR, ZP3, Ross Kinloch, Richard Roller, Carolyn Fimiani, David Wassarman, and Paul Wassarman, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110. The mouse egg's sperm receptor, ZP3, is one of three glycoproteins that comprise the zona pellucida. It has an apparent Mr of 83 kd and consists of a 44 kd polypeptide chain and both N- and O-linked oligosaccharides. Characterization of genomic clones spanning ~15 kb of the ZP3 locus has revealed: 1) ZP3 is encoded by 8 exons that comprise 1318 nt of an ~1.45 kb mRNA and the entire polypeptide chain (424 aa; 46.3 kd); 2) The N-terminal 22 aa comprise a signal sequence, leaving a 402 aa polypeptide chain (43.9kd); 3) The polypeptide chain is serine and threonine (≈17%) and proline (≈7%) rich, is neither very hydrophobic nor hydrophilic, has little α–helix, and has 6 potential N-linked glycosylation sites; 4) ZP3 mRNA has unusually short 5' and 3'-untranslated regions. Characterization of ZP3 gene expression by RNase protection assays has revealed: 1) Fully-grown oocytes (~85 μm) contain 220 ± 20 fg ZP3 mRNA/oocytes, or ≈240,000 copies/oocyte. This represents ≈1.8% of polysomal poly(A+) RNA in fully grown oocytes; 2) ZP3 mRNA expression is detectable solely in oocytes; 3) ZP3 mRNA is detectable in the earliest growing oocytes (≤20 μm) accumulating to ≈300,000 copies/60-70 μm oocyte before falling by ≈20% during late stages of oocyte growth; 4) ZP3 mRNA levels fall by ≈98% during ovulation (≈12hr), such that unfertilized eggs contain only ≈5,000 copies ZP3 mRNA/egg; 5) ZP3 mRNA is undetectable in preimplantation stage embryo's.
We are presently investigating the role of both the 5' and 3'-flanking sequences in the regulation of ZP3 mRNA gene expression.

J 119 GENETIC AND MOLECULAR ANALYSIS OF HETEROCHRONIC MUTANTS IN C. ELEGANS. Zhongchi Liu and Victor Ambros, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138
Mutations of heterochronic genes *lin-4, lin-14, lin-28* and *lin-29* cause temporal transformation of certain cell fates in C. elegans and alter the relative timing and sequence of post-embryonic developmental events. Three developmental events in C. elegans life cycle have been examined in these heterochronic mutants: 1) commitment to dauer larva development, 2) dauer larva differentiation, and 3) stage-specific collagen gene expression. The dauer larva is an anatomically differentiated and developmentally arrested alternative third larval stage formed in response to harsh environmental conditions. In wild type, dauer larva formation is a strictly stage-specific event (only at the second larval molt). Mutations in *lin-4* and *lin-14* lead to dauer larva formation at abnormal stages. Mutations in *lin-4, lin-14, lin-28* and *lin-29* affect dauer larva differentiation. Based on genetic analysis of multiple heterochronic mutants, a hierarchical regulatory pathway for dauer larva commitment and differentiation is proposed. We have identified two larval-cuticle-specific collagen genes col-10 and col-17, and have used them together with adult-cuticle-specific collagen genes (identified by Cox and Hirsh, MCB 5:363, 1985) to analyze these heterochronic mutants. Dot blot and Northern analysis of *lin-14* and *lin-29* mutants have indicated that *lin-14* and *lin-29* regulate temporally the expression of these collagen genes.

J 120 HOMEOTIC TRANSFORMATIONS INDUCED BY *ULTRABITHORAX* PROTEINS IN EMBRYOS AND ADULTS OF *DROSOPHILA MELANOGASTER*, R. Mann and D. Hogness, Department of Biochemistry, Stanford University Medical Center, Stanford, California 94305. P-element mediated germline transformation of various *Ubx* cDNAs driven by the heat inducible HSP-70 promoter has been utilized to analyze the phenotypic consequenses of ectopic *Ubx* protein expression. A 60 minute heat shock of 3 to 6hr embryos capable of expressing *Ubx* forms Ib, Ia, IVa, or two mutant Ia forms containin large N-terminal deletions results in a large number of phenotypic changes which can be summarized as the homeotic transformation of parasegments 1 through 5 into parasegment 6. Parasegments posterior to and including parasegment 6 are completely wild type. Shorter heat shocks result in a less extreme phenotype with the general rule that more posterior thoracic parasegments are more sensitive to transformation by *Ubx* than more anterior ones which are more sensitive than head parasegments. In Df109 homozygotes (where the endogenous *Ubx* and *abd-A* genes are absent) heat shock induced *Ubx* expression results in parasegments 1 through 10 to be transformed into parasegment 6 demonstrating that the presence of the endogenous *Ubx* gene is not required to obtain this transformation. As judged by *in situ* antibody staining, expression of *Antennapedia* protein is greatly reduced as a result of heat shock induced *Ubx* expression. Heat shock induced expression of a mutant *Ubx* form containing a frameshift mutation in the homeodomain (*Ubx*-fs) has no observable phenotype. Heat shock induced expression of *Ubx* forms Ib, Ia, IVa, or one of the N-terminal deletion mutants but not *Ubx*-fs during larval development primarily results in lethality. However, mild heat shocks near the molt between 2nd and 3rd larval instars, results in survivors with an antennal to T3 leg transformation. Rarer transformations of head capsule into thoracic notum and rough eye phenotypes have also been observed.

J 121 The Need for Enhancers Is Acquired Upon Formation of a Diploid Nucleus During Early Mouse Development, Encarnación Martínez-Salas and Melvin L. DePamphilis, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

We are attempting to identify cis-acting signals and trans-acting factors that regulate gene expression and DNA replication in mouse oocytes, fertilized eggs and developing 2-cell embryos. The activity of the polyoma virus origin of DNA replication and the expression of firefly luciferase from the herpes virus thymidine kinase promoter were used as sensitive assays for enhancer function in DNA replication and gene expression. Mouse ova were injected with plasmid DNA containing different enhancer-origin or enhancer-promoter configurations, and then allowed to continue development *in vitro*. The level of promoter activity in oocytes was independent of enhancers. However, both DNA replication and gene expression required an enhancer in developing 2-cell embryos. Competition experiments suggested that enhancers bound specific proteins that were required for replication and transcription. In contrast, DNA injected into 1-cell embryos did not need an enhancer for replication or gene expression, and no competition for replication factors was observed. In fact, the amount of DNA replication and gene expression was at least 10-fold greater in 1-cell embryos than in developing 2-cell embryos. Since 1-cell embryos that replicated injected DNA retained their pronuclei and remained 1-cell embryos, enhancers are not needed in mammalian development until a diploid nucleus is formed.

J 122 REGULATION OF HOMEOBOX GENE EXPRESSION IN HUMAN EMBRYONAL CARCINOMA CELLS. **F. Mavilio, E. Boncinelli and P.W. Andrews.** Istituto Scientifico H.S. Raffaele, Milano, Italy, I.I.G.B., Napoli, Italy and the Wistar Institute, Philadelphia, PA.

The human embryonal carcinoma (EC) cell line NT2/D1 can be induced to differentiate into a variety of cell types, including neurons, by treatment with the morphogen retinoic acid (RA). Accumulation of transcripts from 11 homeobox genes of the HOX 1, 2, 3 and 5 clusters accompains RA-induced differentiation, whereas no transcript is detectable in EC stem cells or after induction with HMBA or BUdR. Expression of different homeobox genes can be differentially regulated by varying either the concentration (10^{-8} to 10^{-5}) of or the lenth of exposure (1 to 14 days) to RA. In particular, expression of two sets of HOX 5.1 transcripts, previously shown to be regulated in a tissue- and stage-specific fashion in embryonic development, is differentially induced by progressively increasing amout of RA. RA α or β receptors are expressed in stem cells and upregulated by exposure to RA. NT2/D1 cells are therefore a model to study RA-responsive regulatory regions in human HOX genes. Transfection of a 1.0 Kb 5' genomic fragment of a HOX 3 gene linked to a reporter gene led to the identification of a putative promoter region, characterized by a canonical TATA element and an octamer consensus sequence previously found in both the Drosophyla Engrailed and the Xenopus Hbox 1 upstream regulatory regions. Studies are in progress in order to define the role of different element in the transcriptional control of the HOX 3 gene in RA-induced NT2 cells.

J 123 EXTINCTION OF GHROWTH HORMONE EXPRESSION IN SOMATIC CELL HYBRIDS INVOLVES REPRESSION OF THE SPECIFIC TRANS-ACTIVATOR, GHF-1, Alison McCormick and Michael Karin, Department of Pharmacology, School of Medicine, M-036, University of California San Diego, La Jolla, CA 92093. Extinction of growth hormone expression in somatic cell hybrids has been attributed to the repression of the pituitary specific factor GHF1 (McCormick et al., Cell 55 379-389).Somatic cell hybridization of non-GH-expressing L cells with pituitary derived GH3 cells usually results in the extinction of GH production. Using in vivo transfections, in vitro transcription, DNase I footprints and immunoblotting experiments, we found that extinction is accompanied by loss of GHF1 protein and mRNA expression. Recently cloned and sequenced, GHF1 has been identified as a homeobox containing protein with sequence homology to human B-cell specific oct-2, yeast mating factor a1, and several drosophila homeobox proteins such as Eve, Prd and IAB-7 (Bodner, et al, in press). Noting the importance of temporal and spatial expression of homeobox containing proteins in determining developmental patterns and cell type specificity, it is of interest to examine the control of GHF1 expression. We believe that this system could be used to study the very limited restriction of GHF1 expression observed in vivo (GHF1 is expressed only in cells of the somatotrophic lineage). Using GHF1 promoter fusions, regions that confer negative and positive regulation in extinguishing and non-extinguishing hybrids, respectively, are being identified.

J 124 PRIMARY STRUCTURE AND DEVELOPMENTAL EXPRESSION OF TWO HOMEOBOX GENES FROM THE ZEBRAFISH. Anders Molven, Pål R. Njølstad, Ivar Hordvik and Anders Fjose, Lab. of Biotechnology, University of Bergen, Bergen, Norway.

In Drosophila it is known that homeobox-containing genes play an important role in the control of embryonic development. These genes are also assumed to be essential for proper development of the vertebrate embryo. As the most primitive group among vertebrates, fish are very suitable for studies on the genetic regulation of embryogenesis. The model system we have selected, the zebrafish (Brachydanio rerio), is a species with rapid embryonic development and transparent embryos. We here report the nucleotide sequence of two zebrafish homeobox-containing genes, ZF-21 and ZF-114, based on the isolation of both genomic and cDNA-clones. ZF-21 is homologous to the murine Hox-2.1 gene, and is activated shortly after the gastrulation process has terminated. In situ hybridization experiments in newly hatched larvae localize the ZF-21 transcripts mainly to the hind-brain region. We could find no obvious murine homologue to the ZF-114 gene. However, ZF-114 is closely related to the Hox-1.5/Hox-2.7 group of homeobox genes and might correspond to a yet unidentified gene in the Hox-3 cluster. Northern blot analysis reveals a temporal expression pattern of ZF-114 similar to that of ZF-21.

J 125 REGULATORY DOMAINS WITHIN THE BITHORAX COMPLEX. Michael B. O'Connor+, Jeff Simon*, and Welcome Bender* , University of California, Irvine Cal. 92177+and Harvard Medical School, Boston Mass. 02115*.
The bithorax complex is responsible for determining the identities of several body segments of the fruit fly *Drosophila melanogaster*. Examination of mutations has shown that approximately 300 kb of DNA is required for normal bithorax function. Despite this large size it appears that there are only three lethal complementation groups (*Ubx*, *abd-A*, and *Abd-B*) within the complex and that most mutations do not map within known exons of these transcription units. Our current working hypothesis is that there are only a few proteins coded for by the complex, but these products are exquisitely controlled in a cell by cell manner by a complex array of cis-acting regulatory elements. We have proposed that these controlling elements are organized into regulatory "domains " which are arranged along the chromosome in the same order as the segments that they transform in the fly. We have been testing the model by fusing putative regulatory sequences to a *lacZ-Ubx* hybrid reporter gene and then reintroducing them into the fly by *P* element mediated transformation. To date, we have found at least four distinct DNA segments each of which activates expression of the reporter beginning at a different parasegmental border in eary embryos. These DNA fragments come from the *abx/bx*, *bxd/pbx*, *iab-2*, and *iab-3* regulatory regions and activate the reporter beginning at the parasegment 5,6,7,and 8 borders respectively. These sequences may form the core of a regulatory domain and are being characterized further.

J 126 FUNCTIONAL ANALYSIS OF THE MURINE HOX 1.3 HOMEODOMAIN PROTEIN. Ward F. Odenwald, Jim Garbern, Bill Greene and Heinz Arnheiter. Laboratory of Viral and Molecular Pathogenesis, NINCDS, NIH, Bethesda, Maryland 20892.
The murine Hox 1.3 homeodomain protein is a nuclear, sequence specific DNA binding protein expressed in many embryonic and adult tissues. The protein undergoes multiple phosphorylation events which increase its avidity for nuclear binding sites. DNase I protection of the Hox 1.3 gene promoter region with nuclear extracts containing recombinant Hox 1.3 protein identifies a binding site 144 bp upstream from the start of transcription. Electrophoretic mobility shift assays performed with a set of synthetic oligonucleotides corresponding to the protected region of the Hox 1.3 gene, homologous sequences of other homeobox promoter regions, and well-characterized viral, yeast and mammalian cis-elements controlling transcription and/or DNA replication allow us to deduce a consensus binding motif of CPyPyNATTAT/GPy. Base substitutions in the core ATTA sequence virtually abolish binding. In the SV40 enhancer, the Hox 1.3 binding motif overlaps both the octamer (Octa-2) and the transactivator protein-1 (AP-1) binding sites. To assay the in vivo significance of these DNA binding sites we are currently co-transfecting Hox 1.3 expression plasmids with plasmids containing different viral promoters such as the HSV immediate early promoter attached to convenient reporter genes.

J 127 GRADIENTS OF EXPRESSION OF TWO HOMEOPROTEINS IN LIMB BUDS

Guillermo Oliver, Christopher Wright, and Eddy M. De Robertis. Department of Biological Chemistry, UCLA School of Medicine, Los Angeles, California 90024.

It has long been proposed that concentration gradients of molecules may be involved in determining positional information during embryogenesis. The vertebrate embryo is subdivided into "fields" of cells acquiring the potential to form certain organs and it has been suggested that morphogenetic gradients may be involved in this process. We have analyzed the expression of two different homeodomain proteins (XlHbox1 and human Hox 5.3) in the developing limb buds of Xenopus and mouse embryos using specific antibodies which cross-react between most vertebrate species. The expression of XlHbox1 protein in Xenopus and mouse forelimb bud mesoderm shows a clear antero-posterior gradient which is strongest in the anterior and proximal region. Hindlimb bud mesoderm is devoid of this protein, representing an early molecular difference between the arm and the leg. When early tadpoles were analyzed, XlHbox 1 expression in the lateral plate mesoderm was detected in the region corresponding to the earliest field of forelimb information but not in the hindlimb field. Similar analysis of the Hox 5.3 antibody showed strong expression both in the fore and hindlimb buds of Xenopus and mouse embryos. Hox 5.3 protein also forms a gradient, with maximal expression in the posterior and distal regions of the limbs. In the forelimb bud the regions of expression of both proteins are exactly complementary, with expression of one homeodomain protein being excluded from regions where the other is expressed.The existence of these homeo protein gradients suggest a common molecular link between morphogenetic fields, gradients, and homeobox genes in vertebrate development.

J 128 APPROACHES TO THE ROLE OF HOMEOBOX GENES IN VERTEBRATE DEVELOPMENT, Nancy Papalopulu, Jim Smith, Robb Krumlauf, Lab. of Mol. Embryology, National Institute for Medical Research, Mill Hill, London NW7 1AA.

A number of murine genes that potentially play an important role in development have been isolated by virtue of their sequence similiarity to known Drosophila morphogenetic loci. Sequence identity was initially thought to be restricted to a 180 bp motif, the homeobox. However homeobox genes have been found in many organisms, and sequence comparisons have revealed additional regions of homology. This work focuses on the mouse homeobox gene Hox 2.1, that has been identified as a member of a complex of genes on chromosome 11. Hox 2.1 is transiently activated in F9 teratocarcinoma cells induced to differentiate with retinoic acid (RA). However the kinetics of expression differ depending on whether the stem cells differentiate into visceral or parietal endoderm. Data on the regulation of expression suggest that post-transcriptional events might be responsible for the very rapid response to RA. Deletion and transfection experiments in EC cells have begun to identify sequences in the 3' end of the gene involved in mRNA stability. Consistent with the idea of homeoboxes being developmentally regulated transcription factors is the nuclear localisation of the Hox 2.1 protein, as shown by antibody staining. None of the Hox 2 genes has so far shown to be allelic with known mouse mutations; therefore efforts are being made to address their function by gene transfer experiments. The strong evolutionary conservation of homeobox proteins permits the use of heterologous systems. Thus preliminary results suggest that the inapropriate expression of mouse homeobox RNA in developing Xenopus embryos is associated with specific phenotypic defects.

J 129 PROTEIN-DNA INTERACTIONS IN THE DEVELOPMENTAL, TISSUE-SPECIFIC AND SPATIAL REGULATION OF THE INDUCIBLE GLUTATHIONE-S-TRANSFERASE Ya GENE

K. Eric Paulson and James E. Darnell, Jr., Laboratory of Molecular Cell Biology, The Rockefeller University, 1230 York Ave., New York, NY 10021. The glutathione-S-transferase Ya gene is expressed in a general distribution in the adult mouse liver. The expression is boosted 10 fold by induction with xenobiotic compounds such as 3-methylcholanthrene (3-MC). However, the induction is specific for cells surrounding the central vein (pericentral cells). In order to determine the molecular interactions involved in inducible pericentral expression, deletion analysis and transient transfection assays were used. The sequences responsible for both basal expression and 3-MC inducibility were identified and shown to function on a heterologous promoter. Using hepatoma and rat liver nuclear extracts, proteins binding to the inducible sequences were shown to be present only in induced extracts. Both induced and uninduced extracts contained proteins binding to the basal elements. The protein-DNA contacts were mapped by methylation protection and methylation interference. Nuclear extracts were prepared from various times during fetal development and the appearence of binding proteins could be correlated with the observed pattern of expression during development.

J 130 NUCLEAR FACTORS THAT REGULATE IN VITRO TRANSCRIPTION OF THE HUMAN CARDIAC α-ACTIN PROMOTER, Howard M. Prentice and Laurence H. Kedes, The Medigen Project, Department of Medicine, Stanford Medical School and V.A. Medical Center, Palo Alto, CA 94304.

A number of upstream elements of the human cardiac α-actin promoter appear to govern tissue specific transcriptional regulation. In particular, the sequences from -113 to -93 bp containing a CC(A+T rich)GG element or CArG box are necessary for the transcription of the transfected gene. This CArG element has been shown to bind the serum response factor (SRF). A second upstream element also implicated in transcriptional regulation which binds NF1 and Sp1 is located between nucleotides -421 and -408. We have developed an in vitro transcription assay for investigating DNA/protein interactions governing transcription of the gene. Experiments employing whole cell extracts from Hela cells indicate an upstream sequence dependence for the cardiac α-actin promoter constructs relative to basal transcription levels. Nuclear extracts prepared from myogenic cell lines were found to enhance basal transcription levels obtained with Hela extracts. The in vitro transcription assay has been further used in reconstitution experiments with transcription factors NF1, Sp1 and the serum response factor in addition to fractionated whole cell extracts from C2 myotubes. Using the in vitro transcription assay it is possible to distinguish the contributions of muscle specific factors from constitutive factors in the transcriptional regulation of this sarcomeric gene.

J 131 AN AP1 ELEMENT IS REGULATED BY A VARIETY OF FACTORS IN A TISSUE SPECIFIC MANNER. John P. Quinn, Rosella A. Farina and David Levens. Lab of Pathology, NCI. NIH. Bethesda MD 20892.

The enhancer element of the Gibbon Ape Leukemia Virus (GALV) contains a perfect consensus sequence for the AP1 regulatory motif which has been demonstrated to bind the two transcription factors c-fos and c-jun. In addition we have demonstrated that this element displays tissue specificity and will bind factors distinct from those above in a cell line in which the enhancer activity is optimal, MLA 144. In these cells the GALV motif binds a complex which contains at least three peptides. Two of these peptides are present in a variety of cell lines in which the enhancer activity varies considerably the other is tissue specific and correlates with high enhancer activity. All three of these peptides are required for specific complex formation at this locus. Thus it would appear that one element, the GALV AP1 motif, can be regulated by a variety of transactivators we are exploring the relationship of these factors to one another and to the AP1 motif in cell differentiation.

J 132 THE PROTEIN PRODUCT OF THE HOMEOTIC GENE *DEFORMED* BINDS TO ITS OWN PROMOTER DNA IN A SEQUENCE-SPECIFIC MANNER, Mike Kuziora, Clare Bergson and William McGinnis, Departments of Biology and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511

Deformed, a homeotic selector gene of *Drosophila* necessary for proper development of the posterior head, codes for a 63.5 kD homeodomain containing protein. E.coli extracts from the strain over-expressing the *Dfd* protein and embryonic nuclear extracts from a transgenic fly strain expressing *Dfd* protein under the control of *hsp70* promoter, were used to test if *Dfd* is a sequence-specific DNA binding protein. Using immunoprecipitations of DNA-protein complexes with anti-*Dfd* serum we have probed 40 kb of genomic DNA in and around the *Dfd* locus. We have identified the highest affinity binding region located 4.5 kb upstream of the *Dfd* gene transcription start site. Footprints of that region will be presented. Results of the genetic experiments with *hsp70Dfd* fusions show that there is an autoregulatory loop involved in the maintenance of *Dfd* ectopic expression in transgenic embryos. Results of the experiments with *Dfd* promoter/*B*-gal fusions suggest that sequences involved in autoregulation map to the region identified through immunoprecipitations as having the highest affinity for *Dfd* protein. We plan to mutate this region to test for its function *in vivo* using promoter/*B* -gal fusions in *hsp70Dfd* background.

J 133 T-DNA MUTAGENESIS AND THE IDENTIFICATION OF GENES INVOLVED IN THE REGULATION OF TOBACCO SEED MATURATION, Bernd Reiss, Csaba Koncz, Christiane Plum, and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, D 5000 Köln 30, F.R.G.

Seed storage proteins accumulate to high levels during development of the seed and provide nutrition for the seedling during germination. The expression of these proteins is tissue specific and developmentally coordinated. Promoters of some of these storage proteins have been characterised recently. We designed a strategy to identify factors participating in the regulation of these genes which takes advantage of transformation by *Agrobacterium tumefaciens*. A selectable marker gene was combined with a seed storage protein promoter and transgenic tobacco plants were generated. These plants expressed the marker gene exclusively in the maturing seed. In the next step, protoplasts will be isolated from these plants and transformed with a Ti-plasmid vector containing a constitutive promoter and a second selectable marker. As the T-DNA inserts randomly into the genome, the constitutive promoter will be inserted occasionally in front of regulatory genes leading to the activation of the first selectable marker and thus to a selectable phenotype. Using the inserted T-DNA as a probe, the tagged regulatory gene can be isolated by conventional molecular biology methods.

J 134 CIS-ACTING REGIONS OF THE HISTONE H5 GENE. Stéphane Rousseau and Adolfo Ruiz-Carrillo, Cancer Research Center and Department of Biochemistry, School of Medicine, Laval University, Québec, CANADA, G1K 7P4

To investigate the elements involved in the expression of the erythrocyte specific histone H5, plasmids containing up to 3,5 kb of 5' flanking H5 gene, including all known DNAseI hypersensitive sites, were fused to a CAT reporter construct and transfected into erythroid and non-erythroid cells. CAT expression measured in transient assays indicated that the H5 promoter had a higher activity in erythroid cells. Deletion analysis defined two distal tissue-specific elements (-3500 to -1155), a negative element (-115 to -90), a functional GC-box (-83 to -74) and a proximal activating element (-59 to -38). A gel retardation analysis revealed that two distinct complexes can be formed by the fragment -97 to -37. The effect of H5 gene 3' flanking regions were analysed on homologous or heterologous promoter. We found an activating erythrocyte-specific element downstream of the gene. Its activity, modest in HD3 cells, is mostly independent of position, orientation, and type of promoter. S1 mapping showed that the transcripts originated at the H5 promoter.

J 135 COOPERATIVITY OF FOS AND JUN ONCOPROTEINS IN TRANSCRIPTIONAL REGULATION, Paolo Sassone-Corsi, Lynn J. Ransone, Jane Visvader, Isabelle Tratner, V.J. Dwarki, William W. Lamph and Inder M. Verma, Molecular Biology and Virology Laboratory, The Salk Institute, La Jolla, CA 92037.

Proto-oncogene fos transcription is inducible with a wide variety of agents influencing growth, differentiation and development. Induction is invariably very rapid but transient, and is carried out by at least two pathways involving either adenylate cyclase or protein kinase C. The serum-induced transcription of the fos gene is under negative feedback regulation mediated by the fos protein. The fos promoter region responsive to repression is also required for serum inducibility and binds a nucleoprotein complex in which the nuclear factor AP-1 is associated with fos protein. Transcription factor AP-1 is the product of the jun proto-oncogene, which is also serum and TPA inducible. The fos-associated p39 has recently been demonstrated to be the product of the jun oncogene. Interestingly, the fos gene product enhances c-jun activation function of a TPA responsive promoter element (TRE). The two oncoproteins fos and jun presumably form heterodimers which bind to a TRE with an affinity one thousand-fold higher than the presumed jun homodimers. Our results to date indicate that the fos "leucine zipper" domain (aa 165 to 193) appears to be essential for binding activity. A distinct domain, localized in the carboxy region of the fos protein, is responsible for transcriptional regulation. We are currently studying the effect of single amino acid mutations in both fos and jun proteins, affecting the "leucine zipper" domains, the putative DNA-binding domains, and the phosphorylation sites.

J 136 EXPRESSION OF THE *DROSOPHILA* TROPOMYOSIN I GENE IS CONTROLLED BY 5' FLANKING AND INTRON DNA SEQUENCES, Joshua Schultz, Terese Tansey, Paul Hanke, Michele Dumoulin and Robert V. Storti. Department of Biochemistry, University of Illinois College of Medicine, 1853 W. Polk St., Chicago, Il. 60612.
Transcriptional control of Tropomyosin I (TmI) gene expression has been investigated by P-element transformation. We have shown previously [Tansey et.al.(1987) EMBO J. 6:1375] that a plasmid containing the TmI gene be can used to successfully transform and rescue *Drosophila* flightless *Ifm(3)3* TmI mutant flies. One copy of the TmI gene is necessary to rescue jumping and two copies of the gene are necessary to rescue flight. To determine the cis-acting DNA sequences that control TmI gene expression, we have made deletions of the TmI gene and have studied their effect on expression by transformation and rescue of flightless mutant flies. The results, based on rescue and measuring mRNA levels by Northern blot analysis, indicate that there are at least two DNA regions that control expression. One of these is located within 800 bps 5' of the transcription start site. The second control region is located within the first intron of the gene and has some properties characteristic of an enhancer element.

J 137 PROTEIN BINDING TO THE PROMOTER OF A DEVELOPMENTALLY REGULATED XENOPUS CYTOKERATIN GENE Alison M. Snape, Erzsebet Jonas and Thomas D. Sargent, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892. The promoter of an embryonic, epidermal cytokeratin gene of X. laevis, XK81A1 (Jonas et al., 1985, P.N.A.S. 82, 5413-5417) has been mapped by injecting a series of 5' deletion clones into the fertilised egg, and monitoring mRNA expression in the embryo. The results suggest that sequence(s) between -487 and +26 control tissue specific expression of the gene. Protein binding to this region has been studied using gel electrophoresis mobility shifts. A crude nuclear extract from tailbud embryos (in which XK81A1 is actively transcribed) produces a mobility shift in a fragment containing promoter sequence from -425 to -143. This shift is efficiently competed by an excess of cold probe, but not by plasmid DNA. When different sections of the probe were used as competitors, the sequence -220 to -143 competed at least ten times more efficiently than other sections, suggesting that a nuclear protein binds to this 77bp region of the promoter. The putative binding site is being mapped more precisely using oligonucleotide competitors in mobility shift assays, and methylation interference. Additional mobility shifts have been carried out on oligonucleotide sequences from the region of interest of the XK81A1 promoter. One of these sequences, which can be shifted by embryonic nuclear extracts, shows sequence similarity to the Factor 2 protein binding site of the c-fos promoter (Gilman et al. 1986, Mol.Cell.Biol. 6, 4305-4316).

J 138 EMBRYONAL CARCINOMA CELL-SPECIFIC NUCLEAR FACTOR(S) THAT BIND TO AN ENHANCER ACTIVE IN EC CELLS, Makoto Taketo and Daniel J. Shaffer, The Jackson Laboratory, Bar Harbor, ME 04609

Murine embryonal carcinoma (EC) cells are refractory to infection by retroviruses because retroviral LTR enhancers have little activity in EC cells. We isolated clonal cell lines that express an integrated neomycin-resistance (*neo*) gene linked to the Moloney murine leukemia virus (Mo-MuLV) LTR [Taketo, M., Gilboa, E. & Sherman, M.I.(1985) *Proc.Natl.Acad.Sci.USA* **82**:2422-2426]. From a vicinity of proviral integration site of one such EC cell line, we isolated a cellular enhancer that allows the viral *neo* gene to be expressed in EC cells [Taketo, M. & Tanaka, M. (1987) *Proc.Natl.Acad.Sci.USA* **84**:3748-3752]. The enhancer sequence contains distinctly characteristic stretches as well as some similarity to various viral and cellular enhancers. Using this enhancer sequence as a probe, we have investigated nuclear binding factors from EC cells by "gel-shift" assays. Three distinct retarded bands have been observed. One of these bands has appeared specific to the enhancer sequence because it has been competed only by its own subsequences. Another band has been competed by a polyoma enhancer-containing fragment whereas the third band has been competed by any DNA sequences, indicating a non-specific binding. The cellular enhancer-specific band has been detected in both PCC4.azalR and F9tk$^-$ EC cell lines whereas the band was not observed with the nuclear extracts from differentiated cells. The result suggest that the binding factor(s) mediates the enhancer activity in EC cells.

J 139 A NEW LOCUS IN C. ELEGANS AFFECTING SEX DETERMINATION AND DOSAGE COMPENSATION Kimberly A. Tanner and William B. Wood MCD Biology, University of Colorado, Boulder 80309

The X-linked ct31 mutation is one of only a few mutations in C. elegans that appear to affect both sex determination and dosage compensation. ct31 was isolated after gamma-ray mutagenesis as a semidominant feminizing mutation. ct31 XX animals are Dumpy (Dpy) hermaphrodites; XO animals are Dpy, variably feminized males. The ct31 mutation also causes a reduction in the level of expression of two X-linked genes in XX animals, suggesting that the ct31 locus is involved not only in sex determination, but also in establishing the hermaphrodite mode of dosage compensation. Genetic evidence suggests that the ct31 mutation may cause a gain of function at the locus, resulting from a chromosomal rearrangement. Quantitative Southern blot analysis using a probe of cloned sequences from the linked myo-2 locus indicates that this region of the chromosome is approximately doubled in copy number in the ct31 mutant, but is restored to normal copy number in ct31 revertants. These results are consostent with the ct31 mutation being a tandem duplication that includes an early-acting feminizing locus on the X chromosome.

J 140 DNA-BINDING SPECIFICITY OF DROSOPHILA HOMEO DOMAIN AND ZINC FINGER PROTEINS, Jessica E. Treisman and Claude Desplan, Howard Hughes Medical Institute, The Rockefeller University, New York, NY, 10021. The segmentation and homeotic genes of Drosophila interact in a complex network to direct pattern formation in the developing embryo. Many of their protein products have sequence-specific DNA-binding activity, suggesting that this interaction occurs at the level of transcriptional regulation. We are examining the specificity of DNA binding in vitro by proteins of these classes containing homeo domains and zinc finger domains. Consensus sequences for binding by the engrailed and Kruppel proteins have been defined, and we are using the engrailed consensus sequence to determine the influence of the amino acid sequence of the homeo domain on its binding specificity. Both comparisons of diverged homeo domain proteins and site-directed mutagenesis will allow us to identify specificity-determining amino acids in the presumed recognition helix. We are also searching for binding sites of potential physiological relevance in the upstream regions of possible target genes, beginning with Kruppel protein binding sites in the hunchback promoter.

J 141 *HOX-1.4* EXPRESSION DURING MURINE MALE GERM CELL DIFFERENTIATION AND EMBRYOGENESIS, C.M. Viviano and D.J. Wolgemuth, Department of Genetics and Development, Columbia University College of Physicians and Surgeons, New York, NY 10032. *Hox 1.4* was isolated from a mouse testis cDNA library using the homeo box region of the *Drosophila Antennapedia* gene and is a member of the Hox 1 Complex of homeo box-containing genes on mouse chromosome 6. We have shown *Hox-1.4* to be expressed abundantly in the testis of the adult animal. Expression within this tissue has been found to be germ cell specific. Northern analysis of poly(A)$^+$ RNA isolated from enriched populations of pachytene spermatocytes and early spermatids has revealed the presence of two *Hox-1.4* transcripts in the testis. A 1.35 kb transcript is detected in meiotic cells while a 1.45 kb transcript appears during post meiotic germ cell stages. RNase H treatment suggests that the difference in transcript size is due to poly A tail length. A third *Hox-1.4* mRNA of 1.7 kb is detected in 10.5-16.5 g.d. embryos. This transcript has been shown to be expressed in the rostral portion of the neural tube in the region of the myelencephalon of the 13.5 day embryo. Preliminary results from RNAse protection experiments suggest that the difference between the embryo and adult transcripts lies in their 5' regions. Studies in progress will further define the basis of the transcript differences with respect to the gene. We are also using antibodies generated to a synthetic peptide located near the COOH terminus of the protein to characterize the *Hox-1.4* protein.

J 142 CROSS-BINDING OF NUCLEAR FACTORS TO PROMOTER ELEMENTS IN THE c-FOS AND MUSCLE ACTIN GENES. Kenneth Walsh, Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106.

A conserved 28 bp element in the skeletal actin promoter is sufficient to activate muscle specific expression when placed upstream of a TATA element. This muscle regulatory element (MRE) is similar in structure to the serum response element (SRE) that is present in the promoters of the c-*fos* proto-oncogene and the non-muscle actin genes. The SRE can function as a constitutive promoter element. Despite this difference in expression both elements bind to the same protein factors *in vitro*. These proteins are the serum response factor (SRF) and the muscle actin promoters 1 and 2 (MAPF1 and 2). The SRF and MAPF proteins are resolved by chromatographic procedures and they differ in their relative affinities for each element. The factors are further distinguished by their distinct, but overlapping, methylation interference footprint patterns on each element. These data indicate that the differences in the expression from the SRE and MRE may be due to a complex interaction of protein factors with these sequences.

J 143 PURIFICATION OF TCF, A HUMAN T-LYMPHOCYTE PROTEIN THAT BINDS TO THE CD3, lck, AND HIV-1 PROMOTERS. Marian L. Waterman and Kathy A. Jones, The Salk Institute, La Jolla, CA 92037

T cells develop into mature immuno-competent lymphocytes in stages defined by the presence and/or absence of various antigens on the cell surface. We have recently identified and isolated from T cell extracts (Jurkat, mature human helper T cell line), a DNA binding protein that interacts with the promoter regions of genes encoding several of these cell surface antigens as well as other genes expressed in lymphoid cells. This protein, designated TCF (T Cell Factor) appears to be greatly enriched in T cells and is not apparent in extracts from a representative B cell line (JY cells; human mature Ig secreting) or a non-lymphoid cell line (Hela, human cervical carcinoma). TCF was purified by sequence specific DNA affinity chromatography using a binding site sequence from the CD3 δ gene, which encodes one of the four CD3 subunits that associate with T cell receptor subunits on the cell surface. SDS-PAGE analysis of purified TCF revealed a tight cluster of 60 kd to 55 kd proteins that were confirmed by southwestern analysis to be responsible for specific TCF DNA-binding properties. DNAase I footprint analysis with purified protein fractions revealed that TCF binds near the transcription initiation sites of the CD3 γ gene and the gene encoding the T cell receptor γ subunit, both of which are coordinately expressed with the CD3 δ gene. In addition TCF binds near the transcription initiation site of the murine lck gene (lymphoid specific tyrosine kinase) and to three regions of the HIV-1 LTR. Work is in progress to determine the influence of TCF binding on the transcriptional activity of the promoters discussed above in *in vitro* transcription systems.

J 144 TRANSCRIPTIONAL CONTROL BY THE *ANTENNAPEDIA* AND *FUSHI TARAZU* PROTEINS IN CULTURED DROSOPHILA CELLS. Gary M. Winslow, Shigeo Hayashi, and Matthew P. Scott. Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347. Two Drosophila homeodomain-containing proteins, *Antennapedia (Antp)* and *fushi tarazu (ftz)* can activate the *Ultrabithorax (Ubx)* promotor in a transient cotransfection assay in cultured cells. We show that a 75bp element in the *Ubx* promotor that is sufficient for the *Antp*-dependent gene activation is located in the non-translated leader, but can function when placed upstream of the normal *Ubx* transcription start site or when attached upstream of a truncated *hsp70* promotor. The *Antp* protein binds to the 75bp element *in vitro* (S. Hayashi, unpublished data) as does the *Ultrabithorax (Ubx)* protein (Beachy, Krasnow, Gavis, and Hogness, personal communication). When a hybrid protein containing an N-terminus from *Antp* and a homeodomain from *Ubx* was tested in the cultured cell assay it activates the *Ubx* and *Antp* promotors in a manner similar to the wild-type *Antp* protein, indicating that in this case related homeodomains are functionally interchangeable.

J 145 THE XlHbox 1 GENE OF XENOPUS: ONE GENE PRODUCES TWO PROTEINS WITH DIFFERENT ANTERO-POSTERIOR BANDS OF EXPRESSION IN THE EMBRYO, Christopher V.E. Wright, Ken W.Y. Cho, G. Oliver, J. Hardwicke, R. Collins, D. Bittner and E.M. De Robertis, Department of Biol. Chem., UCLA School of Medicine, Los Angeles CA 90024-1737.

Immunostaining of sections and whole mounts shows that XlHbox 1 proteins are expressed in a narrow antero-posterior band throughout the CNS and mesoderm of Xenopus and mouse embryos, which might be consistent with XlHbox 1 providing or interpreting positional information during embryogenesis. The two promoters of XlHbox 1 produce transcripts which differentially utilize the same ORF to make two proteins which have the same homeodomain but differ by an 82 aa amino terminal extension (Cho et al., 1988, EMBO J. 7 2139-2149) and in their region of expression in the antero-posterior axis (Oliver et al., 1988, EMBO J. 7 3199-3209). We have synthesized SP6 mRNA for the long and short proteins. Homeodomain point mutations in both proteins serve as controls. Long protein mRNA is translated extremely efficiently and disrupts the entire CNS and mesoderm on the microinjected side of the frog embryo. Short protein mRNA causes lesser asymmetries only over the cervical CNS. Microinjected antibodies delete a subset of the cervical neural crest (dorsal fin, dorsal root ganglia) and cause CNS malformations (sometimes resembling hindbrain structures) restricted to regions that normally express XlHbox 1. SV40 early promoter plasmids which produce the long or short protein ectopically in microinjected embryos (although highly mosaic) and reporter gene constructs are being used to define promoter and enhancer elements in XlHbox 1 functioning both in Xenopus and Drosophila embryos.

Egg Activation; Plant Gene Regulation

J 200 THE PARTHENOGENESIS INDUCTION BY GAMMA-IRRADIATED POLLEN IN ANGIOSPERMS. Sergey V.Andreichenko and Dmitry M.Grodzinsky,Department of Biology, Kiev Medical Institute,Kiev,USSR.

The phenomenon of parthenogenesis provides for rapid inbred line selection in various plants.The present investigation was undertaken to examine the modes and conditions for stimulation egg cell development either in haploid or diploid embryo without previous fertilization.It has been established that the induction of haploid parthenogenesis in plants with long style and binucleate pollen such as petunia and tobacco is possible by their own pollen irradiated by the dose of approximately 100 Gr.The irradiation caused the generative cell mitosis failure and consequently syngamy absence.Meanwhile the pollen tube penetration into embryo sac and triple fusion have occured,moreover the last event resulted in endosperm formation.For plants with trinucleate pollen,for example wheat,similar results were obtained by irradiation of anthers with microspores instead of mature pollen in dry state.The parthenogenetically developed diploid embryos were revealed in pollinations with more heavily irradiated pollen,which contained significantly damaged and disordered chromatin.The pollinations were accompanied by essential slowing of pollen tube growth in the style and full blockade of double fertilization process.The normal seed appearance in this case is explained by existence in pollen of specific substances able to induce mitotic activities of female sex cells in embryo sac.

J 201 EXPRESSION AND SEQUENCE OF AN AUXIN-INDUCIBLE GENE IN SUSPENSION CULTURE CELLS OF ARABIDOPSIS THALIANA. Timothy W. Conner[1], Virginia Goekjian[1], Pawel Strozycki[2], and Joe L. Key[1], [1]Department of Botany, University of Georgia, Athens, GA 30602, [2]Institute of Bioorganic Chemistry, Polish Academy of Sciences, 61-704 Poznan, Poland.

In order to characterize and isolate plant growth hormone regulated DNA binding proteins, we have isolated Arabidopsis auxin-inducible genes homologous to soybean Aux22 and Aux28 genes [Ainley et al.(1988), JBC 263:10658]. Of several different genomic clones isolated, one (AtAux2-27) is expressed in suspension culture cells and hybridizes to 630 nt and 710 nt transcripts; its expression is enhanced in suspension culture cells within 30 minutes after treatment with the auxin indole acetic acid (IAA). The predicted amino acid sequence obtained from DNA sequencing has revealed substantial blocks of amino acid similarity with GmAux22. We have verified expression of AtAux2-27 by S1 nuclease analysis and have identified putative regulatory elements. We will present these results as well as the results from our current work to identify auxin regulated DNA binding proteins as detected by gel retardation analyses of cell extracts on the adjacent upstream regulatory region of this gene.

J 202 BOTH DEVELOPMENTAL AND METABOLIC SIGNALS REGULATE THE EXPRESSION OF PATATIN GENES IN SOLANUM TUBEROSUM. Wolf Frommer, Mario Rocha-Sosa, Meike Köster, Uwe Sonnewald, Xiang Yun Liu and Lothar Willmitzer Institut für Genbiologische Forschung, Ihnestr. 63, 1000 Berlin 33. Patatin is one of the major soluble proteins in potato tubers and is encoded by a multigene family. Based on structural considerations two classes of patatin genes are distinguished. The promoter of a class I gene contained within a 1.5 kb sequence when fused to the ß-glucuronidase reporter gene is essential and sufficient to direct a high level of tuber specific gene activity i.e. 100-1000 fold higher in tubers than in leaves, stems and roots of greenhouse grown transgenic potato plants. Histochemical analysis revealed this activity to be present in parenchymatic tissue but not in the peripheral phellem cells of transgenic tubers. Furthermore the promoter can be activated in leaves under conditions that simulate the need for the accumulation of starch in storage organs i. e. high levels of sucrose. The expression is restricted to mesophyll and epidermal cells in contrast to e.g. vascular tissue. In analogous experiments we could show that the regulatory region of a class II gene directs the patatin expression to a ring of cells in the periderm and to the pericycle of transgenic potato tubers and to the rhizodermis behind the apical meristem in the tips of roots from both transgenic potato and tobacco plants. We find no inducibility of the chimaeric class II gene upon increasd levels of sucrose.

J 203 SEQUENCE-SPECIFIC DNA-BINDING PROTEINS IN DEVELOPING SEEDS OF BRASSICA NAPUS, Anna-Stina Höglund,Mats Ellerström,Ines Ezcurra,Hans-Olof Gustavsson,Kjell Stålberg, Elisabeth Westergren,Lars-Göran Josefsson and Lars Rask. Department of Cell Research,Swedish University ofAgricultural Sciences, Uppsala Biomedical Center, Box 596,S-75124 Uppsala Sweden. Napin is one of the major storage proteins in oilseed rape. It is a small protein coded by some 10-16 genes. Expression of the Napin genes is strictly regulated to a short time period in the developing embryo. Nuclear extracts prepared from immature rapeseed contains several DNA-binding proteins which binds to different sequence motifs in the Napin promotor/upstream region. The binding specificities for these proteins were defined by gel-retardation assay using doublestranded oligonucleotide probes in competition with unlabeled DNA. DNase 1 footprinting experiments performed on a restriction fragment of the Napin promotor region shows distinct protection over some of these protein binding regions. We have started characterization and purification of these DNA-binding proteins and so far the molecular weight for one of them have been determined by DNA-protein UV-crosslinking experiments. In order to study the tissue specific regulation of storage protein genes in developing seeds we are establishing a heterologous template dependent in vitro transcription assay. This will enable detailed analyses of the effects of different DNA-binding proteins as well as mutaions in the Napin promotor region on the transcription of the Napin gene. Similar mutants will also be tested in vivo by analyses of transformed plants, to confirm our results from in vitro studies.

J 204 ORGAN SPECIFIC EXPRESSION IN CORN PLANTS, Jeannine Horowitz, Jeff Rosichan, EniChem Americas, Inc., 2000 PrincetonPark Corporate Center, Monmouth Junction, NJ 08852 Our research has dealt with the characterization of genes which are expressed in different organs of the corn plant. We have identified cDNA clones which represent mRNAs that are induced in specific organs. Of particular interest are those which are expressed only in one week old stalks and not in leaves, since at this age corn stalks are morphologically very similar to leaves. We have also identified a cDNA which hybridizes to 3 mRNAs which are expressed differentially in leaves, stalk, root and kernel. These mRNAs represent between 0.06% and 0.01% of the total mRNA population. We are now using in situ hybridization to determine cell-type specificity within the organs.

J 205 THE *vasa* GENE OF *Drosophila melanogaster*, Paul F. Lasko and Michael Ashburner, Department of Genetics, University of Cambridge, Cambridge, England CB2 3EH. *vasa* is a maternally-active gene involved in the specification of correct anterior-posterior positional information in the embryo. The function of *vasa* is also required at a very early stage in oogenesis, since in females homozygous for a deletion of *vasa*, germ-line development usually becomes aberrant at the time of differentiation of the oocyte. Females homozygous for weaker alleles of *vasa* develop eggs, but these are abnormal; the resulting embryos lack their pole cells and show abnormal posterior development. Males homozygous for a *vasa*$^-$ deficiency (or for the weaker alleles) are indistinguishable from wild-type in their viability or fertility, indicating that the requirement for *vasa* is specific to the female germ line.

We have isolated and characterised the *vasa* gene (Lasko, P. F. and Ashburner, M., Nature 335: 661-667). The predicted vasa protein exhibits 29.1% amino acid identity to murine eukaryotic initiation factor-4A (eIF-4A) over a 376-amino-acid overlap which includes the eIF-4A ATP-binding site. Together with six other proteins, from *E. coli*, yeast, mice, and man, *vasa* and eIF-4A form a new protein family. The family includes a murine protein, PL10, whose expression is specific to the male germ line, and which shares 47% amino acid identity to *vasa* over the same 376-amino-acid overlap.

J 206 ALTERATIONS OF PLANT GROWTH AND DIFFERENTIATION BY MANIPULATION OF PHYTOHORMONE LEVELS IN TRANSGENIC PLANTS. June Medford and Harry Klee, Plant Molecular Biology Group, Monsanto Company, St. Louis, Missouri 63198.

The plant hormones auxin and cytokinin play regulatory roles in the processes of growth and differentiation. Auxin and cytokinin biosynthesis genes from *Agrobacterium tumefaciens* were genetically engineered for expression in plants. A region of the Ti plasmid coding for auxin or cytokinin biosynthesis was fused with an inducible transcriptional promoter (maize hsp70) and transferred to plants. Transgenic *Arabidopsis*, tobacco and petunia plants containing the chimeric genes were obtained. After heat shock (hs) for 2 hours (45°C), transgenic *Arabidopsis* plants containing the hs promoter fused to a gene for auxin biosynthesis had a 15 fold increase in the level of the auxin, IAA. The elevated auxin level resulted in morphological aberrations in various tissues and organs. In transgenic petunia plants these included internode elongation, abnormal leaf expansion, and an increase in vascular tissue. Transgenic plants containing the hs promoter fused to a cytokinin biosynthesis gene had an 8 fold increase in cytokinin levels in the absence of heat shock and a additional 60 fold increase after heat shock. Plants containing the chimeric cytokinin gene showed developmental changes in the absence of heat induction. Despite the large increase in cytokinins following heat shock, there was no further alterations of plant development.

J 207 **DIRECT EVIDENCE FOR A TRANSLATIONAL ROLE FOR THE POLY(A) TRACT OF mRNA**, David Munroe and Allan Jacobson, Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, MA 01655

Translational regulation of specific mRNAs in response to developmental signals in Spisula (**JMB** 166:309, 1983), Dictyostelium (**Cell** 36:1017, 1984), and the rat hypothalamus (**MCB** 8:2267, 1988; **Science** 241:342, 1988) has been associated with changes in mRNA adenylation status. We interpret such regulation in terms of a model for the function of the 3'-poly(A) tract of mRNA which postulates that: a) an interaction between poly(A) and a cytoplasmic poly(A)-binding protein enhances the efficiency of translational initiation; b) mRNAs with relatively long poly(A) tails have a translational advantage over mRNAs with shorter poly(A) tails; and c) the regulatory mechanisms which ensure the efficient translation of poly(A)$^+$ and poly(A)$^-$ mRNAs may be quite different (**NAR** 11:6353, 1983; **Cell** 36:1017, 1984). To test this model directly we have constructed derivatives of pSP65 which direct the synthesis of synthetic mRNAs with different discrete poly(A) tail lengths and compared the relative efficiencies with which such mRNAs are recruited into polysomes and translated in reticulocyte extracts. Using this assay, poly(A)$^-$ mRNAs are found to have a significantly reduced translational capacity when compared to poly(A)$^+$ mRNAs. Poly(A)$^-$ mRNAs are recruited into polysomes less efficiently than poly(A)$^+$ mRNAs and the defect in poly(A)$^-$ mRNAs affects translational initiation. This defect is distinct from the phenotype associated with CAP-deficient mRNAs and appears to have a profound affect on the formation of mRNPs.

J 208 *YEAST GENES IN PLANT DEVELOPMENT, Ioan Negrutiu, Jocelyne Dewulf(Institute of Molecular Biology, St. Genesius-Rode, Belgium), Pierre Hilson, Jean-Marie Jacquemin (Station d'Amelioration de Plantes, Gembloux, Belgium).*
Successful attempts to complement a plant ile⁻ mutant with the corresponding yeast ILV-1 gene(Colau et al, 1987) determined us to use other yeast genes and, following overexpression upon transformation of Nicotiana tabacum and N. Plumbaginifolia protoplasts by direct gene transfer, we assessed their impact on cellular and developmental processes. More specifically, we used several yeast genes involved in the adenylate cyclase system(such as CDC 35, CDC 25, PDE, etc) and show that at least some of them affect important functions in plant cell proliferation and differentiation processes. Both transient and stable transformation experiment have been performed. The results will be discussed in the light of physiological and molecular data, and are considered to bring some new answers to the controversed role of cAMP in plant development.

Colau et al(1987) Molec Cellul Biol 7, 2552 - 2557

J 209 TISSUE SPECIFIC AND HORMONAL REGULATION OF A NOVEL BARLEY GENE IN DEVELOPING AND MATURE GRAINS. **O.-A.Olsen, K.Jakobsen, S. Klemsdal and D.W.Hughes, Dept of Biology, University of Oslo, P.O.Box 1031 Blindern, 0315 Oslo 3 Norway.**
Differential screening of an aleurone cDNA bank from 20 day-old grains resulted in several clones that were either aleurone or aleurone/embryo specific. One clone of the latter class, B22E, a novel barley gene, possesses the following characteristcs: (i) spatial and temporal control of message level, i.e. cell specific expression pattern both in the endosperm and the embryo. The steady state level of the message peaks during mid-maturation and the transcript is missing in mature aleurone and embryos. (ii) Differential regulation in different tissues; In the immature aleurone incubation on basal medium for 20 hrs results in the disappearance of the message, whereas no change is observable in the embryo under similar conditions. Addition of ABA to incubated immature embryos, however, results in the loss of the message. Although absent from mature embryos, transcription is reactivated at the onset of germination.
A genomic B22E-clone is currently under study with the aim to identify sequence elements conferring aleurone and embryo specificity of expression as well as ABA suppression and germinal activation.

J 210 SIGNALS EMANATING FROM THE BLASTOPORE LIP AREA ASSIST IN ORGANIZING THE NEURAL INDUCTION RESPONSE. Carey R. Phillips, Department of Biology, Bowdoin College, Brunswick, ME 04011
Two antibodies, Epi 1 and NCAM, were used to assay the response and the mechanisms of pattern generation of ectoderm during the process of neural induction. Epi 1 recognizes a molecule expressed only on non-neural epithelium. Ventral ectoderm stains positive while dorsal ectoderm does not express the Epi 1 antigen. However, grafting a blastopore lip to isolated ventral ectoderm will cause an area of non-expression adjacent to the animal side of the grafted blastopore lip. Isolated ventral ectoderm does not express NCAM, a neural specific antigen. Combining chordamesoderm, the classic neural inductor, will induce a small amount of NCAM synthesis, however, the cells expressing NCAM are not organized morphologically. Ventral ectoderm, preconditioned by grafting blastopore lip and then co-cultured with chordamesoderm expresses NCAM more intensely and in a higly organized fashion. Thus, the blastopore lip appears to organize the response of both Epi 1 and NCAM expression during the course of neural induction.

J 211 CHARACTERIZATION OF A GENE FAMILY IN *ARABIDOPSIS* WHICH HAS SEQUENCE SIMILARITY TO THE S-LOCUS OF *BRASSICA OLERACEA*, Sara E. Ploense and Robert E. Pruitt, Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108-1095. Identification of different types of individuals in the self-incompatibility system of *Brassica oleracea* is genetically controlled by a single gene known as the S-locus. This gene is involved in a recognition process which arrests self-pollinations at the stigma surface. The product of the gene is a glycoprotein which is abundantly expressed in the stigma of incompatible *Brassica* species but is not easily detected in the pollen or anthers. The gene has recently been cloned and shown to be a member of a large multigene family in *Brassica oleracea*. The possible functions of the other members of this gene family are unknown. We have recently shown that there are a number of genes found in the self-compatible weed *Arabidopsis thaliana* which cross-hybridize with members of the S-locus family. Sequence characterization of the *Arabidopsis* genes demonstrates them to be quite similar to some members of the *Brassica* family. Work is in progress to clone more of the *Arabidopsis* genes and characterize them with respect to nucleotide sequence and their temporal and spatial patterns of expression. In this way we hope to elucidate the roles these genes may play in a self-compatible plant.

J 212 DELETION CLONING APPLIED TO THE ISOLATION OF THE *REG* LOCUS OF *VOLVOX*, Donald Straus and Fred Ausubel, Department of Molecular Biology, Massachussets General Hospital, Boston, MA,02114. The *reg* locus of the eukaryotic alga *Volvox* provides a unique opportunity to dissect the mechanism of germ: soma specialization. *Volvox* individuals are composed of one thousand cells of only two types: somatic and reproductive. These differ greatly in both morphology and function. The most striking functional difference is that somatic cells never divide while reproductive cells cleave to form new embryos. In *reg* mutants, cells that would normally become somatic cells differentiate instead as reproductive cells each of which undergoes embryonic cleavage. Perhaps related to the mechanism of cell fate determination by *reg* is the intriguing developmental stage–specific hypermutability of the locus. To understand how the products of this locus determine cell fate and why it is transiently hypermutable, cloning the gene is essential. Since many *reg* mutants have been isolated, several of which appear likely to be deletion mutants, we developed a method for cloning the DNA that corresponds to the sequences that are missing in deletion mutants. The method involves performing iterative rounds of subtractive hybridization using an excess of photobiotinylated deletion mutant DNA and a small amount of wild type DNA. When we tested the method on a defined yeast deletion mutant every clone isolated contained sequences that were deleted in the mutant. The method is simple and efficient and should prove useful in many systems. Currently, we are analyzing potential *reg* clones that were obtained by using the technique.

J 213 TROPOMYOSIN EXPRESSION IN OOCYTES AND EMBRYOS OF XENOPUS LAEVIS Randall B. Widelitz and Jonathan G. Izant, Dept. of Human Genetics, Yale University School of Medicine, New Haven, Ct. 06510

We have characterized the expression of the actin-binding protein, tropomyosin, in oocytes and developing embryos of the African clawed toad, Xenopus laevis. Two non-muscle tropomyosin cDNAs (each approximately 2kb in length) isolated from a Xenopus ovary library have been sequenced. The predicted 248 amino acid tropomyosin proteins showed 80% homology to horse platelet and human non-muscle tropomyosins. These cDNAs were subcloned into the EMSV-LTR expression vector and the pGEM-3 in vitro transcription vector. The translation products produced by oocytes microinjected with our cDNAs and in vitro transcripts comigrated with endogenous tropomyosin on SDS polyacrylamide gels (relative Mr=33 kD) and were precipitated by anti-tropomyosin antibodies. Two tropomyosin mRNAs of 2.1 and 1.2 kb were found in Xenopus oocytes. The 2.1 kb species was prominant in oocytes and early stage embryos while the 1.2 kb species increased 35 h after fertilization (stage 30/31) and was highly expressed in cardiac and skeletal muscle cells. Tropomyosin was localized to the cortex of mature oocytes (stage VI) by indirect immunoperoxidase staining of whole mount oocytes and by indirect immunofluorescent antibody staining of sectioned oocytes. We are currently altering the pattern of tropomyosin expression to investigate its function in Xenopus oocyte maturation and activation.

Hormonal Regulation; Development of Nervous and Immunological Systems

J 300 REGULATED EXPRESSION OF THE DROSOPHILA HSP22 GENE: CIS-ACTING DNA SEQUENCES AND TRANS-ACTING FACTORS, Edward M. Berger, Christine Morganelli, and Karen Rudolph, Department of Biology, Dartmouth College, Hanover, NH 03755. The Drosophila small heat shock protein (hsp) genes are expressed in response to a variety of stress agents, and they are developmentally regulated. In tissue culture cells ecdysterone also acts as an inducing agent. A transient expression assay was used to identify cis-acting DNA elements, flanking the hsp22 gene, involved in heat shock response and in ecdysterone regulation. Short, non-overlapping DNA sequences required for specific induction by high temperature and hormone, have been defined. Evidence from gel retardation and footprinting studies, in vitro, and from transfection-competition experiments, in vivo, indicates that these elements are recognition sites for specific protein binding. Partially purified heat shock transcription factor (HSTF) appears to associate with the heat shock element concensus sequence, CTNGAANNTTCNAG, to promote hsp22 gene transcription at high temperature. Partially purified ecdysterone receptor, from the dipteran Calliphora, appears to associate with a different DNA sequence in the presence or absence of the hormone. The electrophoretic behavior of recognition site DNA, associated with ecdysterone receptor in the presence or absence of hormone (Ponasterone A), however, differs. This suggests that the free receptor is a sequence specific binding protein, and that hormone binding in situ somehow triggers the onset of transcription.

J 301 ISOLATION AND DEVELOPMENTAL REGULATION OF A XENOPUS β-TUBULIN GENE, James J. Bieker, Brookdale Center for Molecular Biology and Department of Biochemistry, Mt. Sinai School of Medicine, New York, NY 10029

Microtubules are a class of structural proteins involved in a large variety of both general and specific cellular functions. They are composed of filamentous protein polymers of one α- and one β-tubulin polypeptide. Storage of unpolymerized tubulin monomers begins within the immature, developing Xenopus oocyte, reaching approximately 1% of the soluble protein within a stage VI oocyte. These monomers transiently polymerize soon after fertilization, a process that directs appropriate cortical/cytoplasmic rotation specifying the dorsal-ventral axis within the egg. Other β-tubulin isotypes that exhibit tissue-specific (e.g., neural and erythroid) expression become expressed later during embryogenesis.

Although the proper level of tubulin is regulated by a post-transcriptional autoregulatory pathway, transcriptional activation of tubulin message comprises its initial control during cell differentiation. To begin studies related to the regulated expression of cognate β-tubulin gene family members during early development, I have begun to isolate cDNA and genomic clones of a variety of Xenopus β-tubulin genes. My presentation will focus on one particular member of this gene family that is expressed in oocytes, and will describe its isolation, gene structure, oogenic and embryogenic developmental expression, and tissue localization.

J 302 *OCTOPOD*, A HOMEOTIC MUTATION OF THE MOTH *MANDUCA SEXTA*, INFLUENCES THE FATE OF IDENTIFIABLE PATTERN ELEMENTS WITHIN THE CNS. Ronald Booker, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

Octopod is an autosomal dominant mutation of the moth *Manduca sexta*, which results in the homeotic transformation of the ventral surface of the first and less often the second abdominal segments in the anterior direction. The extent of the transformation ranges from a slight deformation of the ventral cuticle, to the appearance of miniature thoracic legs on the first abdominal segment. The effect of this mutation on the fate of identifiable neural elements was assessed by examining the distribution and fate of the postembryonic neuroblasts found within the segmental ganglia of *Octopod* larvae. In each of the thoracic ganglia of wild-type larvae there are a set of 45-47 neuroblasts; a reduced but homologous array of 22 and 10 neuroblasts are found in the first and second abdominal ganglia respectively. The first abdominal ganglia of *Octopod* larvae had 1 to 6 supernumerary neuroblasts, while in 20% of the mutant larvae examined 1 extra neuroblast was found in the second thoracic ganglion. Based on a number of criteria these supernumerary neuroblasts corresponded to identifiable neuroblasts normally found in more anterior ganglia. The *Octopod* mutation also influenced the mitotic activity of neuroblasts normally present in A1. In this case, the neuroblasts generated a lineage of cells that were typical for their thoracic homologs. These data demonstrate that homeotic mutations can influence the fate of identifiable pattern elements within the CNS of insects.

J 303 IL-2Rα INDUCIBILITY OF MURINE THYMOCYTES, Paul D. Boyer, Rochelle A. Diamond, and Ellen V. Rothenberg, Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125. During their development in the thymus, T cells must acquire the competence to respond to appropriate stimuli by inducing the expression of genes required for immune function. One such gene encodes the 55 kD α-chain of the receptor for the T-cell growth hormone, IL-2 (IL-2Rα). By examining the correlation between acquisition of two surface phenotypes characteristic of mature T cells [either CD4 or CD8 and T-cell receptors (TCR)] and ability to induce IL-2Rα expression, we identified three states of competence to express the IL-2Rα gene. The first state is exhibited by a subset of precursor thymocytes. These cells express neither CD4 or CD8 nor surface TCR, but more than half express IL-2Rα *in situ* in response to unknown signals. This IL-2Rα expression is distinguished from that of mature T cells in that *in vitro* stimulation reduces expression on these thymocytes. IL-2Rα expression is extinguished before the precursor cells acquire CD4 or CD8. The second state of competence is an inability to induce IL-2Rα expression in response to *in vitro* stimulation and is shared by cells expressing either CD8 alone or co-expressing CD4 and CD8. These thymocytes include the dividing cells of the thymus as well as small, postmitotic, cortical-type cells. The former are very likely the direct progeny of the precursor cells described above, while most of the latter are fated to die *in situ*. Most have in common low-to-undetectable surface TCR expression. The third state of competence is shared by mature T cells and by thymocytes expressing either CD4 or CD8, as well as high levels of TCR. These cells induce high levels of IL-2Rα expression following stimulation. We are currently investigating the molecular bases which distinguish these three states of competence.

J 304 MOLECULAR GENETIC ANALYSIS OF A HUMAN DEVELOPMENTAL FIELD DEFECT: DIGEORGE SYNDROME, Alisoun H. Carey, Sherry L. Roach, Jan P. Dumanski, Magnus Nordenskjold, Robert Williamson, Peter J. Scambler, Department of Biochemistry and Molecular Genetics, St. Mary's Hospital Medical School, Norfolk Place, London W2 1PG, U.K.
DiGeorge Syndrome (DGS) is a congenital anomaly of the 3rd and 4th pharyngeal pouches. Derivatives of these structures fail to develop normally. This results in absence of the thymus and parathyroid glands, associated with deformities of the ear, nose, mouth and aortic arch, leading to hypoparathyroidism and defects in cellular immunity. DGS can occur sporadically or in families, where it appears to follow autosomal dominant segregation. Data suggests that in both cases, DGS may be associated with a chromosomal aberration involving monosomy for 22q11.1-pter and that the DGS locus is at 22q11.1. In order to analyse DGS, genomic sequences in the physical proximity of the locus have been isolated. Two different techniques were employed: firstly, selecting single copy chromosome 22 probes from a flow sorted library, and secondly, microdissection and microcloning of a small chromosome derived from a translocation between chromosomes 10 and 22. Markers obtained in this way were then screened for hemizygosity in DGS patients with visible cytological deletions. Markers mapping to the deletions were then used to look for hemizygosity or rearrangement in DGS patients with no visible cytological aberration.

J 305 DEVELOPMENTAL REGULATION OF GLUTATHIONE PEROXIDASE GENE EXPRESSION.
Sunil Chada and Peter Newburger. University of Massachusetts Medical Center, Worcester, MA 01605. Viagene Inc. 11075 Roselle St., San Diego, CA 92121.
Selenium is an essential element in the diet, yet the only well characterized mammalian selenoprotein is Glutathione Peroxidase (GPx), which contains a selenocysteine residue in its active site. This enzyme protects cells against peroxide-induced damage to cellular membranes and possibly also DNA. We have isolated cDNA clones for human GPx and found that the selenocysteine in the active site is encoded by a UGA terminator codon. Since GPx is an important component of the anti-oxidant defence system, we have examined GPx gene expression during phagocytic cell development in vitro. We have used the human HL-60 promyelocytic leukemia cell line as a model system. Treatment of HL-60 cells with dimethylformamide (DMF) causes granulocytic differentiation whereas phorbol ester (TPA) treatment leads to macrophagic differentiation. During granulocytic differentiation, GPx enzymatic activity increased linearly from the uninduced level. Cellular mRNA levels initially increased and then returned to uninduced levels. During the period that GPx mRNA levels increased four-fold, the rate of transcription of the GPx gene fell ten fold. Treatment of HL-60 cells with phorbol esters (PMA) induces macrophagic differentiation. During this treatment, GPx enzymatic activity fell markedly, however GPx mRNA levels increased and then returned to uninduced levels. These data indicate that the human GPx gene is regulated at both transcriptional and post-transcriptional levels during differentiation of HL-60 cells.

J 306 ANALYSIS OF DIFFERENTIAL EXPRESSION OF FUNCTION OF MYC-FAMILY GENES IN NORMAL AND TRANSFORMED CELLS. Jean Charron, Renate Dildrop, Tarik Moroy, Kathryn Zimmerman, Ron DePinho, Averil Ma, Steven Goff, and Frederick Alt, Howard Hughes Medical Institue and Departements of Biochemistry and Microbiology, College of Physcian and Surgeons of Columbia University, New York, N.Y. 10032. Expression of the N-, L-, and c-myc genes is differentially regulated during normal murine development. For example, both N- and c-myc are expressed in precursor B and T lymphocytes but only c-myc is expressed after the cells differentiate into mature lymphoctyes. To study the differential control and functions of myc-family genes, we have generated a variety of transgenic mouse lines that contain introduced N- or L-myc genes expressed under the influence of their own regulatory elements or specifically-targeted and deregulated within the lymphoid lineage. In addition, we have disrupted one copy of the endogenous N-myc gene in embryonic stem cells by homologous recombination; these cells have been used for blastocyst injections and resulting chimaeric mice are being anlyzed for germline transmission. We will discuss results of these studies in the context of differential myc-gene regulation and activities in normal and transformed cells.

J 307 CLONING AND CHARACTERIZATION OF A MOLECULAR MARKER FROM NEUROEPITHELIOMA AND EWING'S SARCOMA, Robert G. Collum, Ron Depinho, Scott Mellis, Carol J. Thiele, Mark A. Israel, and Frederick W. Alt, The Howard Hughes Medical Institute and Departments of Biochemistry and Microbiology, College of Physicians & Surgeons, New York NY 10032. Neuroepitheliomas and Ewing's sarcomas are tumors found in children and adolescents, respectively, that share a similar 11;22 chromosomal translocation. We have molecularly cloned a gene expressed in several neuroepithelioma and Ewing's sarcoma cell lines but not expressed in a wide variety of other cell lines and tumor samples, suggesting a common developmental lineage for these two types of tumors. This gene is also expressed in developing eye, spinal cord, and brain but, again, not in any other developing tissues suggesting that this gene has a very limited tissue specificity. Chromosomal mapping data indicates that this new gene is located on chromosome 13 and therefore is not directly involved in the translocation seen in these tumors. In addition to cloning a genomic fragment containing this gene we have cloned an incomplete cDNA copy corresponding to a 4 kb RNA transcript. Preliminary sequence analysis has revealed a large 3' untranslated region preceded by a smaller region open in two reading frames. Further experiments are necessary to determine which of these reading frames codes for protein and to extend the sequence in the 5' direction. Finally, this gene because it is expressed in neuroepitheliomas and not in neuroblastomas provides a useful molecular marker for distinguishing these two tumors (which can present clinically with similar characteristics).

J 308 V-*erbA* FUNCTIONS AS A THYROID HORMONE RECEPTOR ANTAGONIST. Klaus Damm, Kazuhiko Umesono, and Ronald M. Evans, Gene Expression Laboratory, The Salk Institute, PO Box 85800, San Diego, CA 92138-9216. The v-*erbA* oncogene and its cellular homologue, the thyroid hormone receptor, are members of a superfamily of regulatory proteins that include receptors for steroid hormones and the morphogen retinoic acid. V-*erbA*, one of the two oncogenes found in the avian erythroblastosis virus (AEV), interferes with the normal differentiation program of erythroid precursor cells, possibly by modulating the transcription of target genes involved in this process. To understand the molecular details of this mechanism, we analyzed the transcriptional control exerted by the thyroid hormone receptor and v-*erbA* on a thyroid hormone responsive promoter. Utilizing a co-transfection procedure we demonstrate that the thyroid hormone receptor activates transcription in a hormone-dependent manner. The co-expression of v-*erbA*, which has lost its ability to bind hormone, inhibits this induction, suggesting that v-*erbA* functions as a thyroid hormone receptor antagonist. We also show that the retinoic acid receptor can activate gene expression through the same responsive promoter. Additional data, suggesting that retinoic acid, thyroid hormones and v-*erbA* might control overlapping gene networks, will be presented.

J 309 CHARACTERIZATION OF LOCI INVOLVED IN THE DEVELOPMENT OF THE ADULT DROSOPHILA CENTRAL NERVOUS SYSTEM, Sumana Datta and Douglas Kankel, Dept of Biology, Yale University, New Haven, CT 06511

The adult *Drosophila* central nervous system (CNS) is generated during larval growth from previously existing larval neurons and imaginal neurons derived from neuroblasts sequestered early in embryonic development. The *l(1)ogre* locus affects the development of the adult-specific portion of the adult CNS by reducing the proliferation of the imaginal neuroblasts. A null allele of *l(1)ogre* has a lethal period at the larval/pupal boundary, and results in a degenerate brain morphology (large vacuoles in the neuropil throughout the brain).

I am interested in identifying other loci which are involved in the development of the adult CNS. Using the null phenotype of the l(1)ogre locus as a starting point, I have screened P-element induced late larval lethals for aberrant brain morphology. Three lines were obtained which showed abnormal brain morphology, and one line was obtained with a large number of "escaper" mutant males which show abnormal flight and electroretinogram phenotypes. I will present characterization of the phenotypes of some of these mutants as well as preliminary mosaic analysis.

J 310 INDUCTION OF THE ALPHA SUBUNIT OF THE STIMULATORY G-PROTEIN IN MODELS OF NEURONAL DIFFERENTIATION, Kevin P. Dolan, Gabrielle Tjaden, Ann Aguanno, Ravi Kumar, Maryann Buette and Ruth Gubits, Department of Biology, New York University, New York, N.Y. 10003 and Division of Pediatric Neurology, Departments of Neurology and Pathology, Physicians and Surgeons of Columbia University, New York, N.Y. 10977.

PC12 cells are a rat pheochromocytoma derived cell line that are commonly used as a model of neuronal differentiation. When these cells are treated with Nerve Growth Factor (NGF) they stop growing and extend neurite-like processes. We have shown that NGF treatment of PC12 cells results in the induction of mRNA coding for the alpha subunit of the stimulatory guanine nucleotide binding protein (Gs). In this report we show that the induction is specific for Gs in that neither Gi nor Go are induced. The increase in RNA is first detectable at 30 min. after addition of NGF to the medium and reaches a peak after 3-6 hours. The induction of alpha Gs RNA is accompanied by an increase in cholera toxin responsive cAMP synthesis indicating an increase in functional alpha Gs protein. We have also examined a human neuroblastoma derived cell line (SH-Sy5y) that extends processes in response to phorbol ester. Alpha Gs is also induced in this cell line when it differentiates with kinetics similar to that described for PC12 cells.

J 311 REGULATION OF Na^+ CHANNEL GENE EXPRESSION IN THE RT4 FAMILY OF NEURAL TUMOR-DERIVED CELL LINES, Laurel M. Donahue, Sarah D. Meyer and Noboru Sueoka, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder CO 80309.

We are interested in the molecular mechanisms underlying differentiation in mammalian cells. In particular we study (1) the branching in vitro of a neural progenitor stem cell into both neuronal and glial derivatives and (2) the maturation of the derivative cell types. The family of cell lines we use is RT4, which was isolated from an ethylnitrosourea-induced rat peripheral neurotumor and has been extensively characterized. The multipotential stem cell line (RT4-AC) expresses both neuronal and glial properties and gives rise reproducibly to patches of morphologically distinct derivative cell types (RT4-B, RT4-D and RT4-E). RT4-B and RT4-E display only neuronal characteristics and RT4-D expresses only glial properties. Several lines of investigation indicate that the conversion of RT4-AC to the neuronal types, RT4-B and RT4-E, involves repression of the glial pathway. We have been studying the regulation of Na^+ channel gene expression as a marker of neuronal cell identity. The voltage dependent Na^+ channel consists of three proteins in rat brain (α, β_1, β_2). In the rat there are three known genes encoding α-proteins and they are designated R_I, R_{II} and R_{III}. Using a cDNA clone of R_{II} (provided by Dr. Gail Mandel, Tufts-New England Medical Center, Boston, MA) that hybridizes to all three Na^+ channel genes as a probe, we have been able to detect a strongly hybridizing ~10 Kb mRNA on Northern blots using poly (A^+) RNA from the RT4 neuronal derivatives. A much weaker signal is detected using poly (A^+) RNA from the stem cell line (which expresses both neuronal and glial properties but at low levels). Barely detectable hybridization is observed using poly (A^+) RNA from the glial derivative and may be a different molecular weight species. We are currently using gene-specific oligonucleotides to determine which gene(s) is expressed in the RT4 lines so that we may choose which gene(s) to begin deletion anaysis of the 5' upstream region to look for the putative repressor binding site.

J 312 DISRUPTION OF THYMOCYTE DEVELOPMENT BY SV40 T-ANTIGEN, Alex M. Garvin, Kristin M. Abraham, Katherine A. Forbush, Andrew G. Farr and Roger M. Perlmutter, Howard Hughes Medical Institute and Departments of Biochemistry and Biological Structure, University of Washington, Seattle, WA 98195. The *lck* gene encodes a lymphocyte-specific protein tyrosine kinase that is expressed at high levels in the subcapsular region of the thymus where immature thymocytes are undergoing the differentiative events required for T cell maturation. We have identified a lymphocyte-specific transcriptional regulatory element (the *lck* promoter) that directs the expression of heterologous SV40 T-Ag sequences at high levels in immature thymocytes. Transgenic animals bearing *lck*/T-Ag gene constructs have greatly reduced total numbers of thymocytes among which the proportion of cells with immature phenotypes ($CD4^-/CD8^-$) is dramatically increased. 3 of 28 transgenic animals had thymic tumors of lymphoid origin that were capable of growing *in vitro* in the absence of exogenous lymphokines. Cell lines derived from these tumors had immature phenotypes, suggesting that SV40 T-Ag is capable of transforming immature thymocytes.

J 313 GENETIC ANALYSIS OF EMBRYONIC DEVELOPMENT IN THE ZEBRAFISH, David Jonah Grunwald and George Streisinger, Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, Utah 84132. The process of zebrafish embryogenesis is ideal for genetic dissection. We have developed procedures for the induction of germline deficiencies with gamma rays, and point mutations with ethyl nitrosourea or ultraviolet light. Novel recessive developmental mutations are easily detected since homozygous embryos can be produced directly from eggs. The accessibility and optical clarity of the embryo permit detailed morphological analyses of development.

We estimate approximately 10,000 mutable genes contribute to embryonic development in zebrafish. This estimate has been achieved by comparing the frequency with which point mutations arise at any locus that is necessary for embryonic viability and the frequency with which mutations arise at a unique locus. Preliminary results indicate that it is difficult to mutate genes that are essential for body plan. These results are consistent with the interpretation that relatively few genes are devoted to this purpose.

We have examined in detail one mutation that defines a function required by most but not all cells in the embryonic CNS. The mutation causes widespread cell death in the CNS. However, a small set of primary neurons is spared. The surviving cells include sensory neurons, interneurons, and motoneurons. They are among the earliest neurons to arise in the embryo and comprise a simple neural circuit that continues to function in the mutant.

J 314 DEVELOPMENTAL REGULATION OF THE HSP90 GENE FAMILY IN THE MOUSE TESTIS. Carol M. Gruppi, Zahra F. Zakeri and Debra J. Wolgemuth. Dept. of Genetics and Development, Columbia University College of Physicians and Surgeons, 630 West 168 St., NY, NY, 10032. Members of the heat shock protein (hsp) 90 family have been found to be expressed during development and to be associated with the untransformed form of the steroid receptor in hormone responsive tissues. We have used a cDNA probe homologous to a human hsp90 transcript and have observed an abundant 3.2 kb transcript in the mouse and human testis. To determine the developmental specificity of expression of this hsp90 gene in mouse testis, RNA was isolated from mice at different stages of postnatal development. The levels of the 3.2 hsp90 transcript were lowest in day 7 testis, which contain only premeiotic germ cells; increased in day 17 testis, which contain germ cells in all stages of meiotic prophase; and were most abundant in the adult testis, which contains the complete germ cell lineage from spermatogonia to spermatozoa. This suggested that the increased levels of hsp90 mRNA correlated with the presence of differentiating germ cells. Analysis of enriched populations of germ cells demonstrated that this transcript is present in all stages of germ cells, from pachytene spermatocytes to residual bodies. The highest level of hsp90 mRNAs were detected in germ cells that are in meiotic prophase. RNA was also isolated from testes of germ cell deficient mice of the (W/W^V) genotype and from testes of their fertile wild type littermates ($+/W^V$). The presence of the 3.2 kb transcript in the testis of the wild type and mutant mice suggest that the hsp90 transcript is present in both the germ cells and the somatic cells. These observations show that hsp90 mRNA is under developmental regulation in the mouse testis and suggest a role for hsp90 in male germ cell differentiation.

J 315 THE MOUSE HEMATOPOIETIC SYSTEM: CHARACTERIZATION OF PLURIPOTENT STEM CELLS AND RESTRICTED PROGENITOR CELLS *IN VIVO* AND *IN VITRO*, Shelly Heimfeld, Laurie Smith, Donna Rennick, and Irving Weissman, Department of Pathology, Stanford Medical Center, Stanford University, Stanford, CA 94305

Using a panel of monoclonal antibodies directed against "lineage" specific surface antigens expressed on mouse bone marrow cells, along with 3-color FACS, several cell populations have been identified which have precursor activity for one or more lineages. Each subset has been tested for *in vivo* repopulation of irradiated mice, for *in vitro* reconstitution of irradiated Dexter cultures, for Whitlock-Witte pre-B cell capacity, and in growth-factor dependent methylcellulose assays. The correlation between these different assay methods and a possible lineage relationship among the different subpopulations will be presented.

J 316 CONTROL OF CELLULAR DIFFERENTIATION IN HYDRA, S.A.H. Hoffmeister, Zentrum für Molekulare Biologie, University of Heidelberg, Im Neuenheimer Feld 282, 6900 Heidelberg, FRG. Due to the polar organization of hydra which comprises terminally differentiated cells at the head and the foot, the stem cells of the gastric region, require a precise control of their respective differentiation pathways. Head-specific growth and differentiation are induced by head activator and inhibited by head inhibitor, foot-specific development depends on the presence of foot activator and the absence of foot inhibitor. Both activators are peptides with a molecular weight of about 1000; the sequence of head activator being known. The inhibitors are small hydrophilic molecules with a molecular weight of about 500. The four factors mainly act on two responsive stem cell populations: interstitial stem cells which give rise to nerve cells and nematocytes and epithelial stem cells which develop into head- or foot-specific cells. Under steady state conditions and taking the mean for the whole animal, about 10% of the cells in the interstitial stem cell pool differentiate to nerve cells, 30% to nematocytes. Determination of interstitial stem cells to the nerve cell pathway occurs in early S-phase and is only inducible by head activator and antagonised by head inhibitor. Terminal differentiation is initiated in the subsequent G2 phase. At this stage both head activator and foot activator exert a stimulatory effect on the nerve precursor cells hence determining the properties of the newly arising nerve cells, i.e., to become head or foot-specific nerve cells. Head inhibitor acts antagonistically by keeping the nerve precursor cells arrested in G2. Under steady-state conditions ectodermal epithelial cells of the tentacles and the foot are derived from ectodermal stem cells of the gastric region. Both foot activator as well as head activator stimulate mitosis of epithelial stem cells which can be measured as increase in the mitotic index or in the labelling index. Both factors also stimulate differentiation processes which become measurable as an increase in the expression of specific markers like peroxidase activity for foot-specific and cell surface antigens for head specific cells. Therefore although having many properties in common acting on the same set of stem cells and promoting proliferative activity of these cells, head activator and foot activator act completely differently with regard to the specification of terminal differentiation of these stem cells. Sequence analysis of the foot activator will yield some more information about the molecular mechanisms responsible for head and foot activator specificity.

J 317 CELL LINEAGES IN RAT MUSCLE IDENTIFIED BY RETROVIRAL MARKING. S. M. Hughes, M. Cho and H. M. Blau, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.

A modified retroviral vector capable of expressing β-galactosidase but unable to replicate was injected into rat leg and foot muscle at various stages of development. Cells infected by the vector were allowed to continue normal division and differentiation *in vivo* for various periods of time. Muscles were serially sectioned by cryostat and stained for β-galactosidase activity to identify the progeny of infected cells. Well-separated "blue" clones expressing β-galactosidase were detected and their growth and differentiation charted. The retroviral marking system is now being used in conjunction with monoclonal antibodies to myosin heavy chain that are markers of muscle fiber maturity and contractile type. This combined approach should permit an analysis of restriction in developmental fate of subpopulations of myoblasts during normal development *in vivo*.

J 318 X-LINKED CLEFT PALATE: MOLECULAR ANALYSIS OF AN ICELANDIC PEDIGREE, Alasdair Ivens, Olafur Jensson and Robert Williamson, Dept. of Biochemistry and Molecular Genetics, St. Mary's Hospital, London W2 1PG, U.K.

Cleft palate, a common dysmorphology with an incidence of approximately 1 in 1000 live births, results from the non-fusion of the palatine shelves during weeks 6-9 of human embryological development. We have investigated a large Icelandic family in which the associated defect cleft palate and ankyloglossia (tongue-tie) segregate in an X-linked manner through seven generations. Standard linkage analysis, using restriction fragment length polymorphisms (RFLPs), enabled the site of the defect in this family to be localised to a small region of the X chromosome, Xq21.3- Xq22. Further study of deletions within this region tentatively places the "cleft palate mutation" in the region Xq21.31- Xq21.33. The approach we have chosen to identify the defective gene involves chromosome mediated gene transfer; hybrid cell lines containing portions of the X chromosome were screened for the presence of human DNA markers from the "cleft palate" region. A cell line fulfilling these criteria was identified, and a cosmid library incorporating selection for HTF islands was constructed. Human clones were identified against the rodent background by hybridisation to a human repetitive probe. The presence of HTF islands in clones was confirmed by restriction enzyme mapping, and the physical location determined by screening a panel of rodent-human hybrids. In addition, a human cell line with a deletion of the "cleft palate" region was screened to confirm the localisation of candidate sequences. By using this combination of molecular and genetic techniques, the mutation causing cleft palate has been localised to a region of approximately two megabases.

J 319 DISTINCT TEMPORAL REGULATION OF GLUCOCORTICOID AND ESTROGEN RECEPTOR mRNA ACCUMULATION DURING RAT FETAL DEVELOPMENT, Frank P. Johnston and Jack Gorski, Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706

Steroid hormone receptors have been shown to be trans acting activators of transcription. The temporal and spatial organization of their expression is thus of importance to the understanding of the modulation of hormonally regulated genes during development. We have examined the time course of the accumulation of the mRNAs for the glucocorticoid and estrogen receptors during fetal development from day 9 of gestation in the rat. Slot blot analysis of total RNA hybridized to cDNA probes to glucocorticoid and estrogen receptor revealed distinct patterns for the two receptors. Glucocorticoid receptor mRNA increases 3 fold from day 14 to 16, remains constant from day 16 to day 20, and decreases on day 21 just prior to birth. Estrogen receptor mRNA by contrast rises gradually 4 fold from day 14 to 21 of gestation. Estrogen receptor mRNA levels in day 9 concepti are 8 fold greater than those in day 11. Fetal membrane and placental fractions have estrogen receptor mRNA levels greater than those found in 20 day fetal thymus, lung, brain, and heart. A nuclease protection assay localizes estrogen receptor mRNA to the head and viscera with low levels in the trunk in the day 15 fetus. These observations suggest early functions for the estrogen and glucocorticoid receptors in rat development.

J 320 EXPRESSION CLONING OF cDNAS ENCODING SURFACE PROTEINS OF HEMOPOIETIC PROGENITOR CELLS, Robert J. Kay, Graeme J. Dougherty and R. Keith Humphries. Terry Fox Laboratory, University of British Columbia. Vancouver B.C. Canada V5Z 1L3. The interleukin-3 dependent murine cell line B6SUtA is derived from and has many structural and functional attributes of multipotential myeloid progenitor cells. We are identifying surface proteins common to B6SUtA cells and primary murine myeloid progenitor cells with monoclonal antibodies. cDNAs encoding proteins of interest have been cloned by immunoselection of COS cells transfected with B6SUtA cDNA libraries, using shuttle vectors which direct very high levels of expression and facilitate plasmid recovery. One subject of study is the antigen 114/A10, a structurally heterogeneous cell surface protein that is highly expressed on primitive hemopoietic progenitor cells and IL-3 dependent murine cell lines, including B6SUtA. A cDNA directing the expression of 114/A10 in COS cells was isolated by repetitive FACS selection and plasmid rescue. This cDNA encodes an integral membrane protein which has several cysteine rich regions with homology to the EGF-like domains of Notch and Lin-12, and has an N-terminal ser/thr rich repetitive domain. The latter domain serves to present an extensive array of O-linked carbohydrates on the cell surface, which may mediate interactions with the marrow stroma. The effects of the ser/thr and EGF-like domains of 114/A10 on marrow progenitors are being investigated through the production of variant 114/A10 molecules in COS cells.

J 321 EPIDERMAL COMMITMENT AND TRANSFATING DURING EMBRYONIC DEVELOPMENT IN THE LEECH *Theromyzon rude*. G. P. Keleher, Univ. of Calif., Berkeley 94720.

Determinate development of the leech offers an opportunity to test the relative roles of cell lineage and cell-cell interactions in assigning cell fates during embryogenesis. A genealogical analysis and fate map was constructed for an ectodermal o blast cell clone whose descendants take on neural and epidermal fates during the development of the leech nervous system. The results obtained indicate that division of the tertiary o.ap blast cell (the posterior daughter of the anterior daughter of the primary o blast cell) gives rise to a pair of quaternary sister blast cells whose fates differ radically. The anterior sister, cell o.apa, gives rise to a complex set of neural elements and epidermal specializations. The posterior sister, cell o.app, however, gives rise entirely to squamous epidermis. Previous work has shown that the commitment of cell o.app to epidermis requires the presence of the adjacent p bandlet. However, the present work demonstrates that ablation of cell o.apa results in the transfating of its sister, o.app, from strictly squamous epidermis to a variety of cell fates. Thus, both the presence of signals from within the o clone (cell o.apa) and outside it (the adjacent p bandlet) are necessary for epidermal commitment of the o.app sister to its normal fate.

J 322 **EARLY TELENCEPHALIC POSTMITIOTIC NEURONS POSSESS COMMON ADHESION MECHANISM THAT UNDERLIES FOREBRAIN PATTERN FORMATION** L.A. Krushel and D. van der Kooy, Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8.

The earliest born cortical and striatal neurons of the forebrain form separate adhesive compartments *in vivo* in the face of the migration of later born (and not selectively adhesive) neurons. The earliest born cortical and striatal neurons (that form the deep cortical and striatal patch compartments, respectively) will selectively adhere together within their own tissue type, after being embryonically removed, dissociated, and reaggregated. Is this selective adhesion specific to neurons within their own tissue type or is it a common adhesion molecule expressed on the surface of all early born neurons in the telencephalon? To examine this we cocultured dissociated embryonic cortex and striatum in a reaggregate suspension culture. Would early postmitotic cortical and striatal neurons reassociate according to tissue type or to birthdate? Timed pregnant Wistar albino rats were injected with either 1 mCi ^{3}H-thymidine or bromodeoxyuridine (BrdU - 160mg/kg) on embryonic day 13. These injections label primarily the striatal patch and deep cortical layer cells. Two days later the striata and cortices were separately dissected and dissociated into single cells. Striata from the pups of one mother were cocultured with cortices from the pups of another mother so that each culture contained some neurons with a different label: ^{3}H-thymidine or BrdU. After five days in a reaggregate suspension culture, the reaggregates which formed contained relatively small numbers of both cortical and striatal labeled cells, i.e. both BrdU and ^{3}H-thymidine labeled cells. Within the reaggregates all the labeled cells (BrdU or ^{3}H-thymidine labeled) appeared in clumps. Within the labeled clumps, the BrdU and ^{3}H-thymidine labeled cells appeared randomly interdigitated. This suggests the existence of an adhesive factor common to early postmitotic telencephalic cells. To determine if this factor is instrumental in the compartmentalization of the entire forebrain, we are currently culturing early born septal cells (basal forebrain) to see if they are selectively adhesive, and if this putative adhesion factor is the same one present in early born cortical and striatal cells.

J 323 EYE-RELATED EXPRESSION OF HUMAN X-DERIVED COSMID SEQUENCES

Lindsay S,[1,2], Bhattacharya S,[1,2], Sealey P,[2], Ackford H,[1,2], Pierce S,[2], Bower DJ,[2].
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We are characterising sequences selected from a cosmid library with insert DNA from a man/mouse hybrid containing only the human X-chromosome. Two of these cosmids, initially selected for the presence of HTF islands, contain sequence corresponding to transcripts found in a variety of eye-related tissues. Cosmid F5 mapped, using somatic cell hybrids, to Xp22, shows some evidence of differential processing in different tissues. Cosmid C2, mapped to long arm of the X-chromosome, is expressed at a high level in retina, and to a lesser extent in several other tissues tested. We are examining flanking sequences to detect the presence of possible regulatory motifs.

J 324 DEVELOPMENTAL EXPRESSION OF THE MURINE *vg-r1* GENE: A NEW MEMBER OF THE TGFβ SUPERGENE FAMILY, Karen M. Lyons, Ronald W. Pelton and Brigid L.M. Hogan, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

The TGFβ supergene family contains a distinct subfamily of proteins more closely related to the Drosophila Decapentaplegic (DPP) protein than to TGFβ. The members of this subfamily include the Drosophila DPP protein (Padgett *et al.*, 1987, Nature 325: 81-84), the Bone Morphogenic Proteins (BMPs) -2a, -2b, and -3 (V. Rosen *et al.*, 1988, Third Int. Congress on Chem. and Biol. of Mineralized Tissues, p. 76), and the Xenopus Vgl protein (Weeks and Melton, 1987, Cell 51: 861-867). A new member of this subfamily, *vg-r* (for *vg*-related) has recently been isolated from a mouse 8.5d post coital embryo cDNA library (K. Lyons, J. Graycar, A. Lee, P. Linquist, E. Chen, B. Hogan and R. Derynck, in preparation). We have used this cDNA to study the expression of the *vg-r* gene in whole embryos and in tissues isolated at various stages of development by Northern analysis and *in situ* hybridization. In most tissues surveyed, a single 3.5 kb transcript was seen. In adult testes, a 1.8 kb transcript was found in approximately equal proportion to the 3.5 kb transcript. Our results show that *vg-r* is expressed in a variety of tissues, and that the level of expression increases throughout development and into adulthood in many of these tissues. This pattern of expression suggests that, like the *dpp* gene product (St. Johnston and Gelbart, 1987, EMBO J. 6: 2785-2791), *vg-r* may play a role at several stages of development in the mouse.

J 325 PDGF A CHAIN IS EXPRESSED IN EARLY MOUSE EMBRYOS AND IS MATERNALLY ENCODED IN XENOPUS EMBRYOS, Mark Mercola, Clare Brownlee, Doug Melton*, Ursula Drager**, and Charles D. Stiles, Dana-Farber Cancer Institute and the **Department of Neurobiology, Harvard Medical School, Boston, MA 02115, and *Harvard University, Cambridge, MA 02138.

We show that the A chain of platelet-derived growth factor (PDGF) is the predominant form of PDGF in developing mouse and *Xenopus* embryos. In the mouse, A chain and PDGF receptor mRNA are present in embryonic tissue as early as the onset of gastrulation (6.5 days after fertilization). Immunohistochemical localization studies show that the A chain protein is present in the embryonic ectoderm and is most abundant at 6.5 days, decreasing thereafter.

Xenopus oocytes and embryos contain a mRNA encoding the A chain. This mRNA is present throughout the early cleavage stages and increases in abundance at gastrulation following the activation of the embryonic nuclei.

Cloning, sequence, expresseion and mRNA analyses shows that the A chain sequence is remarkably well conserved in both species and that the *Xenopus* protein functions as a mitogen for mouse cells. Also, in both species, alternative splicing gives rise to different forms of the A chain protein. The presence of the A chain and lack of the B chain (the *c-sis* proto-oncogene product) in early mouse development and as a maternally encoded mRNA in *Xenopus* suggests a unique and phylogenetically conserved role for the A chain in early development.

J 326 FIBROBLAST GROWTH FACTOR (FGF) STIMULATES THE PROLIFERATION OF NEURAL PRECURSOR CELLS IN VITRO, Mark Murphy, John Drago and Perry F Bartlett, The Walter and Eliza Hall Institute of Medical Research, Melbourne, 3050, Australia. The mature mammalian central nervous system is made up of two major cell types; neurones and glia. Neuroepithelium as early as embryonic day 10 (E10) has been shown to give rise to all the mature cell phenotypes when cultured in vitro (1,2). The factors that regulate the proliferation and differentiation of the neuroepithelium are unknown and we have developed in vitro assays to examine this problem. Single cell suspensions of E10 neuroepithelial cells which are cytokeratin positive are plated at low density in wells of Terasaki plates in the presence of 1% foetal calf serum. Various recombinant and purified growth factors have been examined for mitogenicity by both visual and ^{3}H thymidine incorporation assays. Of all the factors examined only acidic and basic FGF were found to be mitogenic. Examination of the FGF stimulated population for expression of mature neural cell markers by immunofluorescence revealed that both glial fibrillary acidic protein positive astrocytes and neurofilament positive neurons were generated. Thus, it appears that FGF stimulates the precursors of both neurons and glia to divide, however, it was noted that full differentiation of precursors only occurred at higher concentrations of both acidic and basic FGF.

1. Abney, E.R., Bartlett, P.F and Raff, M.C. (1981) Devel. Biol. 83, 301.
2. Bartlett, P.F., Bailey, K.A. and Wycherley, K. (1987) Neurosci. Lett 27, S42.

J 327 I. STAGE-SPECIFIC B-LINEAGE GENES, Eugene M. Oltz, George D. Yancopoulos, Roberta R. Pollock, Maureen A. Morrow, Kenneth B. Kaplan, Susan A. Prockop, and Frederick W. Alt, Department of Biochemistry, Columbia University College of Physicians and Surgeons, New York, NY 10032. Subtractive hybridization techniques have been employed to isolate cDNA clones specifically expressed in pre-B cell lines but not in myelomas or fibroblasts. Extensive characterization provided 20 independent genes specifically expressed only in the pre-B or in both the pre-B and B stages of B cell development. These genes also display characteristic expression patterns in other lineages. Of particular interest were three clones - PB.65, PB.99 and PB.102. PB.65 is unique in its ability to distinguish bone marrow (+ expression) from fetal liver (- expression) derived Abelson pre-B cell lines. PB.65 is homologous to known regulatory myosin light chain genes. Expression of PB.65 (and a limited number of other pre-B genes) is amplified 50 times upon treatment of panned bone marrow pre-B cells with the only known pre-B growth factor - IL-7. The expression of PB.99 correlates well with available recombinase activity data (in both cell lines and tissues) and with appearance of Abelson targets in placenta and cyclophosphamide treated bone marrow. A partial sequence of PB.99 displays no homology to known genes. PB.102, a gene expressed only in Abelson transformants, maps to the X-chromosome, as do a number of mutations responsible for immune disorders. In addition to the possibility that the pre-B genes are involved in recombination, these genes may prove useful in defining genetic switchpoints and in clarifying gene regulation throughout the B-cell lineage. Expression of these genes has already been employed to stage B-cell tumors that arose in mice transgenic for the N-myc gene. Extension to human systems is likely, as we have defined human homologs for a majority of the characterized genes.

J 328 IN VITRO REGULATION OF PROTEIN SYNTHESIS IN CRAB TISSUES BY 20-HYDROXYECDYSONE, METHYL FARNESOATE, AND JUVENILE HORMONE
Charles R. Paulson and Dorothy M. Skinner, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 and ETSU, Johnson City, TN 37614.
The actions of arthropod hormones on tissues from intermolt individuals of the Bermuda land crab *Gecarcinus lateralis* were studied *in vitro* to investigate the regulation of the molting cycle. Proteins were labeled with [^{35}S]methionine. Total synthesis was measured by TCA precipitation and individual proteins were separated by 1D and 2D gel electrophoresis. The arthropod molting hormone 20-hydroxy-ecdysone (20HE) significantly stimulated the synthesis of 5 proteins from integumentary tissues. For 4 of these proteins, the greatest effect was with a concentration of 10^{-7} M 20HE, characteristic of the late premolt stage in *G. lateralis*. For some of those proteins which increase in synthesis during the premolt stage *in vivo* (Stringfellow and Skinner, Dev. Biol. 128:97 (1988)), 20HE induced similar changes *in vitro*. In midgut gland (hepatopancreas) 20HE inhibited total synthesis; synthesis of 6 proteins from males and 3 from females was significantly reduced. The inhibitory effect of 20HE on midgut gland *in vitro* mirrors the atrophy of this organ which occurs prior to ecdysis. Effects of the putative crustacean hormones methyl farnesoate (MF) and juvenile hormone (JH) were also tested. Total protein synthesis in integumentary tissues was significantly increased by physiological levels of (2E,6E)-MF (the natural isomer) but not by the unnatural isomer (2Z,6E)-MF, or JH. The synthesis of 9 individual proteins was significantly stimulated by (2E,6E)-MF. The synthesis of 3 other proteins was significantly stimulated by JH. No significant effect of (2Z,6E)-MF was found. Studies to further elucidate the mode of action and developmental role of these hormones are continuing. (Supported by NSF and USDOE, under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.)

J 329 NUCLEAR RECEPTORS FOR THYROID HORMONE AND RETINOIC ACID AS SIGNAL MEDIATORS IN DIFFERENTIATION AND DEVELOPMENT, Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037. We have recently described the cloning and characterization of nuclear receptors for thyroid hormone and retinoic acid, two ligands which are important in development and differentiation. The two receptors belong to a multigene family which also includes the steroid hormone and vitamin D_3 receptors. These receptors are intracellular regulatory proteins which when complexed with their ligands recognize specific DNA elements and modulate transcription from linked promoters. Our cloning of the human α thyroid hormone receptor (hTRα) established for the first time that in the human thyroid hormone receptors are encoded by at least two genes. In addition, we have also obtained evidence that subforms of the hTRα are being created by alternative splicing. TRα is expressed in various tissues including the brain. It has long been known that thyroid hormone plays an important role in the central nervous system (CNS). The most striking effects are observed during maturation of the CNS: the absence of thyroid hormones during this period produces multiple morphological and biochemical alterations and in humans leads to irreversible mental retardation. Our analysis of TRα expression during postnatal rat brain development shows a strong temporal and spacial regulation of TRα expression during brain maturation. The human retinoic acid receptor (hRARϵ) recently described by us shows a considerable specificity for proliferating epithelial type tissues. This receptor has a high affinity for other active retinoids and may represent the major mediator of retinoid action in differentiation development and morphogenesis. Although RARϵ shows only limited homology with TRα it is able to activate transcription from thyroid hormone responsive elements.

J 330 TRANSGENIC SHIVERER (shi) AND MYELIN DEFICIENT (mld) MICE: CORRECTION OF A DYSMYELINATING PHENOTYPE, Carol Readhead, Brian Popko, Naoki Takahashi, David Shine, Raul Saavedra, Richard Sidman and Leroy Hood, Division of Biology, California Institute of Technology, Pasadena, CA 91125 and Departments of Neuropathology and Neuroscience, Harvard Medical School, Boston, MA 02115

Shiverer is an autosomal recessive mutation that maps to chromosome 18. Mutant mice have tremors starting at 12 days and by 30 days they have tonic seizures which increase in frequency and severity until their early death between 90 and 100 days. The central nervous system (CNS) of these mice is hypomyelinated (CNS) while the peripheral nervous system (PNS) is unaffected. Myelin basic protein, one of the major proteins of CNS myelin, is undetectable in shiverer mice and this is due to a deletion of most of the structural gene encoding MBP. We have introduced the wild-type MBP gene into the genome of shiverer mice and mice homozygous for the MBP transgene no longer shiver or have seizures and live normal lifespans.

Myelin deficient (mld) is an allelic to shiverer and mice with this mutation have most of the characteristics of shiverer except that they are not as severely affected. MBP is present in very low quantities in the CNS but it is not developmentally regulated. The gene encoding MBP in mld mice has undergone a duplication and an inversion (Popko et al., 1988). The wild-type MBP transgene also corrected the phenotype of these mutant mice.

Popko, B., Puckett, C. and Hood, L. (1988) Neuron 1, 221-225.

J 331 ACCUMULATION OF HOMEOBOX-CONTAINING 2A7 mRNA IS AN IMMEDIATE EARLY RESPONSE TO MESODERM INDUCERS AND IS LOCALIZED TO THE VEGETAL POLE IN THE FROG EMBRYO, Frederic M. ROsa and Igor B.Dawid, Laboratory of Molecular Genetics,Bldg 6,Rm 328, NIH, Bethe da, Md 20892.

In Amphibians, mesoderm is believed to be derived from the induction of the ectoderm region by soluble factors produced by the future endoderm cells. Soluble inducers have been described including FGF, TGFbeta2, and the conditioned medium from the tadpole cell line XTC. We have started to analyse the early phase of mesoderm induction by cloning cDNA corresponding to RNA that are inducible by FGF or XTC-CM in ectoderm explants. One of these clones, 2A7, has been carachterized in detail. 2A7 is inducible strongly by XTC-CM and by the combination of FGF and TGFbeta2. 2A7 is expressed very shortly during embryogenesis, starting around Mid-Blastula transition (Major onset of transcription) and disappearing at the end of the gastrulation. 2A7 is an immediate early response to induction since it appears 15-30 minutes after induction, does not require protein synthesis or cell-cell contact to be expressed. 2A7 encodes for a homeodomain containing protein and the mRNA is localized into the vegetal pole of the embryo (future endoderm + mesoderm). We believe that 2A7 may be involved in the regulation of the development of the vegetal pole cells and might be a second message in response to the inducing signal.

J 332 HIGH LEVEL ERYTHROID EXPRESSION OF HUMAN GLOBIN GENES IN TRANSGENIC MICE, Thomas M. Ryan[1], Richard R. Behringer[2], Richard D. Palmiter[3], Ralph L. Brinster[2] and Tim M. Townes[1], [1]Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama at Birmingham, Birmingham, Alabama 35294; [2]Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104; [3]Department of Biochemistry, Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195. Human α- and β-globin genes were fused downstream of developmentally stable erythroid-specific DNase I super-hypersensitive sites normally located far upstream of the human β-globin locus. These constructs were injected individually or coinjected into fertilized mouse eggs and expression was analyzed in the transgenic animals that developed. All of the mice that contained intact copies of the transgenes expressed high levels of correctly initiated human α1-, $^{A}\gamma$- and β-globin mRNA in both fetal and adult erythroid tissues. A single DNase I super-hypersensitive site was sufficient for high levels of expression of the human β-globin gene. Isoelectric focusing of transgenic hemolysates demonstrate that complete human hemoglobins are synthesized in adult mouse erythrocytes. These experiments provide a basis for the study of the developmental expression and switch of human globin genes and the resulting hemoglobins in transgenic mice.

J 333 ANALYSIS OF DIFFERENTIATION OF SINGLE HEMATOPOIETIC STEM CELLS IN VIVO. Laurie F. Smith, Irving L. Weissman & Shelly Heimfeld. Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305. Previous work in this laboratory has demonstrated that pluripotential hematopoietic stem cells are highly enriched within a population of mouse bone marrow cells (sorted on a fluorescence activated cell sorter) that express low levels of Thy1, lack expression of several other mature blood cell lineage markers, and express a recently identified antigen, Sca-1. $Thy1^{lo}Lin^{-}Sca\text{-}1^{+}$ cells represent approximately 0.05% of mouse bone marrow; 30-50 of these cells injected into lethally irradiated mice confer radioprotection and differentiate to give rise to B and T lymphocytes, myeloid and erythroid cells (Science 241:58-62, 1988). We are now studying the differentiation of single hematopoietic stem cells in order to 1.) demonstrate directly the multilineage differentiation potential of individual cells within our sorted stem cell population, and 2.) examine the magnitude of the contribution of individual stem cells to the total blood cell pool, and the lifespan of single stem cells in vivo. Here we present results of limiting dilution experiments suggesting that approximately 1 in 10 $Thy1^{lo}Lin^{-}Sca\text{-}1^{+}$ cells injected intravenously into irradiated host mice can give rise to a detectable clone in the blood that includes T and B lymphocytes, monocytes, and granulocytes. The sizes and lifespans of these putative clones varied widely. In addition, we report on clones that have developed in irradiated mice injected with a single FACS-sorted $Thy1^{lo}Lin^{-}Sca\text{-}1^{+}$ bone marrow cell.

J 334 XENOPUS D7, A TRANSLATIONALLY CONTROLLED mRNA INVOLVED IN OOCYTE MATURATION, Rosamund C. Smith, Mark B. Dworkin, and Eva Dworkin-Rastl, Ernst Boehringer Institut, Vienna, Austria.

Xenopus D7 RNA is a moderately abundant message whose expression is highest in, and perhaps restricted to, oogenesis and early embryogenesis. D7 mRNA is translationally repressed in oocytes and becomes recruited for translation during maturation. D7 protein is detectable in the unfertilised egg and the early embryo with levels declining after the first day of development. Sequencing of D7 cDNA clones predicts the D7 protein to be a 31kD polypeptide with no homology to any known protein. The putative protein does not possess a standard protein kinase domain and D7 protein in the unfertilised egg does not appear to be phosphorylated. Destruction of D7 mRNA in oocytes was performed by antisense DNA oligomer-mediated degradation. Analysis of injected oocytes by Northern and Western blotting showed site-specific cleavage and subsequent degradation of the D7 mRNA, and the failure of the D7 protein to accumulate during progesterone-induced maturation. The loss of D7 protein affects the maturation process itself, significantly delaying the timecourse of germinal vesicle breakdown. Injection of SP6 D7 RNA transcripts into D7 mRNA-depleted oocytes substantially rescued this phenotype. D7 appears to be a novel gene involved in maturation of the oocyte.

J 335 IDENTIFICATION OF GENES UNIQUELY EXPRESSED IN MOUSE PLASMACYTOMA CELLS, Cynthia R. Timblin, James Battey, and W. Michael Kuehl, NCI-Navy Medical Oncology Branch, Naval Hospital, Bethesda, MD 20814

Mouse plasmacytoma cell lines differ from a mature, immunoglobulin secreting murine B cell line (A20.2J) by several notable features: 1) morphology, 2) lack of expression of certain B cell markers (e.g. surface Ia antigen), 3) lack of endogenous c-myc mRNA expression, and 4) the consistent dominance of the plasmacytoma phenotype in somatic cell hybrids formed with all other types of lymphoid cells. To identify genes which distinguish the plasmacytoma from the closely related B cell lymphoma, we have prepared a subtractive cDNA library. Incorporation of PCR (polymerase chain reaction) technology at several steps has facilitated the construction and analysis of this library. Thus far, we have identified 15 genes which are expressed differentially in both the parental and an unrelated plasmacytoma compared to the subtractive B cell partner. We are continuing to define the extent of difference in gene expression in these cell lines and will soon begin to characterize the genes that are differentially expressed. Our long term goal is to identify and isolate genes which determine or are specific for the phenotype of terminally differentiated plasma cells.

J 336 A MOLECULAR CORRELATE OF CHROMAFFIN CELL PLASTICITY: REGULATION OF NEURAL SPECIFIC GENE EXPRESSION IN CHROMAFFIN CELLS AND ITS RELATIONSHIP TO PHENOTYPIC PLASTICITY, David J. Vandenbergh and David J. Anderson, Divison of Biology, California Institute of Technology, Pasadena, CA 91125. The ability of adrenal chromaffin cells to convert to a neuronal phenotype depends on both the presence of an appropriate environmental signal (NGF) as well as the cell's ability to respond to the signal. We have asked whether chromaffin cells maintain neural specific genes in a conformation that would permit their activation by NGF in the absence of DNA synthesis and cell division. As a test case we have used the SCG10 gene, which is expressed abundantly in sympathetic neurons but not in adrenal chromaffin cells. We have probed the 5' end of the SCG10 gene for sensitivity to DNase I. Two sites that are hypersensitive to DNase I are located around the transcription initiation site in neuronal and adrenal medullary nuclei. These sites are absent from liver, Schwannoma, and fibroblast nuclei. Thus, most or all adult chromaffin cells contain a feature of transcriptionally active SCG10 chromatin that is independent of the absolute level of expression of this gene. PC12 cells, which phenotypically resemble embryonic sympathoadrenal precursors, also contain these two hypersensitive sites. These results suggest that, in terms of the SCG10 gene, chromaffin cells can be thought of as stably determined neuronal precursors.

J 337 EXPRESSION OF myc RELATED GENES IN EARLY XENOPUS DEVELOPMENT, Peter D. Vize and Paul A. Krieg, Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK, and Center for Developmental Biology, Department of Zoology, University of Texas, Austin, Texas 78712, USA.
There are at least 7 different proteins in mammalian cells which share homology to v-myc. These include c, N, L, P, R, and B-myc, and MyoD. Unlike the well characterised c-myc, most of the myc "variants" show tissue restricted expression patterns that are developmentally regulated. One gene in particular, MyoD, has been shown to be involved in specifying myogenic cell differentiation. In the hope of isolating myc variants which may be involved in early developmental decisions we have screened a Xenopus embryonic cDNA library with a mammalian myc probe. We have found Xenopus homologues of both c- and N-myc. Northern analysis indicates that both of these genes are expressed maternally. This is surprising as c-myc expression is in this instance not coupled to cellular replication. N-myc expression in oocytes is also surprising as in mammals this gene is predominantly expressed in neural tissues. Following the MBT the transcripts of both c- and N-myc are destabilised, and from this point onwards display different patterns of expression. The expression of these two proteins is being studied by further transcriptional and immunohistochemical analysis.

J 338 NERVE GROWTH FACTOR ACTIVATION OF PROTEIN KINASE C IN ISOLATED RAT BRAIN CORTICAL NUCLEI, Cheryl L. Weill*, Nancy E. Zorn, Arthur R. Buckley and Diane H. Russell, Department of Pharmacology and Therapeutics, University of South Florida College of Medicine, Tampa, FL 33612 and *Departments of Neurology and Anatomy, Louisiana State University Medical Center, New Orleans, LA 70112

Nerve growth factor (NGF) is known to support neuronal survival and growth in both developing and adult nervous systems, which are rich in protein kinase C (PKC) activity. Recently, mitogenic peptides have been shown to activate a nuclear pool of PKC (nPKC). Using an in vitro assay and isolated rat brain cortical nuclei, NGF was found to activate nPKC maximally at a concentration of 10^{-10}M. The phorbol ester, TPA, which mimics NGF activities in PC12 cells was also maximally stimulating at the same dose. Activation was also effected by PDBu and abolished by NGF co-treatment with NGF antibody or the PKC inhibitors, H7, sphingosine and staurosporine. Specificity studies demonstrated that EGF, insulin, IGF-I, 8Br-cAMP and corticosterone did not activate cortical nPKC, while VIP was half as effective, and FGF and SRIF were equally effective activators relative to NGF. Tissue specificity studies indicated that NGF activated nPKC in the cerebellum, but not in the midbrain or brain stem. As observed in liver and lymphocytes, the activation of nPKC was potentiated by co-treatment with prolactin at 10^{-11}M; potentiation by prolactin was also dose dependent. These data indicate that the archetypical neurotrophic peptide, NGF, activates a nuclear pool of protein kinase C. The molecular mechanism of action of NGF elaborated to date includes, binding to a plasma membrane receptor, and retrograde transport, followed by association of the complexes with the nucleus. Thus, it is suggested that the trophic response of neurons to NGF is mediated, in part, by activation of nuclear PKC. It is inviting to consider, that a general response of neurons to trophic peptides may be the activation of nuclear PKC, and further, that the specificity of the response lies in the PKC isozyme activated, its substrates, and associated gene transcription.

J 339 CHARACTERIZATION OF THE RAT GLUTAMIC ACID DECARBOXYLASE GENE, Russell J. Wyborski, Richard W. Bond, and David I. Gottlieb, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.
Glutamic acid decarboxylase (GAD) catalyzes the synthesis of γ- aminobutyric acid (GABA) which acts as the major inhibitory neurotransmitter in the brain. The high specificity of location of GAD activity to inhibitory neurons makes the regulation of GAD gene expression a good model for the process of neuronal differentiation. Utilizing a rat genomic library in EMBL 3 (obtained from Richard Hynes, M.I.T.) and cultured cell lines, we have begun characterizing the rat GAD gene. Genomic Southern blots with rat GAD cDNA probes indicate a minimum molecular size of 35 kb for the GAD gene. Genomic clones have been isolated which map to the 5' region of the GAD cDNA sequence. Nuclease protection assays have identified the intron/exon structure of this region. GAD gene expression has been identified in the C6 glioma and B65 CNS neural tumor cell lines and the expression pattern is similar to that seen in embryonic tissue. Therefore, the investigation of regulation of GAD gene expression in these cell lines may provide insight into the process of neuronal differentiation.

J 340 MECHANISMS IN REGULATING THE EXPRESSION OF THE CHICKEN α-CRYSTALLIN GENE, KUNIO YASUDA, ISAO MATSUO, MASAHI KITAMURA and MASAYUKI TAKEUCHI, Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan.
Crystallins are lens-specific structural proteins and their expression is highly regulated at site and time during development. To reveal mechanisms involved in regulating tissue-specific expression of the crystallin gene, we analyzed transient expression of chicken α-crystallin gene fused to E. coli CAT gene in various primary culture cells. Deletions in the crystallin promoter revealed that sequence located between -165 and -127 nucleotides upstream of the transcription initiation site contributes to tissue-specific expression. In vivo competition experiment showed a nuclear factor binding the regulatory sequence plays a direct role in crystallin expression in lens cells. Gel retardation assay demonstrated that the binding activity to this regulatory sequence showed tissue-specificity. DNaseI footprinting analysis showed that the binding domain is located between -165 and -141. DNA methylation analysis and gel retardation assay using synthetic oligonucleotides indicated that the DNA binding factor interacts with the sequence 5'-CTGGNNNNNNCCAG-3'. We are now in progress of purification and cloning of this factor.

J 341 MOLECULAR GENETICS OF FASCICLIN I, AN AXONAL SURFACE PROTEIN FROM INSECT EMBRYOS, Kai Zinn†*, Thomas Elkins*, Linda McAllister*, and Corey S. Goodman*,†Division of Biology, California Institute of Technology, Pasadena, CA 91125, *Department of Biochemistry, University of California, Berkeley, Berkeley, CA 94720. Fasciclin I is a surface glycoprotein that is expressed on a specific subset of axons and growth cones in insect embryos, and is thus a candidate for a recognition molecule involved in growth cone guidance during development. It is an extrinsic membrane protein with a signal sequence but no transmembrane domain, and may be attached to the cell surface by noncovalent interactions with another membrane protein (Zinn et al., Cell 53, 577 (1988)). The Drosophila fasciclin I gene is located at 89D, about 20 kb 3' to the Ubx transcription unit. We have identified an existing insertion mutation (TE77), in which a very large transposable element (the Ising TE) is inserted into the second intron of the fasciclin I gene. This mutation appears to completely abolish expression of the gene. Flies homozygous for the TE77 insertion are somewhat unhealthy but have no obvious embryonic defect. Since there is evidence from vertebrate systems that neurite outgrowth and guidance may involve multiple, partially redundant molecular cues, we have tried to define the role of fasciclin I in nervous system development by making double mutant stocks with TE77 in combination with mutations in other genes encoding membrane proteins expressed in the embryonic nervous system; some of these double mutants have embryonic defects.

Spatial Regulation

J 401 The Murine Hox 3.1 Homeo Box Gene: Structural Analysis and Molecular Dissection of Trancriptional *cis* Regulatory Elements. Alexander Awgulewitsch†, Charles Bieberich*, Leonard Bogarad*, Ken Takeshita#, Donna Jacobs†, Frank H. Ruddle*#. Department of †Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425; Departments of *Biology and #Human Genetics, Yale University, New Haven, CT 06511.

The distinct region-specific expression patterns of murine homeo box genes during embryonic development are consistent with the hypothesis of functional similarities between vertebrate and *Drosophila* homeo box genes[1-3]. Certain experimental approaches to prove the suggested morphogenetic functions of murine homeo box genes, as well as analysing the control and regulation of their expression require detailed structural information. We have selected the mouse Hox 3.1 locus comprising a DNA segment of approximately 9 kb as an example, and determined its complete structure on the DNA sequence level. Exon - intron boundaries as well as transcriptional start and termination sites have been determined by Nuclease SI mapping, primer extension studies, and Northern blot analysis. These studies enabled us to start the molecular dissection of Hox 3.1 *cis* regulatory elements that provide region specific expression during embryonic development. DNA constructs containing distinct segments from the Hox 3.1 5'region linked to the *E.coli* ß-galactosidase gene lacZ confer region-specific expression patterns of the reporter gene in transgenic mouse embryos that are strikingly similar to the characteristic expression pattern of the endogenous Hox 3.1 gene.
Ref.: 1) A. Awgulewitsch et al. Nature 320, 328, 1986. 2) M.F. Utset et al. Science 235, 1379, 1987. 3) P.W.H. Holland and B.L.M. Hogan. Genes & Development 2, 773, 1988.

J 402 DEVELOPMENTAL SIGNAL TRANSDUCTION VIA INTEGRIN, David Boettiger, Mindy George-Weinstein, and A. Sue Menko, Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104.

Monoclonal antibodies to the avian integrin beta-1 chain prevent the binding of integrin to the fibronectin substrate and block the signal for withdrawal from the cell cycle and initiation of synthesis of the major muscle specific products in primary avian myoblasts. In the absence of the signal the cells continue to proliferate. The molecular and physical requirements for the initiation of this myogenic differentiation signal have been investigated by manipulation of the substrate components and the construction of artificial substrates using monoclonal antibodies to muscle cell surface components. The experiments demonstrate: (i) that integrin is an essential receptor in the signal transmission, (ii) that the normal extracellular component which participates in signal initiation is fibronectin, and (iii) that the physical nature of the substrate (rigidity) is critical. These results demonstrate a novel pathway for developmental signals, particularly in the context of the cell's physical microenvironment, and provide a pathway for cell shape to control specific gene expression.

J 403 CHARACTERISATION OF ELF 1, A TRANSCRIPTION FACTOR REQUIRED FOR CNS EXPRESSION OF THE *DROSOPHILA* DOPA DECARBOXYLASE GENE. Sarah J Bray, Brian Burke*, and Jay Hirsh. Department of Biological Chemistry and Molecular Pharmacology and *Department of Molecular and Cellular Physiology, Harvard Medical School, 260, Longwood Ave., Boston, Ma 02115.

The expression of dopa decarboxylase (*Ddc*) in the central nervous system (CNS) of *Drosophila melanogaster* is dependent on a short sequence located adjacent to the transcription initiation site. Point mutations in this sequence inhibit expression of *Ddc* in the CNS and inhibit binding of protein present in embryonic nuclear extracts. We have purified the protein(s), named Elf 1, to near homogeneity and isolated monoclonal antibodies that recognise it. Using immunohistochemstry in whole embryos we have shown that the protein is nuclear and found in cells of ectodermal origin, and its expression preceeds the appearance of *Ddc* mRNA by at least 7 hours. Within the CNS only a small number of neurons express Elf 1, the number and distribution of these changes during embryogenesis. Neuronal cells expressing *Ddc* also express Elf 1. The antibodies have been used to clone cDNAs encoding Elf 1 which provide evidence for variations in mRNA structure leading to different Elf 1 isoforms.

J 404 CELL LINEAGE AND EVOLUTIONARY DETERMINANTS OF GENE EXPRESSION PATTERNS IN DROSOPHILA. Douglas R. Cavener, Yue Feng, Diana L. Cox-Foster, Philip A. Krasney, Chavela M. Carr, and Edward L. Organ, Department of Molecular Biology, Vanderbilt University, Nashville, TN 37235 During the development of the reproductive organs of *Drosophila melanogaster*, glucose dehydrogenase (*Gld*) mRNA is abundant in the male ejaculatory duct and ejaculatory bulb and in the female oviduct and spermathecae. Genetic analysis indicated that the expression in the developing reproductive organs was non-essential. Because the oviduct/ejaculatory duct and ejaculatory bulb/spermathecae are developmentally homologous, we argue that the observed expression pattern is a result of common cell lineage. At the adult stage *Gld* expression becomes largely restricted to the male ejaculatory duct. Two other Drosophila species, *pseudoobscura* and *virilis* display different patterns of expression in the developing and mature reproductive organs. Interspecific gene transformation experiments and genetic manipulations of the sex determination pathway suggest a model for *Gld* developmental regulation in the reproductive organs which depends upon cell lineage determination as affected by subtle changes in the expression of the sex determining genes in the genital imaginal disc during the third larval instar.

J 405 MOLECULAR ANALYSIS OF A GENETIC SWITCH, **Steve DiNardo, M. Benedyk and J. Mullen, Rockefeller University, N.Y.C., N.Y. 10021.** During embryonic development a cell's fate must be assigned, not in isolation, but in the context of its neighbors. In molecular terms, cell fate determination involves the initiation of a program of gene expression within a cell, where the products of these genes then regulate the behaviour of that cell. We are using molecular and genetic approaches in the fruit fly to study the regulation of such spatial programming for segmentation genes in Drosophila, and in particular for the developmental switch gene engrailed. The regulatory circuit is comprised of three "pair-rule" genes. We will report our progress on the molecular and genetic analysis of one regulatory gene oddpaired, and its interactions with the other two products, which, acting together, effect **combinatoral** control of engrailed.

J 406 ISOLATION AND CHARACTERIZATION OF A PUTATIVE SEA URCHIN SPICULE MATRIX PROTEIN Nikolaos C. George and Fred H. Wilt*, Dept. of Genetics, *Dept. of Zoology, University of California, Berkeley CA 94720. Spicule formation in sea urchin embryos represents a unique system through which to address questions concerning cell lineage determination and differentiation. Spicules contain an organic matrix of at least ten acidic proteins and are produced by primary mesenchyme cells (PMCs), the descendents of a single well defined cell lineage. The gene encoding a 50 kD spicule matrix glycoprotein (SM-50) has previously been cloned and characterized. We have undertaken to isolate genes that encode other spicule matrix proteins. A lambda gt11 cDNA expression vector library was constructed using mRNA isolated from a PMC enriched fraction of prism stage embryos that was screened with a polyclonal antiserum raised against total spicule matrix proteins. The screen yielded several clones that react to the antiserum but do not cross-hybridize with clones for SM-50. Analysis of two clones, pNG3 and pNG7, indicates that they are homologous and hybridize to a single 2.1 kb transcript. Northern blot studies show pNG3/pNG7 transcripts begin to accumulate to appreciable levels shortly before spicule formation begins and reach peak levels during overt spiculogenesis. In situ hybridization experiments indicate these transcripts accumulate only in PMCs. DNA sequencing, monospecific antiserum production, as well as hybrid selection and arrest studies are being conducted to determine if these clones do in fact encode spicule matrix proteins. This work supported by NIH grants.

J 407 GENETICS of MUSCLE PATTERN FORMATION in *CAENORHABDITIS ELEGANS*, Phuay Yee Goh and Thierry Bogaert, Lab of Molecular Neurobiology, Institute of Molecular and Cell Biology, 10 Kent Ridge Crescent, Singapore 0511 (bitnet MCBOGART@NUSVM Tel 7723380).

To identify the genes/molecules involved in specifying the development of a pattern of muscles and their attachments, we use a set of male specific muscles in the tail and the body wall muscles of *C. elegans* as model systems.

1) We isolated 20 mutants in which the 41 male specific muscle cells make inappropriate attachments and the muscle pattern is disrupted. The pharyngeal muscles and body wall muscle pattern are not affected in these mutants.

2) We also isolated two larval lethals (*e2346* and *e2347*) in which the myoblasts extend processes across quadrants, or are displaced from the quadrants as early in embryogenesis as in the 1.25 - 1.5 fold stage embryos. In L1 larvae many body wall muscles are detached and some are attached across quadrants. *e2346* and *e2347* identify two genes required early in embryogenesis for proper development (possibly attachment) of the body wall muscles.

A progress report on the phenotypic and genetic characterization of these mutants will be presented.

J 408 SIMILARITY BETWEEN *DROSOPHILA* AND MURINE HOMEOBOX NETWORKS, Anthony Graham and Robb Krumlauf, Lab. of Molecular Embryology, NIMR, London NW7 1AA, U.K.

The clustering of homeoboxes appears to be a general phenomenon having been described in *Drosophila*,mouse , human , *Xenopus* and Zebra Fish . It has been previously noted that the physical order of the genes along the chromosome in the *BX-C* and *ANT-C* is related to the antero-posterior order of the segment that each gene affects and to its transcript distribution in the central nervous system (CNS). In this poster we show that a similar situation holds for the murine Hox 2 cluster of homeobox genes. From the 5′ most gene (Hox 2.5) towards the 3′ most gene (Hox 2.7) there are overlapping patterns of expression for each member of the locus in the CNS. It is apparent that the expression of each successive gene in a 3′ direction extends more rostrally along the anteroposterior axis of the animal. What is even more striking is that the correlation between sequence relationship and the relative extent of expression of each gene along the anteroposterior axis holds for both mouse and *Drosophila* . For example the most caudally expressed *Drosophila* genes show the highest sequence identity to the most caudally expressed mouse genes . We suggest that the mouse and *Drosophila* homeobox clusters have both arisen from a common ancient ancestral complex. This cluster of genes would be related to their *Drosophila* and murine counterparts by merit of their sequences and patterns of expression along the anteroposterior axis of the animal.

J 409 DIFFERENTIAL GENE EXPRESSION IN THE ANTERIOR NEURAL PLATE DURING GASTRULATION OF XENOPUS LAEVIS. Milan Jamrich and Sheryl Sato, Laboratory of Molecular genetics, NICHD, NIH, Bethesda, Maryland 20892.

We have isolated three cDNA clones which are preferentially expressed in the cement gland of early Xenopus laevis embryos. These clones were used to study processes involved in the induction of this secretory organ. Results obtained show that the induction of this gland coincides with the process of neural induction. Genes specific for the cement gland are expressed very early in the anterior neural plate of stage 12 embryos. This suggests that the anteroposterior polarity of the neural plate is established during gastrulation. At later stages of development, two of the three genes have secondary sites of expression. The expression of these genes can be induced in isolated animal caps by incubation in 10 mM NH4CL, a treatment which is known to induce cement glands.

J 410 CONTROL OF THE EXPRESSION OF A GUT SPECIFIC ESTERASE DURING C. ELEGANS DEVELOPMENT, Brian P. Kennedy, Eric J. Aamodt, James D. McGhee Dept. of Medical Biochemistry, University of Calgary, Calgary, Alberta, CANADA We are studying the control of gene expression during development in the nematode C. elegans. There is substantial indirect evidence in C. elegans that early cell fates are determined by cell-autonomous, internally-segregating determinants. To identify such determinants, we are concentrating on factors that control a carboxylesterase that is specifically expressed in the gut precursor cells of the embryo as well as in the larval and adult intestine. The esterase gene was cloned by use of an oligonucleotide probe whose sequence was chosen based on the N-terminal a.a. sequence of the purified esterase and the C. elegans codon bias. We have used the cloned gene to transform a strain of C. elegans that contains a null mutation in the esterase gene. Embryos in the transformed line correctly express the esterase both spatially and temporally. The esterase gene appears to be trans-spliced as is true of several other C. elegans genes. We have identified DNase I hypersensitive sites in the 5' non-coding region of the gene, and a 300 bp fragment from this area of the esterase gene is shifted on gels by nuclear extracts. These shifts are competed by the esterase gene but not by a C. elegans actin gene. We have sequenced the esterase gene and are currently sequencing the 5' region of the esterase gene from the closely related nematode species C. briggsae. Comparison of the non-coding regions of the C. elegans and C. briggsae genes should reveal conserved regions that are necessary for correct gene expression. We have developed a transient transformation assay for the esterase gene and are doing deletion analysis to identify the areas of the gene which are required for correct expression.

J 411 EXPRESSION OF GENES CONTROLLING SEXUAL DIFFERENTIATION IN THE MOUSE

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The development of a mammalian embryo as a male normally depends on the action of a gene or genes present on the Y chromosome. A candidate for such a gene in humans encodes a zinc finger protein, and four homologues of this gene exist in the mouse genome. Of these, two are Y-linked and only one is necessary for testis determination. We have isolated probes which allow us to specifically study the expression of these genes, and of the gene for anti-Mullerian hormone which is involved in subsequent steps in sexual differentiation. We describe here the temporal and spatial regulation of these genes, and their co-ordination during mouse embryogenesis.

J 412 THE COMPLEX DEVELOPMENTAL EXPRESSION PATTERN OF *XENOPUS* KERATIN ENDO B: EXPRESSION IN THE NOTOCHORD, CEMENT GLAND, AND PITUITARY AND OLFACTORY RUDIMENTS. Susan LaFlamme and Igor Dawid, Laboratory of Molecular Genetics, National Institute of Child Health and Development, National Institutes of Health, Bethesda, MD.

XK endo B is a type I nonepidermal keratin that was originally identified as a cDNA clone by its preferential expression in the embryonic notochord of the amphibian, *Xenopus laevis* (LaFlamme et al. (1988) Genes & Development 2, 853). A peptide identical to a short region of the predicted amino acid sequence of XK endo B was synthesized and then used to generate antibodies directed against the XK endo B protein. The temporal and spatial expression pattern of XK endo B was then determined by staining sections of *Xenopus* embryos at different stages of development using indirect immunofluorescence. XK endo B expression was observed in the notochord and endoderm as predicted from RNA analysis. In addition, it was observed in the cement gland, and in the pituitary, olfactory, and visceral pouch rudiments. It also showed a nonuniform distribution along the dorsal-ventral axis in the neural tube, as well as along the anterior-posterior axis in the sensorial layer of the ectoderm. Although we find the complex expression pattern of XK endo B intriguing, we do not yet understand the rules that govern its expression. In some cases, this pattern of expression may suggest evolutionary relationships of structures. In others, it may reflect gradients of regulatory molecules along embryonic axes or relationships of structures by cell lineage.

J 414 THE *DROSOPHILA* GENE *KNIRPS-RELATED* IS A MEMBER OF THE STEROID-RECEPTOR GENE SUPERFAMILY, Anthony E. Oro, Jonathan S. Margolis, James W. Posakony, Michael McKeown, and Ronald M. Evans Howard Hughes Medical Institute, Gene Expression Lab, Salk Institute for Biological Studies La Jolla, California, 92138 and University of California, San Diego Department of Biology La Jolla, CA 92093. Molecular cloning studies have demonstrated that the receptors for steroid, retinoid, and thyroid hormones are part of a large superfamily of nuclear regulatory proteins. In vertebrates, these molecules regulate diverse biological processes including pattern formation, cellular differentiation and homeostasis. The universal need for embryonic and adult cells to respond to their external environment suggests that members of this family might pre-date the divergence of vertebrates and invertebrates. Consequently, a *Drosophila* genomic library was screened for steroid receptor homologs with a human retinoic acid receptor (hRAR) cDNA as a hybridization probe. Of several clones recovered, one mapped to chromosomal position 77E1-2, the cytological location of the gap segmentation gene *knirps (kni)*. Sequence analysis of a cDNA clone representing the hRAR homolog revealed homology of the predicted protein to the vertebrate steroid receptors as well as to the predicted *kni* gene product. *In situ* hybridization of a cDNA probe to wild-type embryos revealed a uniform distribution of apparently maternally-derived transcripts. Zygotic transcript accumulation begins prior to cellular blastoderm in a broad antero-ventral domain. At cellular blastoderm, two additional circumferential bands of transcript appear.

J 415 EXPRESSION OF ENGRAILED-RELATED PROTEINS DURING ARTHROPOD DEVELOPMENT, Nipam H. Patel*, Thomas Kornberg#, and Corey S. Goodman*, *HHMI, Dept. of Biochemistry, Univ. of California, Berkeley, CA 94720 and #Dept. of Biochemistry, Univ. of California, San Francisco, CA 94143.

Monoclonal antibody 4D9 recognizes engrailed-related proteins in a variety of arthropods, annelids, and chordates by virtue of its ability to bind to an engrailed-specific epitope in the homeobox domain (N. Patel, E. Martin, K. Coleman, S. Poole, M. Ellis, T. Kornberg, and C. Goodman, manuscript in preparation). The engrailed expression pattern within arthropods is highly conserved and suggests that engrailed serves a similar function during segmentation and neurogenesis in all arthropods. We do find, however, some variation in the generation of the pattern. Early *Drosophila* development involves synchronized cell divisions across the entire embryo, and the individual engrailed stripes appear almost simultaneously. In contrast, the segments of grasshopper and crayfish embryos form one at a time as the embryo elongates from cell divisions occuring at the posterior tip; in these embryos the engrailed stripes appear one at a time. We observe that the later broadening of the engrailed stripe involves new expression in a second row of cells. The nervous system expression pattern is also highly conserved and reveals engrailed-positive neurons that are not part of the posterior compartment.

J 416 DEVELOPMENTALLY REGULATED SEA URCHIN COLLAGEN GENES. F. Ramirez[1], M. D'Alessio[1], H. Vissing[1], H. Suzuki[2], M. Solursh[2], G. Buttice[3], B. Saitta[3], and R. Gambino[3]. [1]SUNY Health Science Center at Brooklyn, Brooklyn, N.Y.,[2]University of Iowa, Dept. of Biology, Iowa City, Iowa, [3]Institute of Development Biology, Palermo, Italy. Extracellular matrices (EMC) influence a variety of cellular activities, including morphogenesis. In the developing sea urchin embyro, collagen expression is believed to play an important role at gastrulation and later during spiculogenesis. To get new insight into the molecular mechanisms modulating collagen expression during early embriogenesis as well as to gather novel information about the evolution of this phylogenetically ubiquitous family of proteins, we have isolated cDNA and genomic clones encoding sea urchin collagens. Briefly, a stage-specific (prism) cDNA library was constructed in λgt 10 vector and screened with a genomic fragment containing several collagenous coding sequences. This identified two distinct sets of cDNAs, both encoding long, uninterrupted Gly-X-Y repeats which are flanked at both ends by non-collagenous sequences. Northern blot hybridizations using RNA purified from different developmental stages revealed that the two genes exhibit uniquely distinct patterns of temporal expression. Experiments are currently in progress to further detail the structural and functional features of this group of genes as well as to elucidate their pattern of spatial expression in developing sea urchin embryo.

J 417 ANAEROBIC TREATMENT ALTERS THE CELL-SPECIFIC EXPRESSION OF Adh-1, Sh, AND Sus GENES IN ROOTS OF MAIZE SEEDLINGS, Lisa J. Rowland, Yen-Ching Chen, and Prem S. Chourey, USDA/ARS, Department of Plant Pathology, University of Florida, Gainesville, FL 32611
We have examined the in situ expression pattern of Sh and Sus which encode sucrose synthase isozymes SS1 and SS2 respectively and Adh-1 which encodes alcohol dehydrogenase 1 (ADH1) in the lower region of the primary root of maize seedlings in response to anaerobiosis. In situ hybridization and/or immunolocalization experiments revealed a unique spatial pattern of expression for each of the three genes. Anaerobic induction of ADH1 RNA was localized to the epidermis and cortex. Induction of Sh was marked by highly elevated SS1 RNA levels in the vascular elements, pith, and epidermis. A significant but less drastic increase in SS protein was found in these same tissues as well as the root cap; the increased level of immunosignal was, however, restricted to cells within about 1 centimeter of the root apex. The specific response of the Sus gene to anaerobic stress was determined using a sh deletion mutant; Sus responded with a slight reduction in SS2 RNA and protein levels except in the root cap where SS2 protein, but not SS2 RNA, was induced. These data indicate that multiple regulatory controls including cell-specific post-transcriptional mechanisms modulate SS levels in anaerobically-stressed seedlings.

J 418 ISOLATION OF DORSAL-AXIS SPECIFIC GENES FROM XENOPUS LAEVIS EMBRYOS, Sheryl M. Sato and Thomas D. Sargent, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892 We have constructed a cDNA subtraction library from Xenopus laevis embryos which is enriched in genes differentially expressed in the dorsal axis during organogenesis. The subtraction cloning scheme utilized "driver RNA" prepared from UV-treated, grade 5 embryos (embryos which completely lack a dorsal axis). Several clones isolated from the subtraction library encoded transcripts that were both UV-supressed and not expressed in muscle. One of these clones, UVS 2, was extensively characterized throughout embryonic development. By in situ hybridization, the transcript was localized in the anterior neural fold of early neurula stage embryos. At the later tailbud stages, expression was restricted to the sensorial layer of specialized head ectoderm (placodal thickenings) located in the most dorsal and anterior portion of the head. The UVS 2 transcript can be induced in explants of animal ectoderm (blastula stage) that have been co-cultured with dorsal mesoderm (early gastrula stage). Tissue-specific molecular markers such as UVS 2 should be very useful in dissecting apart the complicated patterns of embryonic induction, particularly in the later stages of secondary induction.

J 419 Isolation of an α-H2B Histone-Specific Antibody and Immunolocalization in Sea Urchin Embryos.
Barbara Ramsay Shaw*, Daniel J. Thompson*, W. Lee Maloy and Scott C. Chambers, Gross Chemical Lab., Duke University*, Durham, N.C., 27706 and NIH, Bethesda, MD 20205.

We have raised a polyclonal antibody to a synthetic peptide which has been predicted from DNA sequencing to be unique to the *S. purpuratus* α-H2B histone variant. Solid-phase peptide synthesis was used to prepare the α-$H2B_{1\text{-}9c}$ peptide, which corresponds to the first nine amino acids at the N-terminus of the α-H2B histone variant. The peptide was coupled to keyhole limpet hemocyanin via a C-terminal cysteine and injected into rabbits. The virgin antiserum was shown to contain an α-H2B specific antibody, as well as some background reactive antibodies. HPLC-purified α-H2B and other histone variants were used to screen and purify the antiserum. Affinity chromatography with α-H2B histone yielded a monospecific antibody. The antibody reacted specifically with α-H2B by slot immunoblot and TAU- and SDS-western immunoblot assays; it did not react with any other histone variant. Moreover, the monospecific antibody was shown by ELISA to react with whole chromatin. Competition with histone α-H2B or the α-$H2B_{1\text{-}9c}$ peptide abolished the specific reactions, demonstrating that the antibody reacts with chromatin at the α-$H2B_{1\text{-}9}$ epitope. Immunolocalization studies of α-H2B in whole embryos show that the α-H2B monospecific antibody strongly stains nuclei of 32-cell and blastula stage embryo, but not eggs or two-cell embryos, and that immunofluorescent patterns are abolished by competition with α-H2B. The anti α-H2B histone antibody preferentially stains the metaphase plate of 8-cell embryos. The temporal pattern of staining thus follows that expected for the expression of α-H2B: the α-H2B histone first appears in the embryo in early cleavage and its concentration in the nucleus increases up through blastula stage. These results demonstrate the utility of the antiserum to follow the α-H2B histone variant cytologically.

J 420 REGIONAL CYTOKERATIN EXPRESSION IN XENOPUS AND CHICK EMBRYOS, Beverly Smolich, Timothy Charlebois, and Robert Grainger, Department of Biology, University of Virginia, Charlottesville, VA 22901. Our lab studies the mechanisms leading to lens cell determination in Xenopus and chick embryos. We have shown that differences in lens-forming potential exist between regions of ectoderm, prior to any overt lens differentiation. Ectoderm from the presumptive lens area, taken from a 10 somite chick embryo and cultured in vitro, will differentiate into a lens as assayed by synthesis of crystallins; ectoderm from the back of the embryo does not. Hypothesizing that differential gene expression may accompany this difference in potential, a chick embryo cDNA library was screened using probes from head ectoderm and back ectoderm. We have identified a type II cytokeratin (designated T4) whose expression, based on Northern analysis and in situ hybridization, strongly correlates with a loss or absence of lens-forming potential in the ectoderm. In addition, T4 expression delineates a striking regionalization in very early mesoderm (at the primitive streak stage): lateral mesoderm is T4 positive, and dorsal mesoderm is T4 negative. To understand what controls the remarkable regional properties of this gene, we have isolated a homolog in Xenopus, in which embryological data exists defining critical tissue interactions occurring during lens induction and mesoderm induction, and have initiated a spatial and temporal analysis of its expression.

Cytoplasmic Events; Cell-Cell Interaction

J 500 A TISSUE POLARITY GENE ENCODES A PROTEIN WITH 7 TRANSMEMBRANE DOMAINS, Paul N. Adler, Lilly Wong, Woo Jin Park, and Charles R. Vinson, Biology Department, University of Virginia, Charlottesville, VA 22901.

The bristles and hairs that decorate the adult cuticle of Drosophila define a tissue polarity, as they typically point distally on appendages and posteriorly on the thorax and abdomen. Mutations in the *frizzled (fz)* gene disrupt this normally precise pattern. We have found that the parallel alignment of hairs on the wing is in part a consequence of cell geometry. Hairs are formed by large bundles of actin filaments that grow out of the distal vertex of the hexagonally shaped wing cells. We are determining which aspect of this process is affected by mutations that alter normal tissue polarity (eg. *fz* appears to affect the choice of the distal vertex).

Genetic mosaic analysis has shown that *fz* activity is required for the both the transmission of an intercellular polarity signal along the proximal distal axis of the wing, and for the ability of cells to respond to the polarity signal. The results are consistent with *fz* encoding a polarity morphogen receptor that transduces polarity information across the cell membrane.

The *fz* gene has been cloned by transposon tagging, and *fz* mutations found to span 100 kb of genomic DNA. Northern and cDNA clone analyses indicate that *fz* encodes a single 4 kb mRNA in developing adult epidermis. This mRNA encodes a protein with 7 presumptive transmembrane domains. Thus the *fz* protein likely contains extracellular domains that could function in the transmission of polarity information, and intracellular domains that could function in the response of cells to polarity information. Preliminary *in situ* hybridization experiments indicate that *fz* is expressed at similar levels in all regions of the epidermis, suggesting that polarity information is not a result of a gradient of *fz* gene expression.

J 501 OVEREXPRESSION OF THE VIMENTIN GENE IN TRANSGENIC MICE INHIBITS NORMAL LENS CELL DIFFERENTIATION. Y. Capetanaki, S. Smith, & S. Starnes, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The unique expression pattern of the intermediate filament (IF) subunit vimentin motivates the desire to investigate its biological function during proliferation, differentiation and morphogenesis which remains largely unknown. Vimentin seems to interconnect the nucleus with the plasma membrane, possibly contributing in this structural way to the transport processes and communication taking place between the cell surface and the nucleus. Indeed, recent biochemical studies have shown high binding affinity of vimentin, and other non-epithelial IF subunits for nuclear constituents like lamin B, single stranded nucleic acids, particularly DNA, and core histones. However, to date, there is no evidence to support functional roles for any of the demonstrated in vitro structural interactions. To investigate the role of the IF protein vimentin in the normal differentiation and morphogenesis of the eye lens fiber cells, we generated transgenic mice bearing multiple copies of the chicken vimentin gene. The vimentin transgene was expressed in a correct tissue-specific pattern, the transcripts were properly processed to stable mRNAs which were efficiently translated to vimentin protein that could be post-translationally properly modified. In most cases, the vimentin transgene was overexpressed in the lenses of these animals, reaching up to ten times the endogenous levels. This high expression of vimentin interfered very strongly with the normal differentiation of the lens fibers. The normal fiber cell denucleation and elongation processes were impaired and the animals developed pronounced cataracts, followed by extensive lens degeneration.

J 502 IDENTIFICATION OF A 57kDa VIMENTIN-LIKE INTERMEDIATE FILAMENT PROTEIN IN *XENOPUS*. Joe Dent, Andy Polson, Ken Sato & Mike Klymkowsky. Molecular, Cellular & Developmental Biology. University of Colorado, Boulder. 80309-0347. In the course of our studies of vimentin in the *Xenopus* embryo, we have identified a 57kDa polypeptide immunochemically related to vimentin (55kDa). The 57kDa protein reacts with *antiIFA*, a monoclonal antibody that recognizes most intermediate filament subunit proteins. Both vimentin and the 57kDa protein are labelled by the monoclonal antibody *1/h7*. A rabbit anti-vimentin antisera (from R.O. Hynes) reacts preferentially with the 57kDa protein, but also reacts with vimentin, whereas the monoclonal antivimentin antibody RV202 (from Franz Ramaekers) reacts specifically with vimentin. We have examined the expression of the 57kDa vimentin-like protein in various tissues of the adult using western blot. Vimentin and the 57kDa vimentin-like protein are differentially expressed: the 57kDa protein is more abundant the lung and siatic nerve, while vimentin is more abundant in cultured A_6 cells (derived from kidney epithelia). In other tissues, both vimentin and the 57kDa vimentin-like protein are present in roughly similar amounts. In the oocyte, immunocytochemistry with *1/h7* and RV202 suggests that the 57kDa vimentin-like protein is associated with the mitochondrial mass of stage I oocytes. The differential expression of these two intermediate filament proteins suggests that they play somewhat different functional roles within the organism.

J 503 ANTERIOR/POSTERIOR PATTERNING IN *XENOPUS*: TRYPAN BLUE AND SURAMIN CAUSE A CHANGE OF CELL FATE FROM ANTERIOR/DORSAL TO POSTERIOR/VENTRAL. **Tabitha Doniach, Michael Danilchik*, H. Joseph Yost and John C. Gerhart, Department of Molecular Biology, UC Berkeley, CA 94720 and *Department of Biology, Wesleyan University, Middletown CT 06457.**

We have been studying the process of anterior-posterior (A/P) pattern formation in the frog *Xenopus laevis* by examining the effects of the polysulfonated compounds trypan blue and suramin on gastrulation. Injection of these compounds into the blastocoel (an extracellular space) before or during gastrulation results in anterior to posterior truncation of dorsal elements of the body axis, ranging from loss of some head structures to a total loss of all dorsal axial structures, leaving essentially only ventral structures.

In principle, these compounds could truncate the body axis by making prospective anterior-dorsal cells take on more posterior-ventral cell fates, or they could simply eliminate these cells. To distinguish these possibilities, we injected these compounds into embryos that had been pre-treated with lithium. Li^+ alone, applied at the 32-cell stage, causes embryos to develop excessive anterior and dorsal structures with few or no posterior or ventral ones. In contrast, embryos treated first with Li^+ and then with trypan or suramin develop posterior trunk and tail structures and no heads. This indicates that these two compounds cause a change of cell fate toward more posterior-ventral types.

In normal gastrulation, the extent of migration of the mesoderm along the dorsal midline correlates with the degree of A/P development, with anterior mesoderm migrating the farthest, followed by trunk and then tail. Embryos treated with trypan or suramin show reduced mesodermal migration during gastrulation. It is possible that the ultimate position reached by the cells of the migrating mesoderm determines the level of their A/P development, i.e., position could determine fate. If so, these compounds may cause truncation by directly inhibiting migration. Alternatively, the A/P pattern could be set up by signals that are independent of mesodermal position. If so, trypan and suramin could mimic or interfere with such signals. Both compounds have been shown to interfere with the binding of ligands to their receptors; for example, suramin inhibits the binding of FGF, EGF and PDGF to their receptors. Experiments are in progress to distinguish the above possibilities.

J 504 *DITYOSTELIUM* PHOSPHODIESTERASE. TOO MUCH, TOO LITTLE, AND JUST ENOUGH. Michel Faure, Gregory J. Podgorski*, J. Franke, and Richard H. Kessin.

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The gene that codes for the cyclic nucleotide phosphodiesterase has been cloned and used to alter cAMP levels in developing *Dictyostelium* cells. One construct contains about 1.8 kb of coding sequence and 2.3 kb of 5' sequence. A second, which serves as a control, lacks most of the coding sequence. The promoter that lies in the 2.3 kb of 5' sequence produces phosphodiesterase mRNA and enzyme in amounts proportional to copy number. By taking advantage of high copy number transformants we have created a situation in which a large excess of phosphodiesterase is secreted with interesting effects on development. Aggregation is accelerated by excess phosphodiesterase but returns to its normal time course in the presence of a specific glycoprotein that the cells secrete to inhibit the phosphodiesterase. Differentiation of spore and stalk cells is completely blocked by excess enzyme.

The gene has also been used to correct a defect in a mutant that produces niether membrane bound nor extracellular phosphodiesterase. When the gene is introduced into strains carrying the *pdsA* mutation, the cells become capable of aggregation and then form normal fruiting bodies. Only extracellular phosphodiesterase is restored, indicating that under these conditions, the membrane bound enzyme is not essential for formation of fruiting bodies. *pdsA* mutants can be rescued with added phosphodiesterase but form incomplete, irregular fruiting bodies. The transformants produce normal fruiting bodies, which means that the failure at late development is due to the absence of phosphodiesterase and not to a second mutation.

J 505 *DROSOPHILA* EMBRYO CELL DIFFERENTIATION *IN VIVO* AND *IN VITRO*. Liselotte I. Fessler, Talila Volk and John H. Fessler, Molecular Biology Institute and Biology Department, University of California at Los Angeles, Los Angeles, CA 90024

During the development of *Drosophila* embryos the component cells interact with each other and with the extracellular matrix. Gentle dispersal of cellular blastoderm/gastrula embryos yields cells which are determined, but not differentiated, and have lost their cellular neighbors. Subsequent differentiation of these cells *in vitro* requires the presence of appropiate extracellular matrix. We find that the glycoprotein laminin suffices for differentiation of neural cells and formation of myotubes. Fibronectin serves similarly for cell spreading and differentiation (Naidet et al., 1987, Nature *325*, 348-350; Gratecos et al., 1988, EMBO J. *7*, 215-223). We find that myotubes formed in culture express the *Drosophila* integrin beta chain, a component of the integrin group of cell surface receptors. Previously we found that the molecular defect in the embryonic lethal mutant *myospheroid, l(1)mys*, is in the integrin beta transmembrane polypeptide (MacKrell et al., 1988, Proc. Natl. Acad. Sci. USA *85*, 2633-2637). While *l(1)mys* embryos develop muscles, which subsequently detach (Wright, 1960, J. Exp. Zool. *143*, 77-99), Donady and Seecoff concluded that myotubes fail to form from the mutant cells *in vitro*. We suggest that redundant, parallel interactions of cells with each other and with extracellular matrix components provide an assurance of muscular development *in vivo*, which can be partly dissected by differentiation experiments *in vitro*.

J 506 THE DROSOPHILA SEGMENT POLARITY GENE, *patched*, ENCODES A MEMBRANE PROTEIN, Joan E. Hooper and Matthew P. Scott, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309. The segment polarity gene *patched (ptc)* is required for patterning the middle of every *Drosophila* segment. In *ptc*$^-$ embryos, cells in the middle of every segment are transformed to express pattern elements characteristic of the segment margins. Thus *ptc*$^-$ embryos express twice the normal number of segment borders and the ectopic segment borders exhibit reversed polarity. We have cloned *ptc*. Spatial and temporal analysis of *ptc* transcription indicates that it is expressed in wide stripes, one per segment, in embryos at the early extended germ band stage. These stripes are exactly complementary to *engrailed* stripes at this stage. Sequencing of genomic and cDNA clones reveals that the 7 kilobase mature *ptc* mRNA is composed of at least five exons. It encodes a protein of at least 1285 amino acids including seven hydrophobic domains that may be trans-membrane helices.

J 507 MOLECULAR ANALYSIS OF *PAR-2*, A GENE REQUIRED FOR EARLY DEVELOPMENT IN *C. ELEGANS* , D. Levitan and D. Stinchcomb, Department of CDB, Harvard University, Cambridge, MA. 02138

The maternal effect lethal gene, *par-2*, plays an important role in early development of the nematode *C. elegans*.In wild type embryos the early cleavages are asynchronous and asymmetric and produce daughter cells with different developmental potentials. In contrast, embryos produced by homozygous mutant *par-2* hermaphrodites exhibit synchronous and symmetric early cleavages, altered mitotic spindle orientations, abnormal germ-line granule localization, and germ line defects (Kemphues,K.,et.al. 1988, Cell 52, 311-320). We have identified a transposon induced mutation in the gene. Sequence analysis of 800 bp flanking the insertion site indicated the presence of a small open reading frame (105 amino acids).No significant homology has been found between the peptide encoded by this open reading frame and other protein sequences. Additional flanking DNA sequences were used to probe DNA isolated from five other alleles of *par-2* for the presence of restriction fragment length polymorphisms. We have found that one allele is associated with a small deletion located 12 kb from the transposon insertion site. We have microinjected 40 kb of DNA flanking the transposon insertion site into oocytes. Progeny containing the injected sequences were then assayed for the ability to compliment a temperature-sensitive allele of *par-2*. To date, no rescue has been observed. We are currently injecting other overlapping clones to delimit the *par-2* gene by rescuing the mutant phenotype.

J 508 MICROTUBULE DYNAMICS AND CELL DIVERSIFICATION IN THE MOUSE PREIMPLANTATION EMBRYO.
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During preimplantation development, the cells of the mouse embryo undergo both a major subcellular reorganisation (at the time of compaction) and, subsequently, a process of differentiation (as the phenotypes of trophectoderm and inner cell mass diverge). In order to study the role of the microtubule network during these processes, we have examined their distribution using antibodies specific for various post-translationally modified forms of tubulin and tested the effects of the microtubule inhibitors nocodazole (depolymerisation) and taxol (stabilisation) on the various features of compaction. Microtubules redistribute during compaction at the 8-cell stage. As the blastomeres flatten upon each other and cytoplasmic features polarise, an apical-basal asymmetry in the microtubule distribution becomes progressively more marked. microtubules recognised by an anti-acetylated tubulin antibody did not redistribute in the same way, rather they concentrated in the basal cortex. It seems that a less dynamic subpopulation of microtubules exists in these cells with a distinct distribution. Drug experiments indicate that microtubules have a constraining role on various events of compaction: they inhibit flattening and gap junction formation, control the distribution of cytoplasmic organelles and stabilise some components of the surface pole. These observations suggest that the apical redistribution of microtubules may coordinate the various changes taking place during compaction.
When the cells of a compact 8-cell embryo divide, the first two distinct cell types are formed, termed 'inner' and 'outer' cells. Outer cells have a polarised phenotype and tend to envelope the apolar inner cells during the 16-cell stage. We found that acetylated microtubules accumulated preferentially in inside cells. This difference was also seen at the 32-cell stage at the time of blastocyst formation. Differences in nocodazole sensitivity between cell types did not relate to the level of acetylation, although within each cell, acetylated microtubules were more stable. Although the basal accumulation of acetylated microtubules at the 8-cell stage required cell contact, differences between cell types at the late 16-cell stage were maintained in the absence of cell contact. Whether the differences in behaviour of the microtubules between the diverging cell types is important remains unknown.

J 509 LARVAL METAMORPHOSIS OF THE MARINE SPONGE *MICROCIONA PROLIFERA*,
Gradimir N. Misevic, Verena Schlup and Max M. Burger, F.M.I. CH-4002 Basel, Switzerland and M.B.L., Woods Hole, MA 02543 U.S.A.

Assumption about the inversion of embryonal layers during the sponge larvae metamorphosis led to suggestions for reclassification of sponges out of Metazoa. According to this proposal the outer flagellated epithelium of larvae should give rise to choanocytes, endothelial cells which are building up the major organ of flagellated chamber system in the adult organism. Two different evidence are presented that such process does not occur in *Microciona prolifera* larvae:1) electron microscopical autoradiography and time lapse cinematography of different larval stages revealed that the part of outer epithelium migrates into the interior of larvae concomitantly with their settlement and is followed by their destruction and death through phagocytosis by archeocytes, 2) quantitation of the number of different cell types, and autoradiography of pulse and chase thymidine incorporation at various larval stages showed that the choanocytes arise through differentiation of archeocytes without their multiplication. These results support the other cytological and biochemical evidence that sponges belong to Metazoa.

J 510 INHIBITION OF EARLY DEVELOPMENT OF *XENOPUS LAEVIS* USING ANTISENSE RNA FOR MEMBRANE SKELETON α–FODRIN, R.T. Moon and D.H. Giebelhaus, Dept. of Pharmacology SJ-30, University of Washington, Seattle, WA 98195.

Nonerythroid spectrin, also referred to as fodrin, is a dimeric membrane skeleton protein expressed in many cells types. Fodrin appears to be developmentally regulated in myogenic and neuronal cells. In this study, we have begun to use antisense RNA to inhibit the expression of α-fodrin in developing *Xenopus* embryos in an attempt to understand the role of α-fodrin in non-erythroid cells. Plasmids containing oocyte α-fodrin clone *Xen* α 2 (Giebelhaus, et al., 1987, *J. Cell Biol.* **105**:843) in sense or antisense orientation under control of a retroviral LTR were constructed and injected into *Xenopus* zygotes. Transcription of α-fodrin from these constructs is detected after mid-blastula transition and the plasmids persist through development to the tadpole stage. S1 analysis indicates that injection of 100 pg of plasmid expressing antisense α-fodrin RNA leads to a specific reduction in steady state levels of endogenous α-fodrin mRNA. Western blot analysis using an antibody specific for α-fodrin shows that a reduction in α-fodrin mRNA is paralleled by a decrease in levels of α-fodrin polypeptides at gastrula, neurula and tadpole stage. Morphological studies will be presented that indicate a common phenotype was displayed by many embryos injected with antisense plasmid: Antisense-injected embryos became kinked and sessile by tailbud stage whereas sense-injected embryos were not kinked and were capable of normal locomotion. Our data indicate that α-fodrin is required for embryonic development and that skeletal muscle is one of the tissues readily disrupted by reduced levels of α-fodrin protein. These data support the hypothesis that fodrin is required for myogenesis and raise the question of whether abnormal fodrin expression or polypeptides are linked to any disorders of human muscle. A second common phenotype in embryos injected with antisense α-fodrin constructs was the development of spherical embryos containing several differentiated types of tissues. This phenotype suggests that inhibition of fodrin can affect cellular signals involved in spatial information in embryos.

J 511 IDENTIFICATION OF XENOPUS PROTEINS BINDING TRANSLATIONALLY REGULATED LAMINE LI RNA IN VITRO, Marg T. Murray, Georg Krohne, and Werner W. Franke, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, Fed.Rep of Germany. In Xenopus the lamin protein family member LIII is expressed during oogenesis and in the premidblastula embryo, mRNA for Lamin LI and LII are present throughout this period but are not translated until the midblastula and gastrula stages respectively. LI RNA is present as a mRNP of density similar to previously described maternally stored mRNPs in Xenopus. We have used Lamin LI RNA transcribed in vitro in a reconstitution assay to identify RNA binding proteins which lead to particle formation and may mediate the translational repression of LI. Radiolabeled LI RNA incubated with S100 extracts from ovary, oocyte, or early embryos is exposed to UV light of 312 nm, treated with RNases A an T1, and the covalently radiolabeled proteins separated by SDS PAGE. A class of RNA binding proteins is detected who's binding is not competed by tRNA, mRNA cap analog, or poly A. Separation of the reconstituted mix by density gradient centrifugation which is either preceded or followed by the UV crosslinking steps identifies the protein components of the reconstituted RNP. The protein components of the RNP identified by this method are of approximate molecular weight 120 kda. 80 kda, 60 kda, 42 kda, and two proteins of between 50 and 56 kda. This method is being used to identify additional less abundent proteins and their expression throughout oogenesis and embryogenesis.

J 512 EXPRESSION OF TGFβ2 mRNA DURING MURINE EMBYROGENESIS, Ronald W. Pelton, Shintaro Nomura, Harold L. Moses, and Brigid L.M. Hogan, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232. The beta type transforming growth factors (TGFß's) belong to a family of polypeptides which includes the closely related proteins TGFß1, TGFß2, and TGFß3 as well as more distantly related molecules such as Müllerian Inhibiting Substance, the inhibins and activins, the Drosophilia decapentaplegic gene product, and the Xenopus Vg1 gene product. Recent evidence suggests that TGFß2, in addition to its effects as a growth inhibitor and immunosuppresent also plays a role in mesodermal induction in the Xenopus embryo. We therefore set out to investigate the role(s) that TGFß2 may play in embryogenesis in the mouse. Using the techniques of in situ hybridisation and Northern blot analysis we have studied the temporal and spatial expression of TGFß2 mRNA in embryos from 10.5 d.p.c.(days post coitum) to birth (noon of day of plug = 0.5 d.p.c.). The expression of TGFß2 mRNA varies with tissue and developmental stage in a manner consistent with a role in mesenchymal-epithelial interactions. For example, our data shows that at 15.5 d.p.c. there is little or no TGFß2 message expressed in the skin of the head and back. On day 16.5 p.c. the dermis begins to express TGFß2 mRNA and on days 17.5 and 18.5 p.c. transcripts appear throughout the epidermis while the dermal expression appears to fade. Expression is also seen in bone, tendon, and in the mesenchyme of the submaxillary gland, intestine, and esophagus (but not in the epithelial layers of these organs) as well as in the pelvis of the kidney. We are presently investigating the temporal expression of TGFß2 in these organs. In future studies we hope to further characterize the expression of TGFß2 in earlier stages of mouse development and to define the action of this protein on embryonic cell types.

J 513 THE PHOSPHODIESTERASE AND PHOSPHODIESTERASE INHIBITOR: GENES THAT FUNCTION COORDINATELY TO ALLOW AGGREGATION OF *DICTYOSTELIUM DISCOIDEUM*, G. J. Podgorski[1], L. Wu, J. Franke, M. Faure, and R. H. Kessin, Department of Anatomy and Cell Biology, College of Pysicians and Surgeons, Columbia University, New York, NY 10032, and [1]Department of Biology, Utah State University, Logan, UT 84322. Expression of the phosphodiesterase and phosphodiesterase inhibitor (inhibitor) genes is induced soon after the initiation of development in *D. discoideum*. The phosphodiesterase is required to prevent the extracellular concentration of cAMP, the chemoattractant that mediates cell aggregation, from accumulating to levels that saturate the cAMP receptor. The inhibitor is a specific secreted glycoprotein that attenuates the activity of the extracellular phosphodiesterase to sustain chemotaxis through the completion of aggregation. We have obtained genomic clones of both genes. The phosphodiesterase gene produces a non-abundant 1.8 kb constitutive transcript and a developmentally regulated 2.2 kb mRNA that is induced to high levels early in development. Both transcripts share the same two coding exons, but differ in their use of transcriptional start sites separated by 2.4 kb. The developmentally regulated mRNA contains a 0.5 kb 5'non-translated exon not found in the constitutive transcript. The inhibitor gene contains 3 exons, is transcribed into a single mRNA with little flanking non-translated sequences, and encodes a cysteine-rich (16%) protein that binds non-covalently to the phosphodiesterase. Exposure of cells to cAMP induces the mRNA of the phosphodiesterase and represses the inhibitor mRNA. Severe developmental abnormalities result if the phosphodiesterase gene is overexpressed. These experiments are described in the accompanying presentation of Faure *et al.*

J 514 A GENETIC APPROACH TO POLLEN-STIGMA INTERACTIONS, Robert E. Pruitt, Tom Horejzi, Brenda Pierskalla and Sara E. Ploense, Department of Genetics and Cell Biology, University of Minnesota, St. Paul, MN 55108-1095. The first event in the fertilization of the small weed *Arabidopsis thaliana* is the transfer of pollen from the anthers onto the surface of the stigma. Following pollination the stigma transfers water and nutrients to the pollen grains. The pollen grains hydrate, germinate and the growing pollen tubes penetrate the stigma. The transfer of water from the stigma is essential for the germination of the pollen grains and represents the first opportunity for the control of the fertilization process by cell-cell interactions. We are interested in identifying genes and their products which are essential for this first interactive process. To do this we have devised a genetic selection which allows the identification of male sterile mutations which fail to induce the stigma to transfer water to the pollen grains. Pollen from this class of mutant will presumably be deficient for some type of recognition substance needed to properly interact with the stigma. Pollen grains from the one mutant isolated to date are completely sterile in both self- and outcrosses and yet are competent to grow a normal pollen tube *in vitro*. Work is under way to isolate additional mutations of this type as well as to identify the wild-type product of the gene affected by the first mutation we have isolated.

J 515 UV CROSSLINKING OF GTP TO TUBULIN IN *XENOPUS LAEVIS* EGGS , Susan J. Roberts and John C. Gerhart, Department of Molecular Biology, University of California, Berkeley, CA 94720. The events of the first cell cycle following fertilization of amphibian eggs include a 30° rotation of the cortex of the egg relative to the yolky, deeper cytoplasm. This cytoplasmic reorganization predicts the future dorsal-ventral axis of the embryo and correlates with the presence of tracks of parallel microtubules in Xenopus and Rana eggs (Elinson & Rowning, 1988. Dev. Biol. 128: 185-197). Subcortical rotation, specification of the dorsal-ventral axis, and the formation of the microtubule array are all inhibited by agents known to depolymerize microtubules including low temperature, high pressure and microtubule destabilizing drugs. UV irradiation of the vegetal hemisphere also inhibits these processes, but the mode of action of UV light is unknown. We microinjected eggs with [α-^{32}P]-GTP prior to treatment with UV light and observed that UV crosslinks GTP to tubulin under conditions which produce axis-deficient embryos. There is one prominant band incorporating [α-^{32}P]-GTP after UV treatment as determined by autoradiography of egg extracts resolved by SDS-PAGE. This labeled band is immunoprecipitated by anti-tubulin monoclonal antibodies. A similar exposure to UV of partially purified bovine brain tubulin in the presence of [α-^{32}P]-GTP indicates that [α-^{32}P]-GTP becomes crosslinked to the beta subunit of tubulin. The beta subunit of tubulin contains the exchangeable GTP binding site. Previous studies have shown that tubulin containing UV crosslinked GTP fails to polymerize (Nath, Eagle & Himes, 1985. Biochem. 24: 1555-1560). Microinjection of UV treated tubulin will be used to determine if crosslinking of GTP to tubulin is sufficient for inhibition of the subcortical rotation.

J 516 EXPRESSION OF A PROTEIN HOMOLOGOUS TO A MAMMALIAN LAMININ RECEPTOR DURING OOGENESIS AND EARLY DEVELOPMENT IN THE EUCHUROID *URECHIS CAUPO*. Eric T. Rosenthal and Steven C. Benson, Pacific Biomedical Research Center, Kewalo Marine Laboratory, Honolulu, HI 98613

We have identified a maternal messenger RNA in the oocytes and early embryos of the euchuroid worm *Urechis caupo* which encodes a protein sharing extensive homology with laminin receptors from mouse and humans. The *Urechis* protein has a MW of 34,000. There is an unusual alanine rich region at the carboxyl terminus end. The maternal mRNA encoding the protein is transcribed and translated in growing oocytes. Immediately after fertilization the mRNA is removed from polysomes. The concentration of the mRNA declines during the first five hours of development and then begins to increase, presumably due to new transcription in the embryos. In the adult animal the mRNA can be detected in ceolomocytes, and cells of the gut and body wall. Using antibodies and laminin affinity columns we have investigated the accumulation, stability and distribution of the protein in oocytes and developing embryos.

J 517 GENE EXPRESSION DURING LENS AND EYE FORMATION, Margaret Saha, Jonathan Henry, and Robert Grainger, Department of Biology, University of Virginia, Charlottesville, VA 22901. Lens induction is a frequently cited prototype of how cell determination occurs in vertebrate embryos. Extensive transplant and explant experiments on Xenopus laevis performed in our laboratory have raised serious concerns regarding the classic view of the optic vesicle inducing the lens in a single step, and have led to a new model in which lens determination is seen as a multistep process closely linked to neural induction, particularly that of the eye. The detail with which the biology of this particular system is known renders it uniquely well suited for systematic analysis at the molecular level. It is known, for example, that head ectoderm from mid-neurula stages possesses not only the potential of lens formation when placed with the optic vesicle but also the ability to self-differentiate when cultured as an explant; ectoderm from the belly region possesses no such capability. Comparision of protein synthesis patterns on two-dimensional gels indicate that any differences in gene expression are likely to be of a subtle nature. We have, therefore, constructed an enriched cDNA library from dissected pieces of presumptive lens ectoderm and optic anlage and are using selected probes made from head ectoderm and trunk ectoderm to perform a differential screen. In addition, we are in the process of identifying markers which characterize the eye lineage in an effort to unravel the link between neural (specifically eye) induction and lens determination.

J 518 CYTOKERATIN REORGANIZATION AND FUNCTION IN THE DEVELOPING *XENOPUS* EMBRYO: A POSSIBLE ROLE FOR CYTOKERATINS IN GASTRULATION. Dave Shook, Laurie Maynell, Philip Sander, Dan Chu & Mike Klymkowsky, Molecular, Cellular & Developmental Biology. University of Colorado, Boulder. 80309-0347. Based on studies of cultured cells, it appears that the functions of intermediate filaments are primarily non-cell autonomous, that is, that intermediate filaments function at the level of tissues rather than at the level of the single cell. To characterize these functions, we have turned to the developing *Xenopus* embryo. The major morphogenic event of early development, gastrulation, occurs within 10 hours of fertilization in *Xenopus* and involves the controlled movement of epithelial tissues. Cytokeratin-type intermediate filaments form a major epithelial-specific cytoskeletal system. In the late stage *Xenopus* oocyte, cytokeratin filaments are organized in an asymmetric, animal-vegetal network. During both progesterone- and MPF-induced oocyte maturation, cytokeratin organization breaks down. This breakdown appears to be due to the post-translational modification of the cytokeratin proteins. Reassembly of cytokeratin filaments is initiated by fertilization or activation and again appears to be controlled on the post-translational level. The cortical cytokeratin system of the embryo remains asymmetric through gastrulation and its asymmetry correlates with the pattern of morphogenic movement that drives gastrulation. The assembly of the asymmetric cytokeratin filament system is independent of microtubules, but is affected by the Ca^{++} ionophore A23187, suggesting that a gradient in intracellular Ca^{++} plays a role in the asymmetric behavior of the early embryo. To study the functional roles of cytokeratin filaments in the early embryo, we have tested four anticytokeratin antibodies, *AE1, AE3, 1h5* and *antiIFA*, for their effects on development. Of these, *AE3* and *antiIFA* induce specific defects in gastrulation. These defects appear to be due to the generation of insufficient "hoop stress" around the blastopore lip during gastrulation.

J 519 THE EFFECTS OF CELL-CELL DISSOCIATION ON TISSUE -SPECIFIC GENE EXPRESSION IN SEA URCHIN EMBRYOS, Laurie Stephens, Takashi Kitajima and Fred Wilt, Department of Zoology, University of California, Berkeley, CA 94708. The effects of disrupting cell interactions in sea urchin development was investigated by examining the accumulation of a primary mesenchyme specific transcript (SM50) and an ectoderm-specific transcript (Spec 1) in cultures of dissociated sea urchin embryos. SM50 expression is temporally correct and remains restricted to the appropriate cell types, even if the embryo is dissociated as early as the two cell stage and maintained as a suspension of single cells. This strongly suggests that the primary mesenchyme lineage is capable of autonomous differentiation in the absence of cell interactions. Spec 1, however, shows only low levels of expression in otherwise healthy cell cultures, suggesting that expression of this ectodermal character may be positively influenced by cell interactions. This work was supported by an NIH grant, HD105043.

J 520 THE LATERAL BOUNDARY OF THE ORGANIZER IN *XENOPUS LAEVIS*, Ron Stewart and John Gerhart, University of California, Berkeley, CA 94720.
The dorsal marginal zone of the early gastrula *Xenopus* embryo is referred to as the organizer because it can organize a complete set of dorsal axial structures when transplanted into the ventral side of another embryo or into a uv-irradiated embryo. (Uv-irradiated embryos have no organizer and no endogenous capacity for the formation of dorsal axial structures). We have used transplantation experiments combining normal and uv-irradiated half embryos to delimit the lateral boundary of the organizer. Normal stage 9 embryos (late blastulae) are cut along the animal-vegetal axis at varying degrees away from the dorsal midline. These halves are then combined with halves from uv-irradiated embryos that have been cut through the animal-vegetal axis. The resulting recombined embryos are incubated for 2-3 days at which time they are scored for dorsal axial structures. We observe that dorsal axial structures such as eye, otolith, and notochord form only if the normal half contains tissue that is within 30 degrees of the dorsal midline. We conclude that the organizer is less than 60 degrees wide centered on the dorsal midline. Fate mapping experiments have shown that the presumptive notochord is at least 90 degrees wide centered on the dorsal midline. This suggests that lateral regions of the presumptive notochord are not autonomous for the formation of notochord at stage 9. Lineage labelling experiments have shown that notochord can be induced in uv-irradiated tissue in stage 9 lateral half-uv half recombinants. We are investigating the possibility that a similar post-stage 9 induction may be important for the formation of notochord in lateral regions of the presumptive notochord during normal *Xenopus* development.

J 521 PROGRESS IN CLONING THE *C. ELEGANS* MATERNAL EFFECT GENE, PAR-1, Abby Telfer, Ugo Giambarella and Dan Stinchcomb, Dept. of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138
par-1 is a maternally expressed gene of *Caenorhabditis elegans* whose product is required for the correct sorting and movement of cytoplasmic components during early embryogenesis. Some of the defects seen in embryos produced by homozygous *par-1* mothers include a failure to localize germ line-specific P granules to the posterior pole at or before first cleavage, improper spindle movements prior to first and second cleavage, incorrect timing of the early divisions and incorrect differentiation (Kempheus *et al.*, 1988, Cell,52, 311-320) We are cloning *par-1* by chromosome walking from cloned transposable elements located 0.1 map units to the left and 0.5 map units to the right of the gene. With the assistance of A. Coulson and J. Sulston (MRC Cambridge), we have identified overlapping cosmid and YAC clones that connect the flanking sequences. These cover approximately 600 kb. Using cosmid clones as probes, we have identified and mapped several restriction fragment length polymorphisms in the *par-1* region. As we position these RFLPs on our physical map we can narrow our search to a subset of the cloned DNA. We hope to be able to define the locus by rescuing mutant animals with microinjected DNA.

J 522 ANALYSIS OF A GASTRULATION-SPECIFIC GENE, *folded gastrulation*, Ellen T. Wilson, Dari K. Sweeton and Eric Wieschaus, Biology Department, Princeton University, Princeton, NJ 08544-1003. The development of a multilayered embryo depends on the correct completion of *gastrulation*, a precise process of programmed cell shape changes, invaginations and cell movements. In *Drosophila*, comprehensive mutational analyses indicate that few zygotically-active genes are specific to the gastrulation process. Analysis of one of these, *folded gastrulation*, (*fog*), shows that lack of *fog* product causes a specific and obvious early defect. A prominent invagination, the posterior midgut, is not formed. Mosaic studies have shown that the *fog* product is needed only during early embryogenesis. These studies have also shown that *fog* is required in the same cells where phenotypic defects first occur.

We will report comprehensive morphological studies that have more carefully specified defects due to *fog* mutation. These studies have utilized light microscope analysis of stained whole mount embryos, quantitative videotape analysis, definition of early mitotic patterns, and studies of sectioned material.

We will also report progress toward molecular cloning of the *fog* gene, located at 20B in the proximal heterochromatin of the X-chromosome. A molecular entry point to *fog* has been identified utilizing a microdissection library of recombinant clones (G.L.G. Miklos, A.N.U., Canberra). A chromosomal walk (105 kb) initiated from this site crosses a deficiency breakpoint that defines the proximal limit of the *folded gastrulation* gene.