SIGNAL TRANSDUCTION AND GENE ACTIVATION IN DEVELOPMENT

Organizers: Richard Firtel, Judith Kimble and M. Geoffrey Rosenfeld
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Signal Transduction and Gene Activation in Development

Signal Processes Regulating Cell Function

P 001 REGULATION OF THE CELL CYCLE BY CDC2 AND ITS ASSOCIATED CYCLINS, David Beach and Giulio Draetta, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.
In fission yeast, the *cdc*2 protein kinase is required for entry into S phase and M phase. In human cells, forms of *cdc*2 that are active in G_1/S and G_2/M have been identified and biochemically purified. Cell cycle specificity is conferred on the protein kinase by its association with a class of proteins called cyclins. Substrates of G_1/S and G_2/M *cdc*2 are described.

P 002 **GENETIC CONTROL OF cAMP LEVELS IN MAMMALIAN CELLS BY INTRODUCTION OF A LOW K_m cAMP-PHOSPHODIESTERASE GENE.** R.H. Kessin[1], M. M. Van Lookeren Campagne[1], M.M. Gottesman[2], R.D. Fleischmann[2], and S.J. Orlow[3]. [1]Department of Anatomy and Cell Biology, Columbia University, 630 West 168th St. New York, New York 10032, USA; [2]Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD. 20892, and [3]Department of Dermatology, Yale University School of Medicine, New Haven, CN. 06510. The role of cyclic nucleotides in the differentiation of a variety of cells has long been known. Most approaches to investigate the dependence of developmental sequences have relied on pharmacological methods, which in some experiments suffer from lack of specificity. To control the activation of the cAMP second messenger cascade in a genetic way that will allow us to examine which hormones or growth factors require an activation of adenylate cyclase for their physiological effects and which do not, we have developed a method by which the cAMP created after activation of adenylate cyclase is rapidly degraded. The cAMP-dependent protein kinase remains intact and can still be stimulated with appropriate cAMP analogues. The yeast low K_m cAMP-phosphodiesterase gene (SRA5 or PDE2) has been introduced into Chinese hamster ovary (CHO) cells and Cloudman melanoma cells. In both cases the gene is expressed and gives rise to functional enzyme which is specific for cAMP and has an exceptionally low K_m (170nM). CHO cells carrying the gene become resistant to the growth inhibitory effects of cholera toxin, which drives cAMP levels up about 35 fold in control cells, but not in cells carrying multiple copies of the yeast cAMP-phosphodiesterase gene. The transient cAMP elevation that occurs in response to Prostaglandin E1 is also reduced in CHO cells. In Cloudman melanoma cells, melanocyte stimulating hormone (MSH) causes a transient rise in cAMP levels which purportedly lead to a cessation of growth and induction of pigmentation. Introduction of the yeast cAMP-phosphodiesterase gene blocks the rise in cAMP caused by 20 nM MSH. Under these conditions, growth arrest by MSH is less efficient, but in contrast to existing assumptions, melanogenesis is stimulated. Cells carrying the yeast low Km cAMP-phosphodiesterase gene can be studied *in vivo*, where the presence of the yeast cAMP-phosphodiesterase gene causes the formation of melanotic (pigmenting) tumors, in contrast to the amelanotic tumors that are formed by control transfected Cloudman melanoma cells. The effects of expressing the yeast cAMP-phosphodiesterase gene can be bypassed *in vitro* by applying cell permeant cAMP analogues such as N6,O2'-dibutyryl-cAMP or Sp-8-Br-cAMPS, which are not hydrolysed by the yeast enzyme, but which activate the cAMP-dependent protein kinase.

P 003 THE *PERIOD* GENE AND BIOLOGICAL RHYTHS, Michael Rosbash[1,2], Hildur V. Colot[1,2], Mitchell S. Dushay[2], Isaac Edery[1,2], John Ewer[2], Melanie Hamblen-Coyle[2], Paul E. Hardin[1,2], Xin Liu[1,2], Lori J. Lorenz[1,2], Joan E. Rutila[1,2], David Wheeler[2], Laurence J. Zwiebel[1,2] and Jeffrey C. Hall[2], [1]Howard Hughes Medical Institute and [2]Department of Biology, Brandeis University, Waltham, MA 02254

The *period* gene from *Drosophila melanogaster* has been shown to influence circadian and ultradian rhythms. Recent studies have shown that immunohistochemical staining of the *per* protein in fly heads undergoes circadian cycling. Moreover, *per* RNA levels also cycle. As this cycling is influenced by mutants in the *per* protein, *per* RNA levels must be feedback regulated by its own product. The nature of this feedback regulation and its role in generating circadian rhythms of behavior will be discussed. More generally, an updated account of our behavioral studies will be presented.

Abstract Withdrawn

Regulation of Cell Fate-I

P 005 INTERCELLULAR INTERACTION AND THE MOLECULAR PROCESS BY WHICH OCCURS LINEAGE-SPECIFIC GENE EXPRESSION IN THE EARLY 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 11 specific cleavage stage founder cells. However, the egg is initially radially symmetric, and interblastomere interaction as well as an early cytoplasmic polarization are probably required for aboral ectoderm specification. Regulatory sequences of the CyIIIa gene have now been shown to accurately direct spatial and temporal expression of exogenous 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 20 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. 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, cloned, and sequenced, and the developmental appearance of the respective mRNAs determined. In some cases these are maternal, though the regulatory factors so far characterized are all expressed zygotically. A theoretical interpretation of sea urchin embryogenesis integrating classical and molecular evidence will be presented.

P 006 REGULATION OF INTERCELLULAR SIGNALING IN NEURAL AND EPIDERMAL CELL PATTERNING IN *C. elegans*., Cynthia Kenyon, David Waring, and Steve Salser. UCSF Department of Biochemistry, UCSF, San Francisco, CA 94143-0554.

The inhibition of intercellular interactions plays an important role in neural and epidermal pattern formation in *C. elegans*. In wild type animals, anterior epidermal cells generate cuticular structures, whereas posterior epidermal cells instead generate neural sensilla. The cuticular structures formed by anterior cells are induced by intercellular signals; in contrast, posterior sensilla are formed because a gene called *pal-1* specifically prevents intercellular communication. Mosaic analysis indicates that *pal-1* functions on the receiving end of the signalling pathway to inhibit or override signal transduction. A 14 kb DNA sequence with *pal-1* rescuing activity contains a homeobox, suggesting that *pal-1* may influence transcription. Genetic analysis suggests that (directly or indirectly) *pal-1* regulates the gene *mab-5*. The *mab-5* gene, in turn, contains an *Antennapedia*-like homeobox, and is required for many cell types located in the posterior body region to adopt posterior-specific fates.

P 007 ***C. elegans*** **VULVAL INDUCTION**, Paul Sternberg, Min Han, Raffi Aroian, Jane Mendel, Russell Hill, Gregg Jongeward, Helen Chamberlin, Linda Huang, Paul Kayne, Lynn Carta, Phoebe Tzou, Wendy Katz, and Hiro Mori, Howard Hughes Medical Institute, Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125. During induction of the *C. elegans* hermaphrodite vulva, the combined action of an inductive signal and a short-range lateral signal specifies the fates of six multipotent vulval precursor cells [the VPCs]. VPCs can adopt one of three fates: 1° and 2°, which generate vulval tissue, and 3°, which generates nonspecialized epidermis. To understand how intercellular signals specify the VPC fates, we are trying to identify, by genetic analysis and molecular cloning, the genes that are involved in the production of the signals, the response to these signals, and the differentiated properties of the vulval cell types. The action of both an inductive signal from the anchor cell and the products of the Vulvaless genes [defined by Vulvaless mutations in which no vulva is induced: *lin-3, let-23, let-60, etc.*] are necessary to induce the VPCs to become 1° or 2°. The action of the Multivulva genes [defined by Multivulva mutations in which all VPCs become 1° or 2°: *lin-15, lin-1, etc.*] prevents VPCs from becoming 1° or 2° in the absence of inductive signal. *lin-18* appears to act specifically in the 2° VPCs, and thus is a candidate for a gene that is regulated by the intercellular signals. Genetic studies have helped determine the actions and interactions of these gene products. In particular, the level of activity of the *let-60* locus specifies whether a VPC has a vulval [1° or 2°] versus a nonvulval [3°] fate. The activities of *lin-3* and *let-23* are necessary to activate *let-60*, while the activity of *lin-15* and related genes prevent inappropriate *let-60* activity. In addition, extragenic suppressors of *let-23* mutations define several new loci involved in vulval induction [*unc-101, sli-1* and *sli-2*]. Our progress in the molecular cloning of *lin-3, let-23, let-60, lin-15, unc-101* and *lin-18* will be discussed. For example, we have obtained a cosmid that complements the mutant phenotypes of *let-23* when microinjected into *let-23* gonads to create transgenic animals; taken with physical/genetic mapping data, this cosmid likely contains the *let-23* gene. Most of the genes required for vulval induction are required for other aspects of *C. elegans* development. For example, *let-23, let-60, lin-15* and *lin-1* are needed for the correct specification of cell types during development of the male tail; *unc-101* is involved in several other aspects of neural development or function. The multiple functions of these genes are being analyzed by genetic complementation analysis and the isolation of extragenic suppressor mutations.

Nuclear Factors Regulated by Signaling Pathways

P 008 **POU-Domain Gene Products in Development of Cellular Phenotypes in Endocrine and Neural Tissues,** Michael G. Rosenfeld, Xi He, Jeffrey Voss, Maurice Treacy, Vivian Albert, Bryan Crenshaw, Larry Swanson, & Holly Ingraham, Eukaryotic Regulatory Biology Program, Howard Hughes Medical Institute, & School of Medicine, M-013, University of California, San Diego, La Jolla, CA 92093.

The molecular strategies utilized to activate gene expression in specific cell types within an organ have been investigated using anterior pituitary cells as a model. The evolutionarily highly-related growth hormone and prolactin genes are expressed in distinct cell types, referred to as somatotrophs and lactotrophs, respectively. Tissue-specific *cis*-active elements in the growth hormone and prolactin promoters bind to several proteins, including a tissue-specific transcription factor, Pit-1, that contains a POU-domain. Pit-1 can activate transcription of both *rat* prolactin and *rat* growth hormone promoters, even at levels 10-fold lower levels of expression than in *rat* pituitary cells. This data is in contradistinction to reported data comparing *human* growth hormone and an incomplete *rat* prolactin promoter excluding nucleotides beyond -9 in rat pituitary cells, which are, based on the nature of the experimental design, not relevant to the biology of the system.

Analysis of the ontogeny of Pit-1 gene expression reveals it to be expressed in all anterior pituitary cells on e16, but with a lag of detectable protein expression until the time of initial activation of prolactin and growth hormone gene expression. In the mature pituitary, Pit-1 transcripts are present in high concentrations in all cell types, but Pit-1 protein is detectably expressed in only three cell types, apparently reflecting regulation at the level of mRNA translation. Prolactin and growth hormone gene expression in distinct cell types therefore appear to reflect the actions of further restrictive and activating factors in addition to Pit-1. The Pit-1 gene itself exhibits a concentration-dependent pattern of positive and negative autoregulation.

The POU-domain contains a unique region, the 76 aa POU-specific domain that is necessary for high affinity, site-specific binding, and cooperative protein interactions; the POU-homeodomain is necessary for DNA binding, although it alone is capable of only low affinity binding (100-fold lower than with a POU domain) and with highly relaxed intersite specificity. The major transactivation domains reside outside the POU-specific domain.

We have identified a large number of POU-domain genes that are expressed during neurogenesis in mammals and in *Drosophila*. These genes exhibit distinct temporal and spatial patterns of expression, consistent with potential functions in activation of gene transcription. *Cis*-active elements to which several of the brain-specific POU-domain proteins bind have been identified within promoters of genes encoding several different classes of neural proteins. Co-transfection analyses have documented the ability of the POU-domain genes to regulate transcription of these promoters in heterologous cell types. We speculate that the POU-domain gene family exerts important developmental functions in determining neuronal phenotypes in the forebrain of higher eukaryotes.

P 009 TRANSCRIPTIONAL REGULATION BY THE FOS AND JUN ONCOGENE PRODUCTS, Richard Turner, Vijay Baichwal, Karen Perkins & Robert Tjian, Howard Hughes Medical Institute, Department of Biochemistry, University of California, Berkeley, CA 94720

Transcription factor AP-1 consists of a family of proteins which are capable of binding to the enhancer element TGACTCA and activating transcription of contiguous genes. These proteins include the products of the *c-jun* and *c-fos* oncogenes, which form a complex in the nucleus, and similar polypeptides such as Jun B. Interestingly, homologs of both cJun and cFos which function as transcription factors have also been identified in *D. melanogaster*.

The AP-1 DNA binding site imparts inducibility by serum and by TPA on promoters which contain it. Also, various oncogene products which are implicated in cellular signal transduction processes have been shown to activate AP-1 site-containing reporter genes in co-transfection experiments. It therefore appears that one or more components of the AP-1 system are subject to post-translational regulation of their activity in response to extracellular stimuli. We are studying phosphorylation of AP-1 proteins in mammalian cells and in *Drosophila* in an attempt to characterize these regulatory event(s) and to reconcile them with the oncogenic potential of the cJun and cFos proteins.

P 010 FOS-JUN INTERACTION: A PARADIGM FOR TRANSCRIPTIONAL REGULATION, Inder M. Verma, Lynn J. Ransone, Jane Visvader, and V.J. Dwarki, Molecular Biology & Virology Laboratory, The Salk Institute, La Jolla, CA 92037

Gene expression is modulated by the specific interaction of nuclear proteins with unique regulatory sequences in the genome. Nuclear oncoproteins fos and jun (AP-1) cooperate in forming a very stable heterodimeric complex that binds to the AP-1 site with increased affinity. The "leucine zipper" domain of both fos and jun is necessary for the formation of this heterodimer. We have undertaken a mutational analysis of (1) individual leucine residues, (2) neighboring amino acids within the "leucine zipper" domain, and (3) residues in the highly basic domain of both the fos and jun proteins, to examine the contribution of these amino acids to the formation of a stable fos/jun heterodimer, the formation of jun homodimers and the binding potential of the heterodimeric complex to the AP-1 site. Mutations of single residues within the "leucine zipper" domain had no effect on protein complex formation. However, mutagenesis of the first leucine of the heptad repeat in either fos or jun drastically reduced binding of the complex to DNA. Mutations within the fos and jun basic regions and alteration of the spacing between the basic and "leucine zipper" domains indicate that the basic region of fos has a crucial role in determining the DNA binding affinity of the transcriptional complex. This is further substantiated by making site specific mutations in the basic amino acids in fos protein. We have also generated fos-jun chimeras to access the role of "leucine zipper" in the formation of homodimer. Finally, we will also discuss the role of fos/jun complex in transcriptional transactivation.

Regulation of Cell Function via Cell Surface Receptors-I

P 011 SIGNAL TRANSDUCTION PROCESSES CONTROLLING GENE EXPRESSION AND CELLULAR DIFFERENTIATION IN *DICTYOSTELIUM*, Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, Univ. of California, San Diego, La Jolla, CA 92093

The formation of multicellular aggregate and subsequent differentiation in *Dictyostelium* is controlled by a number of extracellular factors, including cAMP. Aggregation is controlled chemotactically through a developmentally regulated cAMP cell surface receptor that activates an intracellular signal transduction pathway regulated by a G protein system. The α subunit of this G protein ($G_\alpha 2$) is also developmentally regulated. In addition to controlling aggregation, the same pathway controls the cAMP relay system that involves the activation of adenylate cyclase and the secretion of cAMP as well as regulating a number of genes that are essential for the aggregation process. Later in development, in the multicellular stages, cAMP mediated signal transduction processes also induce prestalk and prespore genes that are differentially regulated in a cell-type-specific manner. Cells expressing these markers are spatially localized within the migrating slug.

In order to better understand receptor mediated signal transduction processes controlling *Dictyostelium* development, our laboratory, in association with that of P. Devreotes, has cloned and analyzed the expression and function of four G_α protein subunits in *Dictyostelium*. $G_\alpha 1$ is expressed in vegetative cells and during early development. Disruption of the gene has no visible or developmental phenotype; however, overexpression of the gene product results in large, multinucleated vegetative cells that have abnormal development. Disruption of $G_\alpha 2$ blocks all known cAMP receptor mediated developmental events including chemotaxis, cAMP induction of gene expression, and cAMP relay. Appropriate constructs expressing $G_\alpha 2$, but not $G_\alpha 1$, complement $G_\alpha 2$ gene disruptions. $G_\alpha 4$ and $G_\alpha 5$ are preferentially expressed in the multicellular stages during later development. Genetic analysis of both of these G proteins and their effect on developmental events is being investigated.

To better understand the functioning of the G proteins controlling developmental events, specific missense mutations within the $G_\alpha 1$ and the $G_\alpha 2$ proteins have been made in a number of the highly conserved G_α protein domains, including those effected GTP binding and GTPase activity. These mutations are being expressed in wild-type cells as well as cells carrying $G_\alpha 1$ or $G_\alpha 2$ gene disruptions in order to understand the dominant and recessive phenotypes of these mutations and their effect on controlling *Dictyostelium* development. The relationship between the signal transduction processes and cellular differentiation in *Dictyostelium* will be discussed.

P 012 INOSITOL LIPIDS AND PHOSPHATES IN DIFFERENTIATING HL60 CELLS
Robert H Michell, Philip J French, Judith A Creba, Christopher M Bunce, Janet M Lord, Michael A Baxter and Geoffrey Brown, School of Biochemistry and Departments of Immunology and Medicine, University of Birmingham, P O Box 363, Birmingham B15 2TT, UK.

Although there is now clear evidence that Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 play central roles in the regulation of cytosolic [Ca^{2+}] in stimulated cells, there is as yet no good explanation for the presence in mammalian cells at much higher concentrations (generally between 1μM and 100 μM) of several other inositol polyphosphates, notably Ins(3,4,5,6)P_4, Ins(1,3,4,5,6)P_5 and InsP_6. We have been examining the behaviour of these and other inositol metabolites in HL60 cells, a human promyelocytic cell line capable of differentiating towards either neutrophils or monocytes in response to appropriate stimuli, with the following results. 1. Inositol entry is carrier-mediated, with a K_m of approximately 50 μM, and inositol accumulates to a much higher concentration than in the extracellular medium. Its rate of entry is much higher in growing HL60 cells than in mature circulating neutrophils, and during neutrophil differentiation of HL60 cells the rate of inositol entry initially rises and then falls dramatically during the later stages of differentiation. 2. InsP_5 and InsP_6 are present at relatively high concentrations in undifferentiated cells, and they turn over relatively slowly. The concentration of InsP_5 rises substantially during neutrophil differentiation (in response either to DMSO or to a combination of retinoic acid and a polypeptide differentiation factor). 3. By contrast, the concentrations of Ins(3,4,5,6)P_4, InsP_5 and InsP_6 all decline during monocyte differentiation in response to TPA.

Regulation of Cell Function via Cell Surface Receptors-II

P 013 RECEPTORS THAT CONTROL CHOLESTEROL, Brown, M.S. and Goldstein, J.L., Department of Molecular Genetics, University of Texas Southwestern Medical School, Dallas, TX 75209. The low density lipoprotein (LDL) receptor binds plasma lipoproteins that contain apo B-100 or apo E and transports them into cells in coated pits. The ligand binding domain consists of seven cysteine rich repeats, a multiplicity that is often found in surface receptors. To understand the necessity for multiple repeats we have systematically made substitution and deletion mutations in each repeat and measured the ability of the receptor to bind lipoproteins that contain either of its two ligands, apo B-100 or apo E. The results show that apo B-100 binding requires that repeats 3 through 7 are all intact. Apo E binding requires only repeat five. Thus, multiple cysteine rich repeats allow receptors to bind multiple ligands through combinatorial diversity. Through mutagenesis studies we also have found that the internalization of the LDL receptor in coated pits requires the sequence NPXY (where X is any amino acid) in the cytoplasmic domain. We note that NPXY sequences are found in cytoplasmic domains of several, but not all, receptors that move to coated pits. The implications of these findings for receptor biology will be discussed.

P 014 ANALYSIS OF LIGAND BINDING TO G-PROTEIN COUPLED RECEPTORS THROUGH THE USE OF ENGINEERED RECEPTOR PROTEINS, Richard A.F. Dixon and Catherine D. Strader, Departments of Molecular Biology and Biochemistry, Merck, Sharp and Dohme Research Laboratories, West Point, PA 19486 and Rahway, NJ 07065.
The cloning of many small molecule and peptide hormones receptors which couple to G-proteins have revealed that these receptors have common structural features including the presence of seven putative transmembrane segments. A combination of genetic and biochemical studies have demonstrated that the ligand binding site of the beta-adrenergic receptor (BAR) for small molecule agonists and antagonists is contained within these transmembrane domains. Site directed mutagenesis studies of the BAR coupled with structure-activity studies of novel ligands have allowed the identification of several residues within the BAR which are important for ligand recognition and binding. In addition, hybrid receptor proteins have been utilized to identify regions of the receptor important for subtype determination. These studies have led to the construction of a model for the ligand binding site of the BAR.

Control of Cell Differentiation via Signaling Systems

P 015 SIGNAL-TRANSDUCING PROTEIN KINASES AND THEIR TARGETS, Tony Hunter, Bill Boyle, Rick Lindberg, Detlev Jaehner, Dave Middlemas, and John Pines, Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138

Protein phosphorylation is a major mechanism whereby signals are transduced from external stimuli into cellular responses. Many growth factor receptors are ligand-activated protein-tyrosine kinases (PTK), while other PTKs, such as $pp60^{c\text{-}src}$, located on the inside of the plasma membrane may also be involved in signal transduction. We have identified novel PTKs by screening cDNA libraries with oligonucleotide probes to consensus sequences. In this way we have isolated 2 novel receptor-like PTKs. One of these, *eck*, is predominantly expressed in tissues containing proliferating epithelial cells (skin, lung and intestine). The predicted *eck* protein, has 976 residues, and is closely related to the *eph* and *elk* receptor-like PTKs. Antibodies raised against a TrpE-*eck* fusion protein immunoprecipitate a 125 kDa protein from epithelial cell lines. This protein is phosphorylated on tyrosine in an immune complex kinase assay, indicating that the *eck* protein is a PTK. A second putative PTK, *trk*-B, was isolated from a rat cerebellar library. The predicted *trk*-B protein has 810 amino acids, and it is closely related to but distinct from the *trk* receptor-like PTK. *trk*-B is primarily expressed in brain, as a set of RNAs ranging from 13 to 0. 8 kb. The smaller RNAs are too short to encode the intact protein. Analysis of additional *trk*-B cDNAs indicates that there are mRNAs encoding a protein truncated just downstream of the transmembrane domain, with a short novel C terminus. We are investigating the distribution of the two types of mRNA in the brain, and trying to identify their protein products and determine if the full length protein has PTK activity.

Cyclin, is a highly conserved cell cycle-regulated protein that is required in early embryos for entry into mitosis. We have isolated cDNA clones for human cyclin A and B. The mRNA levels of both cyclins vary during the cell cycle, being highest in G2. For cyclin B, this increase is due to an elevated rate of transcription in G2. Using antibodies against cyclin B we find that the level of cyclin B fluctuates during the cell cycle, being high in G2 and M, and falling rapidly as cells enter anaphase. Immunofluorescence staining shows that cyclin B accumulates in the cytoplasm during G2, enters the nucleus at prophase, and is abruptly destroyed at anaphase. During G2 cyclin B associates with the hyperphosphorylated form of the cell cycle regulatory PK, cdc2, and these complexes acquire histone H1 kinase activity late in G2.

To determine how PKs activated at the cell surface induce nuclear events, we are examining the phosphorylation of nuclear regulatory proteins, which could be targets for a surface-initiated PK cascade. The transcription factor c-*Jun*, which associates with c-*Fos*, is a phosphoprotein. c-*Jun* has 4 major sites of phosphorylation. Three of these, which show decreased phosphorylation following TPA treatment, are clustered just upstream of the DNA binding domain. In vitro these sites are phosphorylated by glycogen synthase kinase 3. GSK3 phosphorylation of bacterially-expressed c-*Jun* protein decreases its ability to bind to a TRE. We propose that c-*Jun* function is negatively regulated by phosphorylation, and that TPA activation of transcription from TRE-dependent genes may in part involve dephosphorylation of c-*Jun*. We have isolated 10 cDNAs for mRNAs whose synthesis is rapidly increased following shift of ts $pp60^{v\text{-}src}$-infected NRK cells. These include sequences shown by others to be mitogen-inducible (e.g. c-*fos*, KC, ODC, *Egr*-1). Another $pp60^{v\text{-}src}$-inducible mRNA is *rho*-A. We have shown that the *rho*-A gene is induced by $pp60^{v\text{-}src}$ and by certain mitogens at the transcriptional level.

P 016 MULTIPLE RECEPTORS AND SECOND MESSENGERS REGULATE THE DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM, Alan R. Kimmel and Charles L. Saxe III, Laboratory of Cellular and Developmental Biology, NIDDK, National Institutes of Health, Bethesda, MD 20892.

The early stages of the Dictyostelium developmental cycle are characterized by the transition from a unicellular, growth phase to a complex multicellular aggregate. Secreted cAMP serves as a chemoattractant to direct aggregation and as a hormone-like molecule to modulate gene expression by transmembrane signalling. Secreted cAMP is also associated with cytodifferentiation and morphogenetic movement. Cells within aggregates differentiate and become organized in a defined pattern. Receptors specific for cAMP are present on the surface of Dictyostelium cells throughout the developmental cycle. The activation of the surface receptors by extracellular cAMP promotes the accumulation of a series of intracellular messengers including cAMP, cGMP, Ca^{++} and IP_3. We have isolated members of the multigene family from Dictyostelium which encode cAMP receptors. Each receptor form has unique structural properties and exhibits a specific pattern of developmentally regulated gene expression. Data derived from transformation studies further indicate an association of each receptor with a distinct intracellular signalling system. In addition, we have begun to decribe how the various intracellular messengers interact to regulate gene expression during development. Cyclic AMP, IP_3 and diacylglycerol can act independently and synergistically, transducing events at the cell surface into signals which regulate transcription.

P 017 SIGNAL TRANSDUCTION AND ADAPTATION IN THE YEAST MATING PHEROMONE RESPONSE, Steven I. Reed, Gary M. Cole, Miguel de Barros Lopes and David E. Stone, Department of Molecular Biology, MB-7, Research Institute of Scripps Clinic, 10666 North Torrey Pines Road, La Jolla, CA 92037

In budding yeast, conjugation is initiated by the response of haploid cells to small peptide hormones known as mating pheromones. A trimeric G-protein couples the mating pheromone receptor to an as yet unidentified effector pathway. Ultimately, the response is mediated by the induction of specific nuclear transcripts which encode proteins required for conjugation. The pheromone response has two temporally distinct components: an initial response phase and a subsequent adaptive phase, where cells become desensitized to mating pheromone. This program presumably allows cells which have not conjugated to resume normal growth.

Mutational analysis of the transducing G protein subunits as well as studies of subunit stoichiometry have determined that that the $\beta\gamma$ subunit, encoded by the genes *STE4* and *STE18*, is responsible for the initial response phase of pheromone action while the α subunit has a primary role in sequestering β/γ in the absence of pheromone. In order to further investigate the role of the guanyl nuceotide binding α subunit, a structure/function study was initiated. The gene encoding this subunit, *GPA1*, was subjected to random *in vitro* mutagenesis and mutations which uncoupled the pheromone response were screened. Two classes of mutations were found: those that blocked the signal directly based on interactions with the receptor or with the $\beta\gamma$ subunit or those that induced a constitutive adaptive response. Analysis of the latter class of mutations suggests that the adaptive response is mediated by the activated α subunit of the G protein through a second effector pathway. One probable target of the adaptive response is the β subunit itself. The β subunit is rapidly and completely phosphorylated in response to mating pheromone. Deletion mutations which eliminate the target of phosphorylation on the β subunit do not interfere with the initial pheromone response but inhibit the adaptive response. Thus adaption appears to be mediated, at least in part, by establishment of a negative feedback loop involving the signal transducer itself. Genetic analysis has identified other elements which most likely interact with the G protein and modulate G protein functions. Mutations in the genes *CDC36* and *CDC39* cause constitutive activation of the pheromone response at the level of the G protein. Genetic evidence is consistent with the proteins encoded by these genes having a role in stimulating the intrinsic GTPase of the α subunit or in promoting α/β association. *In vitro* studies are planned to probe the relationships between these various components.

P 018 MOLECULAR GENETICS OF CALCIUM SIGNALLING IN YEAST GROWTH CONTROL. Jeremy Thorner, F. Owen Fields, Mark H. Pausch, Martha S. Cyert, David Kaim and Eva Harris, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720.

We found that Saccharomyces cerevisiae contains a Ca^{2+}-, phosphatidylserine (PS)-, and diacylglycerol (DG)-dependent Ser/Thr-specific protein kinase. This protein kinase C-like activity, purified using protamine phosphorylation as the assay, is stimulated 20- to 40-fold by Ca^{2+} + PS + DG (or phorbol ester). Using probes from mammalian PK-Cs, a homologous yeast gene (PKC1) was isolated. Sequence analysis predicts an open reading frame of 1,151 amino acids, larger than any known mammalian PK-C isozyme. However, the deduced protein possesses similar organization, common structural features, and high homology (50% identity in the catalytic domain) to its mammalian counterparts. A pkc1::LEU2 null allele is a recessive lethal mutation. Haploid cells carrying the pkc1::LEU2 mutation and a plasmid expressing PKC1 from the GAL1 promoter grow on galactose-containing medium, but upon shift to glucose medium arrest growth at a uniform cell cycle stage after DNA replication, suggesting that PKC1 is required for the G2-M transition. PKC1 does not encode the Ca^{2+}-, PS- and DG-dependent activity purified in vitro, indicating that yeast cells must contain at least two different classes of PK-C-related enzymes. Using affinity chromatography on authentic yeast calmodulin (CaM), we purified a Ca^{2+}/CaM-dependent type II (broad substrate specificity) protein kinase. The enzyme closely resembles its mammalian counterparts, being: ∿300,000 MW; comprised of two types of subunits, 55,000 (α) and 60,000 (β) MW, respectively; able to autoin a Ca^{2+}/CaM-dependent manner, to bind ^{125}I-yeast CaM, and to phosphorylate a variety of exogenously-added substrates (including a synthetic peptide containing the regulatory autophosphorylation site found in the rat brain type II enzyme). The structural genes (CMK1 and CMK2) for each subunit were cloned by screening a λgt11 library first with ^{125}I-yeast CaM and then with oligonucleotide probes corresponding to tryptic peptides derived from the purified α subunit. Disruption of the α subunit gene (CMK1) is lethal, providing the first evidence in any system that type II Ca^{2+}/CaM-dependent protein kinase serves a function essential for the growth of a eukaryotic cell. Using yeast CaM affinity, we also purified a Ca^{2+}/CaM-stimulated phosphoprotein phosphatase. Like the corresponding enzyme from mammalian sources, calcineurin (or phosphoprotein phosphatase 2B), the yeast enzyme is composed of a large catalytic (A) subunit and a small regulatory (B) subunit. The gene (CNB1) for the B subunit was obtained by screening the λgt11 library with antibody against the mammalian holoenzyme (provided by C. Klee, NIH). The gene (CNA1) for the A subunit was obtained by screening the collection of CaM-binding phages with a cDNA probe for the human catalytic subunit (also provided by C. Klee). Supported by NIH Grant GM21841 to J.T.

P 019 SIGNAL TRANSDUCTION BY GROWTH FACTOR RECEPTORS, Axel Ullrich, Department of Molecular Biology, Max-Planck-Institut für Biochemie, 8033 Martinsried, West Germany.

A variety of serum factors stimulate cellular responses by interaction with a family of cell surface receptors that possess an intrinsic, ligand-sensitive, protein tyrosine kinase activity. These receptor tyrosine kinases are comprised of an extracellular ligand-binding domain that is linked directly to a cytoplasmic catalytic domain, which is thought to generate a biochemical signal that results in a specific cellular response. Using cloned growth factor receptor cDNAs and structural information of both normal receptors and their oncogenic derivatives, we have investigated functional aspects of normal signal transduction, as well as alternative scenarios that result in receptor-mediated cellular transformation. We find that certain structural alterations, including truncations, deletions and point mutations, in the EGF receptor can contribute to the induction of a transformed phenotype on transfected NIH 3T3 cells. Furthermore, increased expression levels in conjunction with activation by the ligand results in efficient transformation by epidermal growth factor receptor and HER2/*neu*. Interestingly, growth factor receptor gene amplification and overexpression appears to be a frequent characteristic of primary mammary carcinomas and correlates statistically with patient survival and tumor recurrence. Various approaches designed to analyze basic mechanisms underlying normal growth factor receptor-mediated signalling and their subversion in the transformed cell and in malignant cancer will be discussed.

Regulation of Cell Fate-II

P 020 GHF-1: a homeodomain protein which determines somatotroph cell identity

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GHF-1 is a 33KD protein which is a member of the homeodomain protein family. GHF-1 is a specific activator of the growth hormone (GH) gene, which is exclusively expressed in the somatotrophic cells of the anterior pituitary. In addition to the homeodomain, GHF-1 contains a second evolutionary conserved region-the POU domain. Although not directly involved in DNA-binding, the POU domain facilitates sequence recognition by the homeodomain. Near its NH_2-terminus GHF-1 contains a 72 amino acid region which functions as its activation domain. This domain, termed the STA domain, is very rich in hydroxylated amino acid residues and is almost completely devoid of acidic residues or gluatmines. We believe that the STA domain interacts with a specific component of the transcriptional machinery. In addition, it shows certain similarity to the tail of the largest subunit of RNAP-II.

In the mouse, GHF-1 transcripts are first detected on embryonic day (e.d.) 13 in the ventral region of Rathke's pouch-the anterior pituitary anlagen. Their abundance increases dramatically during the next two days but no GHF-1 protein can be detected until e.d. 16. On that day, appearance of GHF-1 protein correlates with activation of GH gene transcription. The mechanism underlying the delayed appearance of GHF-1 protein is unknown but it appears to involve translational control. The GHF-1 gene itself is transcriptionally regulated and its expression is limited to the same cells that will end up as mature somatotrophs. This specificity is due to the action of a promoter region which is recognized by at least two different pituitary specific transcription factors, one of them is GHF-1 itself.

P 021 MATERNAL CONTROL OF SEGMENTATION IN THE DROSOPHILA EMBRYO, Ruth Lehmann, Charlotte Wang, Laura Dickinson, Whitehead Institute and Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, MA 02142. The basic information for the development of the normal segmental pattern in the Drosophila embryo is provided by three groups of maternal effect genes: "the anterior group" that affects the development of head and thorax, "the posterior group" that affects the abdomen and "the terminal group that affects the most anterior (acron) and posterior (telson) structures.

The posterior group of genes (*nanos, pumilio, oskar, vasa, tudor, valois, staufen, cappuccino, spire, Bicaudal-D*) affects the establishment of abdominal segmentation, and in addition seven of these genes — abbreviated *osk, vas, tud, val, stau, capu and spir* — show an effect on germ cell formation: embryos derived from homozygous mutant females do not form the specialized pole plasm and hence lack pole cells, the precursor cells for the germ line of the next generation.

We have dissected the pathway leading to the normal posterior segmentation pattern through genetic experiments as well as cytoplasmic transfers between wildtype and mutant embryos. The abdominal defect of the posterior group mutants can be rescued by transplantation of wildtype cytoplasm into the prospective abdominal region of mutant embryos. Only posterior pole plasm is active while cytoplasm from more anterior regions, including the abdominal region, are ineffective. These experiments lead to the hypothesis that the posterior pole is a source of an activity (signal) which is required in the abdominal region. The molecular and genetic analysis of the posterior group genes indicates that all mutants affect the same developmental pathway and are deficient either in synthesis, storage or transport of the same signal.

The *nos* gene product can be singled out to be essential for the synthesis of the signal. The *nos* gene codes for a single transcript that is synthesized exclusively in the female germ line and persists during early stages of embryogenesis. The *nos* RNA is localized to the pole plasm region. The distribution of *nos* transcript is identical to the spatial profile of the abdominal rescue activity revealed by our previous transplantation experiments. It is thus very likely that the rescuing principle is in fact the *nos* RNA.

P 022 EXTINCTION OF GENE ACTIVITY BY INTERFERENCE WITH THE cAMP SIGNAL TRANSDUCTION PATHWAY G. Schütz, Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG.

The Tyrosine Aminotransferase Gene is specifically expressed in liver cells and is inducible by glucocorticoids as well as via the cAMP pathway. In non-liver cells the TAT gene is subject to dominant negative regulation by the tissue specific extinguisher locus (Tse-1). We have identified a liver specific enhancer 3.6 kb upstream of the transcription start site. This enhancer is responsive to induction by cAMP and is the target for negative regulation by Tse-1. In a hepatoma microcell hybrid line which contains only a small segment of human fibroblast chromosome 17 carrying Tse-1, induction by cAMP is able to overcome extinction thus revealing a functional antagonism between Tse-1 and the signal transduction pathway.

A detailed mutational analysis of the enhancer reveals a bipartite structure: two short DNA sequences are both absolutely essential for function. A multimer of the distal 26 bp sequence behaves as a cAMP-responsive element and is the target for Tse-1 action as expected from the functional antagonism between Tse-1 and the cAMP pathway. In vivo footprinting reveals characteristic changes in dimethylsulfate reactivity at this sequence element which correlates with (i) cAMP induction, (ii) the presence of the Tse-1 carrying chromosome fragment and, (iii) the dominant relief from extinction by cAMP. A multimer of the proximal 18 bp sequence behaves as a cell-type specific activator of transcription. Combination of the 26 bp and 18 bp sequences generates an element with all the regulatory properties of the TAT enhancer. As hormones acting via the cAMP pathway are thought to be critically involved in turning on TAT expression around birth, we suggest that the functional antagonism between Tse-1 and the cAMP pathway is the basis of a molecular switch governing the timed onset of expression.

Signaling; Extracellular Factors; Regulation of Cell Fate

P 100 **Activation of the RAF-1 protooncogene product by colony stimulating factor-1.** Manuela Baccarini, David M. Sabatini, Harald Appt, Ulf R. Rappt, and E. Richard Stanley, Department of Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, N.Y.10461,and †Laboratory of Viral Carcinogenesis, NCI, Frederick, MD 21701. Colony stimulating factor-1 (CSF-1) selectively mediates the survival,proliferation and differentiation of cells of the mononuclear phagocytic lineage. The cell surface receptor for CSF-1 is a protein tyrosine kinase identical with the c-fms protooncogene product. After in vivo stimulation with CSF-1 at 37° and at 4°C, a number of proteins rapidly become phosphorylated in tyrosine. On the contrary, the serine/threonine kinase RAF-1, recently claimed to be one of the substrates of the PDGF receptor, is phosphorylated in intact macrophages in response to CSF-1 treatment at 37°C, but not at 4°C. The phosphorylated RAF-1 does not react with anti-phosphotyrosine antibodies and phosphoserine and phosphothreonine but no phosphotyrosine are detected by phosphoaminoacid analysis. Augmented phosphorylation of RAF-1 is accompanied by an increase in RAF-1 associated serine/threonine kinase activity and is kinetically later than CSF-1-induced protein tyrosine phosphorylation. A small proportion of total cellular RAF-1 molecules can be recovered in anti-CSF-1 receptor immunoprecipitates from both CSF-1-stimulated and unstimulated cells. Thus, although RAF-1 is not a direct substrate for the CSF-1 receptor tyrosine kinase in vivo, its temperature dependent phosphorylation and activation represent an intriguing aspect of CSF-1 signal transduction. This work was supported by NIH grants CA 26504 and CA 32251 (E.R. Stanley), the Albert Einstein Core Cancer Grant, P30-CA 1330 and the Lucille P. Markey Charitable Trust (E.R. Stanley).

P 101 n-DODECANE AS A PROBE OF THE HYDROPHOBIC ENVIRONMENT REQUIRED FOR BINDING AND ACTIVATION OF PROTEIN KINASE C. C. Stuart Baxter, Department of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH 45267-0056 Activation of protein kinase C (PKC) by diacylglycerol released from membrane inositol phospholipids as a result of phospholipase C activation is a signal transduction mechanism common to many growth and differentiation factors. In order to probe the nature of the optimal hydrophobic environment for activation of PKC, the effects of the linear saturated alkane n-dodecane on the binding and activation of the enzyme by the diacylglycerol analog phorbol-12,13-dibutyrate (PDBu) in the presence of phosphatidylserine (PS) were determined. In the presence of suboptimal concentrations of phosphatidylserine, n-dodecane was found to restore specific binding of PDBu to PKC in mouse brain cytosol to levels observed in the presence of optimal phospholipid concentrations. In this way the concentration of phosphatidylserine (PS) required to produce maximal binding was reduced from 100 to 2 µg/ml in the presence of 320 µg/ml dodecane. n-Dodecane alone was not able to reconstitute the binding activity of the kinase, nor did it compete with PDBu for specific binding, nor did it stimulate kinase activity at optimal PS concentrations. The alkane was therefore able to provide most, but not all, of the lipid requirement for binding to the enzyme normally supplied by the phospholipid. These findings therefore suggest that an extensive hydrophobic environment is required for activation of PKC, and that the need for a large excess of phospholipid for activation in vitro is a direct reflection of this requirement.

P 102 PHOSPHORYLATION OF A G PROTEIN SUBUNIT REGULATES THE MATING PHEROMONE RESPONSE IN *S. CEREVISIAE*. Gary M. Cole and Steven I. Reed, Department of Molecular Biology, Research Institute of Scripps Clinic, La Jolla, CA 92037. In *Saccharomyces cerevisiae* peptide pheromones secreted by haploid cells of opposite mating type induce mating responses by means of a G protein-coupled signal transduction pathway. These responses include arrest in G_1 of the cell cycle, transcriptional induction of mating specific genes, and morphological changes preparatory to conjugation. The transducer of this signal is the $G\beta\gamma$ subunit encoded by the *STE4* and *STE18* genes. We have prepared polyclonal antisera to the Ste4 protein to more thoroughly elucidate the events following stimulation of pheromone receptor. We found that in wild-type haploid cells, treatment with pheromone stimulates a rapid phosphorylation of Gβ Ste4. Unstimulated cells exhibit a low but still significant level of Ste4 phosphorylation (~10% of the total Ste4 protein). Signal-induced phosphorylation is predominantly on serine residues, although a small fraction of phosphothreonine is also detectable. An in vitro generated mutation which eliminates both basal and induced phosphorylation of Ste4 without affecting the ability of Ste4 to transduce the mating signal was generated. Cells harboring this mutation exhibit a phenotype of constitutive activation of the pheromone response pathway: they induce a mating-specific transcript and exhibit morphological alterations characteristic of mating cells, although they do not arrest cell division.

Analysis of the interactions of this mutant with other mutants which affect the ability of cells to adapt to pathway stimulation leads us to conclude:

1) In unstimulated cells the pheromone response pathway is active to a low but significant extent.
2) Phosphorylation of free βγ is a major adaptive response to pathway stimulation.

P 103 POSTTRANSLATIONAL MODIFICATION, MEMBRANE LOCALIZATION, AND THE FUNCTION OF YEAST *RAS2* PROTEIN. Robert Deschenes, Karla Hemesath, and Lynn Farh, Department of Biochemistry, University of Iowa, Iowa City, IA 52242.

A number of proteins involved in signal transduction require membrane association. One way this is accomplished is through the interaction of an amino terminal signal sequence that directs the protein to the endoplasmic reticulum. Recent studies on the membrane association of *RAS* oncogene proteins has revealed another mechanism for membrane localization. A short C-terminal sequence, referred to as a CAAX box (C represents Cys, A any aliphatic residue and X, the C-terminal amino acid) has been found to be required for the membrane localization of *RAS* and a number of other proteins. Removal of this sequence, or changing the conserved cysteine to another amino acid, prevents processing and membrane localization. Recent studies from a number of laboratories have revealed that *RAS* processing actually involves a number of steps. They include farnesylation of cysteine, removal of the terminal three residues (-AAX), carboxymethylation, and palmitylation of a second cysteine often found close to the C-terminus. We have examined the importance of each step by creating mutations in the *RAS2* C-terminus by cassette mutagenesis. The criteria used to evaluate the mutants is as follows. First, strains are constructed in which the ability of the mutant *RAS* proteins to support *RAS*-dependent growth is measured. Second, membrane localization is assessed by fractionation of cell extracts and immunoprecipitation of *RAS* protein. Finally, we directly measure the modification of *RAS*. By comparing the level of modification with the degree of membrane localization and *RAS* activity, we have determined the relative importance of each modification. In addition to these studies, recent results suggest that an *in vitro* reconstitution system of the modification steps may be a fruitful approach to isolate and characterize the activities for these posttranslational modifications. The ultimate goal is to understand at a molecular level the function of this unique membrane localization pathway.

P 104 COORDINATE REGULATION OF COLLAGENASE AND HUMAN CHORIONIC GONADOTROPIN (hCG)-α GENE EXPRESSION BY PHYSIOLOGIC STIMULATORS OF ADENYLATE CYCLASE IN PLACENTAL TROPHOBLASTS. Paul Deutsch, Yu Sun, Kalpana Raghavan, and Rachel Glick, Divsion of Endocrinology and Program in Cell Biology and Genetics, Cornell University Medical College, New York, NY 10021

In addition to its synthesis of hCG, the trophoblast is critical in early human development in implantation in the endometrium, involving metalloproteinases of which (interstitial) collagenase is a prototype. Because cAMP stimulates both hCG and, by our recent observations, collagenase gene expression in the model trophoblast cell line JEG-3, we have searched for ligands which might elevate cAMP and coordinately regulate hCG and collagenase gene expression. We report here that both VIP and prostaglandin E2 at nM levels increase cAMP content and activate the expression of CAT reporter genes bearing the hCG-α or collagenase promoter in JEG-3 cells. The nM potency of VIP and specificity for VIP versus related peptides indicates that these are authentic VIP receptors. Sustained exposure to VIP or 8-br-cAMP is required for maximal elevation of hCG-α mRNA and hCG-α CAT activity. However, brief (< 4 hr) exposure to VIP or 8-br-cAMP is sufficient for persistent stimulation of collagenase CAT activity over the ensuing 20 hr, suggesting a different mechanism of activation. Data will be presented indicating that cAMP activates collagenase gene expression in JEG-3 cells via the AP-1 DNA motif, in contrast to the AP-1 motif's cAMP unresponsiveness in HeLa TK- cells. The presence of VIPergic neurons and prostaglandin synthetases in the uterine wall suggest that these observations may be relevant to peri-implantation events.

P 105 "MODULATION OF IL-1 SIGNAL TRANSDUCTON BY TGF-BETA IN RABBIT ARTICULAR CHONDROCYTES"

Giorgio Ferrari and Domenico Rotilio. Consorzio Mario Negri Sud, via Nazionale, 66030 S. Maria Imbaro, Italia.

IL-1 is a cytokine produced by a large spectrum of cells. The well documented roles of this protein are in the induction of a variety of chronic inflammatory diseases. The two cloned forms of IL-1 (alfa-beta) interact with the same receptor on the cell surface. The steps between complex formation and the final event have not been elucidated. Several studies suggest that IL-1 does not affect the levels of intracellular calcium or the breakdown of phosphatidyliositol. In our cellular system IL-1 shows mitogenic activity at low concentrations (1 ng/ml), an effect inhibited by TGF-ß. In a binding assay, the pre-incubation of TGF-ß for 4 h, induces a 45% decrease in specific binding sites for 125I-IL-1ß in rabbit chondrocytes, with no change in the affinity (4,4x10-11M). The treatment of rabbit chondocytes with increasing concentrations of IL-1ß or forskolin, in the presence of isobutylmethylxanthine 2.5mM, causes a rapid increase in adenylate cyclase activity measured as accumulation of c-AMP. Within 20 min after the addition of the stimuli, the system reaches a plateau, the amount of c-AMP produced by IL-1ß or forskolin being similar. In the same conditions no detectable levels of c-AMP are measured after the addition of TGF-ß. IL-1 (1ng.ml) and forskolin (10nM) lead to maximal accumulation of c-AMP in a range of 85-90 pmol/ml and 95-100 pmol/ml respectively. The pre-incubation of cells at two different times (10min, 2h) with increasing amounts of TGF-ß, resulted in a dramatic fall of adenylate cyclase activity induced by IL-1ß but did not affect the direct activation of this enzyme by forskolin. IL-1ß is known to increase c-AMP via a G-protein, whereas forskolin activates cyclase directly. Our results suggest that TGF-ß could inhibit IL-1ß effect through a mechanism in which a G-protein and/or IL-1ß receptor system are involved.

P 106 CHARACTERIZATION OF A DROSOPHILIA HOMOLOGUE OF RAT CALCIUM/ CALMODULIN-DEPENDENT PROTEIN KINASE II, Leslie C. Griffith and Ralph J. Greenspan, Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110

One of the major mechanisms by which calcium transduces information within the neuron is through the activation of protein kinases such as calcium/calmodulin-dependent protein kinase II (CaM kinase). Studies of this enzyme in the rat brain have led to speculation that it may be involved in many synaptic functions, including formation of synaptic connections and plasticity. To address these complex questions genetically we have chosen to study the D. melanogaster homologue of this enzyme.

We have been able to characterize an enzyme activity in flies which corresponds to the rat CaM kinase by many criteria including subunit and holoenzyme size, substrate specificity, regulation by calcium, calmodulin and autophosphorylation, inhibition by autoregulatory sequences of the rat kinase and antigenic cross-reactivity. The Drosophilia CaM kinase consists of a major 50 kDa phosphoprotein and several minor higher molecular weight proteins which may be more highly phosphorylated forms of the 50 kDa protein. Similarity with the rat CaM kinase has allowed development of a specific assay based on phosphorylation of a peptide corresponding to a rat kinase autophosphorylation site. Using this assay we have genetically mapped the Drosophilia CaM kinase gene by means of segmental aneuploidy for eventual mutant studies of the role of this kinase in the function of the nervous system.

P 107 A GENE CONTAINING THE CATALYTIC DOMAIN FOR A PHOSPHOTYROSINE PHOSPHATASE ACTIVITY IS DEVELOPMENTALLY REGULATED IN *DICTYOSTELIUM*, Peter J. Howard and Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, Univ. of California, San Diego, La Jolla, CA 92093

We have begun the investigation of a gene induced with the onset of starvation in *Dictyostelium discoideum*. Initially two messages of 2.2 kb and 2.6 kb appear by two hours of development and are then expressed at moderately low levels throughout development with additional messages of 2.0 and 2.4 kb appearing by ten hours. The gene contains a region of amino acid identity to the catalytic domain of a family of phosphotyrosine phosphatases. Pairwise comparisons of this region with twelve other members of this family reveal that the closest homology (40%) is shared with LAR and the *Drosophila* LAR cognate genes. Full length cDNAs and a complete genomic clone are being obtained and gene disruption experiments are underway to probe function. The spatial distribution of the protein within the multicellular aggregate is being probed using antibodies and β-gal gene fusions.

P 108 TWO DISTINCT AND FREQUENTLY MUTATED REGIONS OF RETINOBLASTOMA PROTEIN ARE REQUIRED FOR BINDING TO SV40 T ANTIGEN, Shi Huang*, Nan-Ping Wang*, Ben Y. Tseng+, Wen-Hwa Lee*, and Eva Y.-H.P. Lee*,*Department of Pathology, M-012, +Medicine and *Center for Molecular Genetics, University of California at San Diego, La Jolla, CA 92093

The retinoblastoma susceptibility gene (RB) encodes a phosphoprotein of 110 kd (pp110^{RB}). Phosphorylation of pp110^{RB} is modulated during the cell cycle and cellular differentiation. The underphosphorylated form of RB protein forms specific complexes with SV40 T antigen. Transforming proteins from several other DNA tumor viruses also interact with RB, and genetic studies suggest that these interactions with RB protein contribute to transformation by these viruses. To help understand the function of these interactions, we have mapped the regions of RB that are involved in binding to T. An in vitro protein synthesis system to produce the full-length RB protein was developed for the mapping study. A 5-10 fold increase in translational efficiency in the reticulocyte lysate was obtained when the 5'-noncoding region of RB mRNA was replaced with that of ß-globin mRNA or a plant viral RNA, alfalfa mosaic virus (AMV) RNA4. A series of mutated RB polypeptides produced from this system were assayed for T binding. Two noncontiguous regions of the RB protein, amino acid residues 394 to 571 and 649 to 773, were found to be necessary for binding to T: mutations in either region abolished T/RB complex formation. These results are consistent with the finding that, in all the cases analyzed so far, mutated RB proteins in human tumor cells also failed to bind to T antigen due to deletions including at least one of the two required regions. Thus the regions of RB defined in vitro as necessary for interaction with T might be physiologically relevant as well, and might play a fundamental role in normal RB protein function.

P 109 A GENETIC ANALYSIS OF SIGNAL TRANSDUCTION MECHANISMS REQUIRED FOR ACTIVATING THE DEFENCE RESPONSE OF TOMATO AND *ARABIDOPSIS*. J. Jones, T. Ashfield, D. Jones and K. Hammond, Sainsbury Laboratory, John Innes Institute, Colney Lane, Norwich, NR4 7UH, UK.

The appropriate response to pathogen challenge is a key recognition/reaction process in the life of plants. Defence mechanisms are probably developmentally regulated, since many pathogens are specialized to attack either root or shoot. Recognition of the incoming pathogen, followed by the induction of different genes that encode proteins required for making a variety of anti-pathogen products, is essential for disease resistance. An additional defence response, the "hypersensitive response" (HR), involves programmed cell death of specific plant cells. These responses are rapid, and specific molecules (elicitors) can induce changes in gene expression in cell culture which mimic changes of gene expression *in vivo* (see Cell **56** 215 for a review).

The nature the of the signal transduction mechanisms (STMs) between pathogen recognition and the defence response is unknown, and is an active area of research. It is clear that plants contain homologues to protein kinases and G-proteins, so both of these pathways could in principle be involved.

We are adopting a genetic approach to identify components of the defence response STM.

(i) We have mutagenized seed of tomato lines which are resistant to a specific race of the pathogen *Fulvia fulva*, in order to identify mutants to disease sensitivity, and to thus identify loci which encode products required for the resistance response.

(ii) We are studying necrotic, or disease lesion mimic, mutants, in which the defence response appears to be activated in the absence of visible pathogens (perhaps analagous to yeast *scg1* mutants).

(iii) We are setting up selections for mutants which fail to induce defence genes by transforming *Arabidopsis* with fusions between defence gene promoters and conditionally lethal reading frames.

Progress in all these areas will be reported.

P 110 CHARACTERIZATION OF PROTEINS SECRETED BY ENDODERM EXPLANTED FROM THE EARLY CHICKEN EMBRYO. Nighat P. Kokan-Moore, John Lough and David L. Bolender, Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI. 53226.

A role for anterior endoderm or its products in terminal cardiocyte differentiation has been supported by recent findings from this laboratory (Lough, et al., Ann. N. Y. Acad. Sci., in press). Studies are now in progress to characterize and test the bioactivity of endoderm products secreted in vitro. Endoderm from stage 4-8 chicken embryos was grown on collagen gels in defined medium, without serum. The cultures were labeled with ^{3}H-leucine and the profile of secreted proteins was determined by SDS-PAGE and fluorography. The pattern of endoderm-secreted proteins was similar, regardless of embryonic age at the time of explantation (stages 4-8) or time in culture (up to seven days). After three days in culture, ten major protein bands were detected having M_r's of 6, 17, 20, 25, 36, 42, 51, 64, 80 and 200 kD; isoelectric focusing revealed that all had pI's in the acidic to neutral range. To date, Western blot analysis has failed to detect the presence of known growth factors. However, a 29 kD band cross-reacted with ES1, an antiserum which recognizes five inductively active macromolecules from the myocardial basement membrane of the developing heart at stage 15 (Krug, et al., Dev. Biol. 120:348; '87). The effects of endoderm-secreted proteins on cardiocyte proliferation and migration are currently being investigated. Supported by NIH grant HL 39829.

P 111 AN INCREASE IN EXPRESSION OF THE APLYSIA HOMOLOG OF BIP/GRP78 ACCOMPANIES THE MAINTENANCE PHASE OF LONG-TERMSENSITIZATION. Kuhl D., Kennedy T.E., Barzilai A.and Kandel E.R., Center for Neurobiology and Behavior and Howard Hughes Medical Institute, New York, NY 10032

Two-dimensional PAGE analysis has shown that long-term sensitization in *Aplysia*, a non-associative form of learning, is accompanied by an alteration in ^{35}S-methionine incorporation into four proteins. Two of these proteins show a similar change in expression in isolated pleural sensory neurons when 5-HT is used to produce long-term facilitation. The changes are blocked by inhibitors of protein and RNA synthesis present during the period of 5-HT application. We have obtained partial amino acid sequence for these two proteins, isolated directly from Coomassie blue-stained preparative two-dimensional gels. In addition to partial amino acid sequence, pI and MW has allowed us to identify one protein as the *Aplysia* homolog of BiP/GRP78. BiP/GRP78 is thought to function as a molecular chaperon of newly synthesized proteins in the lumen of the ER. This is consistent with our finding that the increase of *Aplysia* BiP/GRP78 is first detected at the peak of an increase in overall protein synthesis 3 hours after 5-HT application. Using PCR, we have obtained a partial cDNA clone of *Aplysia* BiP/GRP78, which will serve as a probe to examine the induction of this gene during long-term changes in neuronal function.

P 112 DESENSITIZATION OF CALCIUM MOBILIZATION AND CELL FUNCTION IN HUMAN NEUTROPHILS, Pramod M. Lad, John S. Kaptein, Ching-Kow Lin, Stephen J. Scott, and Cosmas I. Kalunta, Kaiser Regional Research Laboratory, 1515 N.Vermont Avenue, Los Angeles CA 90027.

Neutrophils pretreated with the chemoattractant f-met-leu-phe become unresponsive when reexposed to the same ligand, a process termed desensitization. We have examined whether desensitization of transduction (calcium mobilization) or of other cell functions (superoxide generation, enzyme release, aggregation) occurs synchronously. Simultaneous studies of calcium mobilization and aggregation indicate that under conditions where the aggregation response is abolished, the bulk of calcium mobilization is unaltered. Further studies were then carried out to ascertain whether desensitization of calcium mobilization could in fact be induced. Desensitization was observed dependent on the number of exposures of the cells to the ligand, the concentration of the ligand, and whether the ligand was left in, or washed out of the medium. Under conditions where ligand was continuously present, recovery of the calcium mobilization response was not seen upon subsequent challenge. In contrast, upon removal of ligand, this response showed partial recovery. Whereas complete desensitization of aggregation was noted, enzyme release showed a markedly lesser degree of desensitization and required more frequent exposures to the ligand before it was observed. Little or no desensitization of superoxide generation was observed regardless of the conditions utilized. Studies using PMA as the ligand showed that calcium mobilization and aggregation could be simultaneously inhibited. **Our results suggest that discrete mechanisms of desensitization are possible in human neutrophils and that desensitization of one particular function (aggregation) does not imply concomitant desensitization of other functions.**

P 113 ANALYSIS OF PHYTOCHROME ACTIVATION OF NUCLEAR GENE EXPRESSION IN A PHOTOAUTOTROPHIC CELL SUSPENSION SYSTEM, Eric Lam, AgBiotech Center, Waksman Institute of Microbiology, Rutgers State University, P.O. Box 759, Piscataway, NJ 08855. The study of the signal transduction process between light and its effects in higher plants has been hampered by the lack of an in vitro model system. Plant cells in culture usually have lost their characteristic responses to light. Recently, the expression of light-regulated genes in a photoautotrophic cell suspension derived from soybean leaves, SBP-1, is found to respond to light in a quantitatively comparable manner to soybean seedlings. Moreover, it is shown that this light-dependence is mediated by phytochrome, a well-characterized plant photoreceptor. Inhibitor studies indicate that calmodulin may be involved in mediating the signal transduction process between phytochrome and gene expression. The calcium ionophore ionomycin induces only low expression of the light-regulated genes in the dark, suggesting that calmodulin activation may be required but insufficient to activate these light-regulated genes. Possible involvement of kinases are being investigated by inhibitor studies and labeling with ^{32}P.

P 114 POSITIVE AND NEGATIVE REGULATION OF THE DICTYOSTELIUM DISCOIDIN GENES BY EXTRACELLULAR SIGNALS. Wolfgang Nellen, Piero Morandini, Franz Vauti and Jürgen Blusch, Max-Planck-Institut f. Biochemie, D-8033 Martinsried, FRG

The discoidin gene family is induced 2-3 generations before the onset of development by PSF, an extracellular factor secreted by the cells and accumulated in the medium. Later, discoidin expression is downregulated by extracellular cAMP which is produced by the cells during early development and released into the medium in pulses. By binding to a cell surface receptor, cAMP initiates the signal transduction pathway which finally results in differential gene expression. We have dissected the discoidin I γ gene promoter to investigate the gene expression end of the signal transduction chain. Deletion analysis led to the identification of a short element (TTG-box) necessary for gene induction and a second promoter region (dNCE) required for downregulation by cAMP. A fragment containing the TTG-box is the target for a nuclear DNA-binding protein and probably represents the PSF responsive element. Transfer of this fragment to a heterologous promoter confers developmental induction and PSF mediated gene activation but no cAMP regulation to a reporter gene. Expression of the discoidin genes in regulatory mutants will be discussed.

P 115 *CONCERTINA*: A GENE REQUIRED FOR GASTRULATION IN *DROSOPHILA* ENCODES A G PROTEIN. Suki Parks, Dari Sweeton, Trudi Schupbach and Eric Wieschaus, Department of Biology, Princeton University, Princeton, NJ 08544
Embryos from mothers homozygous for mutations in the *concertina* gene become defective early in gastrulation. Cells in the ventral furrow region fail to undergo the proper coordinated cell shape changes, producing instead a disorganized abnormal furrow. Subsequently, cell shape changes which are required to form a posterior midgut invagination fail to occur. In spite of this early disruption, these embryos express pattern genes normally, including the *twist* gene, which is expressed in cells in the ventral furrow region. Using a P element insertion mutation as a tag, the *concertina* gene has been cloned. Sequence analysis indicates that this gene encodes a novel *Drosophila* G alpha GTP binding protein. These results suggest that signal transduction may be required to orchestrate early morphogenetic movements.

P 116 REGULATION OF THE INDUCTION OF MEIOTIC MATURATION AND MPF IN OOCYTES, Richard Paules,[1] Ira Daar,[1] Noriyuki Sagata,[2] Roberto Buccione,[3] John Eppig,[3] Michael Wigler,[4] and George F. Vande Woude,[1] [1]BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, Frederick, MD 21701, [2]Tsukuba Life Science Center, RIKEN, Tsukuba, Japan, [3]The Jackson Laboratories, Bar Harbor, ME 04609, [4]Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724
The induction of meiotic maturation is dependent on the activation of maturation promoting factor (MPF), a universal autocatalytic activity that drives the transition from G2 through M phase. Two integral components of this regulatory activity are the $p34^{cdc2}$ proteins and the cyclin proteins. We have been investigating the regulation of the activation of MPF in oocytes. We have found that the mos proto-oncogene product is necessary for the normal progression of both Xenopus and mouse oocytes through meiotic maturation. Recently, it has been shown that mos protein is an essential component of cytostatic factor (CSF), the activity responsible for stabilizing active MPF (Sagata et al., Nature, in press). In Xenopus oocytes the induction of meiotic maturation and the activation of MPF by both insulin and progesterone is dependent on the presence of the mos gene product. However, injections of synthetic cyclin $A^{\Delta e}$ RNA or MPF cytoplasmic extracts are both able to induce meiotic maturation in the absence of mos product. While the injection of synthetic mos RNA in fully grown Xenopus oocytes can result in the induction of meiotic maturation and the activation of MPF, this induction can be blocked by the cAMP-dependent protein kinase catalytic subunit.
Research sponsored in part by the National Cancer Institute, DHHS, under contract No. NO1-CO-74101 with BRI.

P 117 THE ACCUMULATION OF THE DEVELOPMENTALLY REGULATED TRANSCRIPTS OF THE *DICTYOSTELIUM DISCOIDEUM* CYCLIC NUCLEOTIDE PHOSPHODIESTERASE GENE IS REGULATED BY THREE DISTINCT PROMOTERS, Gregory J. Podgorski[1], Michel Faure [2], Jakob Franke[2], Anne L. Hall[2], L. Wu[2], and R.H. Kessin[2], 1: Department of Biology, Utah State University, Logan, UT, 84322, 2: Department of Anatomy and Cell Biology, Columbia University, New York, NY 10032 The cyclic nucleotide phosphodiesterase is essential for early and late phases of development in *Dictyostelium discoideum*. The enzyme acts in the intercellular space during aggregation to degrade the chemoattractant and gene regulatory molecule cAMP, and during later stages of development, the enzyme plays a role in pattern formation. The phosphodiesterase gene is transcribed into three mRNAs that are differentially regulated during development: a 1.9 kb mRNA during growth, a 2.4 kb mRNA during aggregation, and a prestalk cell specific 2.2 kb mRNA in late development. All three mRNAs contain the same coding sequence and differ only in their 5' untranslated regions. Each mRNA is transcribed from its own promoter. The pattern of transcriptional regulation conferred by each promoter accounts for the pattern of accumulation of each mRNA. The complex regulation of the phosphodiesterase gene may reflect its multiple roles during development. The structure of the gene suggests the existence of an ancestral gene which may have evolved by the acquisition of regulatory elements which confer three different patterns of transcriptional regulation during development. We have also studied the structure and regulation of the phosphodiesterase inhibitor gene. The single copy inhibitor gene is transcribed during aggregation into a 0.95 kb mRNA which is negatively regulated by cAMP. Treatment with cAMP results in rapid ($t_{1/2}$=30 min.) disappearance of the mRNA. Together, the phosphodiesterase and phosphodiesterase inhibitor genes control the levels of extracellular cAMP during *Dictyostelium* development.

P 118 **KINASE ACTIVITY OF STE11 IS REQUIRED FOR ITS SIGNAL TRANSDUCTION FUNCTIONS,** Nelson Rhodes and Beverly Errede, Department of Chemistry, University of North Carolina at Chapel Hill, NC 27599-3290.
The *STE11* gene from the yeast *Saccharomyces cerevisiae* encodes a component of the pheromone response pathway and is essential for conjugation between the two haploid cell types, **a** and α. STE11 is also required for basal and pheromone inducible transcription of genes required for mating. *In-vitro* kinase assays using immunoprecipitated STE11 protein have demonstrated the kinase catalytic activity of this protein. To correlate kinase activity and *in-vivo* function, we created a point mutation in the conserved catalytic lysine residue using site directed mutagenesis. This mutation does not alter the abundance of the protein or the apparent molecular weight, but does abolish the *in-vitro* kinase activity and results in a nonfunctional protein *in-vivo*. Cells that express this mutation do not undergo G1 arrest and are unable to induce transcription in response to pheromone and, therefore, do not mate. These data show that the kinase activity of STE11 is required for these functions. We are currently looking at how kinase activity may be regulated in the various cell types and under pheromone inducing conditions.

P 119 **CELL CYCLE-SPECIFIC ACTION OF NERVE GROWTH FACTOR IN PC12 CELLS: DIFFERENTIATION WITHOUT PROLIFERATION,** **Brian B. Rudkin,** Philip Lazarovici, Ben-Zion Levi[1], Yuya Abe[2], Ko Fujita and Gordon Guroff, **Section on Growth Factors** and [1]Laboratory of Developmental and Molecular Immunity, Section on Molecular Genetics of Immunity, **National Institute of Child Health and Human Development,** [2]Laboratory of Biological Chemistry, Division of Cancer Treatment, Developmental Therapeutics Program, National Cancer Institute, **National Institutes of Health, Bethesda, MD 20892.** PC12 cells were manipulated in such a way as to permit the study of differentiation-specific responses independently from proliferative responses. Cells were starved for serum then exposed to nerve growth factor (NGF) or serum. Following addition of serum, cells incorporated thymidine in a synchronous manner. Subsequent to the wave of DNA synthesis, the cell number increased approximately two-fold. Addition of NGF to serum-starved cultures had no measureable effect on either parameter. Neurite outgrowth was more rapid and extensive, and appearance of Na^+ channels, measured as saxitoxin binding sites, more rapid than when NGF was added to exponentially-growing cells. Epidermal growth factor receptors were heterologously downregulated by NGF with similar kinetics under both conditions. Induction of the proto-oncogene c-fos by NGF was also greater in the serum-starved cells than in exponentially-growing cultures. These results indicated that serum starvation resulted in synchronisation of the cultures and that NGF action may be cell cycle-specific. Analysis of the cellular response to NGF at different times during the cell cycle showed that c-fos was induced in the G_1 phase but not in S or G_2. Fluorescence-activated cell sorter analysis demonstrated that addition of NGF to exponentially-growing cells, resulted in their accumulation in a G_1-like state. With regard to the study of the mechanism of NGF action, these results illustrate that measurements of NGF effects on specific components in the signal transduction pathway may be confounded by the use of exponentially-growing cultures.

P 120 SECONDARY EMBRYONIC INDUCTION IN THE DEVELOPING HEART IS MEDIATED BY SPATIALLY AND TEMPORALLY SPECIFIC SIGNAL TRANSDUCTION OF A DEVELOPMENTAL STIMULUS, Raymond B. Runyan, Jay D. Potts, Ram V. Sharma, Cleo P. Loeber, Judy J. Chiang, and Ramesh C. Bhalla, Department of Anatomy, Univsity of Iowa, Iowa City, IA 42242.
During heart development, endothelial cells of the atrioventrtricular (AV) canal undergo a cell transformation to form an invasive mesenchymal cell population. This has previously been shown to be due to a complex stimulus produced by the myocardium. One component of this stimulus appears to be a member of the TGFβ family of growth factors (Potts and Runyan, Dev. Biol. 134, 392). We have begun to examine the role of signal transduction mechanisms in this process. Since the quantity of tissue precludes biochemical measurement, our approach initially focused upon the use of activators and inhibitors of potential mechanisms. Activators of protein kinase C, PMA and mezerein, both produce an incomplete transformation in an in vitro bioassay. Inhibitors of both C-kinase (H-7, staurosporine) and tyrosine kinase (genistein) block cellular transformation. Pertussis toxin, but not cholera toxin, can also block cellular transformation. Finally, AV canal endothelial cells loaded with the dye, fura 2, demonstrate an elevation of intracellular calcium in response to the transforming stimulus. This response is specific when compared to non-responsive ventricular endothelia or older AV canal endothelia. These data suggest that signal transduction of the developmental stimulus is mediated by a G protein-coupled receptor, kinase activities (C-kinase and tyrosine kinase), and intracellular calcium. These processes are consistent with a role for the PI cycle in this process. Together, these data show that secondary embryonic induction is mediated by a specific series of signal transduction mechanisms upon receipt of the extracellular matrix stimulus. Supported by NIH HL 38649 (RBR) and HL 35682 (RCB).

P 121 **TRANSCRIPTIONAL REGULATION OF *snap-25* AND NEURONAL DIFFERENTIATION**, Thomas N. Sato and Michael C. Wilson, Department of Neuropharmacology, Res. Inst. of Scripps Clinic, La Jolla, CA 92037. SNAP-25 protein is a neuron specific protein found at the presynaptic terminal whose expression appears induced by the establishment of synaptic contacts between vertebrate neurons. Treatment of cultured neuronal cells with dibutyryl cyclic AMP increases SNAP-25 transcript level by five fold. Hence, this *in vitro* induction may represent a part of synaptogenesis induced transcriptional activation. To examine the hierarchy of gene expression governing nervous system development, the neuron specific promoter of SNAP-25 gene transcription was determined and a molecular mechanism underlying cyclic AMP induced expression was studied. Reporter gene constructs bearing a series of deletions of the 5' upstream regions of the *snap-25* gene were constructed and their activity was tested in several neuronal cells. The minimum neuronal lineage specific promoter activity was identified within 285 bp upstream from the start site. This promoter element contained a CRE element (-10 - -17), three GC boxes but lacked a TATA and a CAAT element. Currently, foot-printing patterns of this upstream region are being examined with nuclear protein extracts from mouse brain and non-neuronal tissue. In order to determine *cis*-acting elements for cyclic AMP induction which requires three days, PC-12 cells were stably transformed with several promoter and enhancer constructs.

P 122 STIMULATION OF c-fos AND c-jun BY GROWTH HORMONE IN DIFFERENTIATING 3T3-F442A CELLS. Jessica Schwartz, G. Gurland, G. Ashcom, Carolyn Hoban, Brent Cochran. Dept Physiology, U Mich Med Schl, Ann Arbor MI 48109; Ctr Cancer Res & Dept Biol, MIT, Cambridge MA 02139 Growth hormone (GH) is one of the major factors required for adipose conversion in 3T3-F442A cells, which differentiate in culture from preadipocyte fibroblasts to adipocytes. To evaluate whether a proto-oncogene associated with differentiation is responsive to GH, we investigated whether GH altered the expression of c-fos in 3T3-F442A cells as they differentiate. Confluent 3T3-F442A preadipocytes were incubated with GH for various times, total RNA was prepared and subjected to Northern blot analysis. The induction by GH of mouse c-fos was evident within 15 min, while control cells showed no response. The response to GH peaked by 30 min and was undetectable after 90 min, parallelling the time course of the effect of 10% bovine serum. GH was effective at concentrations as low as 50 ng/ml, well within the physiological range. In the presence of 10 ug/ml cycloheximide, c-fos expression was superinduced by GH. These findings indicate that GH, like other growth factors, rapidly induces the expression of c-fos in differentiating 3T3-F442A fibroblasts. Furthermore, the inducible DNA binding factor SIF, which binds to an upstream element of c-fos, is rapidly induced by GH. Since Fos protein participates in a complex with the transcription activator Jun to regulate the expression of differentiation-dependent genes in 3T3-F442A cells, we also examined the effect of GH on mouse c-jun. GH increased the expression of c-jun mRNA in 30 min. The stimulation of c-jun peaked at 60 min, and was still evident after 90 min. Thus, GH-induced changes in c-jun are somewhat delayed relative to changes in c-fos. Expression of c-fos and c-jun was negligible or non-existent in the differentiated adipocytes, whether cells were treated with GH or not. These findings are consistent with coordinated expression of c-fos and c-jun playing a role early in responses to GH during differentiation of 3T3-F442A preadipocytes. Induction of c-fos and c-jun are among the earliest responses to GH identified to date, and are likely to be involved in the mechanism(s) by which GH promotes differentiation.

P 123 REGULATION OF THE EXPRESSION OF MURINE HOMEOBOX GENES IN THE HOX-2 CLUSTER. Mai Har SHAM, Nancy Papalopulu, Stefan Nonchev, Martyn Cook and Robb Krumlauf. National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

Homeobox genes are organized in clusters in the mouse, and the Hox-2 cluster is located on chromosome 11. It has been suggested that these clusters are related to each other by the duplication and divergence of an ancestral cluster. We are interested in understanding whether the organization and arrangement of the genes in the Hox-2 cluster are important for the domains of expression of these genes along the axis of the embryo. It is necessary first of all to know how the expression of the Hox-2 genes are regulated. We have observed from Northern blot analyses that there are multiple transcripts for several Hox-2 genes at various embryonic stages. We have mapped multiple transcription initiation sites for the RNA transcripts of Hox2.1. It is possible that some longer transcripts of Hox2.1 are initiated within the Hox2.2 gene. Two different approaches have been employed to identify potential cis-acting elements which regulate the expression of Hox2.1. A range of clones spanning the intergenic region between Hox2.2 and Hox2.1 were linked to the chloramphenicol acetyl transferase gene; these constructs were used for transient and stable transfection assays with F9 embryonal carcinoma cells. In addition, we have made fusion DNA constructs consisting Hox2.1 and E. coli lacZ gene for transgenic mice analysis. We have examined several Hox2.7 cDNA and genomic clones. Comparison with the human Hox2.7 cDNA sequence indicated that the Hox2.7 gene might be regulated by alternative splicing. Sequences at the 3' region of the Hox2.6 gene might be involved in this mechanism.

P 124 REQUIREMENT FOR PLC IN RAS-MEDIATED INDUCTION OF DNA SYNTHESIS, Mark R. Smith and Hsiang-fu Kung, BCDP,PRI and LBP, BRMP, FCRF-NCI, Frederick, MD 21701.
Peptide hormones bind to high affinity receptors on the cells outer membrane surface. The signal is transmitted through the lipid bilayer to inner surface G proteins that integrate many external stimuli into a concerted intracellular response that is mediated by second messengers, such as cAMP, Ca^{++}, and phosphoinositides (PI). Second messengers alter the metabolism of target cells. Several proto-oncogene products serve as intermediates in the signal transducing pathways that feed into mitogenesis and recent studies suggest a link between (PI) turnover and ras transformation. Ras and PI-specific PLC induce DNA synthesis after microinjection into quiescent NIH 3T3 cells. These intracellular mitogens can promote cell growth without hormone-receptor activation. Microinjection of anti-ras monoclonal antibody blocks normal cell division and the mitogenic signal of co-injected transforming ras protein. We have identified a monoclonal antibody that neutralizes the biological activity of both endogenous and injected PLC-γ. Co-injection of ras protein or PLC-γ, with this antibody inhibits induction of DNA synthesis. However, when transforming ras protein or PLC-γ were co-injected with ras neutralizing monoclonal antibody, only the DNA synthesis induced by the ras protein was inhibited, while that induced by PLC was not affected. Our results suggest that ras is an upstream effector of PLC activity in PI-specific signal transduction and that the ras-generated signal flows through PLC. Ras may be the putative G protein that couples polypeptide hormone activation of membrane receptors to PLC and the subsequent generation of intracellular second messengers.

P 125 cAMP STIMULATION OF Dictyostelium discoideum DESTABILIZES THE mRNA FOR 117 ANTIGEN, Gláucia M. Souza, Claudette Klein and Maria Helena Juliani, Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo 01498, Brasil.
Transcription of the 117 gene and changes in its mRNA levels in Dictyostelium discoideum were studied by mRNA hybridization with a cDNA probe. In wild type cells (Ax-2), the expression is developmentally regulated during cell aggregation, while in the aggregateless mutant, Agip 45, 117 mRNA is not detectable during cell starvation. Low concentrations of cAMP, given in the form of extracellular pulses to induce the development of starved Agip 45 cells to aggregation competence, are able to induce the appearance of 117 mRNA. The induction appears to be via the cell surface cAMP receptor and by a mechanism which does not involve changes in intracellular cAMP. Interestingly, high concentrations of cAMP, which down-regulate the cell surface cAMP receptor, elicit a rapid decrease in the level of 117 mRNA in aggregation-competent cells. Nuclear run-off and pulse-chase experiments show that the high concentrations of cAMP selectively destabilize the mRNA for 117 antigen. This destabilization requires both de novo mRNA synthesis and protein synthesis since the addition of inhibitors of these processes eliminates the effects of cAMP on 117 mRNA. The data suggest that a cAMP-induced protein(s) may be involved in the destabilization of selective mRNAs.
Supported by FAPESP, CNPq, FINEP, NIH.

P 126 EXPRESSION OF Dd*ras*G DURING EARLY DEVELOPMENT OF *DICTYOSTELIUM*
George B. Spiegelman, Steve M. Robbins, Meenal Khosla and Gerald Weeks, Departments of Microbiology and Medical Genetics, University of British Columbia, Vancouver, B.C. Canada V6T 1W5. *Dictyostelium* expresses two different *ras* genes at different stages of development. Dd*ras*G is expressed during growth and early development whereas Dd*ras* is expressed during multicellular development. We have examined some requirements for this differential expression.

In the wild type strain V12-M2, Dd*ras*G specific mRNA increased approximately 2 fold during the first 2-3 hours of development and then declined reaching negligible levels by the aggregation stage. The decline in expression did not occur when the cells were developed in the presence of cyclohexamide, suggesting that a developmentally regulated gene product is required for the decline in Dd*ras*G gene expression. In rapidly shaken cell suspensions Dd*ras*G mRNA did not decline during differentiation; however, the decline was restored when the cells were treated with pulses of cyclic AMP. The cyclic AMP mediated reduction of Dd*ras*G mRNA levels did not occur in the presence of caffeine suggesting that the effect required the adenylate cyclase part of the cyclic AMP signal relay pathway. In several axenic strains of *Dictyostelium* Dd*ras*G was expressed abnormally since the mRNA level remained high. Since these axenic strains develop normally, the presence of Dd*ras*G mRNA throughout development does not appear to be a deleterious event.

P 127 CELL CYCLE CONTROL IN HUMAN CELLS: REGULATION OF THE PRODUCT OF THE HUMAN CELL CYCLE CONTROL GENE CDC2Hs IN B-CELLS BY α–INTERFERON AND PHORBOL ESTER, N. Shaun B. THOMAS, Laboratory of Molecular Biology, Department of Haematology, University College and Middlesex School of Medicine, 98 Chenies Mews, London WC1E 6HX, ENGLAND. The product of the cell cycle control gene cdc2 is required in yeast for transition through both G_1 and G_2 control points of the cell cycle. The homologous protein in higher eukaryotes has been shown to be a component of the mitosis promoting factor (MPF) complex and may thus regulate entry through the G_2 control point into mitosis. It is suggested from the work presented here that, as in yeast, the human CDC2Hs gene product ($p34^{CDC2Hs}$) may also play a role in cell cycle control in the $G_1(G_0)$ phase of the cell cycle. Interferon-α (α-IFN) inhibits the growth of the human B cell line Daudi in the $G_1(G_0)$ phase of the cell cycle and prevents cells from entering S-phase. Culturing the cells with α–IFN inhibits the phosphorylation of $p34^{CDC2Hs}$ and causes the down-regulation of CDC2Hs mRNA. Phorbol ester also inhibits the Daudi cell cycle in $G_1(G_0)$ and causes the inhibition of $p34^{CDC2Hs}$ phosphorylation and a reduction of CDC2Hs mRNA. The second messengers which regulate $p34^{CDC2Hs}$ in higher eukaryotes have not been characterised. The studies presented here suggest that the regulation of $p34^{CDC2Hs}$ and the control of Daudi cell cycle progression through $G_1(G_0)$ to S phase may be mediated in part by the action of protein kinase-C. Funded by the Kay Kendall Leukaemia Trust and the Cancer Research Campaign.

P 128 ERYTHROPOIETIN RECEPTOR-MEDIATED ERYTHROID CELL DIFFERENTIATION. Kazuo TODOKORO, Satomi KURAMOCHI and Yoji IKAWA. Tukuba Life Science Center, The Institute of Physical and Chemical Reseach (RIKEN), Tsukuba, Ibaraki 305, JAPAN

Erythropoietin (Epo) is a cytokine that specifically regulates the proliferation and differentiation of erythroid cell lineage. To study the molecular mechanisms of Epo-induced erythroid cell differentiation and proliferation, we have investigated the molecular structure of Epo receptor and its physiological function, and the possible signal transduction pathways in cytoplasm and in nucleus. Several full length cDNA encoding Epo receptor were isolated from Epo-responsive and Epo-unresponsive mouse erythroleukemia cell lines, and their primary structure were determined. Detail analyses of the structural differences and the relationship to its physiological function are now under investigation. Possible signal transduction pathways were also examined, and our results suggest that Epo-induced erythroid differentiation is mediated, at least in part, thouth cAMP-dependent pathway. This conclusion is based on the facts that Epo stimulated cAMP production, and that cAMP-elevating agents could partially substitute for Epo action. Down-regulation of nuclear proto-oncogene c-myb expression is required for Epo induced erythroid differentiation, and its gene expression is regulated during differentiation/proliferation process in a tissue specific manner. Detail analyses of c-myb gene expression, in relation to cell differentiation and proliferaion, will be disscussed.

P 200 REGULATION OF BINDING TO THE c-fos SRE BY A PHOSPHOTYROSINE CONTAINING PROTEIN. S.H. Benedict and B.A. Benson. Division of Basic Science, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO 80206.

Stimulation of human peripheral T lymphocytes (PBL-T) with antigen or mitogenic agents causes the appearance of a DNA binding complex which specifically interacts with the serum response element (SRE) of the c-fos regulatory region. This complex appears within 10 min and is undetectable by 60 min. Nuclear extracts from resting PBL-T contain a protein which prevents the specific DNA binding complex from interacting with the SRE. This negative effector complex is phosphorylated on a tyrosine residue(s). When the cells are stimulated with mitogenic agents or antigen, the negative effector complex is rendered ineffective, and the SRE specific DNA binding complex is then able to bind DNA.

P 201 TARGETED GENE EXPRESSION IN DROSOPHILA MELANOGASTER,
Andrea H. Brand, Marcy Engelstein and Norbert Perrimon,
Department of Genetics, Harvard Medical School, Boston, MA 02115

We have developed a system that allows the selective expression of genes within individual cells or groups of cells in Drosophila. We have taken advantage of the observation that the yeast transcriptional activator, GAL4, activates transcription in flies, and only activates those genes bearing GAL4 binding sites within their promoters. To target gene expression, GAL4 transcription has been restricted to particular cells using two different methods: 1) a promoterless GAL4 gene has been randomly integrated in the Drosophila genome, bringing it under the control of various enhancers and promoters; 2) previously characterized cell- and tissue-specific promoters have been used to direct GAL4 transcription. We are establishing lines of flies in which GAL4 is expressed in a variety of tissue-specific patterns. Next, GAL4 binding sites have been inserted upstream of several genes of interest. We are introducing these genes into flies, which will then be crossed to GAL4-expressing flies. In this way, the genes will be ectopically expressed only in the cells where GAL4 is transcribed. We have placed under GAL4 control the gene encoding the A chain of diphtheria toxin (DT-A), with the aim of selectively killing individual cells or groups of cells. In organisms such as nematodes and grasshoppers the ability to selectively ablate cells has proved valuable in the study of cell lineage, cell-cell interaction and the respecification of the fates of cells surrounding the ablated cell. We will also use the GAL4 system to examine the effect on development of ectopic expression of "switch" genes, which control cell fate or identity. This has previously been approached by expressing switch genes under the control of the hsp70 promoter. Upon heat shock, however, the gene is widely and indiscriminately transcribed. In contrast, using the GAL4 induction system ectopic expression will be targeted to specific cells.

P 202 RESTRICTED EXPRESSION OF SPEC1 GENE HOMOLOGUES IN SEA URCHIN INTERSPECIES HYBRID EMBRYOS,
B.P. Brandhorst, P. Nisson*, M. Filion, M. Gaudette*, and W.R. Crain*, Department of Biosciences, Simon Fraser University, Burnaby, B.C., CANADA V5A 1S6, and *The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The gene encoding Spec1, a calcium binding protein, is expressed only in the aboral ectoderm cells of Strongylocentrotus purpuratus embryos. In reciprocal interspecies hybrid crosses, there is considerably reduced expression of the Lytechinus pictus homologue of Spec1 (LpS1), but normal levels of Spec1 protein synthesis. This restriction is due to reduced LpS1 transcript prevalence associated with reduced transcription of the LpS1 gene in hybrid embryos (based on a run-on assay in isolated nuclei); Southern blots of hybrid embryo DNA indicated no detectable loss of LpS1 DNA. In situ hybridization analysis of serial sections indicates that most embryos express only the Spec1 gene, some (about 1% at pluteus stage in an Lp eggxSp cross) express only the LpS1 gene, and a few (about 1%) express both genes actively; in these, there are patches of aboral ectoderm cells positive for one or the other transcript, but not both. Thus the restricted expression of LPS1 is due to exclusive expression of Spec1 genes in most cells, with a small number of clones expressing only LpS1. At earlier stages some cells appear to include transcripts of both gene homologues, but most cells express Spec1 transcripts exclusively.

P 203 GANGLIOSIDE GM1 SIGNALLING ENHANCES C-FOS PROMOTER ACTIVITY.
Nadia Gabellini, Laura Facci*, Alessandro Negro, Stephen D. Skaper*, Alberta Leon* and Lanfranco Callegaro. Departments of Molecular Biochemistry and Central Nervous System Research*, Fidia Research Laboratories, Via Ponte della Fabbrica 3/a, 35031 Abano Terme, Italy.

Differentiation and proliferation in response to extracellular stimuli require rapid and transient activation of c-fos transcription. When cultured astrocytes are treated with B subunit of cholera toxin, which specifically binds to and cross-links ganglioside GM1, the cells undergo a rapid morphological change and growth inhibition. B subunit can also either inhibit or stimulate growth of 3T3 fibroblasts, depending upon their growth state. In this investigation we asked whether transmembrane signal involving GM1 as a receptor can activate c-fos expression. Northern blot analysis showed that c-fos mRNA rapidly increased after treatment of astrocytes with B subunit. Transient transfections of astrocytes and fibroblasts with a plasmid carrying the bacterial gene chloramphenicol acetyltransferase (CAT) under the control of the c-fos promoter were also performed. A 4.5-fold induction of CAT activity was detected when transfected astrocytes were treated with B subunit, comparable to the induction by isoproterenol. In fibroblasts, B subunit stimulated transcription from the c-fos promoter 2.3-fold. Enhancer elements in the c-fos promotor that respond to serum and growth factors, and others linked to second messengers like c-AMP and Ca^+ have been identified. The c-fos promotor region responsible for induction by B subunit is being investigated by transfection of a series of deletion mutants of the c-fos promotor-CAT gene construct.

P 204 CONCENTRATION OF A MESODERM INDUCING FACTOR IS SUFFICIENT TO INSTRUCT CELL TYPE IN *XENOPUS* J.B.A. Green & J.C. Smith, Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

XTC-MIF, a mesoderm inducing factor from a *Xenopus* cell line, can induce responding animal pole cells to form several distinct cell types in a concentration- dependent manner (1). At least two sharp thresholds in the response of single cells to varying XTC-MIF dose have been found (discussed by the authors elsewhere at this meeting). Cell mixing experiments show that the type of response is essentially cell autonomous and not dependent on population or averaging effects. Thus, cells can record and "remember" the dose of XTC-MIF they have experienced. This demonstrates that concentration alone can carry sufficient information and is instructive in specifying cell type. Plasticity of this specification and strength of "community effects" will be discussed

(1) Green, J.B.A. *et al.* (1990) The Biological effects of XTC-MIF: A Quantitative comparison with *Xenopus* bFGF. *Development* 108 (in press).

DEVELOPING T LYMPHOCYTES, Ellen V. Rothenberg, Thomas J. Novak, Dan Chen, Karen A. Pepper, Julia A. Yang, and Rochelle A. Diamond, Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125

Interleukin-2 (IL-2) is a potent growth and differentiation hormone synthesized transiently by a subset of T cells in response to antigen stimulation. Calcium ionophores (e.g., A23187) with phorbol esters (e.g., TPA) can mimic the natural stimulatory signal sufficiently well to allow polyclonal T-cell populations to express the IL-2 gene in the absence of specific antigens. This has enabled us to explore the developmental process whereby T-cell precursors acquire the capacity to transcribe IL-2 RNA in response to A23187 and TPA, and the extent to which this capacity is segregated to a particular T-cell lineage. Our results show that at different stages, T cells in fact require different signals to induce and maintain expression of this gene. In immature cells, IL-1 must be added as a costimulant, although it has no effect in mature cells. In mature cells, pharmacological stimulation elicits sustained IL-2 production from "non-IL-2 producer" lineages as well as from appropriate T-cell subsets. Thus, development of the ability to express IL-2 appears to reflect alterations in the coupling of transcriptional activation with particular signaling mediators. Induction of IL-2 appears to require a particular class of activation signals, because trans-acting factors that promote IL-4 expression are not sufficient to induce expression of IL-2 regulated recorder genes in transient transfection assays. We have mapped potential regulatory regions, both positive and negative, up to 2 kb upstream of the IL-2 transcription start site, but all seem to be subordinate to a common, but highly modulated, activation signal. Protein-DNA binding studies are now in progress to identify key complexes that may be preferential targets of this regulation at different stages of development.

P 206 CHARACTERIZATION OF EMBRYONIC GENE EXPRESSION IN *C. elegans* BY RUN-ON TRANSCRIPTION, Irene E. Schauer and William B. Wood, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, 80309

We have developed a nucleotide incorporation assay for run-on transcription in *C. elegans* embryonic extracts as an approach to characterizing early transcription. The incorporation is 85-90% alpha-amanitin sensitive and 100% rifampicin resistant, indicating primarily pol II-catalyzed RNA synthesis. The reaction produces transcripts in the size range expected for mRNAs and at rates comparable to those expected *in vivo*. The incorporation is insensitive to inhibitors of reinitiation, indicating that the activity represents primarily elongation of nascent chains initiated prior to extract preparation. Results of hybridizing labeled run-on transcripts from extracts of staged embryos to a set of known cloned genes suggests that the specificity of the *in vitro* reaction accurately reflects developmentally regulated *in vivo* transcription.

Comparative analyses of transcription in extracts from various stages indicates that pregastrulation embryos are already fully active transcriptionally and that the level of transcription per nucleus is approximately constant throughout embryogenesis. Furthermore most embryonically expressed genes are already being transcribed in pregastrulation embryos. We have also used labeled run-on transcripts as probes to screen both genomic and cDNA libraries for sequences that are transcribed preferentially in pregastrulation embryos. There appears to be only a small set of such genes most or all of which are initially transcribed in the germ line prior to fertilization. We have isolated several of these genes for further characterization of their products and their roles in early embryogenesis.

P 207 THE SEGMENT POLARITY GENE, ZESTE-WHITE 3, ENCODES A FAMILY OF SERINE/THREONINE KINASES. Esther Siegfried, Liz Perkins, Theresa Capaci and Norbert Perrimon, Department of Genetics, Harvard Medical School, Boston, MA 02115

zeste-white-3 (zw3) is involved in at least two developmental processes, embryonic segmental patterning and differentiation of adult sensory structures. Lack of maternally provided zw3 product causes embryonic lethality resulting in a segment polarity phenotype identical to mutations of the zygotic gene naked. Homozygous zw3 cells induced within wild type imaginal tissue undergo a change in cell fate where epidermal cells assume a neural fate as seen by the induction of ectopic sensory bristles in the thorax and wing. We have cloned zw3 and determined that it encodes a family of proteins that are serine/threonine protein kinases. These related proteins are encoded by multiple transcripts that are generated by developmentally regulated alternative splicing. All of the transcripts have a common catalytic domain for a serine/threonine kinase. The study of zw3 and other segment polarity genes, and their products, will reveal the processes of cell communication that are necessary to establish pattern in the developing organism.

P 208 CLONING OF TRANSIENTLY INDUCED EARLY mRNAS IN DIFFERENTIATING ADIPOCYTES, Pamela J. Smith, Veterans Administration Medical Center, E. Orange, N.J. 07019 (UMDNJ). Confluent murine 3T3-L1 preadipocytes differentiate into adipocytes when treated with dexamethasone and methylisobutylxanthine (MIX). When grown in hormone depleted medium, treatment with 10-20nM IGF-I (or 2uM insulin) is required to elicit differentiation. It is likely that the process of adipose differentiation involves progression through a differentiation program under the control of one or more "adipogenic" regulatory genes, some of which may be hormone/growth factor dependent. In order to identify potential regulatory genes for adipogenesis, cloning of mRNAs which are transiently induced during early stages of adipocyte differentiation was undertaken. A cDNA library was constructed in the LambdaZapII vector from polyA+RNA isolated from 3T3-L1 cells after 35 hours of treatment with dexamethasone, MIX and IGF-I and screened with a subtracted cDNA probe. Approximately 20 induced signals per 10,000 recombinants were detected. Time course of expression and mRNA sizes were determined by Northern analysis for several representative cDNAs: 3 mRNAs (sizes 1.8, 1.1, and .95 kb) were identified which were expressed in preadipocytes and displayed increased mRNA levels by Day 1, peak induction by Day 2 with return to or below baseline by Day 4. The role of these and other "early" adipocyte mRNAs in the regulation of adipogenesis is now being studied.

P 209 EVIDENCE THAT THREE COPIES OF A SEX-DETERMINING GENE IN *C. ELEGANS* RESULTS IN HERMAPHRODITIZATION AND LETHALITY OF XO MALES,

Kimberly Tanner and William B. Wood, MCD Biology, University of Colorado, Boulder, CO 80309

Duplications including the sex-determination gene *sdc-2* result in a high incidence of XO lethality with the surviving XO animals variably transformed toward hermaphrodites. Three mutations, *ct28, ct30,* and *ct31*, were isolated as gamma-ray-induced suppressors of a masculinizing mutation, *her-1(n695)*. Characterization by genetic analysis and quantitative Southern blots indicates that all three are large X-linked, probably tandem X-chromosome duplications. Each has been shown to include the *sdc-2* locus. Their sizes can be estimated from other known markers that are included: *ct30* duplicates between 14% and 24% of the X chromosome; *ct28* and *ct31* duplicate between 24% and 57%.

The phenotypes resulting from the duplications are virtually indistinguishable, although they probably duplicate different amounts of X chromosome. Hermaphrodites (XX) carrying two copies of the duplication are slow-growing and dumpy. 45% of XO animals carrying one copy of the duplication die as embryos. Of the surviving XO animals, 18% attempt to form a vulva, 8% produce oocytes, and 77% have a defective male bursa.

This phenotype is very different from that of another large X-chromosome duplication, *mnDp10*, that covers much of the same region as *ct28* and *ct31* (between 16% and 34% of the X chromosome), but does not include *sdc-2*. XO animals carrying one copy of *mnDp10* are healthy, mating males; XX animals carrying two copies of *mnDp10* are healthy, nondumpy hermaphrodites. We present evidence that the defects resulting from *ct28, ct30,* and *ct31* are primarily due to increased copy number of the *sdc-2* locus.

P 210 **The Ontogeny of α-fetoprotein Expression in the Mouse Gastrointestinal Tract,** Angela L. Tyner, Roseline Godbout, and Shirley M. Tilghman, Biology Department, Princeton University, Princeton, NJ 08544-1014. The mouse gastrointestinal tract provides an excellent system for studying aspects of epithelial cell differentiation during development. In the adult intestinal tract, monoclonal stem cells localized at the base of the crypts give rise to proliferative cells that differentiate into at least four different cell types: villus columnar cells (enterocytes), goblet cells, enteroendocrine cells, and Paneth cells. In order to gain a better understanding of the mechanisms underlying this differentiation, we have examined the ontogeny of α-fetoprotein (AFP), the major serum protein expressed in the mammalian fetus, in the fetal and adult mouse gastrointestinal tract. AFP mRNA constitutes approximately 0.1% of total mRNA in the fetal gut. The transcripts were localized by in situ hybridization to the most of the epithelial cells lining the villi of the fetal gut. At birth, AFP mRNA declines rapidly to achieve low adult basal levels, which are not affected by different alleles of *raf*, a gene that determines the adult basal level of AFP mRNA in the liver. The basal level in the adult gut is the consequence of continued AFP transcription in a small number of epithelial cells that are distributed infrequently on the villi. These cells were identified as enteroendocrine cells by double antibody staining with antibodies to chromogranin A, a marker for the diffuse neuroendocrine system and AFP. The cis-acting DNA sequences required for expression of the AFP gene in the gut were investigated using Caco-2 cells, a human colon adenocarcinoma cell line. It was determined by transient transfection assays in Caco-2 cells that all of the cis-acting regulatory elements previously identified in hepatoma cell lines are also utilized in the intestinal cells. The Caco-2 cells, which differentiate in culture, provide a unique in vitro system for studying the factors involved in regulating the expression of AFP in the gastrointestinal tract.

P 211 **GENE ACTIVATION DURING EARLY DEVELOPMENT OF THE MARINE MOLLUSC *Patella vulgata*.** André E. van Loon [1,2], Hans Goedemans [1], Ineke Daemen [1], Arno van de Kamp [1], Pierre Colas [2], Pierre Guerrier [2], Jo A.M. van den Biggelaar [1], [1]Dept. Experimental Zoology, State University at Utrecht, Padualaan 8, 3584 CH UTRECHT, The Netherlands, [2] Ecole Normale Superieure, 46, Allée d'Italie, 69364 LYON, France.

We have cloned and sequenced actin, tubulin and cyclin cDNAs from mRNAs which are expressed during the early development of *Patella vulgata*. The three gene families differ drastically in expression patterns. Tubulin mRNAs (both a and b) are present at very low levels during the first 3.5 hrs after fertilization. After the induction of the mesoderm-stem cell (between 3.5 and 5 hrs after fertilization) the level rises 100x. In contrast actin mRNAs are present at very low levels until after mesoderm induction. Then the mRNA level increases steadily to reach a plateau 12 hours after fertilization. The expression of different actin genes could be identified with the 3' untranslated regions of the corresponding cDNAs. These regions were used to show that 2 of the 3 genes examined in this way differ from the general actin expression pattern. Cyclin mRNAs on the other hand are present in large amounts as maternal mRNAs and are less abundant after mesoderm induction. Together these data suggest that mesoderm induction is a key point in the transcription of the zygote genome during early development, similar to the mid-blastula transition in *Xenopus*.

P 212 **Molecular Cloning of a Human gene, A1S9, which Complements the Temperature Sensitive S Phase Specific Mutation ts A1S9 of Mouse L-Cells.** Zacksenhaus E. and Sheinin R. Department of Microbiology, 150 College St. University of Toronto, Toronto, Ont. M5S 1A8. The ts A1S9 mouse L-cell is temperature-sensitive (ts) for growth due to a defect affecting the progression of cells through the S phase of the cell cycle. We used a DNA-mediated gene transfer approach to clone the complementing human A1S9 gene. A human DNA region (42 kb) encompassing the gene, A1S9, which corrects the ts A1S9 cell defect was identified (Zacksenhaus and Sheinin SCMG 1988), cloned (Zacksenhaus and Sheinin SCMG 1989) and mapped to the human X chromosome at Xp11.2-p11.4 (Zacksenhaus et al CCG 1989). A unique fragment, E2.2, derived from the A1S9 locus was found to contain coding sequences. Northern blot analysis indicates that the steady state level of A1S9 transcripts is invariable throughout the cell cycle. The E2.2 probe has been used to isolate cDNA clones from eukaryotic expression libraries. A clone containing a 2.6 kb DNA insert complements the ts A1S9 cell defect following transfection and temperature selection. Preliminary sequence analysis and search in DNA sequence data bank suggests that A1S9 is a novel gene.

Pattern Formation; Cell Surface Receptors

P 300 GENES INVOLVED IN THE DEVELOPMENT OF DROSOPHILA SALIVARY GLANDS, Steven K. Beckendorf, Scott Panzer, Karen Christie and Bing Zhou, Department of Molecular and Cellular Biology, University of California, Berkeley, CA 94720

In Drosophila embryos, cells that will form the salivary glands are first distinguishable at six hours as a circular placode of thickened cells in the ventral part of the most posterior head segment. Shortly afterward, the posterior edge of the placode sinks inward and the cells invaginate to form the tubular salivary glands. After the initial commitment, differentiation to active secretory cells occurs within four hours, suggesting that there may not be very many intervening steps. To explore this developmental pathway, we have started identifying genes acting early in salivary gland development.

So far, we have characterized 20 enhancer trap lines that express ß galactosidase at this early stage. In addition, several genes known to be involved in pattern formation or signal transduction are expressed preferentially in the placode and the developing gland. These include *Notch, Toll* and *forkhead.* Using these genes and the enhancer trap lines we have begun to construct a developmental pathway for salivary gland development.

P 301 THE MURINE PAIRED BOX GENE FAMILY, Urban Deutsch, Gregory R. Dressler, Martyn Goulding, Claudia Walther, Rudi Balling, and Peter Gruss, Max Planck Institute of Biophysical Chemistry, Department of Molecular Cell Biology, 3400 Göttingen, F. R. Germany.

A new family of murine genes similar to Drosophila paired box containing segmentation genes has been identified and 8 members of this family have been isolated. Each of these genes maps to different chromosomal loci. Based on similarities and the presence of a paired type homeo box the paired box gene family can be subdivided into subgroups, analogous to the Drosophila genes. In situ expression analysis of some of these genes reveals their restricted transcriptional activity during mouse embryogenesis. The Pax1 (Paired Box 1) gene is expressed in the developing vertebral column, the sternum and the thymus and has been shown to be mutated in the mouse skeletal mutant *undulated.* Pax2 is expressed in the developing excretory system and shows spatially and temporally restricted activity during murine neurogenesis. Pax3 is also expressed in a restricted pattern in the developing central nervous system and in the dermomyotome of segmental mesoderm. The structure of cDNAs for Pax1, Pax2 and Pax3 and their presumptive proteins will be presented.

P 302 SPATIAL EXPRESSION OF THE *DICTYOSTELIUM DISCOIDEUM RAS* GENE FOLLOWS A DETAILED DEVELOPMENTAL PROGRAM, R. Keith Esch and Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, Univ. of California, San Diego, La Jolla, CA 92093

Dictyostelium contains two *ras* genes. One, *Dd-rasG*[1], is expressed only during growth. *Dd-ras*[2], on the other hand, is expressed at low levels during vegetative growth, ceases to be expressed with the onset of starvation, and is then induced to high levels in multicellular stages and by cAMP in single-cell culture. Expression of an activated *Dd-ras* (*ras*-Thr_{12}) results in abnormal development.[3] Using a *Dd-ras*/lacZ fusion, we have found that *Dd-ras* directed β-gal expression is restricted to the prestalk regions during the multicellular stages of development. During late aggregation, approximately 10-20% of the cells stain. These are randomly located within the developing aggregate. As the aggregate continues to differentiate, these β-gal positive cells sort to the top of the aggregate and are only found in the anterior prestalk zone of migrating slugs. At stages immediately before and after the slug stage, staining is also seen at the base of the multicellular structure. We will show the detailed spatial pattern of *Dd-ras* expression and follow its progression through development. S1 nuclease protection experiments reveal two transcription start sites that show different temporal regulation of expression and respond differently to cAMP in fast shaking cultures. Using deletions, we have separated the promoters and shown that they can be expressed independently in development. Experiments are in progress which will identify any differences in spatial regulation of the two *Dd-ras* promoters.

1. Pawson *et al.* (1985). Mol. Cell. Biol. *5*:33-39.
2. Reymond *et al.* (1984). Cell *39*:141-148.
3. Reymond *et al.* (1986). Nature *323*:340-343.

P 303 DEVELOPMENTAL REGULATION OF pDd63, A PRE-STALK SPECIFIC GENE IN *DICTYOSTELIUM DISCOIDEUM*, Michael J. Gaskell, Donald J. Watts* and Jeffrey G. Williams, Imperial Cancer Research Fund, South Mimms, Potters Bar, Hertfordshire EN6 3LD, England. *Dept. of Biochemistry, University of Sheffield, England.
pDd63 encodes an extracellular protein specific to the pre-stalk zone in developing cell aggregates of the cellular slime mold *Dictyostelium discoideum*. We have been attempting to identify the region(s) in the 5' flanking sequence responsible for the developmental expression of pDd63 by construction of deletion series in both the 5'-3' and 3'-5' directions within the 5' flanking region, using choramphenicol acetyl transferase (CAT) and luciferase as reporter genes. pDd63, in conjunction with a related pre-stalk specific gene pDd56, has been used to investigate the interrelationship of DIF (Differentiation Inducing Factor) and cAMP. DIF is a specific pre-stalk gene inducer, while cAMP acts both as a DIF antagonist and as a pre-spore gene inducer. Finally we have been using the counter current distribution technique to provide further evidence for the existence of sub-populations of cells within the overall pre-stalk zone in developing aggregates of *D. discoideum*. (see Jermyn *et. al.* Nature (1989) 340 144-146.)

P 304 PLASMID RESCUE USING E.COLI METHYLATION-RESTRICTION MUTANTS ALLOWS EFFICIENT CLONING OF TRANSGENIC MOUSE DNA AND IMPLICATES DNA MODIFICATIONS THAT CORRELATE WITH DEVELOPMENTAL TIMING OF TRANSGENE EXPRESSION,S.Grant[1,2], J.Jesse[3], F.Bloom[3], D. Hanahan[1,4]. [1]Cold Spring Harbor Lab. [2]Columbia University, [3]Bethesda Research Lab, [4]U.C. San Franscisco. To "rescue" plasmids integrated in the genome, transgenic mouse DNA is digested with a restriction enzyme, ligated to favor cyclization, transformed into E.coli and selected for antibiotic resistance. Previous experiments suggest that plasmid DNA grown in mammalian cells is modified and subsequently restricted in common E.coli strains used for transformation, resulting in inefficient plasmid rescue. We made mutations in E.coli methylation sensitive restriction systems (McrA, McrB, McrC and Mrr) and assessed plasmid rescue efficiency from four lineages of transgenic mice carrying insulin promoter-SV40 T antigen (Tag) hybrid gene constructs. Two "fast" lineages express Tag from embryonic day 10 and two "slow" lineages express Tag after 10 weeks of age. DNA from "fast" mice evidence efficient plasmid rescue into E.coli strains carrying mutations in both mcrA and mcrB, whereas plasmid rescue from "slow" mice requires mutations in all known methylation sensitive restriction systems. Differential cloning frequencies in the presence or absence of the various methylation dependent restriction genes represents a new way to distinguish modified regions of mammalian chromosomes and implicates these modifications in developmental timing of gene expression.

Abstract Withdrawn

P 306 IDENTIFICATION OF *CIS*-ACTING ELEMENTS THAT ARE INVOLVED IN THE DEVELOPMENTAL INDUCTION, cAMP INDUCTION, AND SPATIAL REGULATION OF SP60, A PRESPORE GENE FROM *DICTYOSTELIUM DISCOIDEUM*; EVIDENCE FOR A SPATIAL GRADIENT OF MORPHOGEN RESPONSE, Linda Haberstroh and Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, UCSD, La Jolla, CA 92093

The gene encoding SP60, a spore coat protein of *Dictyostelium discoideum*, has been cloned and its promoter analyzed. We made SP60/luciferase reporter gene fusions and used these to examine cAMP and temporal regulation during multicellular development in stable transformants. Deletion analysis has revealed three important regions (80 to 100 nt), each containing a CA-rich element (CAEs) of consensus:

$$CACACA^{CC}_{TTA}ACACAC.$$

Sequential 5' deletion of these elements results in quantum 20- to 50-fold drops in SP60/luciferase activity, either during development or in response to cAMP. Spatial regulation of SP60 was studied using SP60/*lacZ* fusions. Unexpectedly, we found that sequential deletion of the CAE containing sequences has profound effects on spatial localization of the fusion protein. While constructs containing 650 nt of 5' flanking region and all three CAEs show the expected uniform staining of the posterior, prespore zone of the slug, deletion of the first (5' most) CAE and surrounding sequence results in a gradient staining pattern from the anterior to the posterior of the prespore zone with very little to no staining in the posterior. Deletion of two of the three CAE containing sequences results in a further restriction of the staining pattern to the prespore zone immediately adjacent to the prestalk/prespore border. Again, staining is present as a gradient. These weaker promoters may be unmasking an extracellular morphogenic gradient reflected as intracellular transcription factor gradient across the prespore zone within slugs.

P 307 CHARACTERISATION OF *ULTRABITHORAX* EXPRESSION IN THE DEVELOPING LOCUST EMBRYO, Robert N. Kelsh[1], Michael Akam[1], Robert O.J. Weinzierl[2], and Rob White[2], Department of Genetics[1] and Department of Anatomy[2], University of Cambridge, Downing Street, Cambridge CB2 3EH, England.

Drosophila is a long-germ insect and is holometabolous (i.e. undergoes complete metamorphosis between immature and adult stages). By contrast, more primitive insects e.g. Orthopterans (locusts, grasshoppers and their allies) are short-germ insects and display hemimetaboly (i.e. incomplete metamorphosis). The homoeotic genes of *Drosophila* encode DNA-binding proteins which function to specify segment identity. They are expressed at high levels in the imaginal discs which will form the adult epidermis. However study of homoeotic expression in the discs is hindered by their complex folded morphology. In hemimetabolous insects there are no imaginal discs, the adult structures such as limbs being formed directly during embryogenesis. We are looking at the expression of homoeotic gene homologues in one such primitive insect, the locust *Schistocerca gregaria*, in order to compare and contrast the timing and position of their expression between these two very different modes of embryogenesis. One monoclonal antibody, FP6.87, generated against *Drosphila Ubx* protein, recognises a homoeodomain epitope shared by both *Ubx* and *abd-A*. This antibody cross-reacts with the *Ubx* and *abd-A* proteins in *Schistocerca*. In the third thoracic limb *abd-A* is not expressed and so FP6.87 can be used to follow *Ubx* expression here. A shifting pattern of *Ubx* expression is observed with an initially distal region of expression developing with time to form a complex pattern of multiple rings around the limb. The hemimetabolan mode of development thus facilitates examination of insect limb development because the complex morphology of imaginal discs is replaced by a simple tube.

P 308 CONTROL OF INTRA-PARASEGMENTAL PATTERNING: ANALYSIS OF THE PAIR RULE GENE, *ODD-SKIPPED*. Janet R. Mullen and Stephen DiNardo, The Rockefeller University, New York, NY 10021.

During early *Drosophila* development, a group of genes known as the segmentation genes, acting in both a hierarchical and combinatorial fashion, catalyze the subdivision of the embryo along the anterior-posterior axis into 14 developmental units called parasegments. Expression in transverse stripes of two segment polarity genes, *engrailed* (*en*) and *wingless* (*wg*), define the anterior and posterior boundaries of each parasegment, respectively. Precise expression of both *en* and *wg* is crucial for generation of the final body pattern. Activation of *en* requires the pair-rule genes *even-skipped* (*eve*) in odd parasegments and *fushi tarazu* (*ftz*) in even parasegments. These activators are transiently expressed across three-quarters of each parasegment, yet *en* expression is restricted to the anterior one-quarter of each parasegment. We are interested in determining how this kind of intra-parasegmental patterning arises.

Embryos mutant for the pair-rule gene *odd-skipped* (*odd*) are unable to restrict *en* expression to the wild type domain and display double width *en* stripes in even parasegments early in development. Later in embryogenesis, while these stripes are narrowing (approaching a normal stripe width), *en* is induced in an ectopic stripe just posterior to the originally widened *en* domain. Recent work in our lab has revealed that *ftz* transcripts show a dramatically altered expression pattern in *odd*$^-$ embryos, indicating that *odd* formally acts as a repressor of *ftz*.

Embryos mutant for the pair-rule gene *odd-paired* (*opa*) also display a defective *en* pattern in that *en* stripes in even parasegments are either very weak or missing. One interpretation of this is that even *en* stripes may require the combinatorial activation of both *ftz* and *opa*, explaining why *en* is not expressed across the entire *ftz* domain. The effect on *en* in *odd*$^-$ embryos may also be explained by hypothesizing that *odd* may repress *opa* as well as *ftz*. In the absence of *odd*, *opa* would then expand posteriorly, leading to activation of *en* in all cells expressing both co-activators (*ftz* and *opa*). We are currently investigating such possibilities in order to unravel the mechanisms responsible for the precise control of *en* in even parasegments.

P 309 IDENTIFICATION AND ANALYSIS OF SEGMENTATION GENES IN A SHORT GERM BAND INSECT (GRASSHOPPER), Nipam H. Patel and Corey S. Goodman, HHMI, Dept. of Molec. and Cell Biology, Univ. of California, Berkeley, CA 94720

During the early development of a long germ band insect, such as *Drosophila*, the interaction of segmentation genes results in a projection of the entire body plan at the blastoderm stage and in the simultaneous appearance of all the *engrailed* stripes. In contrast, the segments of a grasshopper embryo, a short germ band insect, form one at a time as the embryo elongates; in these embryos the *engrailed* stripes appear one at a time. This suggests that the mechanisms generating the *engrailed* pattern might differ between long and short germ band insects. One of the genes required for the proper pattern of *engrailed* expression during *Drosophila* segmentation is the pair-rule gene *even-skipped*. We have isolated the grasshopper homologue of the *Drosophila even-skipped* gene and are determining its expression pattern. *Even-skipped* is also required during *Drosophila* nervous system development and since neurogenesis is almost identical between grasshopper and *Drosophila*, we expect that the pattern of expression during neurogenesis will be conserved. By examining the expression pattern of this gene during grasshopper segmentation, we will be able to determine if pair-rule genes are also utilized during the process of segmentation in both long and short germ band embryos.

P 310 MUTATIONS IN *torpedo*, THE DROSOPHILA EGF RECEPTOR HOMOLOG, INTERACT WITH MUTATIONS IN *Star*. James V. Price and Trudi Schupbach, Department of Biology, Princeton University, Princeton NJ 08544. Mutations in *torpedo* , the Drosophila homolog of the vertebrate EGF receptor (EGFR), cause a variety of defects, including embryonic and postembryonic lethality, and effects on wing vein pattern, eye morphology and bristle pattern of adults. Females homozygous for viable alleles of *torpedo* are sterile due to an alteration of the dorsal/ventral coordinates built into the egg by the female during oogenesis. Mutations in *Star* cause a dominant rough eye phenotype and recessive embryonic lethality. Mutations in *Star* enhance the severity of defects caused by mutations in *torpedo*. Defects in the wing vein pattern, bristle pattern and the dorsal/ventral pattern of eggs laid by adults homozygous for *torpedo* mutations, all become more severe when the wild-type activity of *Star* is reduced. The eye defects caused by mutations in both *Star* and *torpedo* are much more severe than the eye defects produced by mutations in either gene alone. Combinations of *torpedo* alleles that would normally be viable are lethal in the presence of a mutation in *Star*. We have quantified these effects for numerous combinations of *Star* and *torpedo* alleles. Since mutations in *Star* enhance most aspects of the *torpedo* phenotype we believe that the *Star* gene product is a good candidate for a participant in the EGFR homolog signal transduction pathway. We are now using mosaic analysis to determine whether *Star* interacts with *torpedo* in an intercellular fashion (as would be expected for a ligand) or in an intracellular fashion (as would be expected for a substrate or other cytoplasmic components of the EGFR homolog signaling pathway).

P 311 Wing formation in *Drosophila melanogaster* requires *decapentaplegic* gene function along the anterior-posterior compartment boundary. Laurel A. Raftery, Leila M. Posakony*, Ronald K. Blackman, R. Daniel St. Johnston*, and William M. Gelbart, Harvard University, Cambridge, MA 02138.

The *decapentaplegic* gene (*dpp*) acts in dorsal-ventral patterning of the adult fly. All characterized *dpp* transcripts contain the same ORF, which encodes a polypeptide that is related to the vertebrate TGF-ß family of secreted factors (Nature 325, 81, 1987; R.W. Padgett, unpublished data). We describe here the spatial distribution of *dpp* transcripts in the imaginal disks (anlage for adult structures) and compare this to the localized requirement for dpp^+ expression for normal proximal-distal appendage development. In the wing imaginal disk, *dpp* transcripts are expressed in a stripe of cells at or near the known anterior-posterior compartment boundary (A/P boundary). Similarly, *dpp* is expressed in localized regions of all other imaginal disks of the larva. An analysis of flies mosaic for dpp^+ and dpp^- tissues has demonstrated that for normal wing blade development dpp^+ function is required only in the cells on the anterior side of the A/P boundary. We use *engrailed* expression as a marker for the posterior compartment in the disks, in order to assess the precise relationship of the region of *dpp* expression to the A/P boundary. We discuss the possibility that *dpp* plays a role in establishing or maintaining positional information in the disks.

P 312 REGION-SPECIFIC ENHANCERS NEAR TWO MAMMALIAN HOMEOBOX GENES DEFINE ADJACENT ROSTROCAUDAL DOMAINS IN THE CENTRAL NERVOUS SYSTEM
Christopher K. Tuggle*, Jozsef Zakany+, Luciano Cianetti[X], Cesare Peschle[X], and M. Chi Nguyen-Huu*. *Dept. of Microbiology, Univ. of Southern California, 1441 Eastlake Avenue, Los Angeles, CA 90033, +Institute of Genetics,Hungarian Acad. of Sciences, P.O. Box 521, 6701 Szeged, Hungary, [X]Dept. of Hematology-Oncology, Istituto Superiore di Sanita, Viale Regina Elena 299, I-00161 Rome, Italy.

To gain insight into the mechanisms underlying region-specific gene expression in mammalian development, we have investigated the regulatory DNA associated with the proximal promoter of two homeobox genes, murine Hox 1.3 and human Hox 5.1. Using lacZ gene fusions in transgenic mice, we have identified regulatory elements in the 5' flanking sequences of the Hox 1.3 and the Hox 5.1 genes which specifically direct ß-galactosidase expression to the brachial and the upper cervical regions (respectively) of the central nervous system (CNS). These two elements act at the transcriptional level, are active in either orientation, and confer region-specific expression onto unrelated promoters, satisfying the criteria for enhancer elements. The two spatial domains defined by these enhancers are directly adjoining, extend along the rostro-caudal axis for the same span of 6-7 metameres, and represent specific subsets of the overall CNS regions expressing all endogenous Hox 1.3 or Hox 5.1 transcripts. The adjacent domains in the developing murine CNS that express Hox 1.3 and Hox 5.1 gene fusions are strikingly reminiscent of the adjacent stripes of expression in Drosophila embryos seen with Sex combs reduced and Deformed, the two Drosophila homeotic genes most homologous to Hox 1.3 and Hox 5.1, respectively. These findings represent the first demonstration of region-specific mammalian enhancers and raise the possibility that the mammalian CNS may be subdivided into a series of rostro-caudal domains based on the activity of enhancers near homeobox genes.

P 313 IMMUNOLOCALIZATION OF THE wingless PROTEIN IN Drosophila melanogaster EMRYOS.
Marcel van den Heuvel, Peter A. Lawrence*, Norbert Perrimon+, John Klingensmith+ and Roel Nusse. Dept. of Molecular Biology, the Netherlands Cancer Institute, Amsterdam, the Netherlands (as of 3-1-1990: Dept. of Developmental Biology, Beckman Center, Stanford Univ., 94305, Ca); * Medical Research Council, Cambridge, UK; + Dept. of Genetics, Harvard University, Boston, Ma.

wingless is a segment polarity gene that is required for normal development of every segment of the embryo. int-1, the homolog in mouse, is specifically expressed in the developing neural tissue in embryos. We made antibodies against the wingless protein that localize the gene product in Drosophila embryos. In the trunk region every posterior border of the parasegment is marked; double staining with engrailed antibodies confirmed this. Using EM the protein can be seen in different organelles in the wingless expressing cells and in the extracellular matrix (in coll. with Dr. P. Lawrence). The localization of the wingless protein in the matrix and the morphology of the organelles suggest that the protein behaves as a paracrine signal. It seems likely that the protein will bind to a receptor on the cell surface.

We are now studying the possible regulation of engrailed by a wingless signal in different mutants. Gene products specifically involved in the transduction of the signal can be determined. The maternal effect segment polarity gene porcupine indeed shows wingless staining and disappearance of engrailed. This mutant together with dishevelled and armadillo forms a group of segment polarity mutants that give phenotypes indistinguishable from the wingless phenotype in germ line clones. In dishevelled and armadillo mutant embryos both wingless and engrailed antigens are lost during development (in coll. with Dr. N. Perrimon and John Klingensmith).

P 314 TRANSCRIPTIONAL ANALYSIS OF THE MURINE HOX 3.2 HOMEOBOX LOCUS,
L. Venkatakrishnaiah and A. Awgulewitsch, Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425.

Murine homeobox genes have been isolated on the basis of cross-homologies to certain developmental control genes from Drosophila that share a conserved protein-coding sequence motif termed as homeobox. Similar to their counterparts in the fruitfly, the mouse homeobox-containing genes are organized in gene clusters. An important feature of many of the Drosophila homeobox genes is the colinearity of the physical map positions on the chromosome and the distinct anterior expression limits along the longitudinal body axis during embryonic development. Recent reports suggest that such a relationship between the position of an individual homeobox locus within its cluster and its spatially restricted antero-posterior domain of expression in the developing CNS and mesodermal tissues also exists for certain mouse homeobox genes.[1,2] Based on alignment and sequence homologies between individual genes from different murine homeobox clusters it has been proposed that these clusters, originated from a common ancestor. Accordingly, predictions have been made with respect to the anterior expression limit of the Hox 3.2 gene.[1,2] Our data obtained by in situ hybridization analyses demonstrate that the domain of Hox 3.2 expression is in agreement with its predicted anterior limit, positioned posterior to the Hox 3.1 expression boundary in the central nerve cord of 12.5 days p.c. embryos. In addition to comparisons of the Hox 3.2 and Hox 3.1 expression patterns at different embryonic stages, we demonstrate expression of Hox 3.2 in the adult CNS.

References: 1) A. Graham et al. Cell 57, 367, 1989.
2) D. Duboule and P. Dollé. The EMBO J. 8, 1495, 1989.

P 315 ANALYSIS OF GENE REGULATION IN THE MOUSE HOX-2 CLUSTER, Jenny Whiting, Stefan Nonchev, Melissa Rubock*, Martyn Cook, Hans Lerach* and Robb Krumlauf, Molecular Embryology, N.I.M.R., The Ridgeway, Mill Hill, London, NW7 1AA. * I.C.R.F., 44 Lincoln's Inn Fields, London, WC2A 3PX.

The mouse Hox-2 locus contains 9 homeobox genes in 150kb DNA on chromosome 11. The order of these genes along the chromosome shows remarkable conservation both over evolutionary time and with other mouse Hox clusters. In situ analysis has shown that the order of genes is colinear with the anterior boundaries of their expression along the antero-posterior axis of the embryo. The maintainance of the clustered arrangement, the conservation of gene order and the anterior to posterior expression along the cluster suggests that there are complex control mecanisms working within the cluster to correctly regulate the various genes. Because important elements are likely to be some distance from the genes we have decided to clone the lac Z reporter gene in frame into large genomal clones such that all the normal intron and flanking sequences are maintained in their normal configuration. Such constructs have been made for both the most 5' gene, 2.5 and the central gene in the cluster, 2.1. Lines of transgenic mice have been generated and are currently being analysed for their patterns of lac Z expression. It may be necessary to reconstruct larger parts of the cluster to obtain properly regulated expression. To this end a yeast artificial chromosome clone (YAC) which contains unrearranged mouse DNA spanning 8 of the 9 genes has been isolated. This allows longer range analysis using the lac Z reporter as well as testing gene function by altering gene position in the cluster which could generate subtle dominant alterations in the spatial expression of the gene products in transgenic mice.

P 400 MUTATIONS IN DROSOPHILA AND MAMMALS THAT ARE SENSITIVE TO DNA CROSS-LINKING AGENTS EXHIBIT AN ALTERED MITOCHONDRIAL NUCLEASE, James B. Boyd, Paul V. Harris, Kengo Sakaguchi, Manuel Buchwald+ and Malgorzata Z. Zdzienicka*, Department of Genetics, University of California, Davis, CA 95616, USA; +Hospital for Sick Children, Toronto, Ontario, Canada, M5G1X8; *State University of Leiden Department of Radiation Genetics, Wassenaarseweg 72, Leiden, The Netherlands. Current evidence supports the possibility that the *mus308* mutants of Drosophila represent a model for the human congenital disorder Fanconi anemia. Like group A Fanconi cells, the Drosophila mutants are characterized by hypersensitivity to DNA cross-linking agents, chromosome instability and a failure to recover DNA synthesis following mutagen treatment. An enzymatic defect observed in the Drosophila mutants has been extended to cells derived from the A group of Fanconi. Both cell types exhibit a modified mitochondrial nuclease that possesses an altered pI. An analogous modification has also been identified in two Chinese Hamster cell lines that are hypersensitive to DNA cross-linking agents. These observations have stimulated current studies designed to clone the Drosophila gene and to define the nature of the enzyme modification. Preliminary studies suggest that the mutant and control forms of the enzyme differ in the level of their phosphorylation. That possibility potentially associates these mutants with a defective signal transduction pathway.

REGULATION OF p53 EXPRESSION DURING THE DIFFERENTIATION OF MOUSE ERYTHROLEUKEMIA CELLS, Alison Foote, Saadi Kochbin and Jean-Jacques Lawrence, Laboratoire de Biologie Moleculaire du Cycle Cellulaire, Centre d'Etudes Nuclaires, Grenoble, FRANCE.

In murine erythroleukemia cells (MEL) the transformation associated protein p53 is expressed at a relatively high level when the cells are maintained in a proliferative state. Within 2 hours following the induction of differentiation in response to a variety of chemical inducers p53 is found to undergo a substantial (70%) down-regulation. The regulation of the protein occurs at a post-transcriptional but pre-translational level and appears to involve the induced synthesis of an RNA molecule (inRNA). The induction of differentiation is accompanied by the accumulation of a small nuclear RNA molecule which is complementary to a 1.3 Kb sequence located in intron 1 of the murine p53 gene and delimited by Pst I and Hind III restriction sites. The RNA is poly A+ and orientated in an anti-sense manner with respect to the p53 pre-mRNA. In order to determine the importance of this region of intron 1 in the regulation of p53 a series of mammalian expression vectors have been constructed. These contain the first exon of murine p53 (non-coding) and intron 1 with or without the deletion of an Xho I restriction fragment encompassing the Pst I-Hind III region. In order to distinguish the regulated sequences from endogenous murine p53 the region between exon 2 and exon 11 has been replaced by a portion of the albumin gene, not normally expressed in MEL cells. The transfection of MEL cells by these vectors and the effect of this region of intron 1 on mRNA levels during induced differentiation will be reported.

P 402 COMPLEMENTARY DNA CLONING AND CHARACTERIZATION OF A *XENOPUS* HOMOLOGUE OF THE fms-LIKE GENE (FLG), A PUTATIVE FGF RECEPTOR, Robert Friesel and Igor B. Dawid, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892. The heparin-binding growth factors (HBGFs) constitute a family of homologous polypeptides including acidic and basic fibroblast growth factors (FGFs). They participate in a variety of biological processes including angiogenesis, wound healing, neuronal survival, and inductive events in the early embryo. We describe the cloning, cDNA sequence, and embryonic expression of a *Xenopus* homologue of FLG, a member of the HBGF receptor family. The *Xenopus* FLG homologue encodes a transmembrane protein with an intracellular tyrosine kinase domain and a conserved stretch of acidic residues located within the extracellular domain. RNA gel blot analysis demonstrates that the *Xenopus* FLG RNA is expressed maternally and in all embryonic stages including tailbud stage embryos. When stage 8 animal pole explants are cultured in control amphibian salt solutions, *Xenopus* FLG RNA expression declines to undetectable levels by control stage 20. However, if cultured in the presence of bFGF or XTC-mesoderm inducing factor, *Xenopus* FLG RNA continues to be expressed. It is anticipated that other members of the HBGF receptor family will be isolated from *Xenopus*.

P 403 CLONING OF A cDNA HIGHLY HOMOLOGOUS TO THE LOW AFFINITY IL-3 RECEPTOR, Daniel M Gorman, Naoto Itoh, Jolanda Schreurs, Kenichi Arai, and Atsushi Miyajima, Department of Molecular Biology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA 94304. Interleukin-3 (IL-3) binds to its receptor with high and low affinities, induces tyrosine phosphorylation, and stimulates the proliferation and differentiation of various hemopoietic cells. Using the anti-IL-3 receptor antibody, anti-Aic2, we isolated a cDNA (AIC2A) encoding the low affinity IL-3 receptor. In addition, we found a cDNA (AIC2B) highly homologous to this low affinity IL-3 receptor. COS7 cells transfected with the cDNA bound the anti-Aic2 antibody, but did not bind IL-3. The amino acid sequence of the AIC2B protein has 95% identity to the low affinity IL-3 receptor. The amino acid substitutions were dispersed throughout the entire protein, indicating that AIC2A and AIC2B are encoded by distinct genes. However, AIC2B was co-expressed with AIC2A in all the IL-3 dependent cells examined, suggesting some important role of AIC2B in cytokine signal transduction.

P 404 **G-PROTEIN α-SUBUNIT mRNA'S IN DIFFERENT TISSUES FROM THE DIABETIC RAT** **Susanne L. Griffiths and Miles D. Houslay**. Department of Biochemistry, University of Glasgow, Glasgow. G12 8QQ Scotland. U.K.

The present study has sought to determine whether streptozotocin-induced diabetes affects the expression of mRNA for the α-subunits of G-proteins. We have synthesized 33mer oligodeoxynucleotides which distinguish between and specifically hybridise with mRNAs for individual G-protein α-subunits. We have used these to probe Northern blots of RNA isolated from different tissues from control and diabetic rats. The sizes of the transcripts for G-protein α-subunits were unaltered by streptozotocin-induced diabetes but in the major insulin responsive tissues, skeletal muscle, fat and liver we detected some changes in the levels. In hepatocytes, diabetes caused a significant decrease in the levels of mRNA for the α-subunits of Gs (~50%), Gi-2 (~50%) and Gi-3 (~25%) as compared to control levels. mRNA for Gi-1 was not detected. In adipocytes we observed an increase in the number of transcripts for the α-subunits of Gi-1 and of Gi-3 (~4 fold) but no change in Gi-2 or Gs. mRNAs for the α-subunits of Gi-3 and Gs were decreased by ~50 and 40% respectively in skeletal muscle from diabetic animals, with no change in Gi-2. Gi-1 mRNA was not detected. We also detected a decrease in the mRNA for the α-subunit of Gs in RNA from the kidney of diabetic rats but there were no significant changes in the levels of α-subunit mRNAs were observed in brain or heart. We suggest that diabetes causes alterations in the levels of mRNA for the α-subunits of certain G-proteins, in a tissue-specific manner and that this may be relevant to observed alterations in signal transduction mechanisms in the affected tissues.

P 405 G PROTEINS IN *DICTYOSTELIUM* SIGNAL TRANSDUCTION PATHWAYS, Jeffrey A. Hadwiger, Akiko Kumagai, Kumio Okaichi, and Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, Univ. of California, San Diego, La Jolla, CA 92093

Dictyostelium cells grow vegetatively as a solitary amoebae. When starved, cells aggregate to form multicellular fruiting bodies that consist of two major cell types. During this developmental process, cAMP and other compounds are used to transmit signals between cells. Extracellular cAMP signals are detected by cell surface receptors and then transduced to the nucleus where specific gene regulation occurs. To help elucidate the molecular mechanisms responsible for this type of intracellular signal transduction, we have examined genes encoding subunits of G proteins, often found in such pathways. At present, five genes ($G_\alpha1$, $G_\alpha2$, $G_\alpha3$, $G_\alpha4$, and $G_\alpha5$) have been identified that encode proteins homologous to α subunits of G proteins by our laboratory and that of P. Devreotes. The best characterized of these genes, $G_\alpha2$, is primarily expressed during the cAMP-dependent aggregation stage, suggesting that the $G_\alpha2$ protein might interact with a cAMP receptor. Consistent with this are the observations that certain aggregation deficient mutants (*FrigidA*), unresponsive to cAMP stimulation, have chromosomal aberrations at the $G_\alpha2$ locus and that insertional mutagenesis of the $G_\alpha2$ locus results in a phenotype identical to that of *FrigidA* mutants. $G_\alpha2$ null mutants can be complemented by the $G_\alpha2$ gene which restores the normal cellular responses to extracellular cAMP. The $G_\alpha1$ gene is maximally expressed during vegetative growth and early development; however, antisense expression or insertional mutagenesis of this gene does not result in any obvious phenotype suggesting this gene is not essential during these phases of the life cycle. Both $G_\alpha4$ and $G_\alpha5$ genes are maximally expressed late in development, suggesting possible roles in cellular differentiation or culmination. Insertional mutagenesis of $G_\alpha4$ and $G_\alpha5$ is currently being pursued to investigate possible phenotypes associated with null mutations.

P 406 THE ROLE OF THE PHOSPHATIDYLINOSITOL CYCLE IN THE EMBRYONIC CELL CYCLE, Jin-Kwan Han, Richard Nuccitelli, Department of Zoology, University of California-Davis, Davis CA 95616

Many studies suggest that the phosphatidylinositol (PI) pathway is involved in regulation of the early embryonic cleavage cycle. First, it has been shown in sea urchin and frog embryos that an increase in intracellular calcium, possibly by the mobilization of IP_3, is an essential regulatory step required for entry into mitosis. Moreover, fluctuations of intracellular calcium have been monitored by calcium indicator, FURA-2, during the process of cell division. Second, an ativation of protein kinase C by DG may also regulate cell division since phorbol myristate acetate modifyes cell division in preimplantation mouse embryos. Thirdly, lithium which is known to influence the PI cycle by inhibiting phosphatase enzymes affects mitosis in sea urchin zygotes and mouse embryos. The fact that the lithium effect was reversed by myo-inositol further supports a role for PI metabolism in mitosis. Finally, microinjection of the anti-PIP_2 antibody into the cleaving *Xenopus* embyro inhibits cell division. Despite such evidence, it is still unclear whether inositolpolyphosphates (IPs) are made during cell division. Here, using HPLC we have directly measured IPs during the first cell cycle of the *Xenopus* embryos. During the 25 minute window of the first cell cycle we found the IP_1 and IP_2 increased more than 80% when the cleavage furrow was 50% complete and sharply decreased at the end of the cleavage cycle. Different types of isomers of IP_1 and IP_2 appeared throughout the cleavage cycle. IP_3, although we fail to detect large changes, has similar profile of IP_1 and IP_2. Our finding directly demonstrates a cycling of the PI cycle during the early embryonic cleavage cycle.

P 407 REGULATION OF mRNA EXPRESSION OF THE CD18 AND CD11b SUBUNITS OF THE HUMAN LEUKOCYTE ADHERENCE RECEPTOR DURING HL-60 CELL DIFFERENTIATION. Dennis D. Hickstein, Anthony L. Back, and Steven J. Collins. Dept. of Medicine, Univ. of Washington School of Medicine, Seattle, WA 98195.

The CD18 and CD11b subunits of the human neutrophil adherence receptor are noncovalently associated on the neutrophil surface where the complex mediates critical neutrophil adherence-related functions, including adherence to vascular endothelium. Markedly enhanced surface antigen and mRNA expression of both the CD11b and CD18 subunits occurs in HL-60 promyelocytic leukemia cells which have been chemically induced to differentiate with agents such as retinoic acid. Nuclear run-on assays indicate that CD18 mRNA levels are transcriptionally regulated in retinoic acid-differentiated HL-60 cells, whereas CD11b mRNA levels are not transcriptionally regulated. Actinomycin D studies indicated that the CD11b transcript has an extremely short half-life in the undifferentiated HL-60 cells. Incubation of undifferentiated HL-60 cells with cycloheximide resulted in a marked increase in the steady state level of CD11b mRNA, indicating that undifferentiated HL-60 cells contain a labile protein that may be involved in degrading CD11b transcripts. These studies indicate that different molecular mechanisms regulate steady state mRNA levels for each subunit of the CD11b/CD18 receptor during HL-60 cell differentiation.

P 408 EXPRESSION OF PROTEIN KINASE C-ISOFORMS DURING CELL TRANSFORMATION AND HEPATOCARCINOGENESIS, L.L. Hsieh*, C. Peraino**, H. Shinozuka*** and I. B. Weinstein*, Inst. of Cancer Res. and Div. of Envir. Sci., Columbia Univ., New York, NY 10032*, Argonne National Laboratory, Argonne, IL 60439**, Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15261***. It is known that protein kinase C (PKC) belongs to a multigene family and that the different isoforms of PKC vary with respect to: expression during development and differentiation, sensitivity to down-regulation, and certain enzymatic characteristics. For these reasons it was of interest to examine whether the expression of specific isoforms of PKC might be altered during malignant cell transformation and tumorigenicity. We have established derivatives of K16 and K22 rat liver epithelial cell lines which gradually progress to a fully tumorigenic phenotype by introducing stepwise $PKC_{\beta 1}$ cDNA and an activated c-H-ras oncogene (T24). Increased expression of endogenous PKC_{α} and decreased expression of endogenous PKC_{ϵ} was found in the fully transformed derivatives K16PKC-4/T24-2 and K22PKC-2/T24-2 cells by Northern blot analysis, when compared to the parental cells. Increased levels of PKC_{α} were also seen in rat liver tumors induced by BR931, a hepatic peroxisome proliferator, and also in regenerating rat liver after partial hepatectomy. Taken together, these results indicate that alterations in the expression of specific isoforms of PKC may play an important role in liver cell proliferation and hepatocarcinogenesis.

P 409 **CHARACTERIZATION OF TWO FUNCTIONALLY INDEPENDENT MECHANISMS BY WHICH INTERFERON-INDUCED GENE EXPRESSION IS DOWN REGULATED.** Hiroakai Akai, and Andrew Larner, Laboratory of Cytokine Research, Center for Biologics Evaluation and Research, National Institutes of Health, Bethesda, Maryland 20892. Interferons (IFNs) induce the expression of a variety of cellular RNAs. Phorbol esters can inhibit IFN-induced expression of some of these RNAs, including ISG-54K. The actions of phorbol esters on IFN-activated ISG-54K transcription are cell specific and are reversed by inhibitors of protein synthesis. In those cell lines in which phorbol esters inhibit IFN-induced ISG-54K transcription, prolonged IFN exposure induces a "desensitized state" such that further IFN exposure no longer induces ISG-54K expression. IFN-induced desensitization is also reversed by inhibitors of protein synthesis. Experiments are described to determine whether the mechanism by which phorbol esters inhibit IFN-activated ISG-54K expression is the same as the mechanism by which prolonged exposure to IFN makes cells refractory to further induction of ISG-54K expression. Cultured cells treated with TPA for 72 h are desensitized to phorbol esters such that further addition of phorbols does not inhibit IFN-induced ISG-54K expression. In both naive and TPA-desensitized human fibroblasts or WISH cells, prolonged IFN treatment induced a desensitized state that was reversible by cycloheximide. This observation suggests that the mechanisms by which prolonged IFN treatment and phorbol esters inhibit ISG-54K expression are independent.

P 410 PROTOONCOGENE RAS MAY PLAY A REGULATORY ROLE IN THE PROCESS OF ADIPOCYTIC DIFFERENTIATION, [1,2]Ying Lu, [3] Steve Anderson, [2]Hugh Pross, [4] Nobu Hozumi, [4] John Roder, and [1,2] Tina Haliotis, [1]Cancer Research Laboratories, Department of Pathology & [2]Department of Microbiology & Immunology, Queen's University, Kingston, Ont. K7L 3N6 Canada.. [3]Department of Molecular Immunology, Biotechnology Research Institute, Montreal, PQ H4P 2R2. [4]Mount Sinai Hospital Research Institute, Toronto, Ont. M5G 1X5. When cultures of 3T3 or 10T1/2 fibroblasts are propagated in the resting state for several weeks, certain clones are able to convert to adipocytes (Green & Kehinde, 1974). It is clear that environmental or hormonal signals must participate in directing the cell to withdraw from the cell cycle and to progress towards the differentiated state. Despite the apparent clarity of such phenomena, both the nature of the molecular switch that controls the decision to differentiate and the signal transduction mechanisms by which the cell communicates its environmental status to that switch remain obscure. We tested the hypothesis that ras may be involved in the differentiation process, employing the established protocol described above and a set of inducible ras transfectants of 10T1/2 in which the level of ras can be effectively modulated. The following observations were made: i) In agreement with previous reports, 10T1/2 cultures when propagated in the resting state for several weeks, developed at very low frequency (2-3%), centres of fat cell conversion. Furthermore, the frequency of this event could be significantly increased (up to 10-fold) by treatment of the cultures with the demethylating agent 5-azacytidine, ii) 2H1 cultures, which express mutated H-ras showed little, if any, tendency to adipose conversion. This behaviour was entirely refractory to treatment with 5-azacytidine, and the few cells that did appear to convert displayed a highly atypical morphology, and iii) Rev-3 cultures, in which ras is effectively blocked as a result of antisense ras production, displayed a dramatic increase ($\geq$ 10-fold) in frequency of adipose conversion equal to or higher than that observed in 10T1/2 cultures maximally induced with 5-azacytidine treatment. However, while the cells showed clear signs of adipocytic differentiation by all other morphological criteria, they were partially deficient in the ability to fuse their multiple small lipid droplets into one large drop, acquiring the signet ring appearance of mature fat cells. These findings suggest the possibility that p21ras exerts a negative regulatory effect on the decision of the adipogenic stem cell to differentiate, and possibly also influences its progression along that pathway.

P 411 THREE HIGHLY CONSERVED PROTEIN KINASES ARE DEVELOPMENTALLY REGULATED IN *DICTYOSTELIUM*, Sandra K.O. Mann and Richard A. Firtel, Dept. of Biology, Ctr. for Molecular Genetics, Univ. of California, San Diego, La Jolla, CA 92093

We have isolated clones for three developmentally regulated putative serine/threonine protein kinases in *Dictyostelium discoideum*. One of these is expressed for a brief period early in development and a second is expressed at a fairly high level during mid to late development. The third appears to encode two RNAs of different sizes, one expressed during early development, and the other expressed during mid to late development. At the amino acid level, some regions are highly conserved among the three kinases and all show significant homology to the protein kinase A/protein kinase C families in other systems. We are currently investigating the regulation of these three genes at the cellular and molecular levels. We are also investigating the function of these three kinases using transformation and gene disruption studies.

P 412 Structural requirements of the membrane IgM molecule for signalling in mature B-cells. V.S.Parikh ,C.Nakai, and P.W.Tucker. Department of Microbiology, UTSMC at Dallas, Dallas,TX.

The carboxyl-terminus of the IgM molecule is essential for transmitting a tolerogenic signal(Webb, et al).This was shown by creating T15-idiotype specific transfectants which expressed either wild-type or hybrid IgM molecules in which the μ-chain C-terminus was replaced with that of other heavy chains (δ,γ2b,α)and an I-A-alpha chain.All hybrids except μ-I-A delivered a negative signal in CH33 cells. When transfected into another cellline,M12.4,this μ-I-A hybrid was also defective in PC-KLH driven Ca++ mobilization although it effectively mediated endocytosis in the same cells.We have created other hybrid constructs where the various segments of the I-A C-terminus (eg,spacer,transmembranal,cytoplasmic residues) are replaced by that of μ-molecule to determine which domains are essential for signalling. We have also created point mutations in the transmembrane and cytoplasmic domains of IgM molecule to pin-point the amino acid residue(s) involved.The effect of these various mutations on the antigen-induced changes in intracellular Ca++ levels,growth inhibition,and expression of various genes will be discussed.

Webb et al.(1989)PNAS.86:1977

P 413 IDENTIFICATION OF A PLASMA MEMBRANE-ASSOCIATED GTPase ACTIVATING PROTEIN FOR rap, A ras-RELATED GTP-BINDING PROTEIN, Paul Polakis, Bonnee Rubinfeld, and Frank McCormick. Departments of Molecular Biology and Protein Chemistry, Cetus Corporation, Emeryville, CA 94608. rap is a p21 ras-related GTP-binding protein that has been implicated in the reversion of the ras-transformed cell phenotype. We have identified a GTPase activating protein(GAP) specific for rap in the plasma membrane of differentiated HL60 cells. The rap-GAP activity remained quantitatively associated with the membranes following washes with buffered-1M LiCl containing 20mM EDTA but could be solubilized with the detergents Nonidet P40 and deoxycholate. On the basis of molecular size and chromatographic properties the membrane-associated form appears distinct from the rap-GAP present in the cytosolic fraction from HL60 cells. The solubilized rap-GAP was purified to near homogeneity (ca. 2000-fold over plasma membranes) by successive column chromatographies. The rap-GAP activity corresponded to a single polypeptide with a molecular mass of ca. 88kDa. The purified rap-GAP was inactive towards the GTP-bound forms of p21 ras, G25K and rac. Co-incubation of rap-GAP with a 500-fold excess of ras-GAP did not effect the stimulation of rap GTPase activity indicating that they interact on different sites on the rap molecule. Thus, plasma membranes from HL60 cells contain an ca. 88kDa protein that specifically stimulates the GTPase activity of rap.

P 414 A PHOTORECEPTOR-SPECIFIC ANTIBODY WITH UNIQUE SUBCELLULAR LOCALIZATION. John Archie Pollock* and Seymour Benzer, Division of Biology 156-29, California Institute of Technology, Pasadena CA 91125.

Purified rhabdomeres were used as an immunogen to generate monoclonal antibodies (MAbs). One monoclonal antibody identified in this screen, MAb83F6, specifically stains an intense band located intracellularly at the base of the rhabdomeres, the photoreceptive membranes in the eye. This is a region of the photoreceptor cells may be important for the maintenance of the photoreceptive membranes. Structures in this region of the cell may also play an important role in the transduction of signals from the rhabdomeres to the cell body.

Developmentally, the expression commences at 12 - 18 hours after puparium formation (@ 25 °C), associated with the thin retinal epithelium. The antigen continues to be expressed in the developing photoreceptors throughout pupal development into adult life. Expression was also detected in the ocellus and the larval photoreceptor organ. Immunoblotting adult head protein extract revealed an abundant protein of approximately 185 kD. Several different approaches are being applied to cloning the gene that encodes the 83F6 antigen.

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P 415 A CHIMERIC RECEPTOR COMPOSED OF THE EXTRACELLULAR DOMAIN OF CD2 AND THE TYROSINE KINASE DOMAIN OF THE CSF-1 RECEPTOR (*FMS*) ENABLES NIH-3T3 CELLS TO RESPOND MITOGENICALLY TO CD2 ANTIBODIES, Martine F. Roussel,[1] Catherine Transy,[3] Jun-ya Kato,[1,2] Ellis Reinherz,[3] and Charles J. Sherr,[1,2] Department of Tumor Cell Biology[1] and Howard Hughes Medical Institute,[2] St. Jude Children's Research Hospital, Memphis TN 38105, and Dana Farber Cancer Institute,[3] Harvard Medical School, Boston MA 02115

A chimeric receptor containing the extracellular domain of the human T cell CD2 (T11) antigen fused to the tyrosine kinase domain of the human macrophage colony-stimulating factor 1 receptor (CSF-1R) was expressed in NIH-3T3 fibroblasts. Cells plated in semisolid medium were conditionally stimulated to form colonies in response to monoclonal antibodies to CD2 (anti $T11_2$ or anti $T11_3$), and a synergistic response was obtained when both antibodies were used together. The chimeric receptor was phosphorylated on tyrosine after stimulation with anti-T11 and underwent rapid "ligand-induced" downmodulation characteristic of CSF-1R. The ability of the CSF-1R kinase to be fully activated by antibodies to CD2 indicates that CSF-1 *per se* is not required for a mitogenic response and that its primary role may be to induce receptor aggregation. Although transduced CSF-1R is able to trigger a proliferative response in fibroblasts and interleukin 3-dependent myeloid cell lines, neither the intact CSF-1 receptor nor the chimeric CD2/c-*fms* glycoprotein were able to induce T cell mitogenesis or to replace the interleukin-2 requirement of T cell lines after stimulation by CSF-1 or anti-T11, respectively. Thus, T cells may be relatively refractory to growth factor receptor kinases of the CSF-1R subfamily.

P 416 THE MULTIPLE CYCLIC AMP RECEPTORS FROM DICTYOSTELIUM: THE SAME BUT DIFFERENT, Charles L. Saxe III and Alan R. Kimmel, Laboratory of Cellular and Developmental Biology, National Institutes of Health, Bethesda, MD 20892.

We have previously described the cloning of cDNAs for a Dictyostelium cyclic AMP (cAMP) cell surface receptor, CAR1, which is a member of the superfamily of G-protein linked receptors. We now report the characterisation of two additional cAMP receptor-encoding genes, CAR2 and CAR3. The proteins deduced from these genes are predicted to be substantially similar throughout their transmembrane and loop domains but differ in their carboxyl terminal segments.

RNAs encoded by each of these genes display unique spatial and temporal patterns of accumulation and there is temporal regulation of multiple size forms of CAR1-specific RNAs. We have begun to analyse the mechanisms which regulate CAR expression and will report on signalling mechanisms and cis-acting DNA elements important for proper CAR expression.

P 417 ONCOGENIC ACTIVATION OF THE *FMS* PROTO-ONCOGENE PRODUCT (CSF-1 RECEPTOR) THROUGH ALTERNATIVE MUTATIONS IN ITS EXTRACELLULAR DOMAIN, Charles J. Sherr,[1,2] Peter Besmer,[3] and Martine F. Roussel.[2] Howard Hughes Medical Institute[1], Department of Tumor Cell Biology[2] St Jude Children's Research Hospital, Memphis, TN 38105, and Memorial Sloan Kettering Cancer Center,[3] New York, NY 10021

Substitution of serine for leucine at codon 301 in the extracellular domain of the human colony-stimulating factor 1 receptor (CSF-1R), the product of the c-*fms* proto-oncogene, activates its tyrosine kinase activity in the absence of ligand and renders it able to transform NIH-3T3 fibroblasts. CSF-1R molecules bearing glutamic acid, threonine, or proline at codon 301 were transforming, whereas those containing cysteine, lysine, phenylalanine, or methionine were not. With the single exception of glutamic acid, amino acid substitutions at codon 301 did not affect the affinity of CSF-1 binding to the mutant receptors. An activating codon 301 mutation is present in the v-*fms* oncogene encoded by the Susan McDonough strain of feline sarcoma virus (SM-FeSV). In contrast, the recently determined nucleotide sequence of the independently transduced v-*fms* gene of the Hardy-Zuckerman 5 strain of FeSV indicates that it lacks a mutation at codon 301 but contains three other predicted amino acid substitutions that distinguish it from the feline c-*fms* product. Studies with chimeric receptor molecules indicated that the relevant activating mutation within the v-*fms* product of HZ-5 FeSV also resides in its extracellular domain. Thus, the latent transforming activity of c-*fms* can be unmasked by different point mutations within this region of the receptor.

P 418 1,25-DIHYDROXYVITAMIN D3 (D3)-INDUCED REGULATION OF PKC GENE EXPRESSION DURING HL60 DIFFERENTIATION, David H. Solomon, Kevin O'Driscoll*, Gabriel Sosne, I. Bernard Weinstein* and Yvon E. Cayre. Department of Physiology and Institute of Cancer Research*, College of Physicians and Surgeons, Columbia University, New York, NY 10032.

The human promyelocytic leukemia cell line HL-60 differentiates in vitro when treated with inducers. HL-60 responds to 12-O-tetradecanoyl phorbol-13-acetate or to D3 by giving rise to cells with monocytic characteristics. It has previously been shown that protein kinase C (PKC) isozymes increase in abundance during granulocytic differentiation of HL-60 induced by dimethylsulfoxide or retinoic acid (Makowske et al. J. Biol. Chem. 263:3402-3410). Here we demonstrate that treatment of HL-60 with D3 causes a transient increase in the steady state levels of PKC alpha and PKC beta mRNA species with maximal induction at 48 hours. Nuclear run-on transcription and actinomycin D experiments suggest that this modulation of PKC mRNA levels occurs by post-transcriptional mechanism(s). We also observed an increase in PKC protein levels by immunoblotting and an increase in PKC activity. In contrast to the changes in mRNA levels, however, these increases were progressive and increased 7-fold in HL-60 cells treated with D3 for five days. Our data suggest that changes in the level or activity state of PKC may provide a common mechanism by which disparate agents regulate the differentiation of HL-60 cells.

P 419 *TORSO* TYROSINE KINASE AND THE INDUCTION OF TERMINAL STRUCTURES IN THE DROSOPHILA EMBRYO, F. Sprenger, L.M. Stevens and C. Nüsslein-Volhard, Max-Planck Institut für Entwicklungsbiologie, 7400 Tübingen, FRG

The maternal gene *torso* is required for the formation of the unsegmented terminal regions of the Drosophila larva, the acron and telson. Genetic evidence suggests that the activity of *torso* is restricted to the terminal regions of the embryo. The *torso* protein itself, however, is not localized. The predicted structure of the *torso* protein suggests that it functions as a receptor tyrosine kinase and that the activation of the cytoplasmic tyrosine kinase activity is dependent upon binding of a ligand molecule to the extracytoplasmic part of the *torso* protein.

Based on injection experiments using in vitro synthesized *torso* mRNA we present evidence that the activating ligand molecule is localized to the perivitelline space at the anterior and posterior ends of the early embryo. The experiments further indicate that the ligand is diffusible and limited in amount. The localized source/diffusion model for the *torso* ligand predicts that the expression of zygotic target genes like *tailless* and *huckebein* is controlled by a gradient of activated *torso* protein.

P 420 PURIFICATION AND CHARACTERIZATION OF THE MEMBRANE TNF RECEPTOR FROM HL60 CELLS, Bettina Thoma, Matthias Grell, Klaus Pfizenmaier, and Peter Scheurich, Clinical Research Group of the Max-Planck-Society, 3400 Göttingen, F.R. Germany

Using a TFN-alpha affinity column, we have purified the human receptor for tumor necrosis factor (TNF) from HL60 cells about 2000-fold. Enriched receptor material was used for characterization of receptor protein(s) and for production of a monoclonal anti TNF receptor antibody termed H398. This antibody is a TNF antagonist on different human cell lines (K562, U937, HeLa, MCF7 and Colo205) and partially competes with iodinated TNF and LT for cellular binding. In contrast, H398 does not bind to Raji cells, which lack specific TNF binding capacity, or to murine L929 cells. Under nonreducing conditions, H398 precipitates a major protein with an apparent molecular weight of 100 kDa in addition to some proteins with lower molecular weights, all capable of specific binding of iodinated TNF. Similar immunoprecipitates were obtained using cells derived from different tissues, indicating that the H398 antigen is expressed on most if not all TNF receptor-positive cell lines. These results differ from those obtained with various crosslinkers, indicating a major receptor protein with a molecular weight of about 85 kDa, but they are consistent with human TNF ligand binding studies using TNF affinity-purified TNF receptor material.

P 421 **Two Novel Protein Tyrosine Kinases With A Second Phosphotransferase-Related Catalytic Domain Define A New Class Of Protein Tyrosine Kinase.** Andrew F. Wilks, Ailsa Harpur and Raja Kurban. Ludwig Institute for Cancer Research, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

Upon amino acid sequence comparison of 27 putative PTK catalytic domains, the presence of 11 highly conserved motifs is apparent (1). We have generated PTK-specific oligonucleotide primers based on two particularly highly conserved motifs. By applying the Polymerase Chain Reaction (PCR) to genomic DNA, and a variety of cDNA libraries, the catalytic domains of a number of novel PTKs were amplified (2). PCR reactions on a cDNA population produced from the mRNA of a murine haemopoietic cell line FDC-P1, generated clones encoding two novel and related PTKs (JAK 1 and JAK 2). Both genes encode proteins of around 1150 amino acids, which have predicted molecular masses of 130-140 Kdaltons. Sequence analysis confirmed the presence in each, of both a conventional tyrosine kinase catalytic domain and a second, novel phosphotransferase-related domain. This second kinase domain is, as yet, functionally uncharacterised, and differs significantly from other tyrosine and threonine/serine kinase family members. The remarkable structure of this second kinase-related domain, in both JAK family members, is unprecedented in other members of the protein kinase family. The bipotential nature of this class of molecule has implications for signal transduction, and we are currently exploring the properties of the proteins encoded by these cDNAs.

References:

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P 422 LIPOSOME-COUPLED TRANSLATION IN VITRO: A NOVEL APPROACH TO FUNCTIONAL RECEPTOR EXPRESSION, Sergey A. Zozulya, Vsevolod V. Gurevich, Irina D. Pokrovskaya, Ekaterina P. Zerf, Tatyana A. Obukhova,Pavel R. Badalov, Branch of Shemyakin Institute of Bioorganic Chemistry, Pushchino, Moscow Region, 142292, USSR.

Success of protein engineering studies of eucaryotic transmembrane receptors is crucially dependent upon availability of convenient systems for functional preparative expression of their genes, preferably less laborous and expensive than cultured eucaryotic cells or *X.laevis* oocytes.We report a method for the preparative functional expression of bovine visual rhodopsin in vitro in wheat germ extract (WGE) with the principal new feature of cotranslational incorporation of synthesized opsin into artificial liposomes followed by regeneration with retinal. This approach allows to produce hundreds of micrograms of membrane-incorporated rhodopsin with the yield of about 10 μg from 1 ml of WGE in 1-2 working days starting from the cloned gene. Functional (as judged by transducin and cGMP phosphodiesterase activation tests), spectral and immunochemical properties of recombinant,liposome-integrated rhodopsin are very similar to those of its natural counterpart.Functional effects of some amino acid substitutions in rhodopsin molecule, produced by oligonucleotide-directed mutagenesis, including Cys316 → Ser, Asp330,331 → Asn, Asp83 → Asn and Glu134 → Gln have been studied. Applicability of this approach to functional expression of other transmembrane receptors is now under investigation.

Nuclear Factors; Steroid Hormones-I

P 500 THE FUNCTION OF THE *MCM1* AND *STE12* PRODUCTS IN CELL SPECIALIZATION AND SIGNAL TRANSDUCTION IN YEAST. Gustav Ammerer and Michael Primig, Institute for Molecular Pathology, Dr. Bohrgasse 7, A1030, Vienna, Austria.

We present evidence that the *STE12* gene encodes a DNA binding protein which specifically recognizes the PRE (pheromone response element) sequence. This conclusion is based on experiments using different variants of *STE12* products synthesized in an *in vitro* system. Under normal conditions STE12 will bind only weakly to its target site. However, in two cases we observed increased binding: A) The complete STE12 protein can bind cooperatively with MCM1 when a binding site for this protein is adjacent to the PRE. MCM1 is a DNA binding protein with a region of extensive homology to the human transcription factor SRF. MCM1 is also essential for the recruitment of the cell type specific regulators α1 and α2. B) A truncated STE12 which lacks two thirds of the amino acids from the C-terminus can bind to a single PRE with high affinity. Therefore, the deleted region seems to exert negative control over DNA binding. This control can be relieved by interaction of STE12 with MCM1. The C-terminal 200 aa of STE12 seem to be required for this cooperative binding. We find that MCM1 interacts with STE12 through the domain which shows similarity to SRF. The same domain is also involved in MCM1 interactions with the cell type specific repressor α2. We show further that the activator STE12 and the repressor α2 compete for interaction with MCM1.

P 501 A TEMPLATE-BOUND PROTEIN KINASE PHOSPHORYLATES RNA POLYMERASE II DURING THE INITIATION PHASE OF TRANSCRIPTION. Jonathan A. Arias* and William S. Dynan, *Dept. of Molecular, Cellular and Developmental Biology, and the Dept. of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309. The initiation of promoter-dependent transcription by RNA polymerase II requires a β,γ-hydrolyzable adenosine nucleotide (eg., ATP or dATP), in addition to nucleotides incorporated into nascent RNA. We investigated whether this requirement for β,γ-hydrolyzable adenosine nucleotide involves the activity of a protein kinase. Using a unique immobilized template system we purified the transcription activity associated with preinitiation complexes approximately 300-fold. These immobilized transcription complexes contained a tightly-associated protein kinase that incorporates 40-90 phosphate groups into the large subunit of RNA polymerase II during the initiation of transcription. The nucleotide substrate specificity of this template-associated kinase distinguishes it from other enzymes that phosphorylate RNA polymerase II. Moreover, the nucleotide analog ATPγS reversibly inhibited both kinase activity and the initiation, but not preinitiation or elongation, reactions of transcription at similar concentrations. Together, these data correlate this kinase activity with the step during transcriptional initiation that requires β,γ-hydrolyzable adenosine nucleotide. Since ATP or dATP must be present simultaneously with other nucleotides for initiation to occur, we examined whether promoter-bound RNA polymerase II is phosphorylated prior to the initiation of RNA synthesis.We found that transcription from preinitiation complexes was unaffected by incubation with [γ-32P] ATP and washing to remove the radionucleotide. When these complexes were then incubated with initiating nucleotides and nonradioactive ATP initiated complexes were formed that lacked radiolabeled RNAP II. Thus, efficient phosphorylation of the polymerase required the simultaneous presence of initiating nucleotides and ATP. We suggest that phosphorylation by this protein kinase may influence the fate of the polymerase during the early phases of transcription.

P 502 THE IMMUNOSUPRESSANT ***FK506*** SPECIFICALLY INHIBITS MITOGEN-INDUCED TRANSCRIPTION FROM THE 400 BP 5' REGULATORY REGION OR ISOLATED ENHANCER ELEMENTS OF THE IL-2 GENE. S. S. Banerji, P. W. Kwok, J. N. Parsons, M. F. Stinski, N. I. Hutchinson, E. A. O'Neill and M. J. Tocci. Dept. of Molecular Immunology, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065
The macrolide *FK506*, like the cyclic undecapeptide *Cyclosporin A (CsA)*, is a potent immunosuppressant that interfers with transcriptional activation of several EARLY phase activation genes in stimulated T-lymphocytes, including IL-2. To define potential cis-acting sites within the promoter which may mediate this inhibition, we studied the effects of *FK506* on transcription from the 400 bp IL-2 5'UAS, as well as a minimal promoter element (TATA box and CAP site) linked to the defined enhancer elements NFIL-2A, AP-1, NFκB or NFAT-1. Each promoter was fused to the bacterial chloramphenicol acetyl transferase (CAT) gene and used to transiently transfect Jurkat cells. IL-2Rα, CMV-IE and SV40-E UAS were similarly surveyed. Quantitative CAT assays indicated that *FK506* (1nM), like *CsA* (100nM), completely inhibited mitogen-induced CAT gene expression from the 400 bp IL-2 promoter or the NFIL-2A, NFAT-1, AP-1 and NFκB enhancer elements. Transcription from the IL-2Rα UAS was unaffected while transcription from the CMV-IE UAS was inhibited partially (up to 50%) by *FK506* or *CsA* suggesting that *FK-506* was not a general inhibitor of transcription. None of the IL-2 derived constructs were functional in either uninduced or induced MRC-5 fibroblasts, suggesting that the signalling pathway or necessary transcriptional factors may be T-cell specific. Electrophoretic gel shift assays performed with nuclear extracts from vehicle or drug treated Jurkat cells indicated that NFIL-2A, AP-1, NFκB and NFAT-1 DNA binding activities were present in *FK506*-treated cells. These studies suggest that *FK506* may inhibit the transcriptional activation of IL-2 by interfering with a T-cell specific signal transduction pathway and not through the activity of the DNA binding proteins studied.

P 503 **THE ROLE OF CREB IN TRANSCRIPTIONAL ACTIVATION OF THE c-*FOS* GENE BY cAMP,** **Laura A. Berkowitz and Michael Z. Gilman, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, N.Y. 11724** Agents that elevate the intracellular concentration of cyclic AMP rapidly and transiently induce expression of the c-*fos* proto-oncogene in Balb/c 3T3 cells. We have identified three elements in the mouse c-*fos* promoter/enhancer region that confer cAMP responsiveness to the promoter in transient expression assays. All three elements are related to the cAMP-response element (CRE) consensus, TGACGTCA. The relative activities of these elements *in vivo* correlate with their relative affinities for factors present in nuclear extracts. To further investigate c-*fos* induction by cAMP we isolated full-length human cDNA clones encoding the cAMP-response element binding protein (CREB). We have characterized two distinct clones that differ by virtue of a 14 amino acid serine-rich peptide present in one of the clones. This difference is likely generated by alternative splicing. Both forms are highly conserved between and ubiquitously expressed in several cell lines and mouse tissues. *In vitro* translated products of both clones eqivalently bind the major c-*fos* CRE in DNA mobility shift assays. For tests of *in vivo* expression the CREB cDNAs were fused to the DNA-binding domain of the yeast factor GAL-4. Both chimeric proteins were able to confer cAMP responsiveness to a co-transfected reporter gene containing a GAL-4 binding site. However, preliminary experiments in which identical mutations in a phosphorylation site were introduced into these two forms of CREB suggest that their transcriptional activities differ. We are now in the process of determining the basis of their differing activities, and in particular their abilities to affect c-*fos* transcription.

P 504 INDUCTION OF THE HUMAN ACUTE PHASE SERUM AMYLOID A GENES BY IL-1 AND IL-6, Jonathan C Betts, Mark R. Edbrooke, John K. Cheshire and Patricia Woo, Section of Molecular Rheumatology, MRC Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ U.K.

Two of the acute phase serum amyloid A genes, SAA1 and SAA2, are modulated by a number of cytokines including IL-1 and IL-6. Mutagenesis of the binding site for the NFkB transcription factor in the SAA promoter region abolishes binding of an IL-1 inducible factor and transcription of a CAT reporter gene. IL-6 treated HepG2 nuclear extracts contain factors which bind to SAA promoter fragments containing both the NFkB binding site and a putative IL-6 responsive element CTGGGA. However the presence of the IL-6 responsive element is not an absolute requirement for IL-6 induction in functional assays. Cells transfected with CAT constructs containing the mutant NFkB binding site show a 50% decrease in IL-6 induction of CAT compared to wild type constructs, therefore both an NFkB-like factor and at least one other factor is involved.

IL-1 and IL-6 act synergistically to increase SAA transcription, the interaction of their signalling pathways is being investigated in the light of the above findings.

P 505 EXPRESSION OF GHF-1 IN PITUITARY DERIVED GH3 CELLS. Helen Brady, Alison McCormick and Michael Karin. Department of Pharmacology M-036, UCSD, La Jolla, Ca, 92093. The pituitary-specific transcription factor, GHF-1, is largely responsible for expression of growth hormone. GHF-1 has been shown to be a homeobox protein. Other homeobox proteins include B-cell specific oct-2, yeast mating factor A1, and several drosophila proteins involved in developmental pattern formation. Since homoebox proteins are involved in developmental processes as well as cell type specificity elucidation of the mechanism of regulation of these genes is of major importance. A clone of GHF-1 has been isolated from a rat genomic library, using a full length cDNA probe. Further screening identified the 5' flanking promoter region. Tissue specificity of this promoter has been demonstrated in vivo with transient transfections and using in vitro transcription. The minimal tissue specific promoter element has been determined using deletion mutagenesis. DNase footprint analysis clearly demonstrated differences between expressing and non-expressing cells. Mutagenesis of the pituitary-specific footprint regions is being used to better define the important sequences and begin to understand the trans-acting factor(s) binding to this region.

P 506 **ISOLATION OF A BOVINE HOMOLOG TO THE HUMAN MYOGENIC FACTOR MYF-5,** Theodore G. Clark, Julie Morris, Maho Akamatsu*, Royal A. McGraw*, and Robert Ivarie, Department of Genetics, Franklin College of Arts and Sciences, *Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602.

To isolate myogenic factors from bovine skeletal muscle, a 274 bp probe to a conserved region of the mouse MyoD1 cDNA was used to screen a bovine cDNA library from fetal myoblasts. Among a number of positive recombinants isolated, one (*bmyf*) contains a 1933 bp insert with an open reading frame encoding a protein highly related to the human myogenic factor *myf-5*. Human and bovine factors are 92% homologous in their coding sequences, and ~81% homologous in their 5' and 3'-untranslated regions. Similarity ends at the 3'-terminus of human *myf*-5, with *bmyf* extending 470 nucleotides beyond a consensus polyadenylation signal common to both cDNAs. Northern analysis with RNA from different bovine fetal tissues indicates that *bmyf* is expressed exclusively in skeletal muscle. Three different transcripts of 1.5, 2 and 3 kb were detected in skeletal muscle RNA using *bmyf* cDNA as a probe. The 1.5 kb transcript lacks sequences 3' to the polyadenylation signal at nt 1417 in the *bmyf* cDNA. These sequences are present, however, in the 2 and 3 kb RNA species, suggesting that *bmyf* transcripts may be alternatively polyadenylated. Preliminary transient transfection assays indicate that *bmyf* can activate the expression of the myogenic program in C3H10T1/2 fibroblasts.

P 507 X-LIKE PROTEINS EXERT OPPOSITE EFFECTS ON HLA-DRA TRANSCRIPTION BY BINDING TO HOMOLOGOUS PROMOTER ELEMENTS UPSTREAM OF THE X BOX. John P. Cogswell, Patricia V. Basta, and Jenny P-Y. Ting. Lineberger Cancer Research Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599. The X element comprises part of the conserved class II box found in all class II Major Histocompatibility Complex genes. In the B lymphoblastoid cell line Raji, expression of the human class II gene HLA-DRA has been shown to be regulated in a positive fashion by the X box (-108 to -94 bp). Using deletion mutagenesis, substitution mutagenesis, and S1 protection analyses, we identified two additional promoter elements which are homologous to X but exert opposite effects: 1) a positive regulatory W element located between -109 and -135 bp and, 2) a negative regulatory V element located between -179 to -193 bp. DNase I protection and gel mobility shift assays confirmed that proteins bind to these elements and have X-like specificity. We are using cloned proteins to determine whether the same X protein binds to all three elements or V, W, and X bind different proteins of similar specificity. These results have important implications for the transcriptional regulation of the HLA-DRA gene.

P 508 TRANSCRIPTIONAL REGULATION OF GENES DURING THE TRANSITION FROM EMBRYOGENY TO GERMINATION IN HIGHER PLANTS. Lucio Comai and John J. Harada, Department of Botany, University of California, Davis, CA 95616. Embryogeny and germination are separated by a period of metabolic quiescence in which mature embryos are in a desiccated state . To understand the cellular processes regulating the transition between embryogeny and postgerminative growth, we are studying the regulation of genes expressed in late maturation-stage embryos and in seedlings. We used cloned probes representing genes that are primarily expressed in either embryos or seedlings with transcription assays in isolated nuclei and showed that the majority of the genes are regulated at the transcriptional level. This result indicates that transcriptional processes play a major role in the regulation of developmental events in embryo and seedling. Differences in the transcriptional activity of embryo-specific and postgermination-abundant genes in nuclei from embryos, dry seed, and leaves suggest that the transition from an embryonic to a postgerminative program of development occurs after seeds are rehydrated.

P 509 THE T LYMPHOCYTE SPECIFICITY OF THE ENHANCER OF THE RETROVIRUS SL3-3 IS MEDIATED BY MULTIPLE PROTEIN-DNA INTERACTIONS, Brit Corneliussen, Anders Thornell, Bengt Hallberg, Pia Nilsson and Thomas Grundström, Dept. of Applied Cell and Molecular Biology, University of Umeå, S-90187 Umeå, Sweden.
The T lymphomagenicity of the murine retrovirus SL3-3 is mediated by its enhancer which is preferentially active in T lymphocytes. The enhancer is composed of several DNA domains with different importance in different cell types. We have analysed the proteins interacting with the domains which are most important for enhancer activity in T cells. The SL3-3 enhancer factor 1 (SEF1) proteins were found to interact with two different sequences within the enhancer. Mutation of either site resulted in decreased transcriptional activity *in vivo* preferentially in T-cells. A family of proteins denoted SL3-3 enhancer factor 2 (SEF2) was found to bind specifically to a sequence shown to be of importance for transcriptional activity. Mutant analysis separated the SEF2 proteins into two groups which show different nucleotide sequence requirements for DNA binding. cDNA cloning of different SEF2 proteins shows that they belong to the putative helix-loop-helix class of DNA binding proteins. Northern blot analysis of SEF2 mRNA showed that several transcripts exists, and analysis of isolated cDNA's suggests that alternative splicing is a mechanism for creating different such proteins. SL3-3 enhancer factor 3 (SEF3) and nuclear factor I (NFI) site binding proteins were also found to bind different sequences in the enhancer and to show different degrees of importance for its activity in different cell types. Histone H1 was shown to be a NFI site binding protein whose interaction with the DNA was analysed.

P 510 THE ZINC FINGER PROTEIN NGFI-A EXISTS IN BOTH NUCLEAR AND CYTOPLASMIC FORMS IN NGF-STIMULATED PC12 CELLS, Mark L. Day[1], Timothy J. Fahrner[1], Serdar Aykent[2], and Jeffrey Milbrandt[1], [1]Division of Laboratory Medicine, Departments of Pathology and Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110, [2]Monsanto Company, Department of Biological Sciences, St. Louis, MO 63198.
The NGFI-A gene, which encodes a zinc finger protein with a predicted molecular mass of ≈54 kDa, is rapidly activated in PC12 cells by nerve growth factor (NGF). As a possible transcription factor, NGFI-A is a potentially important mediator of the cellular response to NGF. Antisera raised against NGFI-A recognize two predominant NGFI-A species in NGF-stimulated PC12 cells: a 54 kDa form which resides solely in the cytoplasm and an 84 kDa phosphoprotein found exclusively in the nucleus. Both NGFI-A species are rapidly induced in PC12 cells by NGF, phorbol ester, and the calcium ionophore A23187. Pulse-chase analysis revealed no obvious precursor-product relationship between these two forms and demonstrated that both the 54 and 84 kDa species are short-lived proteins. V8 protease digestion of the 54 and 84 kDa forms resulted in the formation of several small peptides that were common to both species. The digest of each species also contained one large, relatively V8 protease resistant fragment that was substantially larger in digests of the 84 kDa form. These two fragments contained common epitopes and were derived from the amino terminal portion of the NGFI-A protein. Collectively, these data suggest that a modification of unknown nature occurring on NGFI-A engenders the nuclear localization of the 84 kDa form.

P 511 MECHANISMS OF GLUCOCORTICOID REPRESSION OF PRO-OPIOMELANOCORTIN GENE TRANSCRIPTION, Jacques Drouin, Yu Lin Sun, Marc Therrien, Alaka Mullick and Mona Nemer. Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal, Montréal (Québec) CANADA, H2W 1R7.
The pro-opiomelanocortin (POMC) gene is specifically expressed in two cell types of the pituitary gland which process POMC into different secretory products. Glucocorticoids are synthesized in the adrenal gland in response to one of these products, ACTH, and they exert a negative feedback on anterior pituitary ACTH secretion and POMC gene transcription. By using DNA-mediated gene transfer into culture cells and in transgenic mice, we have defined a 543 bp fragment of the POMC gene which is sufficient for pituitary-specific expression and glucocorticoid repression in the anterior pituitary. Within this 5'-flanking fragment of the gene, multiple regulatory elements contribute to tissue-specific expression; in particular, a putative corticotroph-specific transcription factor has been identified and shown to bind to a unique sequence of the POMC promoter. A" negative glucocorticoid response element" (nGRE) was also localized at position -63 bp. This nGRE was shown to be devoid of any GRE activity. The nGRE binds the purified glucocorticoid receptor (GR) and GR:nGRE protein:DNA complexes were shown to differ from GR:GRE complexes. Formation of this unique GR:nGRE complex was correlated with repression by the use of point mutations. The nGRE was also shown to bind the COUP transcription factor, suggesting that mutually exclusive binding of COUP and GR may be involved in the mechanism of repression. In conclusion, the mechanisms of glucocorticoid repression at the POMC nGRE may involve 1) the lack of intrinsic GRE activity, 2) the formation of a unique GR:nGRE complex which differs from GR:GRE complexes, and 3) the displacement of COUP transcription factor by GR.

Egr-1 (a.k.a. NGF1A, Krox 24, or zif268) is a sequence specific DNA binding protein containing zinc fingers that is co-regulated with the proto-oncogene c-*fos* during the late differentiation of teratocarcinoma cells. We have now found that egr-1 expression is induced very rapidly by retinoic acid (RA) in the pluripotent P19 cell line. By 20' after RA administration, egr-1 mRNA accumulated to 400% of basal levels. After 90', egr-1 mRNA levels had declined to 20% of basal levels. The pulse of egr-1 message was matched by a corresponding pulse in egr-1 protein synthesis. The lower level of egr-1 mRNA was maintained for at least 24 hours. Thereafter levels increased gradually to 800% of basal levels in fully differentiated nerve and glial cells. Egr-1 message also accumulated in undifferentiated cells treated with the protein synthesis inhibitors cycloheximide or puromycin. The cycloheximide effect is dominant over the normal decline in message seen at 90' post RA administration. The amplitude of responses, both positive and negative, detected by RNAse protection experiments, was greater for the 3' end of the message than for the 5'end, suggesting a steady state accumulation of 5' ends that is significantly greater than the concentration of the whole message. The rapid induction of egr-1 by RA may indicate a crucial role for egr-1 in the initial stages of differentiation.

P 513 COORDINATE EXPRESSION OF TUMOR SUPPRESSOR GENE (Rb) AND MYOGENIC DETERMINATION GENES (MyoD1 & myogenin) DURING MUSCLE CELL DIFFERENTIATION AND DEDIFFERENTIATION, **Takeshi Endo and Susumu Goto**, Dept. of Biology, Chiba University, Chiba, Chiba 260, Japan. Terminally differentiated skeletal muscle cells (myotubes) have been regarded as irreversibly withdrawn from the cell cycle. Using the C2 mouse skeletal muscle cells transfected with the SV40 T antigen genes fused to the Zn^{2+}-inducible metallothionein gene promoter (C2SVTts cells), however, we have demonstrated that the expression of large T antigen in myotubes induces reentry of the terminally differentiated cells into the cell cycle (Endo & Nadal-Ginard, 1989). These myotubes are subject to mitosis and cytokinesis represented by the appearance of condensed chromosomes, mitotic spindles, midbodies, and contractile rings. To elucidate the molecular basis for the terminal differentiation and this dedifferentiation, we examined the expression of three nuclear protein genes: the tumor (growth) suppressor gene Rb and the myogenic determination genes MyoD1 and myogenin. The expression of all these genes was up- and downregulated during terminal differentiation and dedifferentiation of the C2SVTts cells, respectively. These results are remarkable, because the activity of Rb protein has been reported to be regulated by phosphorylation but not by the transcriptional level during the cell cycle or cellular differentiation. In addition, the levels of MyoD1 expression has been shown not to dramatically change during differentiation of parental C2 cells. The results suggest that high levels of these proteins play important roles in the induction or maintenance of a differentiated state. MyoD1 mRNA accumulated several-fold within 2 hr by the treatment of protein synthesis inhibitors. Thus, this mRNA stability is likely to be regulated by short-lived protein(s).

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Coordination of events that are a prerequisite for mating in *Saccharomyces cerevisiae* involves arrest of the mitotic cycle in the G1 phase and transcriptional induction of relevant genes. These responses require that a signal initiated by interaction of a peptide pheromone with a cell surface receptor be transmitted to the nucleus. Genetic approaches showed that the *STE12* gene specifies a component of the signal pathway. Biochemical analyses showed that the STE12 protein forms complexes with the transcriptional control elements of pheromone responsive genes. This finding suggested that STE12 may be a nuclear target of the pheromone induction signal. A further prediction is that STE12 overproduction might be sufficient to cause expression of at least some functions normally observed only under conditions of pheromone induction. Consistent with this prediction, I have found that STE12 overproduction inhibits growth and causes morphological alterations which are characteristic of cells undergoing the mating response. The effects of STE12 overproduction were also examined in strains lacking other components of the signal pathway such as the receptor (STE2), the β-subunit of G-protein (STE4), and two protein kinases (STE7 and STE11). The results do not support a simple model in which these components function in a linear pathway.

P 515 AN IN VIVO SCREENING PROCEDURE IN E. COLI FOR THE ISOLATION OF SEQUENCES ENCODING SPECIFIC DNA BINDING PROTEINS. Günter Feix and Lienhard Schmitz, Institut of Biology III, University of Freiburg, D-78 Freiburg, F.R.C.
A genetic screening procedure in E. coli has been developed for the isolation of cDNAs encoding DNA binding proteins. The method consists basically in first transforming E. coli with an expression plasmid with the DNA target site for the binding protein inserted between a weak promoter and the lac indicator gene. These E. coli cells are then infected with a cDNA expression library prepared from tissue expressing the DNA binding protein. Clones containing a DNA binding protein cDNA may then be recognized by the repressor activity of the synthesized fusion proteins bound to the target site in front of the lac gene. The ß-gal activity is thereby dramatically reduced and can be monitored by plating the cells on McConkey agar. By using repeat units from the external spacer region of a nuclear rRNA gene from maize as recognition site, we were able to isolate several cDNA clones encoding specific DNA binding proteins. The method will be applicable to the isolation of DNA encoding specific DNA binding proteins independent of the organism.

P 516 NFIL-1βA, A NOVEL TRANSCRIPTIONAL FACTOR THAT REGULATES HUMAN IL-1β GENE EXPRESSION, Matthew J. Fenton[1], Fumihiko Shirakawa[2], and Philip E. Auron[2], [1]Dept. of Medicine, Boston Univ. Sch. Med., Boston, MA 02118 and [2]Dept. of Medicine, Mass. General Hospital, Charlestown, MA 02129. Interleukin-1 (IL-1) α and β are pluripotent cytokines with a broad range of biological actions. We have previously reported that human IL-1β gene expression in monocytic cells is regulated at the transcriptional level by at least two distinct stimulus-specific pathways. We have identified a novel nuclear protein (NFIL-1βA) that appears to be both monocyte- and stimulus-specific, binds adjacent to the TATA box of the IL-1β gene, and may interact with the transcriptional factor TF-IID. Several modified IL-1β promoters have been constructed which lack the NFIL-1βA binding sequence, or in which this sequence has been displaced from the TATA box, and subsequently cloned upstream of the CAT reporter gene. These constructs have been transfected into human monocytic cells in order to assess the functional role of NFIL-1βA during transcription. Furthermore, The migration of the DNA-protein complexes generated by NFIL-1βA in gel shift assays has been found to differ depending on the stimulus used to induce IL-1β expression, suggesting that different stimuli can selectively generate distinct post-translationally modified forms of NFIL-1βA.

P 517 ROLE OF THE YEAST STE12 PRODUCT IN THE PHEROMONE RESPONSE PATHWAY, Stanley Fields, Joseph W. Dolan, Celia Kirkman, Ok-kyu Song and Olive Yuan, Department of Microbiology, State University of New York at Stony Brook, Stony Brook, N.Y. 11794. Mating in the yeast Saccharomyces cerevisiae is facilitated by the exchange of peptide pheromones. These pheromones produce their response by causing the dissociation of a receptor-coupled G protein, which leads to arrest in the G1 phase of the cell cycle, changes in cell surface morphology and transcriptional induction. We have analyzed the role of the STE12 gene product in this process, and have shown that it plays a key role in gene induction by binding to the pheromone response element present in the upstream region of inducible genes. Overproduction of the STE12 protein leads to a response similar to that generated by pheromone, suggesting that the principal role of pheromone may be the transcriptional induction of genes whose products are required for mating. In addition, overproduction of the STE12 protein is able to suppress the mating defect of strains deficient in other components of the pheromone response pathway. We have localized the domain of the STE12 protein required for DNA-binding and a domain that responds to the signal generated by pheromone.

P 518 CELL TYPE -SPECIFIC DNase 1 HYPERSENSITIVE SITES IN THE 1st INTRON OF THE HUMAN C-SIS GENE THAT CORRELATE WITH THE TRANSCRIPTIONAL ACTIVITY IN DEVELOPING EXTRAEMBRYONIC TISSUES. Gary Franklin, Susan Pfeifer-Ohlsson, Gail Adam, Mark Donovan, Lars Holmgren and Rolf Ohlsson. Centre for Biotechnology, Karolinska Institute, Novum, S-141 52 Huddinge, Sweden.
The cytotrophoblasts, which are the major cell component of early placenta, exhibit explosive proliferation, invasiveness and evasion of maternal immunosurveillance. These three properties are reminiscent of tumor cell phenotypes and have led to the use of the trem "pseudomalignant" to describe placental trophoblasts. The study of the expression patterns of a number of growth factors/protooncogenes and their receptors has led us to the hypothesis that they may act via autocrine loops, and that control of proliferation is maintained by a "reciprocal" mechanism. The c-sis protooncogene, which is identical to the B-chain of platelet derived growth factor (PDGF), illustrates this "reciprocity". Cytotrophoblasts express the gene at very high levels (with only very low expression of the PDGF receptor); whereas fibroblasts of the placental mesenchymal stroma express high levels of PDGF-B receptor and no detectable expression of c-sis. The JEG-3 cell line, established from a malignancy of the trophoblast (choriocarcinoma) also express c-sis, but at a much more moderate level than normal trophoblasts. We have identified one strong and one weak DNase 1 hypersensitive (DH) site in the 8 kb 1st intron of the sis gene, which are present in trophoblast and JEG-3 DNA but are not present in DNA from 1st trimester human placental fibroblasts. An additional, extremely strong DH site within the 1st intron was also observed exclusively in trophoblasts. The two strongly hypersensitive sites, located 0.5 kb apart in the c-sis1st intron may, therefore, represent a trophoblast-specific enhancer. Sequencing of the relevant region of the intron has revealed the presence of 3 glucocorticoid receptor binding sites concentrated within a region of only 130 bp. This region approximates to within the trophoblast-specific strong DH site; a correlation which is being tested by a precise linear PCR mapping of the DH sites. We have made constructs to test the functionality of the putative enhancer region; both by transient transfection assay and by transgenic mouse experiments. The significance of the glucocorticoid receptor binding sites in the intron with respect to possible hormonal regulation of the c-sis gene , is also under investigation.

P 519 ANALYSIS OF REGULATORY ELEMENTS CONTROLING THE EXPRESSION OF THE HUMAN FAST SKELETAL MUSCLE TROPONIN C GENE, **Reinhold Gahlmann and Larry Kedes, University of Southern California, Los Angeles.**

We have have defined a regulatory element in the human fast skeletal troponin C gene that is critical for the maximal expression of test genes in the myogenic cell line C2. The element is located between -1500 and -1650 bp relative to the transcriptional start site. There are three binding sites for nuclear factors in this regulatory element. Based on gel mobility shift analysis and methylation interference assays it was determined that the nuclear factor 1 (CTF/NF1) binds to one of the three sites. The two remaining sites seem to interact with the same nuclear factor since both binding sites cross-compete for binding in gel mobility shift assays. Both sites have an A/T-rich core in common. The replacement of A/T-pairs by G/C-pairs in the core of both sites eliminates binding of the nuclear factor. In promoter test-constructs transcriptional activity as assayed by reporter gene (CAT) activity was reduced to basal levels when this upstream element was deleted. Most of the 5'-flanking region of the gene between the regulatory element and the transcriptional start site can be deleted without significant impact on expression levels. Muscle-specificity of the upstream element and the requirement of additional promoter elements for the muscle specific expression of the human fast skeletal troponin C gene will be discussed.

P 520 TRANSCRIPTIONAL REGULATION OF THE INTERLEUKIN 2 GENE BY GLUCOCORTICOID HORMONES. A. Gulino, A. Vacca, S. Martinotti, A. R. Farina, I. Screpanti, M. Maroder, M.P. Felli and L. Frati, Dept. of Experimental Medicine, Univ. of Rome and of L'Aquila, Italy.

Dexamethasone (Dex) treatment of Jurkat cells inhibited the enhancing effect of phorbol ester (TPA) and the calcium ionophore A23187 on the interleukin 2 (IL2) mRNA levels and gene transcription from intact nuclei. Dex inhibited also the TPA/A23187-dependent expression of the transfected pIL2CAT, containing the IL-2 gene promoter (-600 bp) driving the expression of the chloramphenicol acetyl transferase (CAT) gene. Transfection of either Dex-sensitive or -resistant Jurkat cells with a human glucocorticoid receptor cDNA significantly increased or induced, respectively, the Dex-mediated inhibition of the TPA/A23187-dependent expression of pIL2-CAT as well as the enhancing effect on the expression of the cotransfected Mouse Mammary Tumor Virus LTR-CAT gene. NIH-3T3 and HeLa cells stably transfected with the intact human IL2 gene expressed constitutively the exogenous gene, whose activity was not further enhanced by treatment with TPA and A23187. Dex was not able to inhibit the levels of the IL2 mRNA in untreated or TPA/A23187-treated NIH-3T3 and HeLa transfectants. Our data suggest that the Dex-mediated transcriptional inhibition of the IL2 gene is T cell specific and is mediated by a glucocorticoid receptor-induced interference with the protein kinase C and calcium-mediated trans-activation of the antigen responsive elements lying in the 5' flanking region of the gene.

P 521 CLONING OF ANDROGEN RECEPTOR cDNA'S WITH THE POLYMERASE CHAIN REACTION (PCR) TECHNIQUES, He, W.W., Zhu, X.P., Young, C.Y.F., Tindall, D.J., Department of Urology Research, Mayo Clinic/Foundation, Rochester, Minnesota 55905

In order to understand the molecular basis of androgen action in different species, we have cloned the mouse and other androgen receptor cDNA's by both the PCR technique and conventional methods. Mouse kidney RNA was isolated and used to synthesize a first strand cDNA which was then amplified using three sets of oligonucleotide primers derived from human and rat androgen receptor cDNA sequences. Three overlapping segments were amplified and sequenced directly. The derived amino acid sequence of the mouse androgen receptor has 96% homology with that of the rat androgen receptor; the DNA and steroid binding domains of the mouse androgen receptor are identical to those of the human and rat androgen receptors. By screening a mouse kidney lambda gt11 cDNA library with a rat androgen receptor cDNA probe, we isolated a 1.2 kb cDNA clone, which covered the steroid binding domain of the mouse androgen receptor. A 1.7 kb genomic fragment covering the entire N-terminus region of mouse androgen receptor was also isolated from an EMBL3 mouse genomic DNA library by screening with a human androgen receptor N-terminus probe. The DNA sequences obtained from the cDNA and genomic clones are exactly the same as that obtained from the PCR products. In order to isolate androgen receptors from other species with the PCR technique, we synthesized two "universal primers" derived from the the DNA and steroid binding regions of receptor and successfully amplified part of the androgen receptors in dog, guinea pig, chicken and frog. These receptor probes are currently being used to address the mechanism of androgen action in different species.

P 522 DEVELOPMENT OF THE ZONAL DISTRIBUTION OF HEPATIC PHOSPHOENOLPYRUVATE CARBOXYKINASE IN NEWBORN RATS, W. Höppner, M. Reiser, A. Schrattenholzer and H.J. Seitz, Physiol. Chem. Inst., University Hospital Eppendorf, 2000 Hamburg, F.R.G.

In the liver of adult rats Phosphoenolpyruvate Carboxykinase (PEPCK) is expressed predominantly in periportal hepatocytes under all nutritional and hormonal conditions investigated. Injection of a high dose of dibutyryl-cAMP in vivo failed to induce the enzyme in perivenous cells. This regulation is achieved at the level of *transcription*. PEPCK is not expressed in fetal rats, but is induced immediately after birth to reach a maximum activity after 24 H. This increase is preceded by a rise in the rate of gene transcription and the amount of hybridizable PEPCK-mRNA. During the first three days of life, all hepatocytes perform gluconeogenesis and express PEPCK as shown by immunohistochemical staining and in situ hybridization. At day five after birth, the protein has disappeared in the perivenous cells and displays a zonal distribution like in liver from adult rats. At this time the PEPCK-mRNA is still detectable in all hepatocytes. The heterogeneous distribution of PEPCK at this state is regulated at the *translational* level. 10 days after birth PEPCK-mRNA and protein show the same heterogenous distribution with a predominant expression in the periportal zone.

Summary: In adult rats gene expression of hepatic PEPCK is regulated at the level of transcription. In newborn rats PEPCK activity is induced by an increase in the rate of transcription in all hepatocytes. Between 3 and 5 days after birth a translational block in perivenous hepatocytes occurs to achieve a heterogeneous distribution. 10 days after birth this developmental process is completed and the gene shows a zonal distribution and regulation like in liver from adult rats.

P 523 HOMEOBOX GENE EXPRESSION IN THE INTESTINAL EPITHELIUM OF ADULT MICE, Robert James and J Kazenwadel, Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Parkville, VIC 3050. The intestinal epithelium is one of the most rapidly proliferating and differentiating tissues yet described. The basic functional unit is the crypt which contains a non-migratory, self-renewing stem cell population that gives rise to all of the different cell types present in the mature epithelium. Although it is clear that for normal development of the epithelium an inductive interaction is required between the mesenchyme and endoderm during embryogenesis, nothing is known about what controls differentiation in the adult crypt. To gain some insight into this complex biological process we have examined whether known regulators of differentiation, i.e. the homeobox gene family are expressed in adult intestine and if so, is this expression restricted to a particular region or cell type. Using a PCR based strategy we have detected nine different homeobox genes including one which has not been previously described. A more detailed study of two of these genes has shown that they are only expressed in the intestine and their mRNA levels vary quantitatively in different regions of the intestinal tract. These graded expression patterns which have been documented in Drosophila and in the developing nervous system in mice suggest a role for these genes in establishing and or maintaining the functional differences between large and small intestine.

P 524 NEGATIVE TRANSCRIPTIONAL REGULATION OF THE HUMAN β-IFN GENE

Andrew D. Keller and Tom Maniatis, Harvard University, Cambridge, MA 02138.

The Human β Interferon (β-IFN) gene is transcriptionally activated by virus for about 8 hours afterwhich it is turned off. Both positive and negative regulatory motifs have been identified in the β-IFN promoter. One of these motifs, PRDI, is capable on its own of conferring both virus inducibility and post-induction shutoff to a heterologous promoter. We have isolated a cDNA clone encoding a protein, termed PRDI-BF1, which may be responsible for the post-induction transcriptional shutoff at the PRDI site. This protein contains five zinc fingers and binds specifically to the PRDI motif in the β-IFN promoter. When PRDI-BF1 is expressed in several cell types, it represses the activity of a cotransfected β-IFN promoter fused to the CAT reporter gene. In HeLa cells, the SV40 enhancer is silenced by the overexpression of PRDI-BF1 provided a PRDI motif is linked in *cis*. In contrast, reporter constructs containing promoters with no apparent binding site for PRDI-BF1 are activated by PRDI-BF1. Experiments are in progress to determine the mechanisms involved in the positive and negative regulatory activities of PRDI-BF1.

P 525 ESTRADIOL REGULATES EXPRESSION OF THE GLYCOPROTEIN HORMONE ALPHA SUBUNIT GENE DESPITE ABSENCE OF AN ESTROGEN RESPONSE ELEMENT, Keri, R.A., Kennedy, J.C., Andersen, B., Hamernik, D.L., Clay, C.M., and Nilson, J.H. Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The glycoprotein hormones (LH, FSH, TSH, and hCG) consist of a common α subunit and a unique β subunit. The single copy α subunit gene is expressed in both pituitary and placenta and responds to a variety of hormones. To further study the molecular mechanisms underlying tissue-specific expression and hormonal regulation of the α subunit gene, transgenic mice were produced with a construct containing 1500 bp of the proximal 5' flanking region of the human α subunit gene linked to the CAT gene. These mice express CAT tissue-specifically in pituitary and placenta and cell specifically in gonadotrophs. *In vivo*, estradiol regulates transcription of the human α subunit gene both positively and negatively. The mechanism of this interaction, however, has not been defined. Using a castration/estradiol replacement paradigm, we observed negative regulation of the αCAT transgene following addition of estradiol. Thus, all of the cis-acting elements necessary for negative regulation by estradiol must be within 1500 bp of the 5' flanking region. Transient expression studies were also used to examine estradiol regulation of the human α subunit gene. JAr cells were cotransfected with an estrogen receptor expression vector and the same construct used to produce transgenic mice. Surprisingly, the αCAT chimeric gene was unresponsive to estradiol in this cell culture system. Furthermore, we have shown, using direct binding studies, that estrogen receptor does not interact specifically with either the 5' flanking region or the entire human α subunit gene. Thus, the 5' flanking region of the human α subunit gene can confer estradiol responsiveness in a whole animal model, but cannot confer responsiveness in a transfection system or bind directly to estrogen receptor. We conclude that while the human α subunit gene is negatively regulated by estradiol, this regulation must occur through an indirect mechanism.

P 526 COORDINATE ACTIVATION OF INTERFERON-STIMULATED GENES REQUIRES CYTOPLASMIC ACTIVATION AND NUCLEAR TRANSLOCATION OF THE HETERODIMERIC TRANSCRIPTION FACTOR ISGF3. Daniel S. Kessler, Susan Veals*, and David E. Levy*, Laboratory of Molecular Cell Biology, Rockefeller University, New York, NY 10021; *Department of Pathology, NYU School of Medicine, New York, NY 10016.

Signal transduction by interferon-α (IFNα) stimulates immediate transcription of a defined set of genes. This coordinate gene activation is dependent upon a conserved cis-acting DNA sequence which is bound by a specific transcription factor, ISGF3. ISGF3 is formed from latent components which pre-exist in cells and are activated following exposure to IFNα. Active ISGF3 initially appears in the cytoplasm of treated cells and is subsequently translocated to the nucleus, a process which can be specifically blocked by treatment with NaF. We have reconstituted *in vitro* one step in the activation of ISGF3. Mixing cytosolic fractions from IFNα treated cells with fractions from untreated cells produces large amounts of active ISGF3. This activation step is non-enzymatic but rather is due to the stoichiometric association of two protein components. One of these components, ISFG3α, is the direct target for activation by IFNα treatment, sustaining a post-translational modification. This modification allows it to associate with the second component, ISGF3γ, and to translocate to the nucleus. The post-translocational modification of ISGF3α has been reproduced *in vitro* in permeabilized cells allowing the characterization of the small-molecule requirements and the biochemical nature of this reaction. In addition, we are studying the nuclear translocation of active ISGF3α component *in vitro* using purified protein and isolated nuclei from cells responsive and non-responsive to IFNα. The levels of the latent forms of ISGF3α and ISGF3γ in these cells and the promptness of the nuclear translocation event correlate with the ability of IFNα to inhibit cellular proliferation in normal and IFN-resistant cells.

P 527 PHOSPHORYLATION OF cAMP-RESPONSE ELEMENT BINDING PROTEIN, CRE-BP1, BY PROTEIN KINASE C AND ITS POSSIBLE INVOLVEMENT IN GENE ACTIVATION, Akira Kishimoto, Atsushi Sakurai, Shunsuke Ishii*, Toshio Maekawa* and Tatsuhiko Sudo*, Department of Biochemistry, Kobe University School of Medicine, Kobe 650 and *The Institute of Physical and Chemical Research (RIKEN), Tsukuba, Ibaraki 305, Japan.

Protein kinases A and C, which are activated by the respective second messenger, cAMP and diacylglycerol, seem to regulate gene transcription by mechanisms not yet understood. The cAMP response element (CRE) is an inducible enhancer of the genes that can be transcribed in response to increase in cAMP concentrations. We have isolated human clones encoding the CRE-binding protein, CRE-BP1, from a human brain cDNA library. The DNA sequence analysis showed that the sequence of CRE-BP1 has a typical leucine zipper structure at its carboxy terminus, but the sequence is totally different from that of the CRE-binding protein, CREB, reported by other research groups (Montminy *et al.* and Habener *et al.*). The purified CRE-BP1, expressed in *E.coli*, was specifically bound to the CRE (TGACGTCA) but not to TPA-response element (TGAGTCAG) nor serum-response element (GATGTCCATATTAGGACATC). The purified CRE thus obtained was efficiently phosphorylated *in vitro* by protein kinase C resulting in the stimulation of its CRE-binding activity (3- to 9-fold). The site of phosphorylation in this CRE-BP1 has been identified, and inactivation of this site by *in vitro* mutagenesis of the CRE-BP1 cDNA abolished CRE-BP1 transcriptional activity. Available evidence suggests that protein kinase C may contribute the regulation of CRE-dependent transcription through the phosphorylation of CRE-BP1 under certain condition (supported by grants from the Ministry of Education, Science and Culture, and the Science and Technology Agency, Japan).

P 528 **Differential regulation of c-fos, c-jun and jun B during in vitro differentiation of mouse P19 Embryonal carcinoma cells.** **Wiebe Kruijer, Rolf P. de Groot, Frank A.E. Kruyt and Paul T. van der Saag. *Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands.***

c-jun, jun B and c-fos encode nuclear proteins involved in the transcriptional regulation of target genes containing the TPA responsive enhancer element (TRE). To study the role of these proteins during development, we characterized their expression during retinoic acid (RA) induced in vitro differentiation of mouse P19 embryonal carcinoma (EC) cells. Undifferentiated P19 EC cells express the c-jun gene at very low levels, while the c-fos and jun B genes are not expressed. RA treatment causes a strong enhancement of c-jun mRNA within 24-48 hours. The RA responsive enhancer in the c-jun promoter has been localized. TPA ails to induce c-jun, jun B and c-fos in undifferentiated EC calls, whereas these genes are transiently induced by TPA in RA-differentiated cells. The differential expression and induction by TPA of these genes correlates with TRE binding activity in nuclear extracts and transactivation of the TRE in transient transfection assays. Both positive and negative regulatory effects were observed by jun gene products on TRE enhancers. To further determine the role of c-jun in the differentiation of EC cells, we transfected P19 EC cells with an SV40 driven c-jun gene and isolated stably transfected cell lines. Both TRE binding and transactivation were strongly enhanced in the c-jun transfected cell lines, but not in control cells. All c-jun expressing call lines had lost the transformed phenotype of undifferentiated EC cells, whereas control cells had retained these properties. Evidence will be presented showing that the differentiation inducing potential of c-jun in EC cells is mediated by the retinoic acid receptor (RAR) α and β genes.

P 529 **The Retinoic Acid Receptor Represses Estrogen-dependent Transcription.** By LIPKIN*, SM, GLASS, CK AND ROSENFELD, MG. University of California, San Diego.

The retinoic acid and estrogen receptors are members of the steroid hormone receptor superfamily of ligand dependent transcription factors, and share a high degree of sequence identity in their respective DNA binding domains. Using an avidin-biotin complex DNA binding assay, we have demonstrated that the retinoic acid receptor (RAR) will bind to the vitellogenin estrogen response element (ERE) with affinity comparable to that of the Estrogen Receptor (ER).

Transient transfection assays using endogenous RAR and ER in rat pituitary tumour cells (GC), and introduced receptors in monkey kidney cells (CV-1), demonstrate that neither the RAR nor a fusion protein consisting of the glucocorticoid receptor tau domain fused to the RAR, activate transcription from promoters containing an ERE. However, the RAR does repress estrogen dependent stimulation of an ERE placed in front of the Tk or SV promoters.

The proliferation of rat pituitary tumor cells (GH_3) is stimulated by estrogen (10^{-11}), and inhbited by cotreatment with retinoic acid (10^{-7}).

These findings suggest that the antagonism of complex estrogen-stimulated events by retinoic acid, such as the proliferation of pituitary and breast tumor cells, may reflect competition between the RAR and ER on estrogen-responsive genes.

Nuclear Factors; Steroid Hormones-II

P 530 EXPRESSION OF HOMEOBOX GENES IN CHICK EMBRYO LIMB BUDS. Susan Mackem[1] and Kathy Mahon[2], [1]Laboratory of Pathology, NCI, and [2]Laboratory of Mammalian Genes and Development, NICHD, NIH, Bethesda, M.D. 20892 Homeobox genes play a key role in specifying the segmented body plan of drosophila, and recent work by several groups indicates that certain homeobox genes may also have an analogous regulatory role during vertebrate limb morphogenesis. We have used degenerate oligonucleotide primers from highly conserved domains in the homeobox to PCR amplify homeobox gene segments from chick embryo limb bud cDNAs. Expression of a number of homeobox genes (at least 16 genes) is detected using this technique. Two homeobox genes have been identified which appear to be new members of the homeobox family in vertebrates. Both of these genes appear to be selectively expressed in developing limb buds between stages 17-24, and one of them is also preferentially expressed in wing buds (4 to 5 fold higher than leg buds), suggesting a possible role in determination of limb-identity. Currently, *in situ* hybridizations are in progress to more exactly determine the temporal and spatial patterns of expression of these genes in chick embryos.

P 531 Characterization of Orphan Nuclear Receptors David J. Mangelsdorf, Estelita S. Ong, and Ronald M. Evans. HHMI, Salk Institute, Gene Expression Lab, PO Box 85800, San Diego, CA 92138. We have begun characterizing vertebrate orphan receptors which are homologous to the superfamily of nuclear hormone receptor genes. These genes were isolated by low stringency hybridization techniques using thyroid hormone receptor and retinoic acid receptor cDNAs as probes. The isolated cDNAs code for proteins that share structural and functional motifs common to other members of this superfamily and have distinct patterns of expression.

P 532 COOPERATIVE INTERACTIONS AND PROTEIN PHOSPHORYLATION AFFECT HIGH AFFINITY BINDING BETWEEN PURIFIED Drosophila HOMEOPROTEINS AND DNA, Enrique Martín-Blanco, Henri-Marc Bourbon and Thomas B. Kornberg, Department of Biochemistry and Biophysics, University of California, San Francisco. San Francisco, CA 94143-0554. The purified homeodomains of the engrailed, even skipped, bicoid and fushi-tarazu proteins bind a synthetic DNA oligonucleotide with different affinities. Using monoclonal antibodies, we found that the homeodomain-DNA interactions are mediated through their helix-turn-helix. The engrailed homeodomain binds as a monomer to a single binding site sequence and sequentially to repeated sites. We have defined a engrailed protein domain that induce a cooperative effect on DNA binding, possibly through protein-protein interactions. The engrailed gene product is a phosphoprotein. We identified the modified residues and the protein kinases involved. We established by gel retardation assays that phosphorilation by purified casein kinase II, the major embryonic kinase in vitro modifying the engrailed protein, increase the affinity of a purified substrate for a specific octamer-like DNA.

P 533 The Effect of cAMP on the Expression of the alpha-L-Fucosidase gene in Early Development of *Dictyostelium discoideum*,

Thomas May, Hedi Kern, Jürgen Blusch and Wolfgang Nellen, Department of Cell Biology, Max Planck Institut of Biochemistry, D-8033 Martinsried, FRG.

The signal transduction pathway leading from the cell surface cAMP receptor to the induction of gene expression in early development of *Dictyostelium discoideum* is being investigated by use of a reverse approach: Analysis of a cAMP regulated promoter, identification of cis-elements and the characterization of the corresponding trans-acting factors should enable us to follow back the path of the signal from the level of gene expression to the extracellular stimulus. For the alpha-L-Fucosidase (aLF) promoter we show by nuclear run-on experiments that cAMP stimulates transcription. A cis-acting element responsible for cAMP dependent induction of the aLF-gene expression is identified by deletion analysis. *In vivo* this element functions in the context of a heterologous promoter sequence as a cAMP responsive element (DCRE) and is target of a developmentally regulated nuclear protein in vitro. Gel-shift and gel-overlay assays demonstrate that the 55 bp element is the target for a developmentally regulated nuclear protein(s). Treatment of partially purified nuclear extracts with phosphatase shows that binding of the protein(s) depends on phosphorylation indicating involvement of a protein kinase in the mechanism of gene induction. Since a mutant defective in Adenylatcyclase activation expresses the aLF gene similar to the wild type, it appears unlikely that Kinase A, which is induced by a rise of intracellular cAMP, mediates factor phosphorylation. With further experiments on the characterization of the binding protein(s) interacting with the DCRE we expect to establish the link between the signal, its transduction pathway and the regulation of gene expression.

P 534 CYTOKINE REGULATION OF MURINE DECIDUAL METALLOTHIONEIN GENE EXPRESSION, M.T. McMaster, S.K. De, S.K. Dey, G.K. Andrews, Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas, 66103. Metallothionein (MT) mRNAs are present at high levels in decidua on days 5-10 of pregnancy (D1=vaginal plug). Expression of the MT genes also occurs in experimentally induced deciduomata which suggests that it is independent of the embryo. In many tissues, metal ions (Zn, Cd), glucocorticoids, or bacterial lipopolysaccharide-endotoxin (LPS) induced cytokines are the most potent inducers of MT mRNA. In order to examine the mechanisms of regulation of decidual MT gene expression, the competence of the D4 pregnant uterus to respond to these agents was determined. MT mRNA levels in D8 decidua are much greater than those in the maternal liver which suggests a local effect on MT genes in decidua. Injection of Zn resulted in only a slight increase in MT mRNA in the D4 uterus, whereas liver MT mRNA levels were maximally stimulated. This establishes that the D4 uterus is not highly responsive to Zn, and suggests that systemic changes in metal ion concentrations do not account for elevated decidual MT mRNA levels. Adrenalectomy on D4 did not effect MT mRNA levels in decidua on D8, indicating that glucocorticoids do not play a significant role in decidual MT expression. Injection of LPS or human recombinant interleukin-1 (IL-1α) rapidly elevated MT mRNA levels several-fold in the D4 uterus. Following injection of LPS or IL-1α, rapid induction of mRNAs for a variety of cytokines (i.e. IL-1α, IL-1β and tumor necrosis factor (TNF-α)) occurred in the D4 uterus. These results establish that exogenous cytokines can enhance MT gene expression in the uterus, and that several cytokine genes in this organ can be up-regulated. Northern blot analysis demonstrated the presence of IL-1α, IL-1β and TNF-α mRNAs, and immunocytochemistry detected IL-1α and TNF-α in untreated decidua. These results lead us to hypothesize that expression of MT genes in decidua is regulated in an autocrine/paracrine manner by cytokines which are produced locally in response to the pro-inflammatory process of implantation and decidualization.

P 535 A DNA STRUCTURAL COMPONENT TO THE cAMP ENHANCENMENT OF THE HUMAN ENKEPHALIN GENE, Cynthia T. McMurray, James O. Dougalss, W. David Wilson Vollum Institute for Advanced Bomedical Research, Oregon Health Sciences University, Portland, Or. 97201. Induction of gene transcription by second-messengers is an important step in the signal transduction process. The site of second-messenger-induced regulation of the human proekephalin gene has been located within an enhancer region, -96 to -114 upstream of the transcriptional start site. Gel electrophoresis and thermal melting analyses combined were used to confirm that the duplex form of the enkephalin enhancer could undergo a reversible conformational flip to two hairpin structures, each containing two mismatched base pairs. The levels of the hairpin can be altered by changes in the DNA concentration,ionic strength, temperature or pH. The conformational flip is mediated by proton transfer to one of the DNA strands which subsequently stabilizes the hairpin form while destabilizing the duplex form. The hairpin structures formed from each strand was confirmed by thermal melting, calorimetry, gel electrophoresis and NMR proton assignment. To test the idea that hairpin formation is important in second-messenger-inducible enhancer function, mutant enhancer elements were synthesized and studied for observable changes in structure and function. Each mutant studied thermodynamically and structurally in vitro has been subcloned into vectors and tested for its ability to act as an inducible enhancer, in vivo. In all cases, the ability to form a good hairpin structure correlates with the ability of the element to act as an enhancer. Additionally, the ability to accept a proton on one of the hairpin forms correlates with the ability to regulate enhancer function.

P 536 **A *DROSOPHILA* HOMOLOG OF THE VERTEBRATE MYOGENIC REGULATORY GENE FAMILY.** A. M. Michelson*, S. M. Abmayr*, A. Martinez Arias#, C. M. Bate# and T. Maniatis*, *Dept. of Biochem. and Mol. Biol., Harvard Univ., Cambridge, MA 02138 and #Dept. of Zoology, Univ. of Cambridge, Cambridge, UK. Muscle development is regulated in vertebrates by a family of transcription factors that share a structural motif consisting of adjacent basic and helix-loop-helix (HLH) domains. We have isolated a homolog of this gene family from *Drosophila melanosgaster* as an approach to undertaking a detailed genetic analysis of myogenesis. The predicted protein product of this gene, designated MDR, is approximately 85% similar to its vertebrate counterparts over 72 amino acids that encompass the basic and HLH domains, a segment that is both necessary and sufficient for the myogenic activation function of mouse MyoD1. The MDR gene encodes a single 1.7 kb polyadenylated zygotic RNA that accumulates in embryos prior to the onset of muscle structural gene expression. MDR mRNA level reaches a maximum in mid-embryogenesis, declines throughout later embryonic and larval stages, and peaks again in pupae coincident with formation of the adult musculature. Homozygous *dorsal* mutant embryos that fail to gastrulate and therefore lack all mesodermal derivatives do not express the MDR gene. Whole mount *in situ* hybridization revealed that MDR transcripts are first localized in 8 hour embryos in segmentally repeated patches of cells in the ventral mesoderm. This corresponds to the time and localization of the earliest sign of muscle differentiation in *Drosophila* embryos, the fusion of the ventral mesodermal cells that are the precursors of the ventral muscles. As the germ band shortens, the spatial expression of MDR becomes more complex and includes cells within the lateral and dorsal parts of the mesoderm, again a pattern that is concordant with that of the developing musculature. At even later times, MDR mRNA is found in a small number of segmentally repeated ventral abdominal and dorsal thoracic muscles. The striking sequence conservation of the MDR gene as well as both its temporal and spatial patterns of expression are consistent with a role in embryonic muscle precursor cell determination.

P 537 THE DROSOPHILA *SEVEN-UP* GENE, A MEMBER OF THE STEROID RECEPTOR GENE SUPER-FAMILY, CONTROLS NEURAL CELL FATES. Marek Mlodzik, Yasushi Hiromi, Ursula Weber, Corey S. Goodman and Gerald M. Rubin, Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720
The Drosophila *seven-up(svp)* gene was isolated as a lethal insertion in an enhancer-trap screen. It is expressed and required in a subset of neuroblasts in the embryonic CNS and photoreceptor cell precursors R1, R3, R4 and R6 during eye development. The absence of svp^+ function causes a transformation of these cells towards an other neural cell fate causing defects in the CNS and embryonic lethality. During eye development the absence of svp^+ function causes a transformation of R1, R3, R4 and R6 towards an R7 photoreceptor cell, as judged by morphology and expression of a R7-specific markers. This transformation depends in part on the *sevenless* gene product, which is required for the formation of the R7 photoreceptor in normal ommatidia. Our result show that *svp* is involved in control of cell fate during the generation of neuronal diversity. Molecular analysis of *svp* reveals that it is a member of the steroid receptor gene superfamily. Interestingly, it codes for two different protein forms generated by differential splicing; one DNA binding domain shares two different potential ligand binding domains. The type 1 *svp* protein appears to be the Drosophila homologue of the human transcription factor COUP. Over the entire COUP protein, 75% of the amino acids are identical in the putative *svp* protein. Within the two defined domains the identity between *svp* and COUP is even more striking; in the DNA binding domain 94% amino acids are identical and the putative ligand binding domains share 93% identity over 222 amino acids. The specific ligand to COUP is not known, nor is the sequence similarity in the ligand binding domain of *svp* to members of the superfamily with known ligands high enough to predict the nature of the ligand. However, the presence of 25 -30% homology of *svp* to the members with known ligand suggests that a specific ligand for svp and COUP is yet to be discovered.

P 538 ANTAGONISTIC INTERACTIONS OF INTERFERON AND OUABAIN ON c-fos EXPRESSION, Yoichi Nakagawa, Hiroakai Akai and Andrew C. Larner, Laboratory of Cytokine Research, Center for Biologics Evaluation and Research, National Institutes of Health, Bethesda, Maryland 20892. Interferons (IFNs) activate the expression of a variety of cellular genes. We showed previously that both ouabain, a specific inhibitor of Na/K ATPase and phorbol esters inhibit the IFN-induced transcriptional activation of the gene ISG-54K. The transcription of the cellular proto-oncogene c-fos is activated by many agents including polypeptide growth factors, phorbol esters, cyclic AMP and increased intracellular calcium. Using nuclear run on assays, we found that ouabain also activates the transcription of c-fos. In contrast to the rapid transcriptional activation of c-fos by most growth factors, activation of c-fos by ouabain requires several hours of exposure to the drug and enhanced transcriptional activity is maintained for more than 16 hours. Ouabain enhances c-fos expression in protein kinase C depleted cells, suggesting that its induction is mediated by pathway(s) which are distinct from c-fos induction by phorbol esters. IFNα inhibits c-fos induction by both phorbol esters and ouabain. The inhibitory actions of IFN occur by a post-transcriptional mechanism. These results emphasize the reciprocal relationship between those cellular genes regulated by phorbol esters and IFNs.

P 539 A CARDIAC SPECIFIC CTF LIKE TRANSCRIPTION FACTOR BINDS TO THE ANF PROMOTER AND IS REGULATED DURING CARDIAC DIFFERENTIATION, Mona Nemer, Ali Ardati, Mona Greenbaum, Jacques Drouin. Laboratoire de génétique moléculaire, Institut de recherches cliniques de Montréal, Montreal (Québec) CANADA, H2W 1R7.
Atrial natriuretic factor (ANF) is a 28 AA peptide hormone with diuretic, natriuretic and vasodilatory properties. The ANF gene is expressed predominantly in the heart where it is under differential control in atria and ventricles. In particular, ventricular expression of ANF is correlated with cardiac cell growth and differentiation: fetal ventricles contain amounts of ANF peptides and mRNA which are equivalent to the atria. Expression of the ventricular ANF gene decreases markedly after the first week of age and the terminally differentiated adult ventricles contain 100 fold less ANF mRNA than adult atria. Using gene transfer into primary cardiocyte cultures, we have identified two regions (a proximal and a distal one) of the rat ANF promoter which are required for cardiac specific activity. We have performed DNase I footprinting experiments on these regions using nuclear extracts prepared from cardiac tissues at various stages of differentiation and from non cardiac tissues. These experiments have identified a DNA element which is present in the human ANF promoter and in both proximal and distal elements of the rat ANF promoter which binds a factor present in cardiac tissues. Gel shift assays using a specific oligonucleotide corresponding to the protected DNA element revealed binding of a cardiac specific nuclear protein to this site. The amount of this nucleoprotein is much higher in 1 day old ventricles compared to adult ventricles. Competition experiments suggest that this protein is related to CTF/NF1 transcription factor although distinct from it as revealed by its chromatographic mobility, its tissue distribution and content and the pattern of methylation interference of its binding site.

P 540 DIFFERENT EVOLUTIONARY PATHWAYS HAVE LED TO PLACENTAL EXPRESSION OF THE GLYCOPROTEIN HORMONE α-SUBUNIT GENE, John H. Nilson, Robert A. Fenstermaker, and Todd A. Farmerie, Department of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

Placental expression of the glycoprotein hormone α-subunit gene occurs in primates and horses only. Tandem cAMP response elements (CREs) in the promoter-proximal region of the human α-subunit gene play an important role in placenta-specific expression. Here, we report that tandem CREs are present only in the genes of higher primates and humans, whereas a single CRE is found in the genes of lower primates. Thus, placenta-specific expression of the α-subunit gene can occur with only one functional CRE, with tandem CREs being a recent evolutionary event. In contrast, the α-subunit genes of non-primates, including the horse, contain a single, non-functional homolog of the primate α-subunit CRE. The presence of a non-functional CRE in the proximal flanking region of the horse α-subunit gene is surprising because this species is the only non-primate known to synthesize *bona fide* chorionic gonadotropin. Thus, different mechanisms underlie the independent evolution of placenta-specific expression of the α-subunit gene in primates and horses.

P 541 **INDUCED TRANSCRIPTION OF THE tPA GENE IN RAT GRANULOSA CELLS,** Monica Ohlsson (1) Pei Feng (1) Xiao-chi Jia (2) Aaron J.W. Hsueh (2) and Tor Ny (1), Department of applied cell and molecular biology, University of Umeå, Umeå, Sweden (1). Department of Reproductive medicine, University of California, San Diego, La Jolla, CA 92093,USA(2).
Tissue Plasminogen activator (tPA) is produced by the granulosa cells in the ovary, under the control of gonadotropins. tPA is thought to initiate a proteolytic cascade at the time of ovulation leading to the rupture of the follicle wall. We have studied the regulation of tPA by Follicle Stimulating Hormone (FSH) and Gonadotropin Releasing Hormone (GnRH) in primary cultures of rat granulosa cells. FSH and GnRH were both found to increase tPA mRNA in a dose dependent manner, but with different kinetics. FSH and GnRH induce tPA mRNA through different intracellular pathways, the protein kinase A and protein kinase C pathway respectively. When granulosa cells were treated with FSH in the presence of the protein synthesis inhibitor cycloheximide, a superinduction of tPA mRNA could be seen. In contrast the induction of tPA mRNA by GnRH was inhibited by cycloheximide treatment, suggesting that the synthesis of an intermediate protein is needed for the induction by GnRH. To further study the regulation of tPA gene expression the rat tPA gene was isolated. Sequence analysis of the promoter region showed that the rat tPA promoter contained a cAMP responsive element, identical to the proposed consensus sequence, in a position were mouse and human tPA have a AP-1 like binding sequence. Studies so far has show that only the rat tPA gene can be directely induced by cAMP. The promoter region of both rat and human tPA has been fused to the firefly luciferase reporter gene and are at present tested by transfection into primary cultures of granulosa cells.

P 542 Function of retinoic acid receptor homologs in Drosophila development Anthony E. Oro and Ronald M. Evans HHMI, Salk Institute, Gene Expression Lab, PO Box 85800,La Jolla, CA 92138. Receptors for the vertebrate morphogen retinoic acid have been isolated that are members of the steroid and thyroid hormone receptor superfamily. Receptors involved in other developmentally important process are postulated to exist. The gene fragment encoding the retinoic acid receptor α DNA-binding domain was used as a probe to pull out cross-hybridizing genomic fragments in Drosophila. One of the fragments map to the X chromosome and thus has been called XR2C8. The genetic and molecular nature of this receptor locus has been studied.

P 543 DIFFERENTIATION LINKED REGULATION OF CHROMOSOMAL PROTEINS HMG-14/-17 IN MYOBLASTS AND ALTERED HMG-14 EXPRESSION IN MOUSE TRISOMY 16 EMBRYO

James M. Pash and Michael Bustin, Laboratory of Molecular Carcinogenesis, NCI, NIH, Bethesda, MD 20892

The expression of chromosomal proteins HMG-14 and HMG-17 was studied in cultured myoblasts. During myogenesis, down-regulation of both HMG-14 and HMG-17 mRNA occurs simultaneously with activation of muscle specific actin mRNA. This process is linked to differentiation rather than to the cell cycle because, unlike histones, inhibition of myoblast DNA synthesis with hydroxyurea had no effect on HMG expression. Protein synthesis was measured by the incorporation of radiolabeled lysine into HMG proteins and histone fractions. Radiolabeling of HMG proteins is significant in myoblasts, but undetectable in myotubes. However, the amount of HMG protein measured by staining is unchanged during differentiation. These results indicate that the regulation of HMG-14/-17 mRNA levels is different from that of the histones and is linked to differentiation rather than to DNA synthesis.

The gene for human HMG-14 has been mapped to the chromosomal region 21q22.3. This region of chromosome 21, which is linked to Down syndrome, is also syntenic with the distal fifth of mouse chromosome 16. Mouse trisomy 16 embryos were examined for a gene dosage effect in the expression of HMG-14. Protein electrophoresis and densitometry indicate that HMG-14 protein is expressed in these embryos at 1.5 times that seen in normal littermates. Aberrant regulation of HMG-14 expression may be involved in the etiology of the syndrome.

P 544 THE FOS PROTEIN COMPLEX: CHARACTERIZATION OF CELL-TYPE-SPECIFIC COMPONENTS, **Karl T. Riabowol, Douglas Q. Girgenti and Michael Z. Gilman, Cold Spring Harbor Laboratory, Box 100, Cold Spring Harbor, NY 11724**

The protein products of the *c-fos* and *c-jun* protooncogenes form stable heterodimers through high-affinity binding via "Leucine Zipper" domains. Such heterodimers have been shown to bind AP1 and related DNA sequences with high affinity *in vitro*, suggesting that this protein complex may serve a role in the transcriptional control of genes containing these motifs. Non-denaturing immunoprecipitations of whole cell lysates with Fos antibodies specifically coprecipitate several isoforms of Jun and a variety of other proteins, several of which are cell-type-specific. We have characterized one such protein which binds to Fos with an avidity similar to that of Jun. Expression of this protein appears to be constitutive, and limited to T lymphocytes. Present evidence also suggests that this protein contains a Leucine Zipper domain since this protein and the Jun protein bind to Fos in a mutually exclusive manner.

P 545 SYNERGISTIC ACTIVATION OF TRANSCRIPTION BY HOMOLOGOUS AND HETEROLOGOUS TRANSCRIPTION FACTORS, Douglas M. Ruden, Marc Lamphier, and Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138. We observe synergistic activation of transcription when two activator binding sites are placed upstream of a target gene. Synergistic activation of transcription means that activation mediated by two activator binding sites is greater than the sum of the activation mediated by the sites individually. The proteins which mediate this synergistic activation of transcription contain either the DNA binding portion of GAL4 (a yeast transcription activator) or the DNA binding portion of lexA (a bacterial repressor) fused to various acidic peptides; the target genes contain either GAL4 binding sites, lexA binding sites, or both. In the case with lexA-transcription activators, synergistic activation of transcription only occurs when the lexA binding sites are less than 50 basepairs apart from each other. The level of the synergistic activation of transcription is much higher when we test weak activators than when we test strong activators.

P 546 ALPHA-AMANITIN RESISTANT TRANSCRIPTION OF PROTEIN CODING GENES IN INSECT AND BLOODSTREAM FORM TRYPANSOMA BRUCEI,

Gloria Rudenko, Joseph A. Smith, Mary G.-S. Lee and Lex H.T. Van der Ploeg,
Department of Genetics and Development, Columbia University, New York City, NY 10032

We are interested in the mechanisms controlling differential expression of the variant cell surface glycoprotein (VSG) genes of the protozoan parasite Trypanosoma brucei. The telomerically located VSG gene expression sites are transcribed by an unusual alpha-amanitin resistant RNA polymerase. All other protein coding genes of T. brucei are transcribed by an alpha-amanitin sensitive RNA polymerase, presumably RNA polymerase II. We have determined that transcription of protein coding genes by alpha-amanitin resistant RNA polymerases is not unique to the bloodstream form of the parasite, in which the VSG gene expression sites are active, but also occurs in insect form trypanosomes. The transcription of the genes encoding ribosomal RNA, the insect form specific surface coat proteins (procyclins or PARPs) and the bloodstream form specific VSGs is equally resistant to alpha-amanitin. Comparison of the PARP and VSG gene families showed that they both produce one of the most abundant mRNAs (1-3% of polyA$^+$ RNA), and they both encode the major cell surface proteins of their respective life-cycle stages. Transcription of a subset of functionally comparable protein coding genes is thus mediated by an RNA polymerase which differs from the regular RNA polymerase II. The PARP transcription units are, in contrast to the other trypanosome genes, relatively small; with the promoter located directly upstream of the PARP coding sequence. The PARP promoter is being analyzed by transfection of promoter-CAT gene fusion constructs in procyclic T.brucei.

P 547 THE MYO D1 GENE PRODUCT IS INVOLVED IN CONTROLLING THE HUMAN CARDIAC α GENE EXPRESSION,

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The human cardiac actin gene (HCA) promoter directs expression exclusively in myogenic cell lines. Although many sequence-specific DNA binding proteins interact with the promoter and control HCA gene expression, the proteins are ubiquitous and the mechanism determining its highly tissue-specific expression has so far not been determined.
A 5' region of the HCA promoter encompassing nucleotides -177 to +68, when linked to a heterelogous reporter gene (choramphenicol acetyl transferase, CAT) sustains high level expression of this gene in rodent myogenic C2 and L8 cell lines but not in non-muscle cells.
By gel retardation, methylation interference and cotransfection assays we show that the Glutathione-Myo D1 fusion protein interacts with nucleotides -57/-50 of the HCA promoter and that the LTR-driven Myo D1 cDNA transactivates the HCA gene. A DNA motif (CArG box) located upstream the Myo D1 binding region and previously found to interact with a protein immunologically and biochemically indistinguishible from the serum response factor (SRF) is sufficient and necessary to mediate the Myo D1-induced transactivation of the HCA gene. This observation suggests a functional cooperation of these proteins in insuring the appropriate expression of the HCA gene. We are currently investigating the functional significance of the Myo D1 binding to the HCA promoter through the use of HCA mutants lacking the Myo D1 binding site.

P 548 IP-1: A PHOSPHORYLATION-REGULATED INHIBITOR OF FOS/JUN. Paolo Sassone-Corsi and Johan Auwerx. Institut de Chimie Biologique, Faculté de Médecine - 11, rue Humann - 67085 Strasbourg Cédex, France.
Addition of TPA to cultured cells causes a rapid and transient induction of fos and jun oncogenes, which is accompanied by an enhancement of nuclear complex formation on a TRE sequence. We analysed several cell types for TPA-induced binding to a TGACTCA sequence and the expected stimulation was observed in the nuclear fractions. The binding activity in the nuclear extract was reproducibly much higher than in the cytoplasm and, interestingly, there was no detectable binding in the cytoplasmic fraction of untreated cells. Induction of specific binding is very rapid upon TPA treatment of HeLa cells, since significant induction of TRE binding is already observed 10 minutes after TPA treatment. The presence of a potential repressor was then analysed by mixing increasing amounts of the cytosolic fraction from untreated cells to the highly active nuclear extract from TPA-induced cells. Our studies indicated the presence of excess amounts of an inhibitory activity for AP-1 conserved among different mammalian species and cell types. We demonstrate that phorbol ester activation of the fos/jun complex involves the inactivation of a specific cellular repressor, termed IP-1. It is a labile protein of 30- to 43-kD which exerts specific DNA binding-inhibitory activity only in its non-phosphorylated form. It is proposed that dissociation of fos/jun oncoproteins from IP-1 presumably allows their translocation into the nucleus and transcriptional activation. cAMP-dependent protein kinase A modulates IP-1 phosphorylation, indicating a cross-talk mechanism of signal transduction at transcriptional level.

P 549 **TRANSCRIPTION REGULATION OF COLLAGEN II GENE.** Savagner P., Miyashita T., and Yamada, Y. Laboratory of Developmental Biology and Anomalies, NIDR, NIH, Bethesda, MD 20814. Collagen II, the major component of cartilage, is synthesized primarily by chondrocytes and certain cells in the eye. Previously, we have shown that both the promoter and tissue-specific enhancer elements located in the first intron are required for a high level of transcriptional activity in chondrocytes. We located two tissue-specific silencer elements which suppress the activity of the promoter in fibroblasts. Using gel shift analysis, nuclear factors isolated from chondrocytes were found to bind specifically to the central region of the collagen II enhancer. Retinoic acid and TGF-ß treated chondrocytes, which show decreased collagen II transcription, showed an altered gel shift pattern. Interestingly, the DNA/protein complex inhibited by retinoic acid was also competed by a 100 bp promoter sequence which also is required for a high level of collagen II transcription. These results indicate that both availability of nuclear factors for the enhancer and silencer binding sites are important for collagen II tissue-specific expression

P 550 **CLONING, STRUCTURAL ANALYSIS AND EXPRESSION OF THE HUMAN SLOW/CARDIAC MUSCLE TROPONIN C GENE,** **Thomas Schreier, Larry Kedes, and Reinhold Gahlmann, University of Southern California, Los Angeles.**

The human slow/cardiac skeletal troponin C gene was isolated, mapped and sequenced. We mapped the transcriptional start site and demonstrated that both skeletal and cardiac muscle use the same start site. A consensus TATA-box is located 29 bp upstream of the transcriptional start site. No canonical CAAT-box was observed. We analyzed the expression of a slow TnC-testgene in the myogenic cell lines C2C12, L8 and H9c2(2-1), in the human lung fibroblastic line WI38 and in the tumor cell line HeLa which all express the endogenous slow/cardiac troponin C gene but at different levels. The endogenous gene is highly expressed in differentiated C2C12, L8 and H9c2(2-1) cells. The gene is also expressed in HeLa cells at high levels compared to the marginal but clearly detectable level found in WI38 cells. Constructs comprising 4.0 kb (pHSTnC4000CAT) of 5' flanking sequences including the genuine transcriptional start site upstream of the chloramphenicol transferase gene as reporter expressed the hybrid gene in differentiated cells of all three myogenic lines tested, and also in Hela cells at significant levels. No expression was detected in fibroblastic WI38 cells. Thus, the human slow/cardiac troponin C construct and the endogenous genes reveals similar levels of expression in these lines.

P 551 DEVELOPMENTAL AND THYROID HORMONE DEPENDENT REGULATION OF A XENOPUS LEAVIS TRYPSIN GENE

Yun-Bo Shi and Donald D. Brown, Department of Embryology, Carnegie Institution of Washington, 115 West University Parkway, Baltimore, MD 21210.

We have isolated two clones encoding Xenopus leavis trypsin from a tadpole cDNA library. Nucleotide sequence analysis reveals that the two clones are generated by alternative polyadenylation and encode a protein with about 70% identity to mammalian trypsinogen. Northern blotting analysis shows that the trypsin gene is expressed in the tadpole pancreas and is under strict developmental regulation. It is activated when the tadpole starts to feed and repressed as metamorphosis reaches climax. Since the endogenous thyroid hormone level peaks at metamorphosis, this suggests that thyroid hormone directly or indirectly regulates trypsin gene expression. The influence of thyroid hormone on trypsin gene expression is supported by treating tadpoles with thyroid hormones, which reduces trypsin gene expression.

P 552 GENERATION OF TRANSGENIC MICE WHICH EXPRESS HIGH LEVELS OF C-*MYB* THROUGHOUT T CELL DEVELOPMENT. Richard M. Siegel, Katsuyuki Yui, Drew E. Tenenholz, Makoto Katsumata, E.P. Reddy, and Mark I. Greene. Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia PA 19104 It has been known for some time that expression of the c-*myb* proto-oncogene is downregulated during development of many hematopoetic cell types. Transfection of a c-*myb* cDNA was shown also shown to inhibit the chemically induced differentiation of an erythroleukemia cell line, (McMahon, et. al. *Oncogene* 3:717) suggesting a casual link between downmodulation of c-*myb* expression and differentiation. To study the role of c-*myb* in T cell development *in vivo*, we have created constructs in which cDNA coding sequences for the mouse c-*myb* oncogene are placed upstream of enhancer elements which confer high level T-cell specific expression in transgenic mice. Unike the situation in normal animals, where c-*myb* expression is downregulated as T cells mature in the thymus and exit to the periphery, all T cells in transgenic mice should express c-*myb* at high levels throughout ontogeny. We are presently in the process of generating transgenic mouse strains with these constructs. Results of our experiments will be presented.

P 553 SYNERGISM BETWEEN THE RECEPTORS FOR PROGESTERONE AND ESTROGEN STUDIED *IN VITRO*, Emily P. Slater and Miguel Beato. Institut für Molekularbiologie und Tumorforschung, Philipps Universität, 3550 Marburg, Federal Republic of Germany.

Steroid hormone receptors can cooperate functionally with each other or with transcription factors when their cognate binding sites are in close proximity to one another. This synergism of action has been extensively studied in transfection experiments using the chicken vitellogenin gene from -720 to -591 containing the hormone responsive element fused to the thymidine kinase promoter and the CAT gene. In gene transfer experiments this construct is expressed under the control of estrogens, glucocorticoids and progestins. At maximal concentrations of hormones these effects are additive and at submaximal concentrations, the effects are synergistic. The dose response curve with estrogen in the presence of progestins is shifted to the left. The mechanism by which synergism is achieved is thought to occur through protein-protein interaction. The HRE of chicken vitellogenin consists of binding sites for the three hormone receptors. We therefore tried to study this effect *in vitro*. In band shift experiments we were able to distinguish complexes arising from interactions of the ER with Vit DNA from those arising from PR. With this system we have shown that when both receptors are present in the incubation mixture with DNA a third complex is also observed, one with slower mobility. Methylation interference experiments demonstrate that both receptors are indeed bound to the DNA isolated from this complex. It would appear that the affinity of one receptor for the DNA is higher in the presence of the other as the PR specific complex becomes visible at a lower concentration when the ER is present. Thus, we can demonstrate the interaction of two different steroid hormone receptors *in vitro* that may allow us to explain the mechanism of synergism of the corresponding hormones *in vivo*.

P 554 TRANSCRIPTIONAL REGULATION OF A XENOPUS EMBRYONIC KERATIN GENE: NUCLEAR FACTORS WHICH BIND UPSTREAM PROMOTER ELEMENTS. A.M. Snape, E.A. Jonas and T.D. Sargent, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health , Bethesda, MD 20892. XK81A1 is a keratin which is expressed specifically in the epidermis of Xenopus embryos. Deletion analysis was used to characterise the upstream regulatory region of this gene (Jonas et al. Development 106, 399-405, 1989). Embryonic nuclear extracts were shown to contain a protein, KTF-1, which binds in vitro to an imperfect palindromic sequence at -157 in the promoter. Alteration of 8bp of the binding sequence abolished KTF-1 binding in vitro and severely reduced transcription of the cloned gene on injection into Xenopus embryos, indicating that KTF-1 is a transcriptional activator of XK81A1 in vivo. The altered gene, although weakly expressed, was still tissue specific. This suggests that other promoter binding factors, currently being characterised, act in concert with KTF-1 to produce high levels of epidermis specific expression. We are now working on the further analysis, and cloning, of KTF-1 and these other keratin-promoter binding proteins.

P 555 ACTIVATION OF THE GM-CSF PROMOTER BY INDUCIBLE AND NON-INDUCIBLE DNA BINDING FACTORS IN JURKAT CELLS, Kenji Sugimoto*, Shoichiro Miyatake, Kenichi Arai+ and Naoko Arai, Department of Molecular Biology, DNAX research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. GM-CSF gene is activated by phorbol-12-myristate-13-acetate (PMA) and calcium ionophore (A23187) in human T cell line Jurkat and the upstream region between -95 and -73 is required for this activation (1). To study the mechanism of GM-CSF gene activation in vitro, nuclear extracts from non-stimulated and PMA/A23187-stimulated Jurkat cells were prepared for transcription and DNA binding assays. Gel retardation assays with mutant oligonucleotides revealed that two DNA binding motifs in the promoter , kB-like sequence and GC-rich sequence, are recognized by inducible and non-inducible factors, respectively. In vitro run-off transcription assays as well as in vivo transfection assays with the mutant promoters showed that the GM-CSF gene is most effectively transcribed when the promoter has both intact motifs. The activation mechanism of the GM-CSF gene by inducible and non-inducible factors will be described.

(1) Miyatake et al. (1988) Nucleic Acid Res., 16, 6547.

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P 556 Accumulation of proto-oncogene c-*erb*-A related transcripts during *Xenopus* development: Association with early acquisition of response to thyroid hormone and estrogen.

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Nuclear receptors for steroid and thyroid hormones and other developmental signals are encoded in the proto-oncogene c-*erb*-A related supergene family. We have studied the expression of genes encoding receptors for estrogen and thyroid hormones, as well as total c-*erb*-A related transcripts, in unfertilized eggs, all stages of embryonic and larval development and in adult tissues of *Xenopus*, by quantitative Northern and slot blot hybridization. DNA and antisense RNA probes complementary to *Xenopus* c-*myc*, cytoskeletal actin and albumin mRNAs served as controls or developmental markers. Hybridization to full-length chicken c-*erb*-A cDNA at moderate stringency revealed a complex biphasic ontogenic pattern for several c-*erb*-A related mRNAs in all tissues and at all developmental stages, an increase of 4-fold in the accumulation of these transcripts occurring before metamorphosis (stages 30 to 40-42), and followed by a gradual build-up after mid-metamorphosis (stage 56). Using full-length or ligand-binding domain fragments of thyroid hormone (TR) and estrogen (ER) cDNAs under stringent hybridization conditions, transcripts of TR and ER were detected from stages 44 and 54 onwards, respectively. The α and β forms of TR mRNAs exhibited different patterns of accumulation during development, the former transcript being present in substantially higher amounts at all developmental stages. The distinct patterns of accumulation of TR-α and ER mRNAs could be correlated with the differential pattern of early developmental acquisition of sensitivity of *Xenopus* larval tissues to thyroid hormone and estrogen, and support the view that receptor genes are activated very early in development.

P 557 DISSECTION OF FUNCTIONAL DOMAINS OF THE PITUITARY-SPECIFIC TRANSCRIPTION FACTOR GHF-1,AND ANALYSIS OF GHF-1 EXPRESSION DURING DEVELOPMENT. Lars E.Theill,Jose-Louis Castrillo,Pascal Dollè and Michael Karin,University of California at San Diego,La Jolla,CA 92093.The specific expression of growth hormone (GH) in the somatotrophic cells of the anterior pituitary is largely attributable to a short 5'-flanking region of the GH gene.This promoter contains two binding sites for the transcription factor GHF-1.GHF-1 is a member of the POU-domain class of proteins that each contain two highly conserved sequence motifs,the homeodomain and the POU-specific domain.Here we report that the homeodomain is sufficient for sequence specific DNA binding,although its activity is stimulated by the POU-specific domain,which does not interact directly with the DNA.Transcriptional activation is mediated by a separate domain rich in hydroxylated amino-acids residues.Similar sequences are present in other cell type-specific transcription factors.GHF-1 expression is specific to cells of the somatotrophic lineage.The relationship between expression of GHF-1,and activation of the GH and prolactin (Prl) genes during development of mouse anterior pituitary was investigated. While GHF-1 expression were detected within 24 hours of the first observable events in anterior pituitary differentiation (embryonic day 13),no GHF-1 protein could be detected until about 3 days later (embryonic day 16).The appearance of GHF-1 protein showed good temporal and spatial correlation with activation of the GH gene. Prl gene expression on the other hand, was observed transiently during embryonic day 16 in two different populations of cells, of which the major one does not contain GHF-1 or GH.These results suggest that expression of GHF-1 is controlled both transcriptionally and post-transcriptionally.The spatial and temporal correlation between the appearance of GHF-1 protein and GH gene activation suggests that GHF-1 is responsible for this very last step in the specialization of the somatotrophic cells.

P 558 PROGESTERONE RECEPTOR STIMULATES CELL FREE TRANSCRIPTION BY ENHANCING FORMATION OF A STABLE PREINITIATION COMPLEX, S.Y. Tsai, L. Klein-Hitpass, G.F. Allan, M. Bagchi, N.L. Weigel, M.-J. Tsai and B.W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Highly purified chicken progesterone receptor (cPR) is shown to stimulate directly RNA synthesis in an in vitro transcription assay. Both form A and form B progesterone receptors activate the target gene and maximum levels of stimulation are obtained at a receptor concentration of ~5 x 10^{-8}M, a level which closely approximates the physiologic concentration in progesterone stimulated oviduct cells. Stimulation of cell-free transcription by cPR requires the presence of progesterone response elements (PREs) in the template and can be specifically inhibited by addition of competitor oligonucleotides containing PREs, indicating that the receptor itself is responsible for activation. Binding of receptor to two PREs is cooperative and leads to a synergistic stimulation of transcription. Using this in vitro transcription system we examined whether the progesterone receptor was required for the formation of a template committed preinitiation complex. Our results indicate that receptor is essential for the assembly of such a stable complex. However, by itself, the receptor is not sufficient for the formation of a template committed complex. Therefore, progesterone receptor acts by enhancing the recognition of the promoter by other factors and thus augments the initiation of transcription by RNA polymerase II.

P 559 UROKINASE-TYPE PLASMINOGEN ACTIVATOR GENE EXPRESSION: REGULATION BY PROTEIN KINASE A AND C PATHWAYS, Dietmar von der Ahe[1], David Pearson[2], Gert Müller-Berghaus[1],and Yoshikuni Nagamine[2], [1]Max-Planck-Gesellschaft, Clinical Research Unit for Blood Coagulation and Thrombosis, D-6300 Giessen, F.R.G., [2]Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel, Switzerland. The association of increased urokinase-type Plasminogen Activator (uPA) production and growth factor activity has suggested a role for uPA in cell division/ proliferation and cell movement (1). A greater understanding of the role of uPA should be given by identification of the gene regulatory elements and the factors that interact with those elements (2,3). In a pig kidney cell line, LLC-PK_1 uPA gene expression is induced by the cAMP-elevating hormone calcitonin, by tumor promoter phorbol esters acting through a cAMP-independent pathway. The uPA gene activation is a primary transcriptional event. Using stably transfected lines we characterized a tripartite cAMP response element (CRE), which confers inducibility in a distance and orientation independent manner, around 3400 bp upstream of the uPA gene. DNase I Footprinting assays reveal that this tripartite enhancer contains two CRE consensus sequences TGACG and a third unique region (footprint C). The activities of the two CRE domains (footprints A and B) depend upon region C, although C has no inducibility by itself. Moreover, we could show that the catalytic subunit of Protein Kinase A augments binding of nuclear factors to site A and B but not to site C. In conclusion, we suggest that cAMP regulate uPA gene expression in part by increasing the affinity of CRE-binding protein(s) to A and B enhancer sequences upon phosphorylation by the C-SU of Protein Kinase A.

(1) Dano, K. et al., Advances in Cancer Research, 44, 139-266 (1985), (2) Nakagawa, J. et al., JBC, 263, 2460-2468 (1988), (3) von der Ahe, D. et al., NAR, 16, 7527-7544 (1988).

P 560 CONTROL OF GENE EXPRESSION AND DNA REPLICATION IN EARLY MOUSE EMBRYOS, Maria Wiekowski, Sadhan Majumder, Encarnacion Martinez-Salas, Miriam Miranda and Melvin L. DePamphilis, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

We are studying gene expression and DNA replication in mouse preimplantation embryos by microinjecting plasmid DNA into the pronuclei of 1-cell or zygotic nuclei of 2-cell embryos. It was previously shown that the expression of microinjected DNA probably is governed by the same control as the genomic DNA, and our prediction was that the requirement of enhancers depended on the zygotic nuclear structure (Martinez-Salas *et. al.*, Genes & Development **2**, 1115-1126, 1988; *ibid* **3**, 1493-1506, 1989). To change the environment of the embryonic nucleus, we are now using parthenogenetically activated eggs, as well as fertilized embryos containing transplanted nuclei. Furthermore, to determine the nature of the transcriptional signal that is present at the beginning of the zygotic gene expression, we are examining the minimal cis-acting sequences that are required for efficient transcription at this stage of development. Based on the above observations, a possible mechanism of control of gene expression and DNA replication in mouse preimplantation embryos will be presented.