GROWTH AND DIFFERENTIATION FACTORS IN DEVELOPMENT

Organizers: Rik Derynck and Brigid Hogan

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Poster Sessions

Growth and Differentiation Factors in Development

Growth Factors Regulating Development-I: The TGF-Beta Superfamily

O 001 REGULATION OF THE FETAL INHIBITOR, MULLERIAN INHIBITING SUBSTANCE, Patricia K. Donahoe, Department of Pediatric Surgery, Massachusetts General Hosptial, Harvard Medical School, Boston, MA 02114
The purification of Mullerian Inhibiting Substance (MIS), the definition of its gene structure, regulation and mechanisms of action, and a description of its classic and newly unfolding functions, as well as attempts to explore therapeutic applications of this fetal regressor/growth regulator will be presented. In addition, we will compare observations made for other members of its enlarging gene family, such as Transforming Growth Factor (TGF) B and the Inhibin/Activins, and of another newly discovered homologous protein which acts as an insulin tyrosine kinase inhibitor, to determine if strategies successful in the study of these proteins can be applied to the study of the MIS gene and molecule. The post-translational processing of holo MIS at its autocrine, paracrine, or endocrine sites of action and potential intracellular processing at the site of production will be considered as a unique form of molecular regulation, dictating the future design of targeted, specifically regulated, possibly chimeric therapeutic agents. Finally, we will explore the upstream regulation of expression of MIS by transacting factors as another avenue of potential growth control.

O 002 TRANSFORMING GROWTH FACTOR-BETA-RELATED GENES AND MAMMALIAN DEVELOPMENT, Karen Lyons, C. Michael Jones, Ron W. Pelton, Riet Van der Meer-de Jong, Victor Fet, Mary E. Dickinson and Brigid L.M. Hogan, Department of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232-2175. There is strong biochemical and genetic evidence that molecules related to polypeptide growth factors and their receptors play important roles in embryonic development in organisms such as Drosophila, Xenopus and Caenorhabditis. Examples include the decapentaplegic (dpp) gene product in Drosophila and the Vg-1 gene product in Xenopus, both of which are related to TGF-β and form part of a distinct dpp-like subfamily. Other members of this subfamily include the Bone morphogenetic proteins -2a, -2b and -3 and the recently described murine VgR-1 (Lyons et. al. (1989) *Proc. Natl. Acad. Sci.* **86**: 4554-4558). As part of a program to study cell differentiation and pattern formation in the mouse embryo we have been following the expression of various members of the TGF-β gene superfamily, including TGF-β1, -β2, -β3, bone morphogenetic protein -2a and Vgr-1, by *in situ* hybridization and Northern analysis. Maternal mRNA for VgR-1 is found at high levels in primary oocytes but the level of RNA declines after fertilization and is not detectable at the 4-8 cell stage. RNA levels subsequently increase above background in the blastocyst and early post-implantation embryo, but do not appear to be localized to any specific region. In contrast, high levels of Vgr-1 RNA are present in differentiating epidermis. Indeed all of the members of the TGF-β gene family so far studied, are expressed in distinct temporal and spatial patterns in the suprabasal cells of the embryonic and post-natal epidermis and hair and whisker follicles. Distinct patterns of expression are also seen in developing teeth and in cartilage and bone. The results suggest that TGF-β related genes may be playing a role in epithelial-mesenchymal interactions at several stages during mammalian development, and in the progression of embryonic stem cells through specific differentiation pathways. More recent experiments suggest that new member(s) of the dpp-subfamily may be present in the mouse genome and may be expressed at the egg cylinder/primitive streak stage when mesodermal induction is taking place as well as at later stages of development.

O 003 CONTROL OF CELL GROWTH AND DIFFERENTIATION BY TGF-ß. Joan Massagué
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TGF-ßs are pluripotent diffusible polypeptides that can act as growth inhibitors, regulators of cell phenotype and regulators of the cell adhesion apparatus. In studies addressing the mechanism of action of TGF-ß, we have identified three widely distributed cell surface components that bind this factor with high affinity. Two of these components are glycoproteins of 53 kDa (type I receptor) and 73-83 kDa (type II receptor). TGF-ß receptors are expressed ubiquitously with the notable exception of human retinoblastoma cells. The selective loss of these receptors in chemically-induced cell mutants resistant to growth inhibition by TGF-ß suggests that they are components of the signaling TGF-ß receptor complex. Their loss of function correlates with loss of all measurable responses to TGF-ß.

We identify the retinoblastoma protein, pRB, as a potential downstream component in the TGF-ß growth inhibitory pathway. The phenotype of hereditary retinoblastoma predicts that one of the functions of pRB is to suppress cell growth. This function has been ascribed to the underphosphorylated pRB form that prevails during G1 phase in the cell cycle. TGF-ß1 added to Mv1Lu lung epithelial cells in mid to late G1 prevents the phosphorylation of pRB scheduled for this point in the cell cycle, and arrests cells in late G1. These findings suggest that TGF-ß1 and pRB may function in a common growth inhibitory pathway in which TGF-ß1 acts to retain pRB in the underphosphorylated, growth suppressive state.

TGF-ß regulates expression of numerous genes that encode components of the cell adhesion apparatus -cell adhesion receptors and extracellular matrix proteins. To determine whether this response may in turn mediate effects of TGF-ß on cell morphology and phenotype, we have used L6E9 myoblasts differentiation as a model test system. This system provides evidence for the presence of two complementary pathways through which TGF-ß can block differentiation. Though one pathway, TGF-ß blocks up-regulation of myogenic differentiation gene expression. Through the other, TGF-ß strongly up-regulates collagen expression, and a collagen-rich matrix inhibits myoblast differentiation. Inhibition of differentiation by a collagen-rich extracellular matrix occurs without blocking up-regulation of the myogenic differentiation gene, myogenin.

Certain growth factors can mediate cell-cell adhesion. As a cell surface glycoprotein, the TGF-α precursor can sustein adhesion of cells that express TGF-α/EGF receptors and can stimulate these cells. By regulating the rate of proTGF-α cleavage, cells might be able to use this molecule to communicate exclussively with adjacent cells or, alternatively, with cells located at some distance in the surounding tissue.

O 004 IN VIVO AND IN VITRO ROLES OF BMP IN SKELETAL FORMATION AND REPAIR, Vicki Rosen, Scott Thies, Leslie Kurtzberg, Paul Cordes, David McQuaid, Marc Bauduy, Ioannis Moutsatsos, Josephine D'Alessandro, John Wozney and Elizabeth Wang, Tissue Growth and Repair, Genetics Institute, Inc., Cambridge, MA 02140. BMP (bone morphogenetic protein) describes an activity derived from bone that is able to direct new bone formation in vivo. Implantation of bone derived BMP protein at an ectopic site in rats results in the attraction of highly proliferative cartilage and bone cell progenitors to the area where they differentiate and produce living bone tissue complete with mature bone marrow. Genetic analysis of the proteins which constitute the BMP activity indicate that they are new members of the TGFb supergene family. While sharing limited homology to the TFGbs, the BMP genes are closely related to Vg1 and dpp, TGFb family members believed to provide positional information during embryonic development. Recent studies from our laboratory suggest that the BMPs may be involved in signaling skeletal formation during embryogenesis. Biochemical and molecular studies focusing on BMP-2 have revealed that BMP-2 mRNA is localized in rat embryo limb buds prior to cartilage and bone formation; BMP-2 protein is present at sites of skeletal formation in the rat embryo; and limb bud cells isolated from rat embryos are able to respond to BMP-2 protein in vitro. In vivo, BMP-2 implantation is sufficient to induce new bone formation. These in vitro and in vivo activities of BMP-2 suggest that it may function as a morphogenetic signal during early bone formation and may be useful as a therapeutic in processes requiring bone repair.

Extracellular Factors Regulating Development (joint)

O 005 MESODERM AND NEURAL INDUCTION IN XENOPUS: GROWTH FACTOR INVOLVEMENT AND GENE EXPRESSION, Igor Dawid[1], Peter Good[1], Anita Roberts[2], Michael Sporn[2], Martha Rebbert[1], Nanette Roche[2], Klaus Richter[3], Naina Bhatia-Dey[1], Frédéric Rosa[4]. [1]Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda MD 20892; [2]Laboratory of Chemoprevention, National Cancer Institute, Bethesda MD 20892; [3]Institute for Molecular Biology, Austrian Academy of Sciences, Salzburg, Austria; [4]Institut Pasteur, Paris, France.

Interactions between vegetal and equatorial cells in early development are involved in the formation of the mesoderm in the amphibian embryo. Culture fluid of XTC cells has mesoderm inducing activity; the major mesoderm inducing factor (MIF) from XTC cells appears to be related to the TGF-ß family of growth factors. We have purified TGF-ß2 and TGF-ß5 from XTC cell medium and separated them from MIF; TGF-ß2 induced mesoderm at high concentration only while TGF-ß5 was inactive. Purification of MIF is being pursued.

Several genes expressed in early development and specific for the nervous system, have been studied. (i) A ß-tubulin isoform is an excellent marker for neural induction. (ii) A gene for nervous system specific ß-subunit of Na/K ATPase has been isolated; it is related to but not the closest frog homolog of the ß1 or the ß2 subunits recognized in other organisms. We propose to identify it as ß3 ATPase. (iii) A gene encoding a putative RNP protein. The nervous system specific expression of this gene suggests possible tissue specific functions in RNA processing or transport.

O 006 Progressive anteriorization of the embryonic axis during Xenopus gastrulation. J. Gerhart, T. Doniach, B. Rowning, and R. Stewart. Department of Molecular & Cell Biology, University of California, Berkeley CA 94720.

In a 50 min period within the first cell cycle, the egg's cortex rotates as a rigid unit by 30° (350 µm) relative to the underlying rigid cytoplasm, around a rotation axis perpendicular to the animal-vegetal axis, transforming the egg's cylindrical symmetry into bilateral symmetry. The egg's meridian of maximal displacement of the cytoplasm in a vegetal direction relative to the cortex coincides with the future dorsal midline of the embryo. Parallel bundled microtubules (MT) occupy the shear zone of the vegetal hemisphere (2-3 µm from the egg surface) and serve as tracks for rotation. All treatments (nocodazole, cold shock, hydrostatic pressure, UV irradiation of the vegetal, not animal, hemisphere) which depolymerize MTs have the effects of arresting rotation, leading ultimately to the development of embryos systematically deficient in dorsal structures: the less rotation, the more posterior is the truncation level of the dorsal anatomy of the embryo. Without rotation, the embryo retains the egg's original cylindrical symmetry and forms only ventral structures.

This truncation series can also be produced at blastula stages by other treatments. If a normal early blastula is surgically deprived of vegetal cells of the Nieuwkoop inductive center, or if a late blastula is surgically operated upon to reduce the size of its organizer, the embryo subsequently develops a body axis truncated from the anterior end, looking just like embryos developing from eggs blocked in cortical rotation. The less the amount of Nieuwkoop center or organizer, the more posterior is the truncation level. If the center or organizer is removed entirely, a ventralized embryo results. To unify these results, we suggest that the amount of rotation determines the amount of inductive activity of the Nieuwkoop center (perhaps its secretion rate), and the strength of this induction determines the size of the Spemann organizer of the late blastula stage embryo.

Still, how might the size of the organizer affect the extent of anterior development? Organizer size correlates with the extent of morphogenesis (especially the convergent extension) of populations of gastrulating cells generating the body axis, and likewise morphogenesis itself correlates with the completeness of anterior dorsal pattern. We can produce the entire truncation series during gastrulation by blocking morphogenesis (using suramin and trypan dyes) in normal embryos at different times after gastrulation begins. The earlier the block, the less complete is morphogenesis and the more posterior the truncation level. We suggest that gastrulating cells do not have their anteroposterior fates defined at the beginning of gastrulation but progressively acquire ever more anterior fates, or progressively acquire the conditions for acquiring these fates, as morphogenesis proceeds.

O 007 EMBRYONIC INDUCTION AND AXIAL POLARITY IN XENOPUS DEVELOPMENT, D. Melton, S. Sokol, G. Thomsen and M. Whitman, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138

The induction of embryonic mesoderm and neural tissue by peptide growth factors will be discussed. Special attention will be given to the roles of the Xenopus Vg1 and activin proteins, both members of the TGFβ family of peptide growth factors. The formation of different mesodermal tissues and CNS will be ocnsidered. In addition, the use of homeobox genes to study the specification of anterior-posterior polarity will be presented.

O 008 **SPECIFICATION OF CELL TYPES IN XENOPUS EMBRYOS BY A MORPHOGEN GRADIENT**, J.C. Smith and J.B.A. Green, Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.

The mesoderm in Xenopus and other amphibian embryos is formed through an inductive interaction in which cells of the vegetal hemisphere of the embryo release a soluble signal which acts on overlying equatorial and animal pole cells. Two classes of 'mesoderm-inducing factors' (MIFs) have been identified. One comprises members of the fibroblast growth factor (FGF) family, and the other some of the members of the TGFβ superfamily (1). The most potent member of the latter class is XTC-MIF, which is derived from a Xenopus cell line (2); we shall discuss the identity of this molecule in our report.

Cell types formed after induction depend both on the class of MIF and on MIF concentration (3). In this report we use a refined technique to analyse this effect at the cellular level. We find that responding cells can sharply distinguish between concentrations of XTC-MIF differing by three-fold or less and that such discrimination operates to give at least two distinct cell-type transitions as assessed by activation of various region- and cell type-specific genes. We compare the pattern of activation of these genes with the fate map of Xenopus and discuss the evidence that body patterning may be caused by gradients of mesoderm-inducing factors.

1. Smith, J.C. (1989). Mesoderm induction and mesoderm-inducing factors in early amphibian development. **Development** 105, 665-677.

2. Smith, J.C. (1987). A mesoderm-inducing factor is produced by a Xenopus cell line. **Development** 99, 3-14.

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

Growth and Differentiation Factors in Development

Growth Factors Regulating Development-II: EGF and TGF-Alpha Related Molecules

O 009 TRANSFORMING GROWTH FACTOR-ALPHA: A POPULATON OF SECRETED FACTORS DERIVED FROM A BIOLOGICALLY ACTIVE TRANSMEMBRANE POLYPEPTIDE. Derynck Rik and Brachmann, Rainer, Department of Developmental Biology, Genentech Inc., 460 Point San Bruno Blvd, South San Francisco CA 94080.

Transforming growth factor-α (TGF-α) is a growth factor that is secreted by many transformed and normal cell types. It is structurally related to epidermal growth factor (EGF) and interacts with the same receptor as EGF. cDNA analysis has revealed that the initially characterized 50 amino acid TGF-α is derived from a longer precursor. Biochemical analysis of the secretedTGF-α indicates the presence of multiple, differentially processed forms of TGF-α, derived from a glycosylated precursor. The most abundant secreted form of TGF-α is an N-glycosylated form of 66 or 74 amino acids. All cells that synthesize TGF-α contain detectable levels of the transmembrane TGF-α precursor, which constitutes a very common TGF-α form in vivo. This transmembrane form is biologically active, since it interacts with the receptor and induces receptor autophosphorylation and subsequent signal transduction. These findings indicate the existence of multiple secreted and non-secreted forms of TGF-α and reveal that a growth factor does not have to be secreted in order to induce the resulting autocrine and paracrine biological effects. This also raises the possibility that the transmembrane TGF-α form serves as a signal transducing receptor.

O 010 EGF-RELATED GENES IN *C. ELEGANS*, John Yochem, Geraldine Seydoux and Iva Greenwald, Dept. of Biology, Princeton University, Princeton, NJ 08544

We will describe three genes from *C. elegans* that are predicted to encode transmembrane proteins containing sequence motifs resembling epidermal growth factor (EGF). Two of these genes, *lin-12* and *glp-1*, have been studied both genetically and molecularly, by our group and others. These two genes encode predicted products that include several identifiable sequence motifs, including multiple tandem EGF-like repeats in their putative extracellular regions; *lin-12* contains thirteen EGF-like motifs, and *glp-1* contains ten. Both *lin-12* and *glp-1* function cell autonomously in distinct cell fate decisions that involve cell-cell interactions, and have been postulated to encode receptors for intercellular signals. Another gene containing EGF-like motifs has thus far been identified only molecularly, by cross hybridization to probes from *lin-12* and *glp-1*. This gene is predicted to encode a transmembrane protein that contains features reminscent of both the mammalian EGF precursor and LDL receptor.

O 011 SIGNAL TRANSDUCTION BY EGF-RECEPTOR AND ITS MUTANTS. Joseph Schlessinger, Rorer Biotechnology, Inc., 680 Allendale Road, King of Prussia, PA 19406

The membrane receptor of epidermal growth factor (EGF-receptor) is composed of a large extracellular ligand binding domain, a single transmembrane region and a cytoplasmic domain containing protein tyrosine kinase activity. We have formulated an allosteric oligomerization model for activation of the catalytic properties of neighboring cytoplasmic domains (1,2). In vitro site directed mutagenesis was used to generate various EGF-receptor mutants. Using this approach, it was shown that the kinase activity of EGF receptor is essential for signal transduction and for normal receptor trafficking, while autophosphorylation is not crucial for receptor signalling (3-6). It was also shown that EGF is able to stimulate tyrosine phosphorylation of phospholipase C-γ (7) and protooncogene c-raf. EGF-induced tyrosine phosphorylation led to activation of c-raf, suggesting that this enzyme may be involved in cascade of kinases initiated by tyrosine phosphorylation. The precise composition of the transmembrane domain is not essential for receptor activity, further supporting the oligomerization model for receptor activation. Binding experiments of EGF to various chicken/human EGF receptor chimera has allowed the identification of domain III of the extracellular domain of EGF-receptor as a major ligand binding domain (8). Some interactions are also provided by domain I which, together with domain III appear to constitute the binding region for EGF. Large quantities of recombinant, extracellular, ligand binding domain of EGF-receptor were generated by either transfected CHO cells or from insect cells using the baculovirus system. The binding of EGF caused the oligomerization of the soluble, extracellular domain indicating that this domain is endowed with at least two functions: ligand binding and oligomerization.

1. Yarden Y. et al (1985) In Growth Factors in Biology and Medicine, Pitman, London pp. 23-45.
2. Schlessinger, J. (1986) J. Cell. Biol. 103: 2067-2072.
3. Honegger, A.M. et al (1987) Mol. Cell Biol. 7: 4567-4571.
4. Moolenaar, W. et al (1988) EMBO J. 7: 707-710.
5. Honegger, A.M. et al (1988) EMBL J. 7: 3053-3060.
6. Honegger, A.M. et al (1988) EMBO J. 7: 3045-3052.
7. Margolis B. et al (1989) Cell 57: 1101-1107.
8. Lax, I. et al (1989) EMBO J. 8: 421-427.

Growth Factors Regulating Development-III: FGF and PDGF Gene Families

O 012 **AUTOCRINE TRANSFORMATION OF CELLS BY FIBROBLAST GROWTH FACTOR,** **Michael Klagsbrun and Avner Yayon, Departments of Biological Chemistry and Surgery, Children's Hospital and Harvard Medical School, Boston MA, 02115**

basic FGF (bFGF) is a potent mitogen for cells of mesodermal origin. bFGF lacks a signal peptide and is not secreted by cells in culture. Many cell types, for example endothelial cells, produce bFGF and have bFGF receptors. Yet these cells appear normal and do not seem to undergo FGF-mediated autocrine transformation. In order to study a possible role for bFGF in autocrine transfomation, bFGF cDNA was transfected into 3T3 cells and cell lines expressing bFGF were established (Rogelj et al, Nature 331 173-175 1988). Cells transfected with native bFGF cDNA grew in monolayers, had a normal morphology and were not tumorigenic in syngeneic mice. On the other hand, cells transfected with a construct of bFGF fused to a signal peptide sequence (spbFGF) were transformed morphologically, and grew as non-adherent aggregates. spbFGF/3T3 cells were highly tumorigenic and metastatic in syngeneic mice. The spbFGF cells appeared to undergo autocrine transformation involving continuous down-regulation of the bFGF receptor. In cross-linking studies with ^{125}IbFGF, spbFGF-transformed cells, unlike control cells, did not have available cell surface bFGF receptors suggesting that continouos down-regulation of receptors was occurring via an autocrine loop. In the presence of suramin, a drug that interrupts growth factor-receptor binding, spbFGF cell surface bFGF receptors reappeared within 8 hours. Reappearance of bFGF cell surface receptors correlated with the reversion of spbFGF cells to a normal phenotype in which the proliferative rate of spbFGF cells was lowered to that of parental 3T3 cells and the cells became fully adherent. Unlike suramin, neutralizing anti-bFGF antibodies had no effect on spbFGF proliferation rate or morphology suggesting that these cells were transformed by an internal autocrine loop. The inability of spbFGF cells to adhere to culture dishes was explored. Compared to cells transfected with native bFGF cDNA, spbFGF cells produced enhanced levels of a 92 kDa gelatinase, an enzyme that degrades type IV collagen. Addition of inhibitors of metalloproteinase (IMP) promoted the attachment of spbFGF-transformed cells to the dish. Taken together, these results suggested that the lack of spbFGF-transformed cell adhesion may be the result of their increased ability to degrade ECM due to an alteration in the balance of proteinases that degrade ECM and their inhibitors. The ability of the spbFGF-transformed cells to degrade ECM might in part explain the invasive and metastatic potential of these cells in vivo.

O 013 EXPRESSION OF GENES IN THE FIBROBLAST GROWTH FACTOR FAMILY DURING MOUSE EMBRYOGENESIS, Jean M. Hébert and Gail R. Martin, Department of Anatomy, University of California at San Francisco, San Francisco, CA 94143.

Evidence is rapidly accumulating that basic fibroblast growth factor (FGF) and related proteins play an important role in embryonic development. In mammals, seven genes (bFGF, aFGF, kFGF, int-2, FGF-5, FGF-6 and KGF) have been identified that share considerable amino acid similarity in commonly aligned regions. To initiate a study of the role of the fibroblast growth factor family in mammalian development, we isolated cDNAs encoding four mouse FGF family members, aFGF, bFGF, kFGF and FGF-5. This was achieved by a process that circumvents the use of cDNA libraries: for each family member, a cDNA fragment containing the conserved portion of the coding region was amplified from a pool of embryonic and teratocarcinoma cell cDNAs using the polymerase chain reaction (PCR) and cloned; the remaining coding sequences 5' and 3' to the conserved region were cloned using the RACE method (1). The cDNA clones obtained were used as probes in Northern blot analyses to examine the expression of these genes at the RNA level in teratocarcinoma cells and embryos at 10.5 to 17.5 days of gestation. Fgfk appears to be specific to undifferentiated teratocarcinoma stem cells. Fgf5 transcripts were detected at every stage and in every tissue tested, but showed a dramatic 15-fold increase in abundance as teratocarcinoma stem cells differentiated to simple embryoid bodies. In situ RNA hybridization analysis has shown that this is due to a transient increase in the level of Fgf5 expression in the core cells of the embryoid bodies. Fgfb expression showed the greatest tissue-specific variability in abundance, with the highest levels detected in the developing limbs and tail. Fgfa showed the least variable pattern of expression, with transcripts detected at roughly equivalent levels in almost all samples analyzed. Based on these data we speculate on some possible roles that the different FGF family members may play in the developing embryo.

(1) Frohman, M. A., Dush, M. K. and Martin., G. R.. (1988). Proc. Natl. Acad. Sci. USA 85, 8998-9002.

O 014 MECHANISMS CONTROLLING THE ACTION OF BFGF AND TGF-β ON ENDOTHELIAL CELLS, Daniel B. Rifkin, Robert Flaumenhaft, David Moscatelli, Natalina Quarto, Ryoji Tsuboi, Yasufumi Sato, and Phillip Dennis, Department of Cell Biology, New York University Medical School, 550 First Avenue, New York, NY 10016. Endothelial cells both produce and respond to basic fibroblast growth factor (bFGF) and transforming growth factor β (TGF-β). The actions of each of these molecules may be controlled by unique extracellular mechanisms. In the case of bFGF, interaction with the matrix glycosaminoglycans may serve to provide a reservoir of growth factor, to stabilize the growth factor against proteolytic degradation and thermal denaturation and to provide a diffusible form of the molecule. Only a small percentage of the bFGF produced by endothelial cells is released. Essentially all of this material is present bound to the extracellular matrix or plasma membrane proteoglycans. All of the soluble bFGF is associated with forms of heparan sulfate. These are released from the cell surface by the action of plasmin. In the case of TGF-β, the molecule is normally secreted as an inactive precursor which does not interact with receptor(s) for TGF-β. Extracellular activation appears to occur through a plasmin-mediated reaction. Activation requires the interaction of two different cell types and may be species specific. Activation also may be self-regulating since one of the proteins whose synthesis is greatly enhanced by TGF-β is a plasminogen activator inhibitor, which effectively blocks the activation of latent TGF-β to active TGF-β. Experiments detailing the properties of the extracellular control of the activity of these two growth factors will be presented.

O 015 BLOOD-BRAIN BARRIER ENDOTHELIAL CELL DIFFERENTIATION,
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The vascular system of the central nervous system is derived from capillary endothelial cells, which have invaded the early embryonic neuroectoderm. This process, called angiogenesis, is probably stimulated by soluble factors. Endothelial cells in the brain differentiate to blood brain-barrier (BBB) endothelium later during embryonic and postnatal development. These endothelial cells are then different from all other endothelial cells in the body, because they have barrier (complex tight junctions, low number of vesicles) and carrier properties (specialized transport systems) comparable to some epithelial cell types. To study the mechanisms involved in the differentiation of BBB endothelial cells we have characterized a novel transmembrane glycoprotein which is expressed in BBB endothelium. Endothelial cells derived from the chick chorioallantoic membrane were induced to express this protein after they had invaded a brain transplant. Therefore, factors produced by the brain (probably astrocytes) seem to be necessary for the differentiation of this highly specialized endothelium.

Pattern Formation (joint)

O 016 EXPRESSION AND EVOLUTION OF IMMUNOGLOBULIN-LIKE CELL ADHESION MOLECULES IN GRASSHOPPER AND *DROSOPHILA*,
Gabriele Grenningloh, Allan Bieber, Michael Hortsch, Jay Rehm, and Corey S. Goodman, HHMI, Dept. of Molecular and Cell Biology, University of California, Berkeley, 94720

We use classical genetic and molecular genetic analysis to study the expression, structure, function, and evolution of cell adhesion molecules in grasshopper and *Drosophila*, with particular emphasis on their role during neuronal development and during the morphogenesis of epidermal structures, including *Drosophila* imaginal discs. Beginning with an immunological approach, we identified and subsequently cloned the genes encoding four different surface glycoproteins in grasshopper and *Drosophila*. These glycoproteins are dynamically expressed on different overlapping subsets of axon fascicles and glia during embryonic development; they are also dynamically expressed outside of the nervous system in other tissues, including the developing epidermis of the embryo and larval imaginal discs. Here we focus on two of these molecules (fasciclin II and neuroglian) which are members of the immunoglobulin (Ig) superfamily. Fasciclin II is related to N-CAM (Harrelson and Goodman, Science, 1988) and neuroglian is related to L1 (Bieber et al., Cell, 1989); both have tandem Ig domains followed by fibronectin type III domains.

The relatively primitive grasshopper (*Schistocerca*) is thought to be separated from the more advanced fruitfly (*Drosophila*) by about 200 million years; between these two organisms is the evolution of imaginal discs and the metamorphosis of a second body form and life style. The *neuroglian* gene was initially isolated in *Drosophila* and the *fasciclin II* gene in grasshopper; we recently isolated the corresponding genes for *fasciclin II* in *Drosophila* and *neuroglian* in grasshopper using the polymerase chain reaction. In *Drosophila*, we have thus far identified a lethal null point mutation in the *neuroglian* gene and overlapping deficiencies which delete the *fasciclin II* gene. We will report on our ongoing genetic analysis of their fuction in *Drosophila*. We will also report on preliminary observations which suggest evolutionary changes in the structure and expression of these molecules between grasshopper and *Drosophila*, changes which may in part reflect the evolution of imaginal discs.

O 017 THE DIF SIGNALLING SYSTEM IN *DICTYOSTELIUM*

Robert Kay, David Traynor, Robert Insall and Mary Berks
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DIF-1 is a novel, chlorinated signal molecule which specifically induces prestalk and stalk cell differentiation during the multicellular stages of *Dictyostelium* development. Two genes have been isolated whose expression is induced by DIF and transformation with promoter fusions from these genes has revealed the existance of at least two sub-types of prestalk cells in the aggregate. Thus *Dictyostelium* amoeba can differentiate into at least 3 cell types - prespore and two types of prestalk cell - and we present evidence that this cell diversification can be accounted for by DIF, acting in a dose-dependent fashion and in combination with cAMP.

Metabolic labelling shows that cells synthesize DIF during development and reveals the existance of similar compounds in other slime mould species. A cytosolic DIF binding protein, with the properties expected of a receptor, has been detected and cells have been found to inactivate DIF by metabolism along a surprisingly complicated pathway. Developing cells therefore appear to acquire a DIF signalling system involving signal production, detection and inactivation. It is our working hypothesis that this signalling system specifies both cell fate and pattern in the aggregate.

O 018 GENETIC CONTROL OF CELL INTERACTIONS IN C. elegans. Judith Kimble, Eleanor Maine, Voula Kodoyianni, and Eric Lambie. Lab of Molecular Biology and Department of Biochemistry, University of Wisconsin, Madison, WI 53705.

During development of the nematode C. elegans, cell interactions play a significant role in controlling cell fate. For example, in the gonad, a somatic regulatory cell called the distal tip cell controls the decision between mitosis and meiosis in the germ line (1). The glp-1 gene acts in the germ line to mediate the signal of the distal tip cell (2). In addition, glp-1 gene product is maternally contributed to the embryo and is required for induction of the anterior pharynx during early embryogenesis (2,3). Mutations in genes encoding collagen suppress the effects of mutations in glp-1, suggesting a role of the extracellular matrix in this cell interaction (4). The glp-1 gene is homologous to lin-12, another gene involved in cell interactions during C. elegans development (5,6). Both glp-1 and lin-12 have a series of repeated motifs including EGF-like and LNG repeats in the extracellular domain and swi-6/cdc10 repeats in the intracellular domain. We have examined the molecular basis of certain glp-1 mutations to learn that the LNG and swi6 repeats are essential to the function of glp-1. Examination of the lin-12 glp-1 double mutant phenotype indicates that these genes may have common functions not observed in either the glp-1 or lin-12 single mutant. We have relied on this double mutant phenotype to search for new genes that may be involved in interactions mediated by both glp-1 and lin-12. To date, we have identifed three new genes, lag-1, lag-2, and lag-3, that have the same phenotype as the lin-12 glp-1 double. We suggest that these lag genes may encode products required for the function of both lin-12 and glp-1 in cell interactions during development.

1. Kimble and White (1981) Develop. Biol. 81, 208-219.
2. Austin and Kimble (1987) Cell 51, 589-599.
3. Priess, Schnabel, and Schnabel (1987) Cell 51, 601-611.
4. Maine and Kimble (1989) Development 106, 133-143.
5. Yochem and Greenwald (1989) Cell 58, 553-563.
6. Austin and Kimble (1989) Cell 58, 565-571.

O 019 SIGNALLING PATHWAYS IN DROSOPHILA OOGENESIS.
Trudi Schupbach, Robert J. Clifford, Lynn J. Manseau, James V. Price,
Biology Department, Princeton University, Princeton, NJ 08544.

During oogenesis the dorso-ventral pattern of the Drosophila egg chamber depends on interactions between the cells of the germline and the somatic follicle cells that surround each egg. We have identified several genes that are required for these cell communication processes. One gene, *torpedo*, constitutes a central element in the dorso-ventral signalling pathway and encodes the Drosophila EGF-receptor homolog (DER)[1,2]. A genetic and developmental analysis of this locus demonstrates that the gene is required in different tissues during development. Although many of the mutant alleles appear to reduce gene activity in all affected tissues in a coordinate manner, some alleles show a differential effect on particular tissues. Studies of mosaic egg chambers have suggested that *torpedo*/DER is active in follicle cells and that this activation depends on the reception of a signal from the germline. We are investigating the role of several other genes that are active during oogenesis and, by genetic criteria, interact with *torpedo*/DER. Some of these genes appear to act "upstream" of *torpedo*/DER and may be involved in generation of the signal, whereas others seem to act "downstream" of the receptor and may be regulated by the receptor tyrosine kinase activity.

1 Schejter, E.D. and Shilo, B.Z. Cell **56**: 1093-1104
2. Price, J.V., Clifford, R.J. and Schupbach, T. Cell **56**: 1085-1092.

Growth Factors in Hematopoiesis and Neurogenesis

O 020 INTERACTIONS OF CYTOKINES IN THE REGULATION OF HEMATOPOIETIC CELL GROWTH, STEVEN C. CLARK, GENETICS INSTITUTE, INC., CAMBRIDGE, MA 02140. The growth and development of hematopoietic stem and progenitor cells in culture is regulated by the interactions of these cells with a number of different cytokines including the hematopoietic colony-stimulating factors (CSFs), the interleukins (IL-ns), the tumor necrosis factors (TNFs) and others as well. Analysis of the purified recombinant factors has revealed that of the known positive regulators of hematopoiesis, IL-3 by itself is the most potent growth stimulator of early cells that can be grown in culture. Granulocyte/macrophage CSF (GM-CSF) supports the development of cells at intermediate stages of development while granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF) act specifically at late stages of the development of the lineages indicated by their respective names. However, combinations of these cytokines reveal very different activity profiles. The stimulatory effects of IL-3 with early cells, for example, are significantly potentiated by other factors including the late acting factors such as G-CSF and M-CSF or the promiscuous factor, IL-6. Interactions of GM-CSF and IL-3 can be demonstrated at the receptor level on target cells having receptors for both ligands. Similarly interactions between IL-3 and GM-CSF in vivo can be demonstrated by sequential administration of the cytokines in a primate model. In this system, pretreatment with IL-3 potentiates the subsequent responsiveness to GM-CSF. Other cytokines including the TNFs may serve as negative stem cell regulators and it is likely that the cycling status of stem cells in vivo is regulated by the net effect of the combined actions of positive and negative regulators. The availability of positive and negative regulators of hematopoietic cell growth provides considerable clinical opportunity for protecting bone marrow from cancer chemotherapy or radiation therapy for cancer as well as for stimulating the recovery of marrow damaged by the same procedures.

O 021 DEVELOPMENT AND REGENERATION IN THE CENTRAL NERVOUS SYSTEM,

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The rat optic nerve is composed of 3 glial cell-types derived from 2 distinct cellular lineages. Type-1 astrocytes appear to be derived from a monopotential neuroepithelial precursor, while type-2 astrocytes and oligodendrocytes are derived from a common O-2A progenitor cell. Type-1 astrocytes modulate division and differentiation of O-2A progenitor cells through secretion of platelet-derived growth factor. Other mitogens also modulate programs of division and differentiation. Moreover, mitogen combinations can elicit programs of differentiation (such as expression of conditional immortalization) not predictable from the effects of single mitogens analyzed in isolation.

In vitro analysis indicates that many dividing O-2A progenitors derived from optic nerves of perinatal rats differentiate symmetrically and clonally to give rise to oligodendrocytes, or can be induced to differentiate into type-2 astrocytes. Still other O-2A^{perinatal} progenitors differentiate to form a further O-2A lineage cell, the O-2A^{adult} progenitor, which has properties specialized for the physiological requirements of the adult nervous system. In particular O-2A^{adult} progenitors have many of the features of stem cells, in that they divide slowly and asymmetrically and appear to have the capacity for extended self-renewal.

The apparent derivation of a slowly and asymmetrically dividing cell, with properties appropriate for homeostatic maintenance of existing populations in the mature animal, from a rapidly dividing cell with properties suitable for the rapid population and myelination of CNS axon tracts during early development, offers novel and unexpected insights into the possible origin of self-renewing stem cells and also into the role that generation of stem cells may play in helping to terminate the explosive growth of embryogenesis. Moreover, the properties of O-2A^{adult} progenitor cells are consistent with, and may explain, the failure of successful myelin repair in such conditions as multiple sclerosis, and thus seem to provide a cellular biological basis for understanding one of the key features of an important human disease.

O 022 BIOLOGY OF AMYLOID BETA PROTEIN PRECURSOR, David Schubert, The Salk Institute for Biological Studies, La Jolla, CA 92037

A major protein component of CNS plaques in Alzhemier's disease is a 40 amino acid peptide (β-protein) derived from a family of precursor molecules (amyloid β-protein precursor, ABPP) about 700 amino acids in length. At least one form of ABPP contains a protease inhibitor, while another form of the protein contains no inhibitor. The structure and biology of both forms of this protein will be discussed. It will be argued that ABPP is a ubiquitous secreted protein which is primarily associated with the extracellular matrix, and that ABPP is involved in the regulation of normal cell growth and that it can mediate neurite outgrowth. The modulation of ABPP synthesis and secretion by known CNS growth factors will also be discussed.

Growth and Differentiation Factors in Development

O 023 ROLE OF COLONY STIMULATING FACTOR-1 IN HEMOPOIESIS AND PLACENTAL DEVELOPMENT, E. Richard Stanley, Robert J. Areci*, Manuela Baccarini, Anna Bartocci, Wei Li, Jeffrey W. Pollard and Philip Roth+ Department of Developmental Biology and Cancer and Department of Pediatrics+, Albert Einstein College of Medicine, Bronx, NY 10461 and* Division of Pediatrics, Hematology and Oncology, Dana Farber Cancer Institute, Boston, MA 02115.

Colony stimulating factor-1 (CSF-1) is a circulating glycoprotein growth factor which regulates the survival, proliferation and differentiation of mononuclear phagocytes (progenitor cell- monoblast- promonocyte- blood monocyte- tissue macrophage). It can be expressed as transmembrane cell surface molecule or be rapidly secreted and its effects are mediated by a membrane spanning tyrosine kinase receptor that is the c-fms proto-oncogene product. Circulating CSF-1 appears to be predominantly synthesized by endothelial cells and is selectively cleared from the circulation by sinusoidally located macrophages via CSF-1R mediated endocytosis and intracellular destruction. As circulating CSF-1 regulates macrophage production, its clearance by macrophages, the rate of which is proportional to their number, represents an elegant feedback control on monocytopoiesis. During pregnancy, under endocrine control, CSF-1 is synthesized in large amounts by the uterine epithelial cells. The increased production of CSF-1 is temporally associated with the proliferation of uterine decidual and trophoblastic cells that express high levels of CSF-1R mRNA. Despite the 1,000-fold increase in uterine CSF-1 concentration during pregnancy, the circulating concentration only increases by 1.4-fold, indicating that the uterine production of CSF-1 is a local phenomenon. These and other observations strongly suggest CSF-1 plays at least two distinct regulatory roles, one humoral (non-endocrine regulated, monocytopoiesis) and the other local (endocrine regulated, placental development). Several aspects of recent studies of the initial events in CSF-1 signal transduction will also be discussed. Supported by NIH grants CA25604, CA32551 and a grant from the Lucille P. Markey Foundation.

Cell-Cell and Cell-Matrix Interactions in Development

O 024 SYNDECAN, A MEMBRANE PROTEOGLYCAN MEDIATOR OF MORPHOGENESIS, Merton Bernfield, Joint Program in Neonatology, Harvard Medical School, Boston, MA 02115.

Syndecan is a cell surface proteoglycan that contains heparan sulfate and chrondroitin sulfate chains attached to a 31 kDa core protein. Based on the cDNA, the syndecan core protein has a cytoplasmic, a hydrophobic transmembrane, and an extracellular domain that contains 5 potential Ser-Gly GAGylation sites. Both 5' and 3' untranslated regions are highly homologous to identical regions of the human insulin receptor cDNA. Syndecan likely represents a family of integral membrane proteoglycans that differ in extracellular domains, but share cytoplasmic domains.

Syndecan is found predominantly on epithelia in mature tissues where it exhibitis both a structural polymorphism and a difference in localization. Simple epithelia show syndecan solely at their basolateral surfaces and produce a larger molecule (increased number and size of heparan sulfate chains) than stratified epithelia which show syndecan over their entire cell surfaces.

Syndecan behaves as a matrix receptor: It binds mouse mammary epithelial cells to interstitial matrix molecules via its heparan sulfate chains. Binding is to types I, III and V collagens, fibronectin and thrombospondin and is of high affinity (K_d~1nM for type I collagen). It associates with the actin cytoskeleton when crosslinked at the cell surface. When cultured cells round up, they rapidly shed the extracellular domain, loosening the cell's association with the substratum. Syndecan also binds basic FGF via its heparan sulfate chains.

Altered syndecan expression correlates with change in cell behavior. Mesenchymal cells contain 1/100 the amount of cell surface syndecan than epithelial cells. Neoplastically transformed mammary epithelial cells show reduced levels of syndecan mRNA and of cell surface syndecan. Syndecan-deficient mammary epithelial cells, produced by transfection with an anti-sense cDNA construct, show varying levels of cell surface syndecan. Cells showing less than 10% of normal cell surface syndecan become fibroblastic and grow as individual fusiform cells with extensive filopolia that under- and overlap adjacent cells, while cells showing greater than 40% normal retain their epithelial phenotype.

Syndecan changes in amount, location and structure during development: it appears initially on 4 cell embryos (prior to its matrix ligands), becomes restricted in pre-implantation embryos to the cells that will form the embryo proper, changes its expression due to epithelial-mesenchymal interactions (e.g. induced in kidney mesenchyme by the ureteric bud), and with association of cells with extracellular matrix, (e.g. during B-cell differentiation) and ultimately, in mature tissues, becomes tissue-specific.

Its interactions with major extracellular effector molecules that influence cell behavior, its role in maintaining cell shape and its spatial and temporal changes in expression during development indicate that syndecan is involved in morphogenesis.

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O 025 CELLULAR ARCHITECTURE & BASEMENT MEMBRANE ARE IMPORTANT DETERMINANTS OF MILK PROTEIN GENE EXPRESSION. Mina J. Bissell, Charles H. Streuli and Li-How Chen, Division of Cell & Molecular Biology, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720 USA.

We have shown previously that a basement membrane preparation derived from EHS tumors allows mammary cells from mid-pregnant mice to form alveoli-like structures in culture and to synthesize and secrete high levels of milk proteins vectorially (1). Formation of these structures also allows expression of whey acidic protein (WAP) by inhibiting production and/or accessibility of a WAP-specific inhibitor (2). TGF-β inhibits WAP production as well, but it is not yet clear whether or not the WAP inhibitor and TGF-β are the same molecule. Mammary cells cultured on floating type I collagen gels do not express significant quantities of WAP, although in other respects they differentiate to the same extent as cells cultured on EHS matrix. Functional differentiation on floating gel correlates with the deposition of an endogenous basement membrane containing laminin, type IV collagen and entactin. Formation of the basement membrane itself may be dependent on cell-cell interactions and establishment of polarity. Cells on tissue culture plastic express high levels of mRNA for laminin and type IV collagen and secrete these and other matrix proteins. However, when a basement membrane is formed by cells cultured on floating gels, there is a negative feedback on the expression of extracellular matrix components (3). We conclude that tissue-like multicellular architecture and basement membrane, as well as hormones, are necessary for the establishment and maintenance of a fully lactational phenotype.

This work is supported by the Office of Health and Environmental Research, Office of Energy Research, U.S. Department of Energy, under contract #DE-AC03-76SF00098 for MJB and an EMBO fellowship for CHS.

REFERENCES

1. Barcellos-Hoff, MH, Aggeler, J, Ram, TG and Bissell, MJ (1989) Development **105**:223-235.
2. Chen, L-H and Bissell, MJ (1989) Cell Regulation **1**:45-54.
3. Streuli, CH and Bissell, MJ (1990) J. Cell Biology. In press

O 026 CADHERIN-MEDIATED SELECTIVE CELL-CELL ADHESION AND MORPHOGENESIS, Masatoshi Takeichi, Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan

Cadherins are a family of Ca^{2+}-dependent cell-cell adhesion molecules, in which about 50% amino acids are conserved among different members. Evidence has been accumulated that this molecular family is essential for selective cell-cell adhesion: Cadherins bind cells by a homophilic interaction, and, in this interaction, each type of cadherins preferentially bind to the identical type, thus conferring adhesive specificities on cells (1, 2).

In order to determine which regions of cadherin molecules are responsible for their binding specificities, we constructed chimeric molecules of P- and E-cadherin and examined their adhesive specificities. The results showed that the amino terminal 113 amino acid region of the extracellular domain was important for the specificities of these molecules. We then identified an especially important site within this region, containing a characteristic sequence, involved in the specific cadherin binding.

The intracellular domain of cadherins is associated with cytoskeletons. The cadherin molecules in which the carboxy-half portion of the intracellular domain has been deleted, however, cannot bind to cytoskeletons, nor exhibit cell binding activity (3). Thus, the association of cadherins with cytoplasmic components seems essential for the cell binding action of the extracellular domain. Immunoprecipitation analyses showed that a 94kd protein was tightly associated with the carboxy-half region of the intracellular domain of E-cadherin, suggesting that this molecule may play a role in mediating the interaction of cadherins with cytoskeletons. The cadherin-mediated cell adhesion, thus, seems to be intracellularly controlled.

Experiments to investigate the role of cadherins in morphogenesis will also be described.

References

1. Nose, A., Nagafuchi, A. and Takeichi, M. (1988). Cell, 54, 993-1001.
2. Miyatani, S. et al. (1989). Science 245, 631-635.
3. Nagafuchi, A. and Takeichi, M. (1989). Cell Regulation 1, 37-44.

Retinoids

O 027 EMBRYONIC TERATOCARCINOMA CELL DIFFERENTIATION AS A MODEL SYSTEM FOR THE MOLECULAR ANALYSIS OF RETINOIC ACID ACTION, Lorraine J. Gudas, Joseph D. Gold, Joseph F. Grippo, Betsy A. Hosler, Lan Hu, Gregory J. LaRosa, Melissa B. Rogers, Carol M. Stoner, and George W. Vasios, Dept. of Biol. Chem. and Molec. Pharm., Harvard Medical Sch., and Dana-Farber Cancer Inst., 44 Binney St., Boston, MA 02115

This lab is utilizing murine F9 cells to analyze mammalian cell differentiation at a molecular level. F9 embryonic teratocarcinoma stem cells differentiate into extra-embryonic parietal endoderm cells in response to retinoic acid (RA), a vitamin A derivative; this differentiation process is enhanced by cyclic AMP analogs. RA action in this system is possibly mediated by three RA receptors, RAR α, β, and γ, and a RA binding protein designated CRABP. We have shown that the RARα and RARγ mRNA levels remain relatively constant over time after RA addition. In contrast, the RARβ mRNA level increases within 12 hr. after RA addition and reaches a 20-fold higher level by 48 hr. Strikingly, this RA-associated RARβ mRNA increase is not prevented by protein synthesis inhibitors but is prevented by the addition of cyclic AMP analogs. The CRABP mRNA level decreases by 3-6-fold after RA addition. In addition to characterizing the expression of the RARs and CRABP, we have cloned and characterized several RA-responsive genes in this differentiation system. REX-1, a gene that encodes a putative protein with four repeats of the zinc-finger DNA-binding motif, exhibits a 5-8 fold decrease in its transcription rate within 24 hrs. after RA addition. ERA-1/Hox 1.6, a gene that encodes two putative proteins, one containing a homeobox domain and one without this domain, is rapidly induced by RA in a protein synthesis independent fashion. ERA-1/Hox 1.6 mRNA is also expressed in the primitive streak in day 7.5 mouse embryos, as assessed by in situ hybridization. The laminin B1 gene is a basement membrane protein gene which we've previously shown to be positively regulated by RA at the transcriptional level; in collaboration with Dr. Pierre Chambon's lab, we've now identified a RA response element (RARE) in the promoter of the laminin B1 gene that functionally interacts with the RARs. Through the use of deletion and mutation analyses, this RARE has been defined as a 46-bp element between -477 and -432 of the laminin B1 5' flanking region. Insertion of this RARE in either orientation into a thymidine kinase promoter/CAT expression vector causes CAT expression to be activated 5- to 9-fold by the co-transfected human RAR α, β, or γ constructs in RA-treated F9 cells, and this RARE also functions in HeLa cells. Further analysis of teratocarcinoma cell differentiation at the molecular level should provide more information about how retinoic acid acts during early mammalian development.

O 028 RETINOIC ACID AND PATTERNING AND MORPHOGENESIS IN DEVELOPING CHICK LIMBS

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Retinoic acid application to chick limb buds brings about striking changes in pattern that mimic those produced by the polarizing region. The polarizing region is a small group of mesenchyme cells located at the posterior margin of the bud. This provides the opportunity to use a defined chemical to explore the molecular basis of patterning and to investigate how patterning is co-ordinated with growth and morphogenesis in developing limbs.
The specification of pattern could involve homeobox containing genes. In collaboration with Drs Oliver and de Robertis (University of California, Los Angeles) we examined how retinoic acid and polarizing region grafts affect expression of XIHboxI gene in wing buds.
The apical ectodermal ridge, the thickened epithelium at the tip of the limb bud, plays a central role in co-ordinating patterning with morphogenesis and growth in developing limbs. Retinoic acid acts directly on the limb mesenchyme and modifies epithelial-mesenchymal interactions.

Steroid Hormone Regulation of Cell Differentiation (joint)

O 029 RETINOIC ACID RECEPTORS P. Chambon, P. Dollé, P. Kastner, A. Krust, M. Petkovich, E. Ruberté and A. Zelent, LGME/CNRS and U.184/INSERM, Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg - France.

Retinoic acid receptors (RARs) belong to the family of nuclear receptors (1) which include steroid and thyroid hormone receptors and act as ligand-inducible transcriptional enhancer factors by binding to specific cis-acting responsive DNA elements. A comparison of the structure of the three human and mouse RARs alpha (RAR-α), beta (RAR-β) and gamma (RAR-γ), which have been recently cloned in our laboratory (2-5), led us to suggest that they may specifically control the expression of different subsets of genes and thus account for the multiple effects of retinoic acid during embryogenesis and cell differentiation. Results of in situ hybridization studies on mouse embryo sections will be presented, which strongly support this suggestion. These results (6, 7) demonstrate that RAR-α, RAR-β and RAR-γ exhibit specific spatio-temporal patterns of expression, which are also distinct from those of the cellular retinoic (CRABP) and retinol (CRBP) binding proteins.

Ref. 1. S. Green and P. Chambon (1988) Trends in Genetics 4, 309-314. 2. M. Petkovich, N.J. Brand, A. Krust and P. Chambon (1987) Nature 330, 444-450. 3. N. Brand, M. Petkovich, A. Krust, P. Chambon, H. de Thé, A. Marchio, P. Tiollais and A. Dejean (1988) Nature 332, 850-853. 4. A. Zelent, A. Krust, M. Petkovich, P. Kastner and P. Chambon (1989) Nature 339, 714-717. 5. A. Krust, P. Kastner, M. Petkovich, A. Zelent and P. Chambon (1989) Proc. Natl. Acad. Sci. USA 86, 5310-5314. 6. P. Dollé, E. Ruberté, P. Kastner, M. Petkovich, C.M. Stoner, L.J. Gudas and P. Chambon (1989) Nature, in press. 7. E. Ruberté, P. Dollé, A. Krust, A. Zelent, G. Morriss-Kay and P. Chambon (1989) Development, in press.

O 030 PATTERN FORMATION IN LIMBS AND IN THE DEVELOPING CENTRAL NERVOUS SYSTEM OF VERTEBRATES, Gregor Eichele, Susan M. Smith, Olof Sundin, Christina Thaller, Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115USA.

Broadly speaking, embryonic development can be divided into two processes. One leads to the formation of terminally differentiated cell types (e.g. muscles, chondrocytes, neurons,), the other assures that these differentiated cells form a proper pattern (e.g. a hand, a properly wired nervous system). It has long been proposed that the establishment of the patterns depends partly on morphogens. Morphogens are compounds that are locally generated in the embryo and diffuse into the surrounding tissue, where they elicit in a concentration-dependent manner, a developmental program (e.g. specification of cell fates along an embryonic axis). Retinoic acid (RA) is a candidate morphogen in the developing vertebrate limb. Earlier studies have shown that locally applied RA induces dose dependent duplications in the developing chick wing. It was further shown that limb buds contain endogenous RA and that RA is distributed in the form of a concentration gradient. Limb buds can synthesize RA from its biosynthetic precursor retinol in situ at a rate that can account for the steady-state levels of endogenous retinoic acid. By in situ hybridization we have begun to study the expression pattern of beta RA receptor transcripts in the early chick embryo. Taken together our studies support the view that RA is a morphogenetic signalling compound locally produced the early limb bud and that it specifies in a concentration-dependent manner, through the RA receptor, the developmental fate of limb bud cells along the anteroposterior limb axis.

Gross anatomical and histological investigations have revealed that the hindbrain of vertebrates consists of eight segmental units known as rhombomeres. Studies performed predominantly in the developing chick embryo, show that the cranial motor- and sensory nerves are anatomically and functionally associated with a particular rhombomeres. Neural crest cells that form a major part of the skeleton and of the soft tissue of the vertebrate head, arise in part from rhombomeric neuroectoderm. It thus appears that the segmental organization of the hindbrain can provide neurons and neural crest cells with cues directing their fate and their function. We have isolated a chick homeobox-containing gene, Ghox-lab, that is related to the *Drosophila* gene *labial*. Transcripts of Ghox-lab are found in rhombomere 4. By immunohistochemistry of embryo sections and whole mounts, we found that Ghox-lab protein is expressed in rhombomere 4 and in the neural crest cell population that derives from this rhombomere. In analogy to the segment specification mechanisms operating in *Drosophila*, we propose that Ghox-lab provides segmental identity to rhombomere 4 and to the neural crest cell population that derives from it.

O 031 MOLECULAR GENETICS OF STEROID AND THYROID HORMONE RECEPTORS, Evans, R. M., Howard Hughes Medical Institute, The Salk Institute, La Jolla, California 92037

We have identified receptors for steroid and thyroid hormones and for the vitamin A-derived morphogen retinoic acid. The homology of these receptors and the cDNAs which encode them define the existence of a superfamily of related regulatory proteins. We demonstrate that these molecules contain homologous structures that include domains required for DNA binding, ligand binding and trans-activation. Site-directed mutagenesis was used to identify the DNA-binding specificity of the glucocorticoid receptor. We have demonstrated that a single amino acid change can redirect the GR so that it now recognizes an estrogen response element (ERE). Interestingly, this mutant receptor retains GRE specificity. These results indicate that it is possible to generate a receptor that can recognize and activate through two different sequences. A change in the second zinc finger transforms the specificity to that of the thyroid hormone receptor.

Recent studies indicate that the thyroid hormone receptor can, in the absence of its ligand, suppress activity of a responsive promoter. Addition of thyroid hormone, however, results in the stimulation of expression. The oncogenic derivative of the thyroid hormone receptor, v-erbA, acts as a constitutive repressor and when co-expressed with the receptor, blocks activation by thyroid hormones. Thus, v-erbA is an example of a dominant negative oncogene.

Recent studies from our lab have shown that the retinoic acid receptor is capable of binding to and activating thyroid hormone responsive promoters. This suggests a potential relationship between retinoid and thyroid hormone receptors. To pursue this hypothesis we have examined the action of the erbA oncogene on retinoic acid receptor activity. As with the TR, v-erbA functions as a retinoic acid receptor antagonist and in principle, may be used as a molecular tool to dissect the inductive events of retinoids in cell culture and in transgenic animals. Studies on the relationship of thyroid and retinoid receptors will be presented.

Growth and Differentiation Factors in Early Mammalian Embryogenesis

O 032 GERM-LINE MUTATIONS IN THE c-*kit* GROWTH FACTOR RECEPTOR: THEIR EFFECTS ON GROWTH AND DEVELOPMENT IN *W* MUTANT MICE, Alan Bernstein, Alastair Reith, Robert Rottapel and Lesley Forrester, Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada M5B 1X5.

Mutations at the mouse *W* locus lead to intrinsic defects in stem cells of the melanocytic, hematopoietic and germ cell lineages that can result in white spotting, severe macrocytic anemia, mast cell deficiency and sterility. Previous genetic and molecular studies have suggested that *W* is allelic with the transmembrane receptor tyrosine kinase proto-oncogene c-*kit*. To establish that the pleiotropic developmental defects in *W* mutants are the result of mutation in this growth factor receptor, we have analyzed the c-*kit* locus and the c-*kit*-associated kinase activities, in 5 independent *W* alleles. In addition, we have reproduced in cell culture the deficiency in mast cell growth conferred by both mild and severe *W* alleles. Mast cell cultures derived from mice or embryos homozygous for each of 5 *W* alleles were deficient in c-*kit* kinase activity, the extent of which paralleled the severity of phenotype conferred by a given *W* allele. Mice bearing the W^{37}, W^{55} or W^{41} mutant alleles contain point mutations within the kinase domain of the c-*kit* polypeptide. The nature and location of these amino acid substitutions provide direct evidence that the pleitropic developmental defects associated with the *W* locus arise as the result of dominant loss-of-function mutations in the c-*kit* transmembrane receptor tyrosine kinase. (Supported by MRC and NCI of Canada, and NIH.)

O 033 THE *int-1* GENE FAMILY IN VERTEBRATE DEVELOPMENT, Andrew P. McMahon, Brian J. Gavin, Jill A. McMahon, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110. Until recently, the *int-1* gene family consisted of only two members; *int-1*, a proto-oncogene implicated in mammary tumorigenesis, and *irp* (*int-1*-related protein), which shares 38% of amino acids with *int-1*. Both proteins contain a hydrophobic leader sequence, suggesting that they are secreted, as well as a number of absolutely conserved cysteine residues. Although *int-1* and *irp* are expressed during mouse development, their patterns of expression are quite different. *int-1* is restricted to a small subset of cells at the dorsal midline of the mammalian neural tube, while *irp* is expressed in the mesenchyme of the ventral lateral body walls, the allantois, and the pericardium of the heart. Although the roles of *int-1* and *irp* are unknown, the finding that the Drosophila homologue of *int-1* encodes the segment polarity gene *wingless* suggests that like *wingless*, *int-1* may be a secreted factor involved in cell-cell communication. We have now isolated five new *int-1*-related genes that encode proteins with a similar structure to *int-1* and *irp*. All are expressed during mouse development, and expression is localized to specific regions. Thus, it is likely that the *int-1* family constitutes a major group of related proteins, all of which are involved in different aspects of cell-cell communication during vertebrate development.

O 034 THE EXPRESSION AND FUNCTION OF GROWTH FACTORS DURING EARLY DEVELOPMENT OF MOUSE EMBRYOS, Zena Werb and Daniel A. Rappolee, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750. Growth factors regulate cell proliferation, differentiation, migration and invasion. Mouse embryos grow autonomously during preimplantation development, and then, upon implantation, the cells proliferate and differentiate into extraembryonic and specialized embryonic tissues. We have been interested in identifying and characterizing the role of embryonic growth factors in these early periods. We have used reverse transcription coupled with the polymerase chain reaction (RT-PCR) (1,2) initially to identify, unambiguously, the accumulation of mRNA transcripts for growth factors and their receptors in early embryos. Transcripts for transforming growth factor (TGF)-α, insulin-like growth factor-II (IGF-II), platelet-derived growth factor (PDGF) A chain, Kaposi's sarcoma-type fibroblast growth factor (kFGF), TGF-β_1 (1,3), and interleukin-6 were all found soon after the onset of zygotic transcription, increasing to the blastocyst stage when differentiation into the first two tissue layers takes place. The outer cells give rise to the extraembryonic trophectoderm, and the inner cell mass (ICM) give rise to both extraembryonic tissues and the embryo proper. By immunofluoresence TGF-α, PDGF, and IGF-II were distributed in all cells of the blastocyst, whereas TGF-β_1 appeared to be absent from some ICM cells, and kFGF apparently was confined to the ICM only. We next analyzed embryos for the presence of functional receptors for some of these endogenous growth factors. Evidence for the presence of mRNA transcripts for receptors by RT-PCR was obtained for receptors recognizing IGF-II ligand. Transcripts for the IGF-II/mannose-6-phosphate receptor appeared at the two cell-stage, whereas mRNA for the insulin and IGF-I receptors, which also recognize this ligand, did not appear until compaction. That some of these receptors are functional was shown by the metabolic response of embryos cultured without or with purified IGF-II from the two-cell stage to blastocyst. The embryos also responded to exogenous TGF-α and FGF. To show that endogenous growth factors play a role in embryonic growth, we cultured embryos from the two cell stage to blastocyst in the presence of sense or antisense oligonucleotides designed to coding sequences. Antisense, but not sense, oligonucleotides for IGF-II and TGF-α markedly retarded the rate of growth of the preimplantation embryos in culture. These data provide the first direct evidence that endogenous and exogenous growth factors function on intraembryonic targets in early development. Supported by NIH (HD 23530 and ES 07106) and US-DOE OHER (DE-AC03-76-SF01012).

1. Rappolee, D.A., C.A. Brenner, R. Schultz, D. Mark & Z. Werb (1988). *Science.* 241:1823-1825.
2. Rappolee, D.A., A. Wang, D. Mark, & Z. Werb (1989). *J. Cell. Biochem.* 39:1-11.
3. Rappolee, D.A., K.S. Sturm, G.A. Schultz, R.A. Pedersen & Z. Werb (1989). *In:* Early Embryo Development and Paracrine Relationships. Edited by S. Heyner & L. Wiley. A.R. Liss Inc., N.Y. In press.

Growth and Differentiation Factors in Development

Transgenic Mice as Model Systems to Explore the In Vivo Role of Growth and Differentiation Factors

O 035 EXPRESSION OF MULLERIAN INHIBITING SUBSTANCE GENES IN TRANSGENIC MICE
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Mullerian inhibiting substance (MIS), also known as anti-Mullerian hormone is a glycoprotein expressed specifically in Sertoli cells of the fetal and adult testis and granulosa cells of the postnatal ovary. During fetal development both males and females possess Mullerian ducts, the anlagen of the uterus, oviducts, and upper vagina. In the male fetus, MIS production brings about the regression of the Mullerian ducts. MIS has also been implicated in testicular differentiation, as suggested by in vitro studies of fetal ovaries exposed to purified MIS. These ovaries lose germ cells and develop seminiferous cord-like structures.

We have generated transgenic mice chronically expressing human MIS under the influence of the mouse metallothionein-1 promoter to investigate its role in sexual development. Nine founder mice were generated (2 females and 7 males). Seven of these mice including both females had circulating levels of human MIS ranging from ~50-600 ng/ml. In comparison, MIS is undetectable in plasma from human adults. Both founder females were sterile and possessed a blind vagina, and no uterus or oviducts. Surprisingly, ovaries were also not detected. All of the expressing founder males were fertile and transmitted the transgene to progeny. Female progeny that inherited the transgene from these males exhibited the same phenotype as the founder females. A developmental study revealed that transgenic females did possess ovaries at birth; however, germ cells were not as prevalent as in controls. Subsequently, the ovaries became devoid of germ cells and this was followed by the organization of the somatic components of the ovary into structures bearing a striking resemblance to the seminiferous tubules of the male gonad. A proportion of males from the 2 highest expressing lines exhibited feminization of the external genitalia, impairment of Wolffian duct development, and undescended testes.

These results demonstrate in vivo that MIS negatively influences the differentiation of the Mullerian ducts. In addition, our findings support the notion that this factor may be actively involved in testicular morphogenesis. MIS may also affect androgen or androgen receptor biosynthesis perhaps by influencing Leydig cell differentiation. Finally, MIS appears to be involved in testicular descent.

O 036 EMBRYONIC LETHAL MUTATIONS CAUSED BY DNA INSERTION IN TRANSGENIC MICE, Frank Costantini, So-Wun Cheng, James Lee, Christopher Perkins, William Perry III, Glenn Radice and Hyeung Jin Son, Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY 10032. The molecular analysis of mutations affecting mouse embryogenesis represents a powerful method to identify new genes involved in mammalian development. Insertional mutations caused by the integration of foreign DNA in transgenic mice can be readily analyzed, using the transgene as a probe to clone the locus of insertion. We are currently investigating three embryonic lethal mutations caused by transgenic insertions. Embryos homozygous for the ßS12 mutation develop normally to the morula stage of pre-implantation development, but fail to form a blastocyst. Homozygous embryos in line Hß58 develop normally through the early egg cylinder stage and undergo gastrulation, but develop only a rudimentary neural axis and a beating heart, as well as extra-embryonic membranes. Homozygous embryos in line HE46 die at day 9.5 with neural tube abnormalities. DNA from all three mutant loci has been cloned, and a disrupted gene has been identified at the Hß58 locus. The properties of this gene and its relation to the Hß58 mutant phenotype, as well as attempts to identify genes at the other loci, will be discussed.

O 037 MOUSE GERMLINE MANIPULATION USING EMBRYONAL STEM CELLS,
M.L.Hooper*, S.Thompson+, A.R.Clarke*, A.M.Pow+ and D.W,Melton+, *Department of Pathology, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, Scotland, and +Department of Molecular Biology, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JR, Scotland.

The introduction of planned modifications into specified genes in the mammalian germline is a powerful strategy with a broad range of applications in biology and medicine. We describe progress in the development of such an approach using cultured mouse embryonal stem cells which retain the ability to contribute to somatic and germ cell lineages of chimaeric animals produced by injecting the cells into blastocyst-stage embryos. Genes can be mutated in the cultured cells by gene targeting, a process involving homologous recombination with exogenous DNA. We describe the correction of a partial deletion in the gene coding for hypoxanthine phosphoribosyltransferase (HPRT) in mouse embryonal stem cells by gene targeting and the introduction of the corrected gene into the mouse germ line by breeding from chimaeras produced from the corrected cells. The corrected gene has the same qualitative pattern of expression as the wild-type gene, with the characteristic elevated level of expression in brain tissue. This demonstrates the feasibility of introducing planned modifications into mice by germline transmission of mutations introduced into embryonal stem cells by gene targeting.

O 038 A TRANSGENIC MOUSE ANALYSIS OF VARIANT FORMS OF GROWTH HORMONE AND INSULIN LIKE GROWTH FACTORS,
Timothy A. Stewart, Philip G. Hollingshead, Denny Liggitt, Laura Martin, Heather Oakley, Sharon Pitts and Timothy Terrell, Genentech, Inc., 460 Point San Bruno Blvd., South San Francisco, CA 94080

Growth hormone and the insulin like growth factors are the principal mediators of post-natal axial growth and development. An analysis of the activities of these proteins in an intact animal is complicated by the presence of variant forms of these proteins that are produced either by differential splicing of the primary transcript or by post translational modification. We have produced transgenic mice that have altered expression of these variant forms of these growth mediators. Physiological and histopathological analyses of these mice will be presented.

Nuclear Factors Regulating Cell Function (joint)

O 039 HOW TRANSCRIPTION FACTORS CONTROL WHERE AND WHEN CELLS DIVIDE DURING EMBRYOGENESIS, Patrick H. O'Farrell, Bruce A. Edgar and Christian F. Lehner, Department of Biochemistry, University of California, San Francisco CA 94143

The timing of embryonic cell divisions in Drosophila is precisely controlled. The pattern of the division 14 has been particularly well characterized. All cells start cell cycle 14 at the same time after 13 rapid and synchronous divisions but cells in different positions in the embryo differ in the length of cycle 14. The lengths of cycle 14 are regulated by evolutionarily conserved regulators that govern entry into mitosis to produce a precise spatio-temporal pattern. One of these regulators, the products of the string gene, is limiting. The time of string gene transcription is regulated in an intricate pattern that predicts the time of mitosis (1). Induced premature expression of string induces premature mitoses. The string gene is homologous to and can substitute for the S. pombe cell division cycle gene, cdc25. The time of string transcription, and thus the time of cell division, is regulated by combinations of known transcriptional regulators that control pattern formation in the embryo.

Cyclin A and cyclin B, two homologous proteins were previously thought to be the best candidates for the regulation of the timing of cell division because of the striking change in their levels during the cell cycle. In many species these proteins accumulate throughout the cell cycle and are abruptly degraded at mitosis. We have shown that the levels of cyclins do not determine the time of mitosis 14 (2).

During development the regulation of the cell cycle changes. At different times different regulators are limiting so that the pivotal regulator changes (3).

1) Edgar B.A. and O'Farrell, P.H. (1989) Cell 57, 177.
2) Lehner, C.F. and O'Farrell, P.H. (1989) Cell 56, 957.
3) O'Farrell et al (1989) Science 246, 635.

O 040 THE SEX-DETERMINING FUNCTION OF THE MAMMALIAN Y CHROMOSOME, Douglas Vollrath, Peggy Beer-Romero, Laura G. Brown, Elizabeth M.C. Fisher, Shiuh-Wen Luoh, Graeme Mardon, Rebecca Mosher, Jody Pringle, Anne Ridley, Ansbert S. Schneider-Gädicke, Elizabeth M. Simpson, Steven Swendeman, and David C. Page, Whitehead Institute and Dept. of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142

In mouse and man, the presence of the Y chromosome determines whether an embryo develops as a male or female. By deletion analysis of human XX males and XY females, we found that sex is determined by the presence of much less than 1% of the Y chromosome. In particular, an essential portion of the sex-determining function was mapped to a 140 kb portion of the short arm of the human Y chromosome. Having cloned the sex-determining region, we set out to completely define its genetic content. Within this region we identified a gene, *ZFY*, encoding a protein with thirteen Cys-Cys/His-His "zinc fingers" and a highly acidic domain. *ZFY* may be the pivotal sex-determining signal.

A closely related gene, *ZFX*, exists on the human X chromosome. *ZFY* and *ZFX* diverged from a common ancestral gene as evidenced by similarities in their gene structure and exon DNA sequences. The human *ZFY* and *ZFX* genes are transcribed in a wide variety of XY and (in the case of *ZFX*) XX cell lines; *ZFX* escapes X inactivation. Like *ZFY*, *ZFX* encodes a protein composed of a highly acidic amino-terminal domain, a basic putative nuclear localization signal, and a carboxy-terminal zinc finger domain. By analogy to proteins such as yeast GAL4 and GCN4 and the human glucocorticoid receptor, this combination of features suggests that the ZFY and ZFX proteins are sequence-specific activators of transcription. The ZFY and ZFX proteins may bind to the same nucleic acid sequences and regulate transcription of the same gene(s). Alternative splicing generates *ZFX* transcripts encoding isoforms of 575 and 804 amino acids. These ZFX isoforms differ in the length of their acidic domains and may be functionally distinct.

Homologs of the human *ZFY* and *ZFX* genes are found on the Y and X chromosomes of all placental mammals tested. In contrast to other placental mammals, the mouse genome contains four homologous zinc-finger loci: *Zfy-1*, *Zfy-2*, *Zfx*, and *Zfa* (on autosome 10). The two homologs on the mouse Y chromosome, *Zfy-1* and *Zfy-2*, are closely related, the result of an intrachromosomal duplication that occurred during mouse evolution. Both *Zfy* loci map to the sex-determining region of the mouse Y chromosome. However, *Zfy-1* and *Zfy-2* are not *both* required for testis determination. Both *Zfy-1* and *Zfy-2* are transcribed in mouse adult testis. Analysis of mouse cDNAs demonstrates that *Zfy-2* and *Zfx* encode, respectively, 783 and 799 amino acid proteins, each with a large, highly acidic domain and 13 zinc fingers; the mouse Zfx protein is 70% identical to the mouse Zfy-1 and Zfy-2 proteins. In contrast to the mouse *Zfy* genes, *Zfx* is widely transcribed in embryos, newborns, and adults, both male and female. Moreover, *Zfx* transcripts contain long 3' untranslated sequences which are phylogenetically conserved. *Zfa* is a processed gene derived from *Zfx*. When fused to the DNA-binding domain of GAL4, the acidic domains of Zfx and Zfy-2 activated transcription in yeast.

O 041 TRANSCRIPTIONAL REGULATION BY THE ANTENNAPEDIA HOMEOTIC GENE OF DROSOPHILA, Matthew P. Scott, Shigeo Hayashi, Gary M. Winslow, Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309-0347.

The ectopic expression of the *Antennapedia* (*Antp*) protein in the head primordia of the fly, due to either a mutation or to the use of a heat shock promoter, leads to the development of legs in lieu of antennae. *Antp* normally controls the formation of thoracic structures in the fly. The presence of essentially a single protein in the head primordia is therefore capable of completely altering the fates of a large and complex array of cells. We have shown that the *Antp* protein is capable of sequence-specific DNA binding and that in cultured cells the protein is capable of activating transcription. We have studied the properties of *Antp* protein by modifying its structure and testing its ability to affect transcription in cultured cells. The protein contains a homeodomain which is sufficient for DNA binding. The homeodomain alone, without the rest of the protein, can compete with the intact protein and thus prevent transcriptional activation. In addition we have introduced modified proteins into the fly under the control of a heat shock promoter to assess their abilities to induce antenna to leg transformations. The sites upon which the protein acts have been altered to learn about the specificity of DNA binding and transcriptional activation by the protein. The protein has been partially purified from cultured cells and tested for DNA binding using band shifts and DNAse I foolprinting. In vitro the protein binds to both a repeated TAA sequence and to the sequence TCAATTAAATGA with a dissociation constant of $< 10^{-9}$. We have also found that serine phosphorylation is important in controlling DNA binding in vitro.

Growth and Differentiation Factors in Development

TGF-Beta; Extracellular Factors

O 100 THE TGF BETA GENE FAMILY AND MURINE EMBRYOGENESIS. Rosemary J. Akhurst, Fergus Millan, David Fitzpatrick, Elizabeth Duffie and Sigrid A. Lehnert. Duncan Guthrie Institute of Medical Genetics, Glasgow University, Glasgow, Scotland, UK.

We have examined the expression pattern of TGF beta-1 RNA during murine embryogenesis from implantation to birth. TGF beta-1 RNA is produced at high levels in epithelia and endothelia involved in morphogenetic interactions (Lehnert and Akhurst, 1988) and is correlated with the presence of TGF beta protein in the underlying mesenchyme (Heine et al 1987). Sites of epithelial TGF beta-1 gene expression are also correlated with localisation of tenascin subepithelially giving support to the theory that one function of epithelial TGF beta-1 is modulation of the extracellular matrix.

We have also examined expression sites of TGF BETA-2 and beta-3 and gene manipulation experiments are underway to test the functions of these growth factors in vivo.

Lehnert and Akhurst (1988) Development 104 263-273
Heine et al (1987) J. Cell. Biol. 105 2861-2876

O 101 CHARACTERISTICS OF MEMBRANE AND SOLUBLE FORMS OF THE TGF-ß BINDING PROTEOGLYCAN, BETAGLYCAN, Janet L. Andres, Sela Cheifetz, and Joan Massagué, Department of Cell Biology and Genetics, Sloan-Kettering Institute, 1275 York Ave., New York, NY, 10021.

Betaglycan (formerly, the TGF-ß type III receptor) is a heparan sulfate/chondroitin sulfate proteoglycan of apparent molecular weight 250-400 kDa. The core protein, which appears as a doublet or triplet after chemical or enzymatic deglycosylation, has a molecular weight of 110-130 kDa. Betaglycan specifically binds TGF-ß1, TGF-ß2, and TGF-ß3 through its core protein. Studies with cell mutants defective in glycosaminoglycan (GAG) synthesis show that the GAG chains are not required for betaglycan expression on the cell surface or TGF-ß binding. Betaglycan is found in many cell types and is particularly abundant in embryonic fibroblasts, but is not detectable in skeletal muscle myoblasts, hematopoetic progenitor cells, or vascular endothelial cells, despite the fact that all of these cell types respond to TGF-ß. The function of betaglycan is not known but it does not appear to be directly involved in signalling. It has been shown to exist in both soluble and membrane-bound forms and may affect availability of TGF-ß to the signalling receptor. Proteoglycans are able to interact with extracellular matrix components and some betaglycan can be extracted from the extracellular matrix of fibroblasts. Both soluble and membrane-bound forms have been found in vivo. Peptide maps indicate that the binding sites of these two forms are identical and distinct from the type I and type II receptors, the TGF-ß receptors implicated in signal transduction by this factor. The betaglycan that has been found associated with the extracellular matrix resembles the soluble form in its characteristics on SDS-PAGE. Betaglycan has been extensively purified and the ability of this highly purified form to associate with individual extracellular matrix components is under investigation.

O 102 INSULIN AND GROWTH FACTOR MODULATION OF CELL PROLIFERATION IN RAT SKELETAL MYOBLASTS. Najma Begum and Jaswant S. Bhorjee, Division of Biomedical Sciences, Meharry Medical College, Nashville, TN 37208. Treatment of serum-starved L6 cells and L8 derived fusion-defective fu-1 myoblasts (Mb) for 24 hrs with insulin, IGF1, dexamethasone (dex), PDGF and EGF resulted in 60% increase in [^{3}H]TdR incorporation. In L6 cells the effects of these growth factors in combination were not additive. H7, a PKC inhibitor, or TGFß completely blocked the growth promoting effects of dex, PDGF and EGF, while only partially blocking insulin effect. By contrast, TGFß stimulated DNA synthesis in fu-1 Mb; H7 neither blocked the effect of TGFß nor of the other growth factors in fu-1. PGE2 reversed the growth promoting effects of dex and indomethacin in L6 cells, suggesting a role for endogenous PGE2 in limiting cell proliferation. Continuous presence of growth factors promoted cell proliferation initially, but on initiation of fusion, they promoted myotube formation. TGFß alone or in combination with insulin, PDGF and EGF inhibited the promotion of cell proliferation and differentiation. The above growth factors also induced expression of c-myc, c-fos and c-ras oncogenes in L6 Mb. TGFß did not inhibit growth factor induced oncogene expression, suggesting that induction of oncogenes alone is not sufficient for stimulation of growth. Additional studies on the effect of several growth factors on synthesis of the high mobility group (HMG) class of the nuclear proteins, revealed stimulation of HMG-1 in both L6 and fu-1 cell lines; this effect was blocked by TGFß in L6 but not in fu-1 cells. The mechanism(s) of effects of insulin, growth factors and TGFß on cell proliferation will be discussed. [Supported by NIH award HLO1979 to JSB. NB was supported by a PDF from NSF-MRCE award RII8714805.]

O 103 CHICKEN TRANSFORMING GROWTH FACTOR BETA-2 GENE STRUCTURE AND FUNCTION, D.W. Burt, I.R. Paton, Department of Molecular Genetics, AFRC Institute of Animal Physiology and Genetic Research, Roslin, Midlothian EH25 9PS, UK.

Transforming growth factor beta (TGF-B) is a multi-functional regulator of cellular differentiation and proliferation. Recently, it has been suggested that members of the TGF-B gene family also control differentiation and morphogenesis in embryonic development. Since chicken embryogenesis has been so extensively studied, we chose to clone chicken TGF-B homologues to facilitate a more detailed analysis of the developmental roles of TGF-B. Using a simian TGF-B2 cDNA (Hanks *et al*, 1988) we have cloned the gene for TGF-B2 from a White Leghorn chicken genomic library. Using chromosomal walking methods we have isolated the entire TGF-B2 gene from overlapping genomic clones. The gene is greater than 50 kb in length. DNA sequencing has revealed seven exons and six introns, the first two introns are very large. Comparison of mammalian and avian TGF-B2 precursor sequences show an exceptionally high degree of homology at the amino acid level, implying that TGF-B2 has a highly conserved function, maintained by strong selection pressures. Flanking sequences also show extensive homology, possibly of regulatory significance. The intron/exon structure is also conserved amongst the different members of the TGF-B gene family. This conservation adds to the view that these genes evolved from a common ancestor by gene duplication some time before the divergence of birds and mammals. However, a major divergence is an extra exon (exon 1a) within the TGFB-B2 gene. By a process of alternative splicing, mRNAs with or without exon 1a are produced. The extra amino acids encoded by this exon are probably surface residues, the significance of these multiple TGF-B2 polypeptides is unknown. It is clear, by using alternative splicing, multiple promoters and poly-adenylation signals that the TGF-B genes can give rise to multiple mRNAs and proteins. The significance of these multiple products is unclear.

O 104 TRANSFORMING GROWTH FACTOR β_1 INHIBITS THE DIFFERENTIATION OF CHICKEN ADIPOCYTE PRECURSORS AND STIMULATES THE DEDIFFERENTIATION OF MATURE ADIPOCYTES, Simon C. Butterwith, Agricultural and Food Research Council, Poultry Department, Roslin, Midlothian EH25 9PS.

Transforming growth factor β (TGF-β) has been shown using 3T3-L1 cells to inhibit the expression of glycerol-3-phosphate dehydrogenase and lipid filling in 3T3-L1 cells. However this was the case only if TGF-β was added prior to the start of the differentiation process. We have been examining the role of TGF-β in the regulation of chicken adipocyte precursor differentiation using lipoprotein lipase as an alternative marker of the differentiation process. We have found that TGF-β_1 can reduce by up to 80% the activity of lipoprotein lipase in fully differentiated adipocytes. This required exposure of the cells to TGF-β for 6 hours suggesting an effect on the synthesis of the enzyme. The reduced lipoprotein lipase activity remained for at least 24h after removal of TGF-β from the cells. These results suggest that TGF-β may be capable of inducing a dedifferentiation process in these cells.Weiner et al.(Biochemistry, 1989, 28, 4094) have recently demonstrated the presence of TGF-β mRNA in proliferating 3T3-L1 cells and its marked reduction in differentiating cells. The possible role of TGF-β as an autocrine regulator of adipocyte development will be discussed.

O 105 HIGHLY PURIFIED BOVINE BONE-INDUCTIVE ACTIVITY CONTAINS MULTIPLE PROTEIN SPECIES RELATED TO BMP-2, Anthony J. Celeste, James A. Iannazzi, Robin C. Taylor, Rodney M. Hewick, Vicki Rosen, Elizabeth A. Wang and John M. Wozney, Tissue Growth and Repair Program, Genetics Institute, Inc., 87 Cambridge Park Drive, Cambridge, MA 02140.

Characterization of the polypeptides present in bone-inductive protein extracts from bovine bone has led to the cloning of seven novel regulatory molecules, six of which are distantly related to TGF-beta. The three recently discovered human proteins we describe here, BMP-5, BMP-6 and BMP-7, show extensive sequence similarity to BMP-2, a molecule which by itself is sufficient to induce de novo bone formation in vivo. The additive or synergistic contribution of these BMP-2 related molecules to the osteogenic activity associated with demineralized bone is strongly implicated by the presence of these growth factors in the most active fractions of highly purified bone extract.

O 106 **TGF-B1 INHIBITS THE GROWTH OF CCL64 CELLS AND ALTERS THE AP-1 COMPLEX, Chojnicki, E., Perez, M., Lacomis, E., Kozick, L.*, Racker, E., and Leister, K.*, Department of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853; *Analytical Research, PRDD, Bristol-Myers Squibb Co., Syracuse, NY 13221. Mammalian activator protein (AP-1) consists of a complex mixture of proteins that include heterodimers of c-jun/c-fos as major constituents that control transcription by binding to AP-1 responsive DNA elements (AREs). In order to explore the effect of TGF-B1, confluent CCL64 cells were exposed to 5 ng/ml TGF-B1 in complete media for 0 - 8 hr.. Nuclear extracts were prepared by the method of Prywes and Roeder (1986, Cell, 47:777-784) and were assayed for mobility shift in 6% acrylamide gels prepared with TBE buffer using a synthetic ^{32}P-labelled oligonucleotide (24 bp) which contained a single ARE. There were marked changes in binding patterns particularly within the first hour of exposure. The amount of TGF-B1 that altered the formation of the AP-1 complex was inhibitory to growth of CCL64 cells as measured by radioactive thymidine incorporated into acid insoluble material. We conclude that TGF-B1 is influencing the binding of the transcription factor to DNA and propose that this finding is relevant to the inhibition of growth by TGF-B1. (Supported by PHS grant CA-08964)**

O 107 Vg1 A TGF-β HOMOLOGUE IN EARLY *XENOPUS* DEVELOPMENT, Leslie Dale, Glenn Mathews and Alan Colman, School of Biochemistry, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K. Vg1 is a maternal mRNA localised to the vegetal hemisphere of *Xenopus* oocytes and embryos, that encodes a protein homologous to the mammalian growth factor TGF-β. In oocytes and early blastulae most Vg1 molecules contain 3 N-linked oligosaccharide side chains (M_r 45 kd), but in late blastulae and gastrulae a 2-chain form (M_r 43.5 kd) becomes the predominant newly-synthesised Vg1 protein made from the endogenous maternal mRNA. Following injection of synthetic Vg1 mRNA both proteins are over-expressed in all regions of the embryo, however the 45kd form predominates at all stages, and in gastrulae the 43.5 kd protein is only found in the vegetal hemisphere. This suggests the emergence in the embryonic secretory pathway of a vegetally-localised and saturable quantity of a Vg1 binding protein which can influence the degree of Vg1 glycosylation. An *in vitro* assay for the analysis and cloning of this protein has been developed.

By analogy with TGF-β the protein is expected to be processed and secreted as a dimer of M_r 25-30 kd, so far we have been unable to observe the expected processing or secretion of this protein which appears to remain within the E.R. lumen as a monomer. Over- and ectopic-expression of the protein appears to have no effect on the development of the embryo and isolated animal hemispheres do not differentiate mesoderm. These data suggest that Vg1 alone cannot be responsible for mesoderm induction in amphibian embryos.

O 108 EFFECT OF GROWTH FACTORS ON MCF-7 AND MDAMB-231 CELLS IN THE PRESENCE AND ABSENCE OF SERUM. G. Di Fronzo, C. Ruedl, V. Cappelletti, G. Granata, D. Coradini. Istituto Nazionale Tumori, Milano (Italy).

Human mammary tumor cells are known to produce growth factors and in the case of the hormone dependent ones the growth factor synthesis is under estrogenic control. We studied the effects of estradiol alone or in combination with different growth factors (EGF, IGF-I bFGF and insulin) on the proliferation of two human breast cancer cell lines: a hormone dependent one (MCF-7) and a hormone independent one (MDAMB231). The mitogenic action was tested under completely serum free conditions, in presence of 5% dextran coated charcoal-treated fetal calf serum (DCC-FCS) and finally in the presence of 5% growth factor-denaturated DCC-FCSd. Independently of the cell responsivity to each single growth factor. The intensity of the proliferation stimulus decreased when tested in 5% DCC-FCS compared to 5% DCC-FCSd. The weakest stimulation was observed in both cell lines under completely serum free conditions. The present results would suggest that some still unknown factor which is present in the serum may be responsible for modulation of the growth factor mitogenic activity.

Supported in part by grant no. 88.01127.44, CNR, Rome - Italy.

O 109 MOLECULAR CLONING OF THE cDNA FOR RAT PROTEIN PHOSPHATASE INHIBITOR-1. Alex Elbrecht[1], James DiRenzo[1], Roy G. Smith[1] and Shirish Shenolikar[2]. [1]Department of Growth Biochemistry and Physiology, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065 and [2]Department of Pharmacology, University of Texas Health Science Center, Houston, TX 77025.
Protein phosphatase-1 is involved in cell cycle regulation and therefore growth in general. It is inhibited specifically by a peptide termed inhibitor-1. We have cloned the cDNA for rat muscle protein phosphatase inhibitor-1 using a 60 bp oligonucleotide probe based on the published peptide sequence for rabbit inhibitor-1. The cDNA codes for a peptide of 171 residues with 80% homology to the rabbit peptide. The homology is particularly striking at the amino-terminus with residues 4 to 61 being identical between these two species. The region between residues 9 and 54 has previously been shown to be the minimal active fragment for inhibitor activity and also contains the threonine residue (position 35) whose phosphorylation is necessary for activity. The highest level of expression for inhibitor-1 mRNA in both rat and rabbit is in skeletal muscle, however, it is also expressed in kidney and brain. Northern blots showed that inhibitor-1 mRNA was expressed at very low levels in heart and was undetectable in lung. A major difference in expression of the message between the two species was its presence in rabbit liver and its absence in rat liver.

O 110 INDUCIBLE EXPRESSION OF ERYTHROID DIFFERENTIATION FACTOR/INHIBIN βA CHAIN mRNA IN CULTURED HUMAN MONOCYTES, Marja Erämaa,* Olli Ritvos,*" Mikko Hurme,§ and Raimo Voutilainen*#, *Department of Pathology, §Bacteriology and Immunology, University of Helsinki, 00290 Helsinki, Finland; and the Departments of "Obstetrics and Gynecology, and #Pediatrics, Helsinki University Central Hospital, 00290 Helsinki, Finland.
Erythroid differentiation factor (EDF), a polypeptide secreted by human monocytic leukemia THP-1 cells, promotes erythrodifferentiation in both human bone marrow cultures and cultured erythroleukemia cells. It is structurally a member of the family TGF-β-like molecules and identical to activin A, a homodimer of two βA-chains of the gonadal hormone inhibin. Inhibin itself is a heterodimeric hormone composed of an α-chain combined to one of two β-chains, βA or βB, yielding inhibin A and inhibin B, respectively. Inhibins suppress and activins stimulate pituitary follicle-stimulating hormone secretion. We now show that human peripheral blood monocytes, when activated by PMA, LPS or vitamin D_3, express the about 7 kb, 3 kb and 2 kb βA-chain mRNAs, but not that of α- or βB-chains, suggesting that monocytes may be a site of EDF/activin A production (cDNAs for α-, βA- or βB-chains were obtained from Dr. A. Mason, Genentech, and subloned into pGEM-4Z vectors for synthesis of riboprobes used in Northern blotting). Also, in HL-60 promyelocytic leukemia cell line induced toward monocytic differentiation with PMA, the same transcripts of βA-chain mRNAs are detected within 72 h, whereas no inhibin subunit expression occurs during their granulocytic differentiation with DMSO. Protein kinase inhibitor studies suggest that the induction of βA-chain mRNA expression by LPS in monocytes is mainly protein kinase C dependent. As human monocyte/macrophages are involved in a variety of tissue events such as inflammation and repair and hemotologic development, their ability to produce EDF/activin A implies that this polypeptide may have a wide spectrum of biologic roles beyond its known effect on the control of reproductive and hematopoietic processes.

O 111 MODULATION OF GROWTH FACTOR MEDIATED TYPE II PNEUMOCYTE PROLIFERATION BY TGFβ, Jacob N. Finkelstein, and Mary E. Brandes, Department of Pediatrics and EHS Center, University of Rochester School of Medicine and Dentistry, Rochester NY 14642.
Proliferation of alveolar type II pneumocytes is a crucial step in recovery from lung injury. Alveolar macrophages have been shown to produce two growth factors for type II cells: transforming growth factor α (TGFα) and the partially characterized macrophage-pneumocyte growth factor (MPGF). The synthesis of these growth factors by alveolar macrophages activated *in vitro* or during *in vivo* lung injury is accompanied by the production of transforming growth factor β, TGFβ. This study examined the interaction of TGFβ and the type II cell growth factors in modulating type II cell growth. TGFβ (20 pM) was found to completely inhibit the proliferation of type II cells stimulated by MPGF, TGFα, and EGF. Type II cells were shown to contain both low and high affinity EGF/TGFα receptors. In addition, type II cells were found to express the low molecular mass TGFβ receptors (53 and 85 kD) which have been associated with its inhibitory effects on growth. Prolonged treatment of type II cell cultures with TGFβ resulted in a slow decline in the amount of EGF bound, plateauing at a 40% reduction in total EGF bound per cell in 16 h. Scatchard analysis revealed that this decrease was due to a loss of high affinity EGF receptors and a drop in the number of low affinity EGF receptors. The TGFβ effect on type II cell EGF receptor number is a possible mechanism by which TGFβ may mediate its growth inhibitory effects. (Supported by HL32476, HL36543, HL37388, CA27791, and ES07026)

O 112 LUNG MORPHOGENESIS IS ALTERED BY TRANSFORMING GROWTH FACTOR β; POSSIBLE ROLE FOR MATRIX DEGRADING METALLOPROTEINASES AND THEIR INHIBITIORS. Gail L. Ganser, Ron W. Pelton, Brigid L.M. Hogan, and Lynn M. Matrisian. Dept. of Cell Biology, Vanderbilt University, Nashville TN 37232.

Extracellular matrix remodeling by matrix degrading metalloproteinases (MMPs) has been implicated in the early morphogenesis of branched organs. Growth factors such as TGFβ are known to regulate the expression of MMPs and their inhibitors. We therefore examined the effects of TGFβ and an inhibitor of MMPs, TIMP, upon branching of the embryonic lung. Lung rudiments dissected from day 11.5 p.c. mice undergo extensive growth and repetitive branching during a 48h period in culture. Lungs treated with 10 ng/ml TGFβ for 48h were smaller and displayed fewer branches with narrower lumina compared to controls. Enzymography of conditioned medium from the TGFβ-treated lungs revealed a marked induction of a MMP which most likely corresponds to the 72kD type IV collagenase/gelatinase which specifically degrades basement membrane collagens. Lungs treated with 5μg/ml TIMP exhibited an altered morphology, including a broadening of the luminal space at the ends of the bronchial trees. These observations suggest that growth factors such as TGFβ may influence the morphogenesis of branched organs at least in part by regulating the expression of MMPs and/or their inhibitors.

O 113 EXPRESSION OF THE TRANSFORMING GROWTH FACTOR TYPE BETA GENE FAMILY IN HUMAN EMBRYOGENESIS Derek Gatherer, David Baird * and Rosemary J. Akhurst. Institute of Medical Genetics, Glasgow, UK and * University Department of Obstetrics and Gynaecology, Edinburgh, UK. The TGF-β gene family consists of genes encoding proteins which regulate growth and differentiation. TGF-β 1, 2 and 3 are known to be important in murine embryogenesis and we present data from similar studies in human embryos. At 56 days post-fertilization, TGF-β1 mRNA is expressed in some haematopoietic progenitor cells, zones of intramembranous ossification, cardiac valves and the endothelia of large blood vessels. At the same stage TGF-β2 mRNA is expressed in perichondria, bronchioles and many mesenchymal areas, and TGF-β3 in bronchioles and trachea, perichondria, intervertebral discs, cardiac valves, the submucosa of the gut, and the tunica intima of large vessels. By contrast at 44 days, TGF-β1 mRNA is confined to liver haematopoietic progenitors and TGF-β2 mRNA is found in several mesenchymal areas, which we judge to correspond to zones of developing muscle and limb cartilages. This data suggests that the TGF-β gene products have different and multiple functions in embryogenesis, sometimes acting individually and sometimes in a synergistic fashion. Experiments are currently in progress to extend the mRNA data to other embryonic stages and to determine the expression patterns of TGF-β proteins.

O 114 IN VIVO INDIRECT ACTION OF TAMOXIFEN ON TGF-β mRNA

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TGF-β is a multifunctional growth factor, the nature of its action depends on many variables as the target cells its differentiation state, growth conditions and presence of other growth factors. In human breast cancer cells TGF-β is a hormonally regulated negative growth factor. In vitro the secretion of TGF-β is induced up to 5 fold under treatment of MCF-7 cells with an entiestrogen as Tamoxifen (Cell. 48: 417-428, 1987). The growth regulatory action of antiestrogen probably involve two mechanisms: antagonism at the estrogen receptor level and induction of the TGF-β. The preseent study was undertaken to verify in vivo the TGF-β induction by Tamoxifen already observed in a cell culture system. We collected biopsies from canine spontaneous mammary tumors before and after treatment with 0.7 mg/kg of Tamoxifen for 10 days. Poly (A) RNA samples isolated from biopsies, submitted to the histologic examination, were analyzed by Northern hybridisation for TGF-β and TGF-α. We observed that TGF-β mRNA levels increased up to 2 fold after the treatment whereas TGF-α mRNA levels were unchanged. The results will be discussed starting from the hypothesis that estrogen-dependent breast cancer growth may be regulated by a combination of negative growth modulators as TGF-β and positive growth modulators as TGF-α.

O 115 TRANSFORMING GROWTH FACTOR β1 mRNA EXPRESSION IN HUMAN DEVELOPING TOOTH AND ODONTOGENIC TUMORS, Kristiina Heikinheimo[1,4], Minna Sandberg[2], Marja Erämaa[4], Ismo Virtanen[3], Olli Ritvos[4], Departments of Oral Pathology[1] and Medical Biochemistry[2], University of Turku, 20520 Turku; and Dept of Anatomy[3] and Pathology[4], University of Helsinki, 00290 Helsinki, Finland.

In situ hybridization studies using ^{35}S-CTP labeled single stranded antisense TGF-β1 cRNA probe (λC1 cDNA, Derynck et al., Nature 316:701, 1985) were carried out on frozen tissue sections of 13-24 weeks-old human fetal mandibular (n = 6) tooth germs together with odontogenic tumors (n = 6) of both odontogenic epithelial (ameloblastoma) and epitheliomesenchymal (ameloblastic fibroma) origin. Extracted total cellular RNA from human fetal tooth germs and odontogenic tumors were analyzed simultaneously using Northern blot hybridization with the TGF-β1 cDNA probe (0.8 kb EcoRI-BamHI fragment of λC1, which does not hybridize to ribosomal RNA). The purpose of our investigation was to study the role of TGF-β1 in the growth regulation of developing tooth and odontogenic neoplasms. *In situ* hybridization studies showed positive hybridization in the mesenchyme underlining the fetal oral epithelium, surrounding the dental lamina and the tooth germ proper. Ameloblastic fibromas showed a high copy number of TGF-β1 mRNA in their epithelial compartment at the epithelium-mesenchyme interphase as well as to a lower extent in their connective tissue stroma. By contrast, ameloblastomas showed positive expression of the mRNA only in the connective tissue stroma. Northern blot analysis confirmed that in each of these tissues the transcript size was 2.5 kb corresponding to that earlier reported in other human tissues. In conclusion, TGF-β1 seems to be involved in the regulation of human tooth development as well as in the epithelio-mesenchymal interaction during tumor progression of odontogenic neoplasms.

O 116 A cDNA FOR A TGFβ-LIKE FACTOR ISOLATED FROM AN XTC cDNA LIBRARY, Geoffrey Howes and J.C. Smith; The Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, LONDON. NW7 1AA., U.K.

The Xenopus tissue-culture cells XTC produce a small factor that acts as a potent inducer of mesoderm in Xenopus animal pole cells. This mesoderm-inducing factor (MIF) has been purified and, on the basis of biochemical properties, appears to belong the TGF β-like superfamily.

I have been using the strategy of screening cDNA libraries with TGF β-like probes and I have isolated a cDNA from an XTC cDNA library that encodes a TGFβ-like factor.

O 117 Processing of the fertility hormone (pro)activin A or Erythroid Differentiation Factor (EDF), a member of the TGFß -superfamily, expressed by recombinant vaccinia virus, Danny Huylebroeck [1,2], Kristien Van Nimmen [1], Abdul Waheed [3], Kurt von Figura [3], Henk Stunnenberg [2] and Hugo Van Heuverswijn [1], [1] Innogenetics, B-9710 Ghent, Belgium, [2] EMBL, D-6900 Heidelberg, F. R. Germany and [3] Biochemie II, Georg-August Universität, D-3400 Göttingen, F. R. Germany

Inhibins and activins are gonadal protein hormones that regulate FSH, but not LH, secretion. The smallest biologically active form of inhibin is a heterodimer composed of an 18 KDa α-chain and a 14 KDa ß-chain. Two ß-chains are known ($ß_A$ and $_B$). Activins are disulfide -linked homodimers of the ß-chains which are also found in inhibin. Several groups, including ours, have isolated cDNA clones encoding the inhibin subunits from different species. Both inhibin subunits show structural and sequence homology to an emerging list of polypeptides with growth or differentiation-regulating properties belonging to the TGF ß -family. We have assessed the biosynthesis and the intracellular processing of the $ß_A$-proprotein in a wide spectrum of cell types. Most cell lines constitutively secrete both disulfide-linked prohormone and mature hormone as homodimers composed of two 54 to 61 KDa subunits and of 14 KDa subunits, respectively. Heterodimers composed of one 14 KDa and one 54-61 KDa chain are also found. Pro-parts cleaved out from the prohormone are secreted in an equimolar ratio compared to mature chains and are not disulfide-linked. NH_4Cl experiments suggest that intracellular processing is acid pH-dependent. Addition of glycosylation inhibitors does not inhibit secretion of activin from the cells. We have investigated whether proactivin A is a ligand for the 215 KDa M6P/IGF-II receptor and are documenting activities of activin in diverse systems.

O 118 ROLE OF TGF-B1 IN ADHESIVE BESPONSES OF MYELOID CELLS. Ronald A. Ignotz, Department of Cell Biology, University of Massachusetts Medical Center, Worcester, MA 01655
TGF-B has been reported to elevate the expression of several integrins on fibroblasts and LFA-1 on monocytes. Further examination of th e effects of TGF-B1 on promonocytic cell lines, THP-1 and U937, reveal that TGF-B1 is growth inhibitory for these cells. In addition, treatment of these cells with TGF-B1 results in a change in morphology from round cells to ovoid or irregular shaped cells. TGF-B1 treated cells also begin to extend pseudopodia-like processes and bind to culture plates at several fold higher levels than the untreated cells. The synthesis of several integrins and a protein of approximately 115 kDa is elevated following incubation with TGF-B1. The 115 kDa protein may be ICAM-1. Physiologically, the up-regulation of integrins on leukocytes by TGF-B may be relevant to the adherence of leukocytes to the endothelium at sites of trauma. Preliminary data suggests that TGF-B1 treated monocytes can bind more effectively to endothelial cell cultures. Taken together, the change in morphology and binding to endothelium suggests that TGF-B1 may play a role in diapedisis of monocytes and their maturation into macrophages. Specific markers of macrophage differentiation are being examined. These cells express the type I receptor for TGF-B but not the type III receptor, betaglycan. The type II receptor may be present but at a very low copy number. Thus, the effects of TGF-B upon monocytic cells appear to be mediated through the type I receptor.

O 119 EXPRESSION OF TGF-ßs IN CHONDROCYTES AND MYOCYTES OF CHICKEN EMBRYOS, Sonia B. Jakowlew, Pamela J. Dillard, Thomas S. Winokur, Belinda A. Marascalco, Kathleen C. Flanders, Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892. cDNA probes and antibodies for TGF-Bs 1, 2, 3 and 4 were used to study TGF-ß mRNA and protein levels in chicken embryo chondrocytes and cardiac myocytes. TGF-ß 2, 3 and 4 mRNAs, but not TGF-ß1 mRNA were detected in cultured chondrocytes and myocytes. TGF-ß 2 and 4 mRNA expression increased with increasing age of chondrocytes from 12 to 17 days, while expression of TGF-ß3 mRNA was independent of developmental age. TGF-ß 2, 3 and 4 mRNAs were all expressed constitutively in cultured myocytes extracted from 7 to 10-day-old embryonic hearts. Immunoprecipitation of [^{35}S]cysteine-labelled chondrocytes with a polyclonal antibody against human TGF-ß1 demonstrated 2-fold higher levels of TGF-ß secreted into the media than found in the cell lysates. In contrast, immunoprecipitation of [^{35}S]cysteine-labelled myocytes with the same TGF-ß1 antibody demonstrated 5-fold higher expression of TGF-ß in the cell lysates than in the media. Immunoprecipitation of chondrocytes and myocytes with a polyclonal antibody against porcine TGF-ß2 showed 4-fold and 20-fold higher levels of TGF-ß in the cell lysates than in the media of chondrocytes and myocytes, respectively. Immunohistochemical staining of paraffin sections of cultured chondrocytes with polyclonal antibodies against TGF-ß 1, 2, 3 and 4 showed immunoreactive TGF-ß 2, 3 and 4, while no TGF-ß1 was detected. In situ hybridization of paraffin sections of cultured chondrocytes with chicken TGF-ß riboprobes showed hybridization with TGF-ß 2, 3 and 4 riboprobes, while no hybridization with TGF-ß1 riboprobe was detected.

O 120 **Isolation and Initial Characterization of Vgr-2: A New Member of the TGF-β Gene Family,** C. Michael Jones and Brigid L. M. Hogan, Department of Cell Biology, Vanderbilt University, Nashville, TN 37232.

There is good evidence that members of the TGF-β gene superfamily play important roles in early vertebrate development. A subgroup of this superfamily includes such genes as *Xenopus* Vg-1, *Drosophila* Decapentaplegic, the Bone Morphogenetic Proteins, and Murine Vgr-1. We have used a probe derived from the 3' portion of Xenopus Vg-1 mRNA which codes for the conserved C-terminal part of the protein to screen at low stringency a mouse 6.5 day *post coitum* cDNA library (kindly provided by David Weng, Johns Hopkins Medical School). We report the isolation of a novel 1.2 kb cDNA, provisionally called Vgr-2, having extensive homology to all members of the aforementioned TGF-β subgroup. Upon genomic Southern analysis, it was found that the cDNA hybridizes to DNA fragments distinct from all other members of the subgroup, confirming the identification of a novel gene. Comparisons between the encoded amino acids of Vgr-2 and *Xenopus* Vg-1 show approximately 70% homology over the conserved C-terminal domain but only 48% homology over the amino-terminal region. Our initial results show that murine Vgr-2 is expressed in F9 teratocarcinoma cells, embryonic stem cells, and in mouse embryos at 6.5 days of development. A wide range of adult tissues were examined and no expression was detected. These data suggest that Vgr-2 plays a role in establishing the initial body plan during early murine development.

O 121 A POTENTIAL MECHANISM FOR THE INCREASED PRODUCTION OF TGF-ß IN ADULT T-CELL LEUKEMIA, Seong-Jin Kim, John H. Kehrl*, Hy De Lee, David Danielpour, Michael B. Sporn, and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, and the *Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Activation of various genes in HTLV-1 transformed T cells has been ascribed to Tax, since Tax alone or in combination with lectin activates genes for IL-2 receptors (p55), IL-2, GM-CSF, IL-3, and c-fos. Recently, elevated excessive amounts of TGF-ß1 have been reported to be secreted by freshly isolated adult T-cell leukemic (ATL) cells. The overproduction of TGF-ß by these cells is intriguing since TGF-ß has been shown to have potent immunosuppressive properties and to affect calcium metabolism in vitro. To elucidate the mechanism for the increased production of TGF-ß1 in ATL cells, we examined the effect of the HTLV-I Tax gene product on the human TGF-ß1 promoter. Transfection of deleted constructs of the TGF-ß1 promoter revealed specific sequences in the 5' regulatory regions which were required for Tax-induced transactivation of the TGF-ß1 promoter. In addition, we confirmed the overproduction of TGF-ß by freshly isolated ATL cells and have demonstrated that the TGF-ß isoform secreted by these cells is exclusively TGF-ß1. Long term ATL cell lines expressed significant amounts of TGF-ß1 mRNA as well as detectable levels of TGF-ß1 protein. These results suggest a role for Tax in the up-regulation of TGF-ß1 in HTLV-I infected cells.

O 122 CLONING AND CHARACTERIZATION OF THE CDNA FOR AN EVOLUTIONARILY CONSERVED GROWTH FACTOR DEGRADING ENZYME, Wen-Liang Kuo[1], Barry Gehm[1], Robert Holmgren[2] and Marsha R. Rosner[3], Ben May Institute[1], and Ben May Institute and the Department of Pharmacological and Physiological Sciences[3], University of Chicago, Chicago, IL 60637; and Department of Biochemistry and Molecular and Cell Biology, Northwestern University, Evanston, IL 60208[2].

We have previously identified and characterized a metalloproteinase from *Drosophila* that cleaves insulin and transforming growth factor-alpha (TGF-alpha), but not epidermal growth factor (EGF), at physiological concentrations. On the basis of enzymatic properties and substrate specificity, this enzyme was presumed to be the *Drosophila* homologue of the mammalian insulin degrading enzyme (IDE). We now report the cloning and sequencing of the cDNA coding for the *Drosophila* IDE. Northern blot analysis indicates that the *Drosophila* IDE is translated from a 3.6 kb transcript similar in size to one of the two human IDE transcripts. The gene for the *Drosophila* IDE has been mapped to chromosome 3L (77B). The sequence of the *Drosophila* IDE is very similar to that of the human IDE, and both enzymes share limited but significant identity with the bacterial metalloproteinase Protease III. Comparison of the amino acid sequences of the dIDE with those of other metalloproteinases suggests that the amino acids involved in zinc binding include histidine residues 81 and 85 of the dIDE. These results indicate that the IDE is a highly conserved enzyme evolutionarily, and may represent either a new branch or a new family of metalloproteinases that spans bacteria, *Drosophila*, and humans.

O 123 EXPRESSION OF THE BMP-2A GENE DURING MURINE DEVELOPMENT, Karen M. Lyons and Brigid L.M. Hogan, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Bone Morphogenetic Protein 2a (BMP-2a) is a member of a distinct subfamily of TGFß-like molecules that display more homology to the Drosophila DPP and Xenopus Vg1 gene products than to TGFß1. BMP-2a was originally isolated based on its ability to induce ectopic cartilage formation *in vivo* (Wozney *et al.*, 1988, Science 242: 1528-1534). A murine cDNA encoding BMP-2a was isolated (kindly provided by M. Kobrin and R. Derynck) and used to construct a specific probe to examine the expression of BMP-2a during murine development by Northern analysis and *in situ* hybridization. A single 3.5 kb transcript is detected by Northern analysis. *In situ* hybridization shows that in the developing skeletal system, BMP-2a transcripts are first detected in condensing precartilaginous mesenchyme and at later stages within the osteogenic zone, consistent with a role in both cartilage and bone formation during development. BMP-2a transcripts are also detected in other developing tissues, in both epithelial and mesenchymal cells. For example, BMP-2a transcripts are present in the mesenchyme and epithelial odontoblasts in the developing tooth. BMP-2a expression was also detected in developing hair and whisker follicles, deciduum, heart, palate, and in the mesenchyme underlying the developing nasal cavity. A comparison of the expression of several members of the TGFß superfamily in developing whisker follicles reveals that these genes are expressed in temporally and spatially distinct patterns, in both epithelial and mesenchymal components. These observations suggest that the BMP-2a gene product is involved in a variety of developmental processes, and that the coordinate expression of several members of the TGFß superfamily is involved in the morphogenesis of specific organs.

O 124 SEQUENCE REQUIREMENTS FOR PHOSPHORYLATION OF THE REGULATORY TYROSINE RESIDUE IN $p60^{c\text{-}src}$. Alasdair MacAuley and Jonathan A. Cooper, Dept. of Cell Biology, Division of Basic Sciences, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104.

Wild type $p60^{c\text{-}src}$ is present in fibroblasts in a stoichiometrically tyrosine phosphorylated form with low specific activity. The tyrosine phosphorylated is tyr 527, 6 amino acids from the carboxy terminus, and dephosphorylation of this residue is associated with activation of $p60^{c\text{-}src}$ activity. The protein sequence of this regulatory domain is very similar in all members of the *src* gene family described so far. I have been using cassette mutagenesis to probe the sequence requirements for phosphorylation of tyr 527. Most residues between tyr 519 and the carboxy terminus appear to be able to withstand a variety of nonconservative changes without altering the regulation of $p60^{c\text{-}src}$. Two residues appear to be essential, glu 524 and asp 518. These residues appear to be able to tolerate only a conservative change to the other acidic amino acid. Radical changes at two other residues, ser 522 and thr 523, will activate $p60^{c\text{-}src}$ activity, suggesting steric and perhaps charge limitations on the amino acids at these positions. The reason for the sequence conservation of the carboxy terminal domain within the *src* family is not clear, but may be related to regulation of the protein products in cell types other than fibroblasts.

O 125 EXPRESSION OF TGFß, PDGF AND IGF DURING THE BIPOTENTIAL DIFFERENTIATION OF EMBRYONIC STEM CELLS. C.L. Mummery, A. Feyen, E. Freund, W.Kruijer, A.J.M. van den Eynden, Hubrecht Laboratory, 3584 CT Utrecht, The Netherlands. Embryonic stem (ES) cells can now be maintained in culture in an undifferentiated state provided they are grown in the presence of a differentiation inhibitor (LIF/DIA). Bipotential differentiation in monolayer can be induced either by (i) addition of retinoic acid (RA) to LIF/DIA containing medium; the cells then resemble those of parietal endoderm in terms the expression of specific marker proteins (1), or by (ii) deprivation of LIF/DIA; ES cells then resemble P19EC cells in monolayer +RA (1). We have compared changes in the expression of several growth factors by Northern blotting during differentiation of ES cells with that in F9 and P19EC cells. Transcripts recognized by a hTGFß1 probe are found in all cells, but a 1.8kb transcript is specifically reduced by differentiation; a chicken TGFß4 probe recognized a similar transcript. TGFß2 mRNA is induced by differentiation and the protein becomes detectable by immunofluoresence (2) using an anti-TGFß2 antibody (3); anti-TGFß2 also stains endoderm-like cells of blastocyst outgrowths in vitro, but not the ICM. TGFß3 and PDGF-B (c-sis) are induced in ES cells deprived of LIF/DIA and P19+RA; PDGF-A is unchanged. PDGF-A is reduced in ES and F9 +RA. IGF2 is induced in all cases. These studies have clarified conflicting results from different EC cell systems and provided more details of growth factors involved in early development.(1) Mummery et al 1990, Cell Diff. (in press); (2) Mummery et al 1990 Dev. Biol. (in press) (3) Van den Eynden et al, submitted.

Abstract Withdrawn

O 127 MODULATION OF HUMAN KERATINOCYTE GROWTH AND DIFFERENTIATION BY TGF-ß2, David R. Olsen, Roberta W. Rhudy, Pedro Carrillo, and Karen Persichitte, Department of Cell Biology, Celtrix Laboratories, Collagen Corporation, Palo Alto, CA 94303

The growth and differentiation of human keratinocytes is influenced by several growth factors including transforming growth factor ß. In this study we examined the effects of TGF-ß2 on ornithine decarboxylase (ODC) gene expression and ^{3}H-thymidine incorporation and the levels of keratin 5 and involucrin mRNA in normal human epidermal keratinocyte (NHEK) cultures. ^{3}H-thymidine incorporation and ODC mRNA expression were down-regulated following exposure of NHEKs to 1-50 ng/ml TGF-ß2. Inhibition of NHEK growth following TGF-ß2 treatment was not accompanied by morphologic changes resembling those seen in cultures induced to undergo terminal differentiation, suggesting growth inhibition was not associated with differentiation. However, the same concentrations of TGF-ß2 which inhibited proliferation and ODC expression induced an increase in involucrin expression, a marker of keratinocyte differentiation. Expression of keratin 5 (a basal cell associated keratin) mRNA was unaltered by concentrations of TGF-ß2 as high as 50 ng/ml. These results suggest that TGF-ß2 induced arrest of keratinocyte proliferation, in itself, does not supply the appropriate signal to commit these cells to terminal differentiation even though specific markers of differentiation may be induced.

O 128 OP-1, AN OSTEOGENIC PROTEIN IN THE TGF-BETA FAMILY, Engin Özkaynak, T. Kubera Sampath, David C. Rueger, Eric Drier, Clare M. Corbett, Richard J. Ridge, and Hermann Oppermann, Creative BioMolecules, 35 South street, Hopkinton, MA 01748

We have cloned a new gene belonging to the TGF-beta super family of genes that encodes an osteogenic protein and named this protein OP-1 (osteogenic protein one). OP-1 was cloned by first obtaining partial peptide sequences from highly purified osteogenic protein preparations extacted from bovine tibia and femur. These peptide sequences revealed close relationship with the developmental genes DPP of Drosophila and Vg-1 of Xenopus. Building upon this information we constructed a synthetic consensus gene encoding a consensus protein sequence which mimicked the C-terminal 103 amino acids of the TGF-beta like domain that is the hallmark of this protein family. We later used this synthetic gene as a probe and screened a human genomic DNA library which resulted in the cloning of three interrelated genes the products of which are involved in cartilage and bone formation. Two of the genes, BMP-2 and BMP-3, have been described by others while the third gene, OP-1, has not been identified before and shows extensive homology to the recently described Vgr-1 gene. OP-1 was expressed in E.coli and upon refolding it was found to be capable of bone induction when implanted subcutaneously in rats.

O 129 THE EMBRYONIC EXPRESSION PATTERN OF TGFβ3 RNA DIFFERS FROM TGFβ1 AND TGFβ2. Ron W. Pelton, Brigid L.M. Hogan, and Harold L. Moses, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232. The β-type transforming growth factors (TGFβs) are an increasingly large family of proteins known to have a multitude of effects on cells of both embryonic as well as neoplastic origin. These effects include the regulation of proliferation, differentiation, and extracellular matrix production. In addition, some family members have been implicated in the modulation of inductive events. By comparing sequence similarities, this family, which at present contains at least 14 members, can be divided into various sub-families such as the TGFβ-like subfamily (TGFβ1-4), the DPP-like subfamily (decapentaplegic, the bone morphogenetic proteins, Vg-1, Vgr-1) and others (Müllerian inhibiting substance, the activins and inhibins, XTC-mesoderm-inducing factor). In light of recent *in vitro* studies which demonstrate mostly quantitative, and in a few cases qualitative, differences in the biological activities of TGFβ1-3, we are attempting to use mRNA expression analysis to identify systems in which the TGFβs are differentially regulated in order to gain insight into the biological functions of these proteins *in vivo*. Using Northern blot analysis and *in situ* hybridization, we have compared the expression in mouse embryos of TGFβ3 mRNA to the closely-related genes TGFβ1 and TGFβ2. *In situ* hybridization studies demonstrate that TGFβ3 RNA is expressed in distinct spatial patterns when compared to adjacent sections probed with either TGFβ1 or TGFβ2. This is illustrated in sections of the snout from a 13.5 d.p.c. (days post coitum) mouse embryo. TGFβ3 RNA is found at very high levels in the epithelial edge of the non-fused palate, at low levels throughout the mesenchyme and not at all in the epithelial component of the vomeronasal organs. In contrast, TGFβ1 RNA is found to be expressed only at low levels in the epithelium of the fusing palate, at very high levels in scattered individual cells in the mesenchyme, and at relatively high levels surrounding the epithelium of the vomeronasal organs. Lastly, TGFβ2 RNA is found in the epithelium of the palate at levels comparable to TGFβ1, at a relatively high level throughout the mesenchyme and into the upper dermis, and at high levels in the epithelium of the vomeronasal organs. Hence, these proteins may possess differences in activities *in vivo* which are not seen *in vitro*. We are using further studies to approach this hypothesis.

O 130 BINDING OF TGFβ TO THE MAJOR HUMAN PREGNANCY-ASSOCIATED PLASMA PROTEIN (PREGNANCY ZONE PROTEIN, PZP), Anie Philip, Lena Bostedt*,Torgny Stigbrand* and Maureen O'Connor-McCourt, Biotechnology Research Institute, National Research Council Canada, Montreal, Quebec, Canada H4P 2R2, and *Department of Physiological Chemistry, University of Umea, S-901 87 Umea, Sweden

We have previously shown that in the non-pregnant state TGFβ is bound to serum alpha2-macroglobulin (α2M), and that this interaction accounts for the latency of serum TGFβ. PZP and α2M are homologous proteins which have similar proteinase-binding properties. To test whether PZP is able to bind TGFβ in a manner similar to α2M, 125I-TGFβ was incubated with purified PZP and analyzed by SDS-PAGE. We found that 125I-TGFβ comigrated on non-reducing SDS-PAGE with the characteristic 360 kDa PZP dimeric species, and on reducing SDS-PAGE with the characteristic 180 kDa PZP monomeric species, although approximately 50% of the bound counts were lost upon reduction. This binding was specific as demonstrated by inhibition in the presence of excess unlabeled TGFβ. Furthermore, when the chemical cross-linker (BS3) was added to the TGFβ/PZP incubation mixture, the binding increased more than 100-fold. These results demonstrate that 125I-TGFβ binds to PZP in a predominantly noncovalent manner with minor reductant-resistant and reductant-sensitive covalent bonding components. These *in vitro* binding characteristics are identical to those which we have observed between TGFβ and α2M, and are also similar to the binding between proteases and α2M. We suggest that the binding of TGFβ to PZP may regulate the action of TGFβ during pregnancy.

O 131 AN EXTRACT FROM SKELETAL MUSCLE STIMULATES NEOVASCULARIZATION IN THE RABBIT CORNEA AND MONOCYTE CHEMOTAXIS IN VITRO, Gregg D. Phillips and David R. Knighton, Department of Surgery, University of Minnesota, Minneapolis, MN 55455

The object of this study was to determine if damaged skeletal muscle releases a factor(s) capable of stimulating revascularization. Extracts were prepared from rabbit hindlimb muscles and incorporated into Hydron, a slow-release polymer, at concentrations ranging from 10-500 ug per 20 ul pellet. Pellets of Hydron containing the test extract were implanted between the collagenous layers of the rabbit corneal stroma 2-3 mm from the limbal blood vessels as an assay for angiogenesis. The normally avascular corneas were examined daily for one week for the presence of blood vessels directed toward the implants. Two and 7 days postimplantation, the corneas were photographed and removed for histological examination. The areas covered by the vascular responses were subsequently quantified with a digitizing pad (Zidas, Carl Zeiss, Inc.). The crude extract stimulated dose-dependent inflammation associated angiogenesis. Four hundred to 500 micrograms were required to produce maximum vessel ingrowth. The control, Dulbecco's phosphate buffered saline in Hydron, failed to stimulate neovascularization. The crude extract was dialyzed against 0.1M acetic acid and analyzed by cation exchange chromatography. Angiogenic activity was restricted to a small series of peaks generated by eluting the column by raising the pH. The pooled fractions from these peaks stimulated angiogenesis in the rabbit cornea and monocyte chemotaxis in vitro, but failed to induce endothelial cell chemotaxis. Therefore, the angiogenesis factor(s) released by skeletal muscle appear to act in an indirect manner.

O 132 EXAMINATION OF TGF-RELATED MOLECULES IN THE DEVELOPING HEART, Jay D. Potts, Dan L. Weeks, and Raymond B. Runyan, Departments of Anatomy and Biochemistry, University of Iowa, Iowa City, Iowa 52242.

The cells of the atrioventricular (AV) canal of the embryonic heart undergo a temporally specific epithelial-mesenchymal cell transformation. Our lab has previously demonstrated that a member of the TGFβ family is present, in the embryonic heart, and is partially responsible for this transformation. Subsequent molecular biological experiments, using both Northern analysis and RNase protection assays, have demonstrated the presence of multiple members of the TGFβ family, in the embryonic chick heart. Using a cDNA probe to chicken TGFβ3, several unique transcripts have been identified from embryonic chick heart mRNA. These messages appear to be heart specific. A cDNA probe to chicken TGFβ 2 recognizes a single band on a Northern blot of both heart and embryonic mRNA. RNase protection assays have confirmed that chicken TGFβ2 mRNA is present in the heart at the time transformation is taking place. With the recent identification of antisense messages to certain growth factors, we have begun to look at the possible presence of antisense transcripts for several members of the TGFβ family at varying points in cardiac development. Our preliminary experiments suggest that such an antisense message may be present in the heart. Future experiments will be aimed at an identification of each of the TGFβ-related messages found in the heart during cell transformation. Supported by NIH HL 38649 (RBR) and GM 40308 (DLW).

O 133 CYCLOHEXIMIDE SENSITIVE CYCLIC AMP-DEPENDENT INDUCTION OF TRANSFORMING GROWTH FACTOR β1 mRNA IN HUMAN CHORIOCARCINOMA CELLS, Olli Ritvos[1,3], Marja Erämaa[3], and Raimo Voutilainen[2,3], Departments of Obstetrics and Gynecology[1] and Pediatrics[2], Helsinki University Central Hospital; and Department of Pathology[3], University of Helsinki; 00290 Helsinki, Finland.
Transforming growth factor β1 (TGF-β1) has previously been purified from the human placenta (Frolik et al., PNAS 80:3676, 1983) and its mRNA is abundantly expressed there (Derynck et al., Nature 316:701, 1985). However, the cellular origin and the biological role of TGF-β1 in placenta are not known. We found that the 2.5 kb TGF-β1 mRNA is expressed in the human choriocarcinoma cell line JEG-3, which has been widely used as a model system for studying the regulation of trophoblast hormone secretion. Cholera toxin, which elevates the cellular levels of the second messenger cyclic AMP (cAMP), increases the secretion of chorionic gonadotropin and steroids in these cells being thus a potent inducer of trophoblast functional differentiation. Cholera toxin also stimulated TGF-β1 mRNA levels in JEG-3 cells in a concentration and time-dependent manner as studied by Northern blotting (with λC1 cDNA, Derynck et al., Nature 316:701, 1985). The maximal effect (5-fold increase above basal levels) occurred within 12-24 hours of stimulation with 1.0 ng/ml of cholera toxin. These results were reproducible also with the cell permeable cAMP-analog 8-bromo-cAMP, suggesting that the effect of cholera toxin was indeed mediated through cAMP. Cycloheximide, an inhibitor of protein synthesis, prevented the effect of cholera toxin on TGF-β1 mRNA expression suggesting that a protein mediator may be involved in the mediation of its effect. Although *in-situ* hybridization experiments for cellular localization of the mRNA in placenta and studies on the effect of TGF-β1 on trophoblast growth and hormone secretion are still required, these preliminary results suggest that TGF-β1 may have a role as a regulator of trophoblast differentiation.

O 134 INHIBITION OF THE GROWTH OF MURINE BALB/MK KERATINOCYTES BY TRANSFORMING GROWTH FACTOR ß1 IN A SERUM-FREE MEDIUM, Donald J. Sarubbi and Michael J. Newman, Department of Biochemistry, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110. TGF-ß1 is an inhibitor of the growth of epithelial cells, including the nontransformed Balb/MK murine keratinocyte cell line. A serum-free medium for Balb/MK cells has been reported (Falco, J.P. *et al.*, Oncogene 2, 573-578, 1988), but was not able to support the growth of our particular sample of these cells. Therefore, the previously described medium was used as a starting point for the development of a new serum-free medium for these cells. Tissue culture wells (2 cm^2) were pre-coated with 5 µg fibronectin and then 5000 cells were plated in MEM/F12 medium containing 50 µM calcium, insulin, transferrin, epidermal growth factor, ethanolamine, sodium selenite, 1 mg/ml bovine serum albumin-linoleic acid complex, and 25 µg/ml high density lipoprotein. This medium supported growth of the Balb/MK cells comparable to that observed with 8-10% fetal calf serum (approximately 120,000 cells after 5 days). Addition of 30 pM TGF-ß1 on the day following cell plating resulted in a 60-80% inhibition of growth of cells plated in serum or serum-free medium. This new serum-free medium for the growth of Balb/MK cells should be useful for the study of calcium-induced cell differentiation and the mechanisms of growth regulation by TGF-ß.

O 135 THE EFFECT OF TYPE I TRANSFORMING GROWTH FACTOR β ON THE DIFFERENTIATION OF OSTEOCLASTIC CELLS IN MURINE BONE MARROW CULTURES, Doron M. Shinar and Gideon A. Rodan, Department of Bone Biology and Osteoporosis Research, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486
Multinucleated cells with osteoclastic characteristcs can be induced in bone marrow cultures of mice, humans and other species by treatment with 1,25- dihydroxyvitamin D_3. TGF-β1, which promotes bone formation and remodeling in experimental animals, enhanced the generation of osteoclastic cells in murine bone marrow cultures at concentrations of 3 to 100 pg/ml, whereas above 200 pg/ml it had a strong inhibitory effect: 100% inhibition at 4 ng/ml. TGF-β1 treatment increased the production of prostaglandin E_2 in these cultures in a dose-dependent manner throughout that concentration range. The generation of the osteoclastic cells was completely inhibited by the prostaglandin synthesis inhibitor, indomethacin (250 ng/ml), and this inhibition was overcome by the addition of prostaglandin E_2 (1 µM). These findings suggest that the stimulatory component of the biphasic effect of TGF-β1 on osteoclast generation in this system is mediated by stimulation of prostaglandin E_2 synthesis.

O 136 THE INVOLVEMENT OF PHOSPHATIDYL INOSITOL TURNOVER DURING GROWTH AND DIFFERENTIATION OF BC3H1 CELLS, Gwendolyn Spizz and Linda J. Pike, Howard Hughes Medical Institute and Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110. BC3H1 mouse cells proliferate rapidly in the presence of high serum-containing medium and exhibit fibroblast-like morphology. These cells can be induced to withdraw from the cell cycle and differentiate into muscle-like cells either by the withdrawal of serum from cells grown at subconfluence or by allowing the cells to reach confluence in high serum. The turnover of inositol phospholipids, which has been implicated in events associated with growth regulation of different cell types, has been examined in BC3H1 cells under conditions of growth or differentiation. Within the first 24 hours after the removal of serum from BC3H1 cells, the incorporation of [^{3}H]-inositol into phosphoinositides decreased by 65% to 85%. The decrease could not be attributed to reduced uptake of the radiolabel, reduced PI synthase activity or to a reduced mass of PI. The decrease appears to be due to a general decline in the metabolism of phosphoinositides. This decrease in phosphoinositide metabolism is not due simply to the removal of serum as differentiation of BC3H1 cells by growth to confluence in high serum is associated with a 35% to 45% decrease in the incorporation of [^{3}H]-inositol into polyphosphoinositides. TGF-β, which blocks differentiation of BC3H1 cells, partially blocks the decrease in the rate of PI metabolism. These findings are consistent with the interpretation that withdrawal from the cell cycle and differentiation of BC3H1 cells is associated with a decline in phospholipase C activity.

O 137 TGF-β_1 REGULATION OF ANTIBODY PRODUCTION IN THE MOUSE, Mark Tepper, Barbara Petty, Isia Bursuker, Richard Pasternak, George L. Spitalny, and Bernice Schacter, Department of Immunology, Bristol-Myers Squibb, PRDD, Wallingford, CT 06492. Transforming growth factor beta (TGF-β) is a polypeptide which has been shown to inhibit both B and T lymphocyte proliferative responses in vitro. The actions of TGF-β on the immune system in vivo have been less well documented. We have examined the in vivo effects of TGF-β on the antibody response elicited by T-dependent antigens in the mouse. 500 µg of Keyhole Limpet Hemocyanin (KLH) was administered IP on day 0 and TGF-β_1 was injected IP on day 1-3 at doses of 25, 10, 5, and 1 µg each day. Animals were bled on day 4, 7, and 14 with anti-KLH IgM and IgG antibody titers determined by ELISA. In two separate experiments, TGF-β_1 had a dose dependent inhibitory effect on the titer of anti-KLH IgM antibodies on day 4 with no effect on day 7 or 14 levels. TGF-β_1 had no effect on the titer of anti-KLH IgG antibodies on any day examined. The effect of TGF-β on anti-KLH IgM levels was observed at doses of 5 µg per day and above, resulting in 2-3.5 fold inhibition of IgM production. 15-Deoxyspergualin, a known immunosuppressant, inhibited both IgM and IgG production. The results of these experiments show that TGF-β_1 administered IP results in a transient decrease in the anti-KLH IgM levels.

In a related system, we have looked at the effect of TGF-β_1 on antibody production from spleen cells of sheep red blood cell (SRBC) immunized mice. TGF-β in these experiments also inhibited antibody production to SRBC in vivo. This effect of TGF-β is selective for the IgM component of the antibody response, as no effect was seen on IgG levels. This selective effect of TGF-β is being investigated further.

O 138 ISOLATION OF MULTIPLE NEW MEMBERS OF THE TRANSFORMING GROWTH FACTOR BETA FAMILY FROM *XENOPUS*. Gerald H. Thomsen and Douglas A. Melton, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge MA, 02138. Members of the TGF-beta growth factor family, such as the maternally-expressed Vg1, have been implicated in the induction of mesoderm in *Xenopus*. To search for other *Xenopus* TGF-beta related growth factor genes with potential roles in embryonic induction, we have employed the polymerase chain reaction (PCR) and have cloned more than 10 new members of the family. These genes display varying degrees of relatedness to other members of the family. In addition, several genes have been cloned directly from the cDNA of *Xenopus* XTC cells, a cultured line that secretes a mesoderm inducing factor. The PCR clones have been used to isolate several complete cDNAs which display distinct patterns of maternal and zygotic expression. The details of these studies will be presented.

O 139 TRANSFORMING GROWTH FACTOR BETA DOWN-REGULATES THE FETAL RAT LUNG FIBROBLAST GLUCOCORTICOID RESPONSE, John S. Torday,Department of Pediatrics(Physiology),Harvard Medical School,Boston,MA 02115

Classically,glucocorticoids accelerate the differentiation of a wide variety of fetal tissuses (lung,liver,kidney,gut) via fibroblast-epithelial cell interactions which are mediated by soluble growth factors.In lung fibroblasts,glucocorticoids must first be activated by the enzyme 11-oxidoreductase(11OR) and subsequently bind to specific glucocorticoid receptors (RGc) which mediate the steroid-nucleus interaction resulting in expression of fibroblast-pneumonocyte factor which stimulates epithelial differentiation.A TGFb homolog produced by immature lung fibroblasts(Torday,Pediatr.Res. 25,61A,1989) blocks glucocorticoid stimulation of both 11OR and RGc in fetal rat lung fibroblasts,though it does not affect baseline or previously-stimulated activities of either 11OR or RGc,suggesting that TGFb blocks the acute stimulatory mechanism.Based on these observations it would appear that endogenous TGFb inhibits lung differentiation by down-regulating the fibroblast glucocorticoid response mechanism.Supported by NIH grant HL34616.

O 140 HUMAN HEPATIC STIMULATOR SUBSTANCE: ITS PURIFICATION, CHARACTERIZATION & TRANSLATION IN VITRO, Qiang Tu, Fucu He, Guichun Xing and Ctutze Wu, Department of Experimental Hematology, Institute of Radiation Medicine, Beijing 100850 Human hepatic stimulator substance(HSS) was found in human fetal liver cytosol,which displayed a capacity to stimulate DNA synthesis of normal and malignant cells of liver origin. The factor is a heat and acid stable protein with a MW of approx. 10-30KD and is liver specific. More than 10,000 fold purification of the protein has been achieved using membrane ultrafiltration, DEAE cellulose, sephadex G75 and FPLC ion exchange chromatography. The poly(A)+ mRNA encoding HSS of human fetal liver origin was translated in Xenopus Laevis oocytes. The activity of HSS was detectable both in oocytes cytosol and in its supernatant. The HSS translated in vitro showed similar activity and physical-chemical characterization to the HSS purified from fetal liver cells. Our experiment suggests that HSS is the product of gene expression of human fetal liver cells.

O 141 XENOPUS ACTIVIN RELATED GENES: IDENTIFICATION OF A GENE THAT IS EXPRESSED DURING EARLY EMBYOGENESIS, Naoto Ueno, Shin-ichiro Nishimatsu, Atsushi Suzuki, Makoto Asashima*, Kazuo Murakami, Institute of Applied Biochemistry, University of Tsukuba, Ibaraki 305, *Yokohama City University, Kanagawa 236, JAPAN
Activin, a dimer of TGF-β related peptides, is a multifunctional factor that stimulates not only FSH secretion but also erythrocyte differentiation. To investigate the role of activin in early development, human recombinant activin was tested for mesoderm inducing activity in Xenopus *laevis*. Activin showed potent mesoderm inducing activity at a concentration of 50 ng/ml and the morphological change observed was similar to that by TGF-β. Xenopus liver genomic DNA library was screened using a rat activin βA cDNA as a probe to identify Xenopus homologue of mammalian activin gene. Overall, 5 independent genomic DNA clones, tentatively named Xar3, Xar4, Xar5, Xar9, Xar14 were isolated. The deduced amino acid sequence of these genes all showed almost perfect conservation of the distribution of cysteine residues. Northern blotting analysis of mRNA from oocytes and embryos at various stages of development showed Xar14 mRNA is maternally transcribed and retained until stage 10-11 embryos, suggesting the activin related protein may have a crucial role in the embryonic induction in Amphibia.

O 142 EFFECT OF TRANSFORMING GROWTH FACTOR BETA (TGFß) ON THE PROLIFERATION OF C3H 10T1/2 MOUSE FIBROBLASTS. Charles E. Wenner, Alan J. Kinniburgh, Ann Neilson, and Anthony F.Cutry, Departments of Exp'tl Biology and Human Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263.

Transforming growth factor beta (TGFß) increases by greater than 50% the cell number of postconfluent, quiescent C3H 10T1/2 cells 46 hours after administration. In the presence of EGF and insulin, which also produces an increase in cell number, TGFß was found to potentiate this stimulation further. These findings were corroborated by simultaneous measurements of DNA synthesis as measured by ^{3}H-thymidine incorporation. Therefore, it was of interest to examine the role of 'immediate-early gene' expression in TGFß-induced DNA synthesis and cell proliferation.

TGFß induced c-fos expression as early as 15-30 minutes. Since c-fos induction by TGFß has nearly identical kinetics of induction by other mitogens in 10T1/2 cells, it would be surprising if the induction of this gene was dependent upon PDGF production. However, an increase in c-myc expression was not observed until 6-8 hours. Further, TGFß delayed EGF-induced c-myc expression until 7-9 hours post EGF addition. The delay of c-myc expression implies that TGFß modulates regulatory events which result in alteration of c-myc mRNA levels.

TGFß has been observed to delay cell cycle kinetics as measured by its effect on EGF-induced DNA synthesis. TGFß added one hour after EGF did not delay the course of EGF-induced ^{3}H-thymidine incorporation. We are studying whether the TGFß induced delay in cell cycle kinetics is correlated with the TGFß mediated delay in c-myc expression and the role of G-proteins.

O 143 TGF-ß LIKE FACTORS AND MESODERM INDUCTION. M. Whitman, D.Tannahill, D.A. Melton. We have been studying the possible role of members of the TGF-ß family of growth factors in specification of cell fate during early development. Our laboratory has previously reported the identification and cloning of a developmentally localized mRNA, designated Vg-1, with significant sequence similarity to TGF-ß. We have also shown that this mRNA is translated during embryogenesis; its protein product appears to be localized to the vegetal region of the early embryo. Evidence concerning the structure of endogenous Vg-1 protein and its possible function in the induction of mesoderm in the early embryo will be presented. In addition, the identification of other TGF-ß like mesoderm inducing factors in the early embryo will be discussed.

O 144 INCREASE OF GLOBIN mRNAs IN HUMAN ERYTHROID CELLS BY ACTIVIN, John Yu, Normand L. Frigon, Jr., Li-en Shao, Lina Maderazo, Joan Vaughan, Wylie Vale and Alice Yu, Research Institute of Scripps Clinic, The Salk Institute for Biological Studies and University of California at San Diego, La Jolla, CA 92037

A new regulatory control for erythropoiesis has recently been proposed (J. Yu et al., Nature 330:765, 1987). Activin, a TGF- β family protein, was shown to potentiate the proliferation and differentiation of erythroid progenitors in human bone marrow cultures. It was indicated that this potentiation effect is restricted to erythroid lineage (Blood 73: 952, 1989). Present studies have used a human erythroleukemic cell line, K562 and purified human erythroid progenitor cells as two model systems to investigate the effect of activin on various globin mRNA transcripts. The human blood burst-forming units- erythroid (BFU-E) were partially purified from peripheral blood and, after 7-8 days culture, the generated cells that consisted largely of colony- forming units- erythroid (CFU-E) were used for studying the effects of activin. When these purified progenitors were cultured with or without picomolar concentrations of activin, the number of cell progeny and the size of erythroid colonies were largely similar in these two cultures. However, the amount of globin mRNAs was significantly increased among erythroid progenies after treatment with activin in the culture, with a very small increase in the degree of maturation. Similarly, globin transcripts were also increased in the K562 cells after three days of incubation with activin. Hence, in addition to its indirect effect on DNA synthesis and cellular proliferation of erythroid progenitors, activin may exert its effect on potentiating the globin mRNA contents in human erythroid cellslargely independent of other accessory cells.

EGF, TGF-Alpha, FGF and PDGF

O 200 bFGF in the nucleus of endothelial cells, François Amalric, Véronique Baldin, Isabelle Bosc-Bierne, Gérard Bouche, Béatrix Bugler, Hervé Prats and Anna-Maria Roman, Centre de Recherches de Biochimie et Génétiques Cellulaires du CNRS, 118 Route de Narbonne, 31062 TOULOUSE Cedex. Primary cultures of adult bovine aortic endothelial (ABAE) cells require bFGF to grow. G1-arrested cells, obtained after 48h without serum and bFGF were found to enter S phase and grow synchronously for at least two generations on addition of bFGF. In growing cells bFGF was detected both in the cytoplasm (90%) and in the nucleus (10%) where it accumulates in the nucleolus. It was not detected in the nucleus of confluent cells. bFGF uptake was continuous in the cytoplasm throughout cell with a maximum in G2, while nuclear uptake occured only in late G1. Cytoplasmic bFGF (18.4 kDa) is cleaved into a 16.5 kDa peptide in G1 ($t_{½}$: 30mn). In the nucleus, the 18.4 kDa form was the only form detected 2h following bFGF addition and was then cleaved into the 16.5 kDa in early S phase. These results are consistent with the possibility that in addition to the classical pathway of signal transduction, exogenous bFGF is directly translocated to the nucleus in late G1, and could play a role in replication and/or in transcription of rDNA. Another form of bFGF (22.5 kDa) was detected in human hepatoma cells and was shown to be initiated upstream at a CUG (Prats et al. PNAS 1989). This form is biologically active as the 18 kDa form. After transfection into COS cells and expression, the 22.5 kDa is recovered in the nucleus. The additional NH_2-terminal 53 residues of the 22.5 kDa form contains a signal for nuclear targeting. We are currently investigating whether according to the initiation codon used, the endogenous synthesized molecules could present different routing in the cell.

O 201 TGFa EXPRESSION AND CELLULAR LOCALISATION IN NONMALIGNANT AND MALIGNANT TISSUES. Thomas Bauknecht, Friedrich Kommoss, Olaf Wintzer, Roger Walker*. Universitäts-Frauenklinik, D 7800 Freiburg, FRG. *Triton Corp. San Francisco, USA.
TGFa is thought to be expressed in tumors and to stimulate tumor growth in an autocrine manner through the EGF signal transduction pathway. Using a TGFa c-DNA probe (plasmid PTRP7) and a monoclonal antibody (Tab-8, both Triton Corp.) we analyzed TGFa mRNA expression by northern blotting and cellular distribution of TGFa by immunohistochemistry. TGFa protein levels in acetic tissue extracts were quantified using the EGF receptor assay. A clear correlation exists between the relative amounts of TGFa mRNA, TGFa levels in tissue extracts, and the semiquantitative results of immunohistochemistry. Using the latter method, TGFa was detected in various nonmalignant specimens from stomach, small and large bowel, kidney, endometrium fallopian tube, ovary, skin. Only the epithelial components are TGFa(+), whereas stromal cells were TGFa(-). Different amounts of TGFa were detected in carcinomas, mesenchymal tumors were consistently TGFa(-). In some carcinomas TGFa expression correlates with the c-myc expression indicating the existence of both TGFa sensitive and insensitive tumors.

O 202 AMINO ACID AND cDNA SEQUENCES OF A VASCULAR ENDOTHELIAL CELL MITOGEN THAT IS HOMOLOGOUS TO PDGF, Marvin L. Bayne, Greg Conn, Denis D.Soderman, Perry W. Kwok and Kenneth A. Thomas, Department of Biochemistry, Merck Sharp and Dohme Research Laboratories, Rahway, N.J. 07065. Glioma-derived vascular endothelial cell growth factor (GD-VEGF) is a 46 kDa dimeric glycoprotein mitogen with apparently greater specificity for vascular endothelial cells than the well characterized fibroblast growth factors. The GD-VEGF cDNA sequence encodes a 190 amino acid residue subunit that is converted by the removal of an amino terminal hydrophobic secretory leader sequence to the mature 164 residue subunit characterized by direct protein sequencing. The GD-VEGF homodimeric subunit is homologous to the platelet derived growth factor (PDGF) A and B chains and its oncogene homolog v-sis.

O 203 EGF RECEPTOR EXPRESSION IN A MOUSE MODEL WITH A HOMOZYGOUS LETHAL DELETION. M.A. Behzadian[1,2], J.B. Whitney[3], M. Black[1] and S. Maghsoudlou[3]. Department of Obstetrics and Gynecology[1], Anatomy[2] and Cell and Molecular Biology[3]. Medical College of Georgia, Augusta, GA 30912.

Mouse embryos homozygous for α-Thalassemia die at 5-6 days post conception. In one of the mutant strains, 27HB, 3 1/2 days old homozygote embryos are still at the morula stage, while at this age the normal embryos reach the blastocyst (Differentiation, 17:205). Thus, the embryonic growth retardation in mutants begins before onset of globin gene expression in normal embryos.

When heterozygote 27HB mutants were mated with normal mice, the number of heterozygous pups were fewer than was anticipated and their sizes were smaller than their normal littermates. This was true whether they were born to normal or to a heterozygous mother. ^{125}I-EGF binding was assayed in liver membrane preparations from ten randomly selected pairs of normal and mutant animals of both sexes. It was found that heterozygous mutants bind 40-50% less EGF than normals; moreover, females bind approximately half as much as males in each group. Scatchard analysis indicates that the reduced binding is the result of a decline in the number of binding sites rather than in binding affinity. The low expression of EGFR gene in heterozygotes was further confirmed by RNA analysis. Equal amounts of polyadenylated liver RNA extracts were hybridized with a genomic DNA probe specific for mouse EGFR (pMEO.5). These observations together with the fact that erb-B has been mapped close to the α-globin locus may indicate that the deletion in the α-globin locus on chromosome 11 of mouse 27HB somehow influences the EGF receptor locus.

O 204 OVEREXPRESSION OF THE EGF-RECEPTOR GENE AND LACK OF EXPRESSION OF THE EGF-GENE IN HUMAN RENAL CARCINOMAS, Susanne Bock[1], G.Jakse[2] and Petro E. Petrides[1,3], [1]Molecular Oncology Laboratory D-8000 Munich, University School of Medicine, [2]Klinikum Rechts der Isar D-8000 Munich, [3]GSF, D-8000 Munich, Germany. Growth factors and their receptors play an important role in tumor formation and progression. Since the kidney is a major source of EGF we study human renal carcinomas for the expression of this growth factor and its receptor (R) by the Northern blotting technique. Tumor tissues as well as normal parts of the same kidney were obtained by nephrectomy. In our studies 4µg of Poly A$^+$ RNA was analysed. In all tumor samples we identified a 10 kb EGF-R mRNA. The majority of these tissue specimens also contained 4.6 and 3.3 kb mRNAs. For comparison adjacent normal tissue of the same kidney was tested. The gene for the EGF-R was expressed in most of the normal tissues as well but the expression was significantly lower than in transformed regions. In the untransformed part of the kidney we identified the 5 kb EGF mRNA, however, no EGF expression could be shown for carcinomas. Southern analysis of genomic DNA with ECO RI reveals no gene deletion for the EGF gene or gene amplification for the EGF-R gene. These results suggest that the EGF-R is overexpressed in human renal carcinomas, but must have an other ligand instead of EGF, since no EGF expression could be found. Since the large molecular EGF-precursor structurally resembles transmembrane receptors, it may bind a regulating signal. In such a case, EGF-precursor down regulation may cause insensibility to such a signal which may be important for the differentiated state of the cell. There are two functions after different processing of the EGF discussed. The unprocessed protein could be a receptor and the processed protein could be a growth factor. Supported in part by a grant from W.Sander foundation.

O 205 PRODUCTION OF TRANSFORMING AND FIBROBLAST GROWTH FACTORS BY F9 STEM AND F9 DIFFERENTIATED VISCERAL AND PARIETAL ENDODERM CELLS, Susan J. Braunhut, Lorraine J. Gudas, Joanne Pasquale and Patricia A. D'Amore, Depts of Ophthalmology, Surgery and Biol. Chem. & Molec. Pharm., Childrens Hosp. and Harvard Medical School, Boston MA 02115.

In recent years, the fibroblast growth factors (FGF) have been shown to be morphogenic agents, capable of inducing mesoderm in ectoderm explants derived from amphibian embryos. We have previously reported that F9 murine teratocarcinoma cells synthesize biologically active factors with properties characteristic of acidic and basic FGF only after differentiation into extra-embryonic parietal endoderm (J Cell Biol, 108:2467-2476). Using heparin affinity chromatography, mink lung epithelial cells, 3T3 cells and endothelial cells we have now also assayed F9 aggregate cultures and differentiated F9 visceral endoderm cells for the presence of FGF and transforming growth factor-beta (TGF-B). We have detected biologically active cell-associated acidic and basic FGF in retinoic acid (RA)-treated F9 aggregate cultures that contain visceral endoderm cells but not in aggregates cultured in the absence of RA. In contrast, F9 stem cells, grown in monolayer culture or as aggregates, and differentiated F9 parietal and visceral endoderm cells contain, in near equivalent amounts, proteins with characteristics of TGF-B. In preliminary studies, we have also detected activated forms of TGF-B-like factors in the serum-free conditioned media of these cells. These studies provide evidence that the extraembryonic endoderm may serve as an endogenous source of FGFs and TGF-B in the developing embryo.

O 206 IMMUNOAFFINITY PURIFICATION AND cDNA CLONING OF A PUTATIVE RECEPTOR FOR ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS, Laura W. Burrus, Bruce A. Lueddecke, Michael E. Zuber, Kafryn W. Lieder, and Bradley B. Olwin. Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

We have recently published the isolation of a putative receptor for acidic and basic fibroblast growth factors from embryonic chick (Burrus and Olwin, JBC 264:18647). This partially purified preparation was utilized to generate monoclonal antibodies that recognize a 150 kDa polypeptide that specifically binds both acidic and basic FGF. Chromatography of solubilized membranes prepared from 7 day embryonic chick on a wheat germ agglutinin column followed by the immunoaffinity column yields a nearly homogeneous preparation of the putative FGF receptor. All of the material in the wheat germ agglutinin eluant that can be crosslinked with ^{125}I-bFGF is effectively adsorbed by the immunoaffinity column. A mixture of three monoclonal antibodies that recognize distinct epitopes of the 150 kDa polypeptide were used to screen a λgt 11 cDNA library from 13 day embryonic chick brain. Eleven clones were selected from ~10^6 plaques screened. Nine of these clones, ranging in size from 2.7 kb to 3.6 kb, possessed all three of the epitopes recognized by the antibodies. In addition, these nine clones cross hybridized on a Southern blot, suggesting that all nine clones are representative of a single gene. It has recently been published that the *flg* oncogene encodes a receptor for acidic FGF. By inference, it seems probable that a highly homologous chick gene (Lee *et al.* Science, 245: 57; Pasquale *et al.* PNAS, 86: 5449) also encodes an FGF receptor. Preliminary cDNA sequencing indicates that our putative FGF receptor clone is distinct from the protein tyrosine kinase isolated by both Lee and Pasquale. Furthermore, none of the clones isolated hybridized with *bek* (Kornbluth *et al.* Mol. Cell. Biol. 8: 5541), a mouse homologue of the *flg* oncogene. Northern and Southern analysis as well as cDNA sequencing are in progress.

O 207 FATE AND FUNCTION OF BASIC FGF INJECTED INTO XENOPUS LAEVIS.
Marino BUSCAGLIA, John FULLER, Ana-Maria GONZALEZ, Michael ONG, Pamela KARLIN and Andrew BAIRD. The Whittier Institute for Diabetes and Endocrinology. Dept. of Molecular and Cellular Growth Biology. 9894 Genesee Avenue, La Jolla, CA 92037

Human recombinant basic FGF (bFGF) locally injected (1 ng to 1 µg) in the mesenchyme of tails of non-metamorphosing, perchlorate treated (0.1%) Xenopus laevis tadpole induces a local increase in the number of small neoformed capillaries. The angiogenic response begins between days 5 and 7 after injection, it reaches its maximum between day 10 and 20. bFGF does not affect the normal metamorphosis in non-perchlorated tadpoles, nor does it show any effect on T4-induced tail regression. At different times after injection, the localization of topically injected mammalian bFGF was studied by immunohistology using a specific polyclonal antibody showing only a weak cross-reaction with the corresponding endogenous Xenopus bFGF or by histoautoradiography following a local injection of ^{125}I-labeled bFGF. We show that immediatly after the injection, the immunoreactive signal is positive at the level of the intercellular spaces in the area of the injection. From day 1 to day 10 the signal becomes progressively and then exclusively (day 10 to 15) associated with the extracellular matrix. The amount of active mammalian bFGF remaining at the site of injection can be assesed after long term incubation by Western blotting and in bioassays. Under these conditions, the results suggest that the actual half-life of bFGF is very long and that its binding to the extracellular matrix plays a role in its biological availability. The study of the the fate of exogenous bFGF supports the notion that its biological availability and activity are regulated by the extracellular matrix. We propose that this local concentration of bFGF may act as an "organic" pellet to allow for the slow release of the heparin binding growth factor and to mediate its angiogenic response *in vivo*.

O 208 RAPID, COMPLETE, AND REVERSIBLE TRANSFORMATION BY v-sis PRECEDES IRREVERSIBLE TRANSFORMATION, Philip Carpenter, Susan Oatley, Cora Humberson, Mark Mercola and Dan Mercola, Department of Pathology, University of California, La Jolla, CA 92093.

NIH-3T3 cells were used to develop a model of reversible sis-dependent transformation. The cells were transfected with pSV2neo alone (control cells) or with a plasmid containing an abbreviated mouse metallothionein (MT) promoter and v-sis (NIH-neoMTsis cells) and selected for neomycin resistance. Multiple clones were characterized by Southern analysis which confirmed full-length insertion at variable copy numbers. In the presence of zinc (50 µM), NIH-neoMTsis cells - but not control cells - exhibit a >10 increase in sis transcript levels, a 2-2.5 fold increased growth rate which is first apparent in 48 hours, morphological changes and efficient anchorage-independent growth. NIH-neoMTsis cells are tumorigenic in athymic mice with a growth rate that increases with zinc stimulation. The high *in vitro* proliferation and transcription rates reverse upon removal of zinc. Further cells isolated from soft agar colonies revert to a normal phenotype but again exhibited the complete transformed phenotype in the presence of zinc. We conclude that zinc induces much increased v-sis transcription which leads to rapid, complete and reversible transformation in these immortal cells. However cells isolated from tumors 9 days following inoculation exhibit a somewhat increased basal growth rate with a reduced (*ca.* 1.5 fold) zinc-stimulation of growth and cells from 18 day old tumors grow as fast as transformed cells, show no increase with zinc and exhibit a significantly ($p \leq 0.01$) decreased mean chromosomal number. The results suggest that sis-dependent transformation can be resolved into two stages: an exclusively sis-dependent and reversible stage and an irreversible stage requiring further steps.

O 209 ALTERNATIVE SPLICING GENERATES AT LEAST TWO FORMS OF mRNA CODING FOR HUMAN HEPARIN-BINDING GROWTH FACTOR 1, Ing-Ming Chiu, Wen-Pin Wang, and Kirsten Lehtoma, Department of Internal Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio 43210

Human class 1 heparin-binding growth factor (HBGF-1), also known as acidic fibroblast growth factor, is a mitogen for a variety of mesoderm- and neuroectoderm-derived cells in vitro as well as an angiogenic factor in vivo. Several oncogenes and growth factors have been shown to be homologous to HBGF-1. Four overlapping cDNA clones coding for HBGF-1 have been isolated from a human brain stem cDNA library. Nucleotide sequence analysis revealed that alternative splicing generated at least two different forms of HBGF-1 mRNA. Since the difference occurred at the 5'-untranslated regions, these transcripts may result from the usage of alternative promoters. One of the cDNA clones contained the polyadenylation signal, AATAAA, and a poly(A) tail, representing the 3'-end of an HBGF-1 mRNA. RNase protection assays suggested this cDNA clone corresponds to a minor transcript, and the majority of the HBGF-1 mRNA terminates at 3.1 kbp downstream from the translation termination codon. The biological significance of this unusually long 3'-untranslated sequence is not known. To study the HBGF-1 gene structure, we have isolated 50 kbp of contiguous genomic DNA clones coding for the HBGF-1 protein. Both restriction enzyme mapping and nucleotide sequencing established that the distance between the first and second protein-coding exons is 13.6 kbp while that between the second and third is 5.3 kbp. By using the HBGF-1 cDNA as a probe, we showed that human fetal heart expresses high levels of HBGF-1 mRNA. Thus, HBGF-1 may be involved in mediating processes such as embryonic development and vascular growth in the heart.

O 210 COMPARATIVE LOCALIZATION OF GROWTH FACTOR RECEPTORS AND ADHESION MOLECULES IN EMBRYONIC INDUCTION: FEATHER DEVELOPMENT AS A MODEL. Cheng-Ming Chuong[1], Wei-Ping Shen[2], Jung San Huang[3]. [1]Department of Pathology, University of Southern California, Los Angeles, CA 90033; [2]Department of Pediatrics, Univ. of California, Irvine, CA 92715; [3] Department of Biochemistry, St. Louis Univ., St. Louis, MO 63104.

To explore the molecular basis during embryonic induction, we have used chicken feather development as a model because of its distinct pattern. We have previously shown that N-CAM appears to be one of the major components of induction because it is transiently expressed in the feather placode as well as lens and otic placode (Chuong and Edelman, 1985. Expression of Cell Adhesion Molecules in Embryonic Induction. I. and II. Morphogenesis of Nestling and Adult Feathers J. Cell Biol. 1009-1043). We have now followed up to study the roles of growth factors in the induction of placode. Here we observed that during feather induction PDGF receptor is highly expressed in the epithelial cells induced to undergo active cell proliferation: the placode and collar epithelia, both are epithelia facing dermal papilla, the inducing mesenchyme. Neu receptor has similar expression pattern. The results are compared with BrdU incorporation for mitotic activity. On the contrast, tenascin, an adhesion molecules containing one domain homologous to EGF, is highly expressed in the inducing dermal papilla. These results demonstrate that multiple molecules are involved to achieve induction. We are now establishing their expression sequence and will try to sort out their regulatory relationship and differential function.

O 211 K-fgf GENE EXPRESSION IS MEDIATED BY 3' ELEMENTS WHICH ARE SPECIFICALLY ACTIVE IN EMBRYONAL CARCINOMA CELLS, Anna Maria Curatola and Claudio Basilico, Dept. of Pathology, NYU Medical Center, 550 First Ave., New York, NY 10016.

The expression of the K-fgf protooncogene is restricted in a variety of tissues and organs. K-fgf RNA is detectable in cells of the early stages of development and in undifferentiated embryonal carcinoma (EC) cells, but not in their differentiated counterpart as well as in any murine and human "normal" cell line tested so far or in adult mouse tissues. By constructing chimeric plasmids in which CAT gene expression is driven by the K-fgf promoter region and transfecting them into undifferentiated EC cells and Hela cells, we have identified cis-acting DNA elements which are responsible for the cell and developmental specific expression of the gene. These elements are located in the 3' non-coding region of the K-fgf third exon and are present in a comparable location in both human and murine K-fgf genes. They appear to have the properties of developmental-specific enhancers since they are able to stimulate transcription from K-fgf promoter, that by itself is essentially inactive, as well as from heterologous promoters only in undifferentiated EC cells but not in Hela, NIH3T3 or differentiated EC cells. Further characterization of the K-fgf-enhancer elements will be discussed.

O 212 HUMAN AND MOUSE CRIPTO GENE. Dono, R. and Persico M. G. International Institute of Genetics and Biophysics (I.I.G.B.), CNR, Naples, Italy.

An emerging body of literature suggests that growth factors, or related molecules, may play a role as morphogenetic and differentiation signals during mammalian development. "EGF family" corresponding growth factors share domains of homology with the C. Elegans homeotic locus lin 12 and Drosophila loci Notch and Slit. We described the isolation of the human gene, provisionally named by us CRIPTO, coding for a protein of 188 residues (Ciccodicola et al. 1989 EMBO J. 8 : 1987-1989) whose central portion contains a sequence similar to Epidermal Growth Factor. The CRIPTO mRNA is present in undifferentiated teratocarcinoma cells of human (NT2D1) and mouse (F9) origin but not in the same cells induced to differentiate with retinoic acid. The corresponding mouse gene is expressed in the stem cells derived from three day-old embryos. It codes for a 171 amino acid-long protein, showing 93% homology, respect to the human protein, in the "EGF like" corresponding region. Lower extended homology is found in the surronding regions. Proteolytic target sites are retained in the same position in both human and mouse proteins.

O 213 GROWTH FACTORS IN SKIN DURING HAIR FOLLICLE DEVELOPMENT AND AT MATURITY, Diana L. du Cros, Pilas Pisansarakit and G. Philip M. Moore, CSIRO Division of Animal Production, Blacktown NSW 2148, Australia

The close apposition of the epidermis and mesenchyme of skin during follicle development and growth indicates that interactions amongst these cell populations and matrices mediate these processes. The nature of the interactions between cells of the mesenchyme and various epithelial components of the skin were studied using immunochemical procedures to identify growth factor activity. EGF-like molecules were detected in developing wool follicles of foetal sheep skin, particularly in the sebaceous glands and outer root sheath. This activity persisted in the mature follicle. EGF immunoreactivity was also detected in the capsule of vibrissa follicles and in fibroblasts derived from interfollicular regions of the skin. These results, together with evidence of EGF receptors in the sheep skin (Wynn *et al.* 1989 J. Endocr. 121, 81-90) are consistent with a specific autocrine or paracrine function for EGF in the follicle. FGF-like immunoreactivity was found in the epidermis of sheep skin (Pisansarakit *et al.* 1989 Arch Dermatol. Res. in press). As well as being mitogenic for mesenchymal and neuroectodermal cell-types, FGF has recently been implicated in mesoderm induction, acting during early development of the amphibian embryo (Slack *et al.* 1987 Nature 326, 197-200). This provides a different perspective on the functions of FGF, and its presence in the skin suggests a possible role in the development and maintenance of the follicle.

O 214 PLATELET DERIVED GROWTH FACTOR RECEPTOR ALPHA-SUB-UNIT HOMODIMERS CAN MEDIATE SIGNALLING FOR DIRECTED CELL MOVEMENT Ferns G.A.A., Sprugel K.H., Seifert R., Bowen-Pope D, Raines E., Ross R, Dept. of Pathology, University of Washington, WA 98195 and Zymogenetics, Seattle, WA98105.

The cellular responses to platelet derived growth factor (PDGF) are mediated by high affinity interactions with membrane bound receptors. The functional receptor is a homo-, or hetero-dimeric combination of two sub-units, termed alpha and beta respectively, which are encoded by separate gene loci. We have recently transfected and expressed both genes separately in a baby hamster kidney (BHK) cell line. We have now investigated the migration of the parental cell (570.2) and alpha-(1-10) and beta-(5) transfectants, to the three PDGF dimeric forms. The beta-sub-unit transfectant responded only to PDGF-BB, with a 10-fold peak response. While clone 1-10 (the alpha-sub-unit transfectant) responded to all three isoforms, with a similar peak response in each case. A checker-board analysis showed the migratory response was both chemotactic and chemokinetic. For the transfected BHK cells, a positive chemotactic response to PDGF correlated with changes in actin organization as demonstrated by rhodamine-phalloidin staining. In a study of smooth muscle cells, we found that isolates differ in their ability to respond chemotactically to PDGF-AA; this correlated with their mitogenic responsiveness to this PDGF isoform, and to their relative proportion of the two PDGF receptor sub-units. Hence, both receptor sub-units are capable of mediating a chemotactic and mitogenic signal. However, the degree of cellular responsiveness depends on receptor sub-unit distribution. This work was supported by grants from the NIH (HL-18465) and British Heart Foundation. GAAF is a reciprocal fellow of the BHF and American Heart Association.

O 215 SYNTHESIS, POST-TRANSLATIONAL PROCESSING AND COMPARMENTALIZATION OF bFGF, *Robert Z. Florkiewicz, Ana-Maria Gonzalez and Ann Becker, MCGB, The Whittier Inst., La Jolla, Ca 92037.* We characterize multiple forms of human bFGF and describe their novel molecular origin. "Multiple" larger molecular weight forms of bFGF are co-expressed along with the 18kD bFGF polypeptide and are encoded by a single mRNA *in vitro* and *in vivo*. Oligonucleotide-directed mutagenesis and transient expression in COS-1 cells show that: 1) the three larger bFGF proteins are co-linear, amino terminal extensions of the 18-kD protein, 2) the amino terminal extensions do not constitute a "signal sequence", 3) there is no precursor-product relationship, 4) translation initiation of the larger proteins begins at CUG (leucine) codons rather than AUG (methionine) codons, and 5) translation initiation of the 18-kD protein begins at an AUG-methionine codon. We have extended these studies and demonstrate that rat bFGF is also expressed as a set of multiple polypeptides In this case there are three HS-binding, co-expressed, co-linear polypeptides with sizes 17.8 (AUG), 20.9 (CUG) and 21.9kD (CUG). These three polypeptide are present when cDNA is expressed transiently in COS cells and in primary rat brain extracts. This suggests that CUG codons initiating translation of multiple forms of human bFGF is not an exception to the rule but may in fact be a general feature of bFGF gene expression. Indirect immunofluoresence, pulse-chase immunoprecipitation and cell surface iodination data indicate that at least one molecular form of bFGF is directly targeted into the nuclear environment, as well as to the cell surface, and suggests that bFGF reaches the cell surface in a dynamic (active) manner, not (only) as a result of cell death. It seems possible, then, that some of the proposed autocrine activities of bFGF may not require prior interaction with cell surface receptors but may occur as a direct result of bFGF nuclear uptake of bFGF polypeptide synthesized *de novo*. Finally, it is not known how the physiological activities previously attributed to one (18kD) form of bFGF is divided between the multiple forms we have described.

O 216 **PROLIFERATION VERSUS DIFFERENTIATION IN POLYOMA LARGE T ANTIGEN TRANSGENIC LIENS CELLS.** Anne E. Griep. Department of Oncology, University of Wisconsin-Madison, Madison, Wisconsin 53706.
To test the ability of an immortalizing oncogene to promote transformation of the ocular lens, we created transgenic mice carrying a gene fusion of the murine alphaA-crystallin promoter to the coding sequences for polyoma large T antigen (PyLT, Griep et al., 1989, Genes Dev. 3:1075-1085). Expression of PyLT specifically in the lens led to abnormal lens development, however, no evidence of hyperplasia or tumor formation was found in these PyLT lenses.
To address the nonproliferative nature of the PyLT lens cells, cells from neonatal normal and transgenic mice were established in culture. Unlike normal lens cells, transgenic lens cells proliferated indefinitely in culture. Transgenic lens cells continually expressed PyLT and alpha crystallins by immunofluorscent staining. Beta and gamma crystallins, usually found only in differentiated lens cells, was also detected in transgenic lens cells. Treatment of transgenic lens cells with high levels of basic fibroblast growth factor (bFGF), a factor known to influence lens cell differentiation in vivo, resulted in morphological alterations suggestive of differentiation and to growth inhibition. Thus, our results suggest that proliferation of PyLT lens cells is suppressed in vivo by specific factors in the ocular environment, most notably bFGF.

O 217 GENES INDUCED BY DOUBLE-STRANDED RNA INCLUDING A CA^{2+}-TRANSPORTING ATPASE ARE DIFFERENTIALLY REGULATED BY RECOMBINANT PDGF-ISOFORMS AA, AB AND BB, AND OTHER CYTOKINES LIKE TNF-α AND IFN-β, G. Gross, C. Koch, U. Buwitt, A. Habenichts, H. Gründel, J. Hoppe*; Gesellschaft für Biotechnologische Forschung (GBF), Mascheroder Weg 1, 3300 Braunschweig; *) Universität Würzburg, Würzburg, F.R.G.
PDGF and the double-stranded RNA poly r(I):r(C) induce expression of genes belonging to the competence-class of the cell-cycle using different signal-pathways. To study this phenomenon 10 poly r(I):r(C)-induced cDNA clones were isolated by differential screening of a induced human fibroblast(FS4) cDNA bank, four of these have not been described before . Among these are two potential Zn-finger proteins and the plasma membrane Ca^{2+}-transporting ATPase which reconstitutes one of the essential control elements of intracellular Ca^{2+}-levels in most, if not all, animal cells.
The various poly r(I):r(C)-induced genes respond in a differential way to the various recombinant (*E. coli*) PDGF-isoforms: the PDGF-AA and -AB isomers in comparison to PDGF-BB efficiently induce several poly r(I):r(C)-induced genes which is in contrast to the PDGF-receptor situation since about 7x more PDGF-receptor β-subunits than α-subunits are present on the cell surface suggesting alternative pathways for gene induction by the various PDGF-isomers. Furthermore, it should be pointed out that we were able to induce IFN-β by the PDGF-AB isomer, repeating an effect described by Stiles and collaborators, 1985.

O 218 THE CHARACTERIZATION OF RECOMBINANT AND NATURAL TRANSFORMING GROWTH FACTOR ALPHA SPECIES BY GEL ELECTROPHORESIS AND IMMUNOBLOTTING, Richard N. Harkins and Sharon L. Metcalf, Department of Protein Chemistry, Triton Biosciences Inc., Alameda, CA 94501. A discontinuous Tricine based sodium dodecyl sulfate-polyacrylamide gel electrophoresis system (SDS-PAGE) has been adapted (Schagger and Von Jagow, Anal. Biochem (1987), 166, 368-379) for the analysis of various Transforming Growth Factor Alpha (TGF-α) species from several sources. Samples are alkylated with iodoacetamide prior to electrophoresis to minimize the formation of disulfide oligomers. With this technique we have been able to separate low molecular weight (less than 10 kilodaltons) TGF-α species and partially processed forms of TGF with excellent resolution. We have also developed an immunoprecipitation and immunoblotting method which utilizes antibodies raised against a recombinant human TGF-α. Coupled with the Tricine SDS-PAGE system TGF species can be resolved and detected at the nanogram level. Several applications of these techniques have been performed in the analysis TGF species from recombinant (E. coli, yeast) and natural (cell media, human urine) sources.

O 219 A GROWTH FACTOR-TOXIN FUSION PROTEIN PROLONGS SURVIVAL OF TUMOR-BEARING NUDE MICE. D. C. Heimbrook, J. Ahern, N. Balishin, G. Edwards, D. Patrick, S. Stirdivant, I. Pastan and A. Oliff, Department of Cancer Research, Merck Sharp and Dohme Research Labs, West Point, PA 19486.

Transforming growth factor-alpha (TGFα) - Pseudomonas exotoxin-40 (PE40) is a chimeric protein consisting of an N-terminal TGFα domain fused to a C-terminal 40kDa fragment of Pseudomonas exotoxin A. TGFα-PE40 exhibits the receptor binding activity of TGFα and the cell killing activity of PE40. These properties make TGFα-PE40 an effective cytotoxic agent in vitro for cells that possess epidermal growth factor receptors (EGFr), while cells lacking the receptor are resistant. In the current study, we demonstrate the in vivo efficacy of this fusion protein. TGFα-PE40 significantly ($p < 0.001$) prolongs the survival of nude mice bearing tumors derived from A431 or HT-29 cell lines. These studies suggest that a therapeutic window exists in vivo for the use of some growth factor-toxin fusion proteins as anticancer agents.

O 220 EGF RECEPTORS ARE INDUCED IN EPTIHELIAL AND STROMAL CELLS AS A RESPONSE TO CORNEAL INJURY, Fred J. Hendler, Brian Hawkins, Kelly O'Neill, Richard Eiferman, Departments of Medicine, Biochemistry, Surgery, and Opthalmology, University of Louisville, and Louisville VA Medical Center, Louisville, KY 40292.
EGF receptor is overexpressed following injury in surgical wounds and disruption of cultured epithelial cells. The increased expression occurs at the disturbed epithelial surface or the monolayer. To determine if the same process occurred after corneal abrasion, we studied the effect of scraping corneal epithelium in cats. One half of the corneal epithelium was removed by scraping while the remainder of that cornea and the other served as control tissue. 24 hrs following injury cats were sacrificed and the corneas were frozen in liquid N_2. Cryosections were prepared which included tissues from both untreated and injured regions of the corneal as well as the control cornea. The sections were reacted with [^{125}I]-EGF-R1, a murine monoclonal antibody which specifically binds the EGF receptor using a competitive binding assay. Specific binding to the receptor which represented a significantly increased expression was detected at the epithelial edge of the wound. Focal binding was detected at the denuded wound surface and in the anterior stromal cells near the denuded wound surface. No significant binding was detected in the endothelial cells. These observations suggest that the injury is causing the stromal cells to express EGF receptors and that these cells have a significant role in corneal wound healing. (Supported in part by VA research grants.)

O 221 THE C-SIS GENE AND HUMAN EXTRA-EMBRYONIC DEVELOPMENT: FUNCTIONAL RESTRICTIONS IN LOCAL STIMULATORY LOOPS BY THE RECIPROCAL DISTRIBUTION OF ACTIVE PDGF-B LIGAND AND RECEPTOR GENES.

Lars Holmgren, Susan Pfeifer-Ohlsson, Lena Welch, Carl-Henrik Heldin and Rolf Ohlsson. Centre for Biotechnology, Karolinska Institute, Novum, S-141 52 Huddinge, Sweden.

The earliest recognizable cell type of mammalian embryogenesis, the trophoblast, emerges already in preimplantation blastocysts. Following implantation in humans, the trophoblasts attain an explosive proliferative phenotype that will produce the basic structure of the future placenta. By examining the putative existence of short-range growth stimulatory loops, we sought to define a general principle for how this dramatic process might be regulated in the absence of embryonic blood circulatory systems (before 4 weeks postconception). To this end, we have analyzed the spatiotemporal pattern of the transcriptionally human placental PDGF-B ligand and receptor genes. By performing in situ hybridization we have shown that the highest levels of c-sis mRNA were confined to the most proliferative structure of the early human placenta, the trophoblastic shell. In contrast, the PDGF-B receptor mRNA was most abundant in low-proliferative cytotrophoblasts and mesenchymal stromal cells, including endothelial cells, of an adjacent section. Taken together, the data suggest that a low proportion of cytotrophopblasts will, at any developmental stage, coexpress both the growth factor ligand and corresponding high-affinity receptor genes. The specific induction of PDGF-B receptor expression in human trophoblasts, triggered by cell-substratum interaction in vitro, demonstrates one potential mechanism that may act transiently to increase the proliferative potential. Based upon these and other observations, we have put forward the hypothesis of reciprocity, that states that placental growth factor ligand and receptor genes are independently controlled to restrict the formation of autocrine/short range paracrine growth stimulatory loops.

O 222 REGULATION OF HSP90 BY SERUM AND INSULIN, V. Jérôme, J. Léger, E.E. Baulieu and M.G. Catelli, INSERM U33, Lab. Hormones, 94275 Bicêtre Cedex, FRANCE.

Hsp90 is a heat-shock protein constitutively expressed in most cells. Besides regulation by thermal stress, the expression of hsp90 is also positively regulated during the early embryo development, by estrogens in mouse uterus, and by mitogen agents. The effect of serum and insulin on protein and hsp90-mRNA levels has been studied in the chicken hepatoma cell line DU249. The culture of cells in serum free medium followed by a serum restimulation shows a decrease of the hsp90-mRNA level, followed by a transient increase after 6h of serum restimulation. Insulin alone also increases the expression of hsp90 gene. This effect is dose dependent. Futhermore, the insulin-induced expression of the hsp90 gene is transient, being maximum form 6h to 9h after insulin addition. The insulin-induced increase of hsp90-mRNA is supressed by cycloheximide (30 µg/ml), but not by the inhibitor of DNA synthesis Ara C. Other growth factors (IGF1 and EGF) demonstrate positive effect on hsp90-mRNA level, and IGF1 and EGF effects are additive with that of insulin. We are currently investigating the mechanisms implicated in the regulation of hsp90 expression by growth factors.

O 223 DEVELOPMENTAL REGULATION OF THE k-FGF ONCOGENE AND TRANSFORMING GROWTH FACTOR BETA (TGF-β) BY EMBRYONAL CARCINOMA CELLS AND THEIR DIFFERENTIATED CELLS. D. Kelly, J. Tiesman, W. Campbell and A. Rizzino. Eppley Institute for Research in Cancer, Univ. Nebr. Med. Ctr., Omaha, NE 68105.

Embryonal carcinoma (EC) cells provide a useful model system for studying growth factor production and regulation during mammalian embryogenesis. We have demonstrated previously that the mouse EC cell lines F9 and PC-13 and the human EC cell line NT2/D1 produce a growth factor belonging to the fibroblast growth factor (FGF) family and that production of this FGF-related growth factor is suppressed when the EC cells differentiate (Rizzino et al., Dev. Biol. 129:61-71, 1988). Using northern blot analysis and a combination of reverse transcription plus polymerase chain reaction (RT-PCR), we have shown that F9, PC-13, and NT2/D1 EC cells express transcripts for the k-FGF oncogene, a member of the FGF growth factor family. In addition, we have shown that the transcript levels of k-FGF are drastically reduced when EC cells differentiate. In contrast to the expression of k-FGF, we have determined that differentiation of EC cells up-regulates the expression of several members of the TGF-β growth factor family. In the mouse EC cell line, F9, differentiation leads to a 2-fold increase in the release of TGF-β1. In the human EC cell line, NT2/D1, differentiation leads to the expression of TGF-β2 and, in F9 and PC-13 EC cells, differentiation leads to the expression of Vgr-1, a distant member of the TGF-β growth factor family. This work was supported by grants from NIH (19837, 21568) and the Council of Tobacco Research (2520).

O 224 ADVANCED GLYCOSYLATION ENDPRODUCT (AGE) BINDING TO ITS SPECIFIC RECEPTOR STIMULATES INCREASE IN EGF AND EGF RECEPTOR mRNAs: ROLE IN TISSUE REMODELING. Martina Kirstein, Sander van Deventer and Helen Vlassara, Department of Medical Biochemistry, The Rockefeller University, New York, NY 10021.

Normal tissue homeostasis requires finely controlled interactions between cells, including macrophages and mesenchymal cells, for remodeling in response to protein senescence or local injury. In vivo, proteins undergo nonenzymatic reactions with glucose which over time form irreversible advanced glycosylation end products (AGEs), implicated in senescence. Recently it has been shown that monocytes/macrophages possess receptors specific for AGE-modified protein uptake, which in addition can stimulate synthesis and release of the growth-promoting monokines TNF, Il-1 and IGF-1, thus contributing to tissue repair. We hypothesized that fibroblasts might be themselves capable of processing AGE-proteins via specific receptors. Using a human fibroblast cell line (FS4), binding of a model ligand, radioiodinated bovine serum albumin glycosylated in vitro by 50 mM glucose-6-PO_4 (AGE-BSA), was measured at 4°C. Scatchard analysis indicated 2 x 10^5 receptors/cell, and a Ka of 3.15 x 10^7 M^{-1}. Fibroblasts both synthesize and respond to EGF, a potent growth factor. By polymerase chain reactions (PCR) and Northern blotting techniques we observed that both EGF and EGF receptor mRNA expression was significantly enhanced in quiescent fibroblasts after exposure to AGE-BSA (150 μg/ml, 1 hr, 37°C). EGF mRNA was approximately 3-fold, and EGF receptor mRNA was 5-fold greater than unmodified BSA control. These data indicate that ubiquitous tissue AGEs can interact with specific binding sites on human fibroblasts, inducing both EGF and EGF receptor mRNAs. AGE-induced secretion of EGF protein may contribute to normal tissue remodeling, and autocrine upregulation of the EGF receptor may lead to excessive fibroblast proliferation common in diabetes.

O 225 REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR) DETECTION OF BASIC FIBROBLAST GROWTH FACTOR (bFGF), ACIDIC FGF (aFGF), AND KERATINOCYTE GROWTH FACTOR (KGF) EXPRESSION IN THE RAT OVARY DURING FOLLICULAR DEVELOPMENT. Robert D. Koos, Richard H. Seidel, and C. Erik Olson, Department of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Development of the ovarian follicle involves the rapid proliferation of several cell types, particularly granulosa cells, epithelioid cells which enclose the oocyte, and endothelial cells, which constitute the rich thecal capillary plexus that surrounds the granulosa cell layer. The factors regulating this proliferation and differentiation are unknown at this time. It seems likely that heparin-binding growth factors, such as aFGF and bFGF, which are thought to play important roles in angiogenesis, and KGF, which is specific for epithelial cells, could be involved in these processes. The purpose of this study, therefore, was to determine if these factors are expressed in the rat ovary during follicular development. RNA was extracted from whole ovaries or isolated granulosa cells from gonadotropin-primed immature rats. One μg of total RNA, obtained using the guanidine isothiocyanate extraction/lithium chloride precipitation method, was reverse transcribed using M-MLV reverse transcriptase. A sample of the RT mixture was then amplified by PCR, using primers specific for rat aFGF, rat bFGF, or human KGF. Transcripts for all three factors were detected in whole ovarian extracts. By contrast, neither bFGF nor KGF mRNA was found in granulosa cells. Amplification of aFGF message was obtained from two of four granulosa cell preparations, but even when it was present the yield of aFGF PCR product was low. These results indicate that all three growth factors are expressed by cells in the interstitial/thecal compartments of the ovary, but, with the possible exception of aFGF, not by granulosa cells. We conclude that both bFGF and aFGF could be involved in the development of the thecal vasculature. KGF, on the other hand, which is synthesized with a signal sequence and probably secreted, could be produced in stromal tissues of the ovary and act on the adjacent epithelioid granulosa cells. Supported by CA45055.

O 226 RELATIONSHIP BETWEEN c-*sis* EXPRESSION AND SENSITIVITY TO SURAMIN IN HUMAN GLIOMA CELL LINES. La Rocca, R.V., Danesi, R. Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892.

Variations in the pattern of proto-oncogene expression within a specific tumor type may denote an underlying difference in the biology and clinical behaviour of those cancers. Suramin sodium, which contains six reactive sulfonic acid carbonyl groups, may inhibit the binding of several growth factors to their cell surface receptors, including PDGF, bFGF and TGF-ß. In addition this compound has been demonstrated to have antitumour activity in humans. In the present study we have studied the sensitivity of six glioma cell lines (SNB 19, SNB 78, U-87 MG, U-343 MG, U-373 MG, U-343 MGa Cl 2:6) to the cytotoxic activity of suramin. We have previously shown that detectable levels of c-*sis* expression were present in SNB-19 and U-343 MGa Cl 2:6, U-87 MG, U-373 MG, and U-706T but not in SNB 78 and U-343 MG. Morphologic analysis of these cell lines revealed three different growth patterns: bipolar, epithelial, and pleomorphic-glial; detectable levels of c-*sis* mRNA were observed in glioma cell lines with either an epithelial (U-87 MG, U-706T, U-343 MGa Cl 2:6) or pleomorphic-glial morphology (U-373 MG, SNB 19). The sensitivity to the cytotoxic activity of suramin were evaluated by colony formation assay. Cells exposed to increasing doses of suramin (75-600 μg/mL) for 6 days were subsequently plated in drug-free medium and the colonies counted after 14 days. The results revealed that the most sensitive cell lines were SNB 19 and U-343 MGa Cl 2:6 ($LD_{50} \leq 75$ μg/mL); U-87 MG and U-373 MG showed intermediate sensitivity (LD_{50} : 150 μg/mL) while SNB 78 and U-343 MG had an LD_{50} of >300 μg/mL of suramin. From the data of the present study we conclude that the glioma cell lines show heterogeneity in c-*sis* expression and the presence of detectable levels of c-*sis* mRNA may be a marker for those cells which will be sensitive to the cytotoxic activity of suramin at doses clinically achievable in humans. Evaluation of the role of other growth factors such as FGF and TGF-ß in the biology of these cell lines is underway.

O 227 CLONING OF NOVEL ONCOGENES BY TRANSFECTION OF FIBROBLASTS WITH GENOMIC DNA AND A TAGGED PROMOTER/ENHANCER ELEMENT.

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A defined media assay[1] has been used in our laboratory to isolate a novel oncogene, FGF-5[2]. This oncogene was shown to have been fortuitously activated by a cotransfected strong promoter/enhancer element. In a search for additional transforming genes we have cotransfected human placental DNA and a molecularly tagged promoter/enhancer sequence into NIH3T3 cells. Several transformed cell lines were isolated and one of these was demonstrated to contain the Human hst gene. We are currently proceding with molecular cloning of the transforming genes from the other cell lines.

1. Human oncogenes detected by a defined medium culture assay. Zhan,Xi et.al. Oncogene (1987) 1, 369-376.

2. The Human FGF-5 Oncogene Encodes a Novel Protein Related to Fibroblast Growth Factors. Zhan, Xi et.al. Molecular and Cellular Biology (1988) **8** (8) 3487-3495.

O 228 ROLE OF bFGF IN PROLIFERATION OF SMOOTH MUSCLE CELLS IN INJURED ARTERIES IN VIVO,

Volkhard Lindner, Richard A. Majack and Michael A. Reidy, Dept. of Pathology, University of Washington, Seattle, WA 98195 and Dept. of Vascular Biology, Synergen, Inc., Boulder, CO 80301.

Removal of endothelium with a balloon catheter in the rat carotid artery leads to medial smooth muscle cell (SMC) proliferation and migration with subsequent intimal thickening. Recently it was shown that the initial wave of SMC replication in this model occurs in the absence of platelets and therefore release of platelet mitogens cannot alone explain this proliferative response. We have investigated whether bFGF, which is present in the arterial wall and thought to be released by injury, can stimulate SMC proliferation in vivo. The administration of bFGF (120μg) following balloon catheter injury caused a significant increase in SMC proliferation (17.1% vs. 4.5%) as measured by 3H-thymidine administration (2 doses 24 and 32 hours after injury) and autoradiography. Using an alternative denuding technique, which causes little or no trauma to the medial SMCs, bFGF administration (120μg) again resulted in a highly significant increase in SMC proliferation (16.4% vs. 0.4%). When bFGF was injected intravenously (12 μg daily for 2 weeks) into balloon catheterized rats a two fold increase in the intimal lesion of the carotid artery was observed. This increased thickening was the result of increased medial and intimal SMC proliferation. bFGF also stimulated endothelial replication and endothelial outgrowth in these arteries. These data demonstrate that bFGF if released by injury could profoundly influence the proliferation of SMCs in the arterial wall. (Supported by Deutsche Forschungsgemeinschaft (Li 429/1-1) and NIH grants HL-03174 and HL-41103).

O 229 Tissue-specific expression of human kallikrein and growth factor genes

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Human homologues of the murine EGF and NGF-binding kallikreins have not been described to date. We have examined the tissue-specific expression of two human kallikreins (PSA and GK1) for which biological functions have yet to be elucidated. cDNA prepared from a variety of human tissues was subjected to PCR amplification with primers specific for PSA, GK1, EGF and NGF sequences. Our results indicate distinct regulatory mechanisms for PSA and GK1. In addition, there are splicing variants of GK1 exhibiting tissue-specific distribution. The sequence of one of these variants predicts a frameshift within the GK1 coding region. Our results allow us to identify human kallikrein species which exhibit a pattern of tissue-specific distribution similar to that of EGF or NGF. A cDNA clone for each human kallikrein species has been isolated and configured for high-level expression in E.coli.

O 230 **Development of Mammary Adenocarcinoma in Transgenic Mice Overexpressing a MMTV-TGFα cDNA Construct.** Matsui, Y.+, Halter, S.A.^, Holt, J.T.+^, Hogan, B. L. M.+, and Coffey, R. J., Jr.+* Department of Cell Biology+, Medicine*, and Pathology^, Vanderbilt University School of Medicine, Nashville, TN 37232 In order to study the role of TGFα in mammary gland development and its possible contribution to mammary gland neoplasia, we have generated transgenic mice in which human TGFα expression is under the control of the MMTV promotor/enhancer region. The construct consists of 1.5 kb of the MMTV LTR and a 0.9 kb human TGFα cDNA inserted into the 3rd exon of the rabbit β-globin gene. In preliminary experiments, this DNA was stably transfected into the TGFα non-producing human breast concer cell line, Hs578T. Dexamethansone and estradiol induced expression of the predicted 1.4 kb mRNA which hybridized to both the TFGα and rabbit β-globin probes. This construct was then introduced into one cell eggs of (C57BL x DBA) F1 females fertilized by (C57BL x DBA) F1 males. One transgenic line, designated 29, has been studied in detail. Males (4 months of age) show expression of the 1.4 kb TGFα transcript in salivary gland, testis, seminal vesicle, lung, muscle and bladder. A founder female developed adenocarcinoma in several mammary glands at age 10 months, after a number of pregnancies. The intensity of expression of the 1.4 kb TGFα transcript correlated with the degree of atypia in the gland. No expression was detected in other tissues. These preliminary results suggest that upregulation of TGFα in mammary tissue may contribute to mammary gland neoplasia.

O 231 **FGF MODULATES THE PDGF-DRIVEN PATHWAY OF OLIGODENDROCYTE DEVELOPMENT.** R.D.McKinnon[1], T. Matsui[2], S.A. Aaronson[2] and M. Dubois-Dalcq[1]. LVMP/NINDS[1], LCMB/NCI[2], NIH, Bethesda MD 20892. O-2A glial progenitor cells from the rat optic nerve are bipotential, differentiating into either myelin-forming oligodendrocytes or type-2 astrocytes under different culture conditions. Platelet-derived growth factor (PDGF) drives progenitor cell division and regulates the timing of their differentiation (Raff, Science 243:1450, 1989). In defined media containing low levels (50 ng/ml) of insulin, purified rat brain O-2A progenitor cells differentiated into oligodendrocytes and accumulated myelin basic protein mRNA transcripts within 48 hrs. PDGF (AB heterodimer) was mitogenic and delayed MBP transcript accumulation, and Western analysis demonstrated that O-2A cells express PDGF-α but not PDGF-ß receptors. Basic fibroblast growth factor (bFGF) was a more potent mitogen for O-2A progenitors and completely blocked differentiation, either alone or in the presence of PDGF. FGF also increased both the levels of the PDGF-α receptor and the sensitivity of O-2A progenitor cells to PDGF. Our results demonstrate that FGF can control the response of cultured O-2A glial progenitor cells to PDGF, and suggest that a subtle interplay between these polypeptide growth factors may set the timing of myelination in specific tracts in vivo.

O 232 REGULATION OF TISSUE-TYPE PLASMINOGEN ACTIVATOR GENE TRANSCRIPTION BY PHORBOL ESTER, CYCLIC AMP AND EPIDERMAL GROWTH FACTOR. Robert Medcalf, Marlies Rüegg and ¶Wolf-Dieter Schleuning, Central Hematology Laboratory, CHUV, 1011 Lausanne Switerland, and ¶Schering, A.G, Biochemistry Institute, 650311, Berlin, D-1000, Germany. Tissue-type plasmingen activator (t-PA) is a principal mediator of fibrinolysis. An increase in t-PA activity has also been correlated with embryonic development, cell migration and tumor metastasis. We have investigated the modulation of t-PA biosynthesis by the tumor promoter, phorbol 12-myristate 13-acetate (PMA), cAMP agents and epidermal growth factor (EGF) in HeLa cells. Based on nuclear "run-on" transcription assays, t-PA is modulated by PMA and EGF on the level of transcription. The cAMP agonist, forskolin, and 8-br-cAMP do not induce t-PA gene transcription alone, but do so in cooperation with PMA. Transient expression assays with deletion mutants of the t-PA gene promoter fused to a reporter gene in HeLa cells indicate that constitutive and part of the PMA and cAMP mediated increase in transcription requires sequences downstream of position -145. An increase in CAT activity by EGF could not be demonstrated. DNase-1 protection analysis reveals two protein binding sites: one located between position -102 to -115 which differs from the consensus sequence of the cAMP-responsive element (CRE) by a single base, and another located in the first exon (position +60 and +74), displaying homology to the consensus sequence of the AP-2 binding site. Using site directed mutagenesis, we demonstrate that these two elements are responsible for constitutive and part of the PMA and cAMP mediated increase in t-PA biosynthesis.

O 233 PDGF EXPRESSION DURING EMBRYOGENESIS AND ITS STUDY BY DOMINANT NEGATIVE MUTATIONS. Mark Mercola[1], Prescott Deininger[2], Julie Porter[1], and Charles Stiles[1], [1]Department of Microbiology and Molecular Genetics and the Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, and [2]Department of Biochemistry and Molecular Biology, LSU Medical Center, New Orleans, LA 70112.

We have isolated cDNA clones encoding *Xenopus* PDGF A and the PDGF α receptor. These have been used as hybridization probes for mRNA expression in early *Xenopus* embryos. Furthermore, to explore the function of PDGF during embryogenesis, we have created dominant negative mutations of PDGF A which efficientently block growth factor action.

Expression studies show that maternally-derived mRNAs for PDGF A and its receptor are present in eggs and early embryos. Embryonic transcripts for both these genes first appear at gastrulation, somewhat after the onset of zygotic transcription. Culture of dissected tissues indicate that PDGF A mRNA is expressed primarily in presumptive ectodermal derivatives whereas the α receptor is expressed in presumptive mesoderm derivatives. These results suggest that the determinants dictating differential spatial expression are localized in the early embryo and that PDGF may be involved in mesoderm-ectoderm interactions.

To create mutant embryos incapable of PDGF expression, we have designed dominant negative mutations of PDGF. Two mutant genes, that encode biologically inactive proteins, inhibit the activity of a co-transfected wild-type gene in COS cells. Inhibition occurs because the wild-type PDGF (which normally dimerizes) dimerizes with the mutant, and, depending on the mutant is either degraded or incompletely processed. A seven-fold excess of mutant to wild-type gene is capable of 90% inhibition. We are currently injecting synthetic mRNA and genes encoding the mutants into 1-cell *Xenopus* and mouse embryos.

O 234 TRANSGENIC MICE OVEREXPRESSING TGFα EXHIBIT IMPEDED DUCTAL MORPHOGENESIS IN THE MAMMARY GLAND AND DUCTAL METAPLASIA IN THE PANCREAS. G. Merlino[1], C. Jhappan[1], C. Stahle[1], R. Harkins[2], D. Liscia[3], and G. Smith[4]. [1]LMB, NCI, NIH, Bethesda, MD; [2]Triton Biosciences, Alameda, CA; [3]Ospedale San Giovanni Antica Sede, Torino, ITALY; [4]LTIB, NCI, NIH, Bethesda, MD.

Transforming growth factor alpha (TGFα) is a potent mitogen that has been implicated in cellular transformation, and may play an important role in cellular differentiation and development as well. Transgenic mice were made using the human TGFα cDNA driven by the mouse MT1 metallothionein gene promoter. Four lines of transgenic mice were made: One of these (MT42) exhibited strong zinc-inducible RNA expression in liver, pancreas, kidney, and stomach by ribonuclease protection analysis. Lower expression was detected in colon, brain, lung, bone, testes, and breast. TGFα protein was detected in the blood (~2.5 ng/ml) and urine (~3.7 ng/ml) of transgenic mice by a radioimmunoassay. The ability of ductal epithelial cells to invade the mammary fat pad is greatly reduced in pubescent MT42 transgenic mice. Furthermore, the majority of transgenic mammary stromal lipoblasts at that age fail to fully differentiate into adiopocytes. It is likely that the resulting perturbation of the interaction between epithelial and mesenchymal cells results in altered morphogenetic development of the branching ducts. A sclerous reaction in the pancreas of adult mice was also observed, with extensive development of fibrotic interstitial tissue, and a concomitant degeneration in acinar cells. In addition, the pancreas typically exhibited a marked ductal metaplasia. Experiments are underway to determine mechanisms by which TGFα induces these effects.

O 235 EGF UPREGULATES ITS OWN mRNA SYNTHESIS IN FETAL JEJUNAL EXPLANT CULTURES. Päivi J. Miettinen, Raimo Voutilainen. Departments of Pediatrics and Pathology, University of Helsinki, Haartmanink. 3, SF-00290 Helsinki, Finland.

Immunoreactive epidermal growth factor (EGF) has been demonstrated in human fetal kidney, intestine and placenta. To clarify the function of EGF on the development of fetal intestine we studied the expression and regulation of EGF mRNA in human fetal jejunum.

Tissues were obtained from legal prostaglandin induced abortions at 13, 18 and 19 gestation weeks. Jejunum was dissected from the bloodvessels and surrounding fat, rinsed in ice cold PBS and cut into 3mmx5mm explants. The explants were grown in Leibowitz-medium supplemented with EGF 100 ng/ml for 7 days. The medium was renewed every other day. For Northern blots whole jejunum was freezed in liquid nitrogen and stored in -70°C until used for RNA preparation . Total RNA from the cultures and jejunum was isolated with guanidine isothiocyanate method by ultracentrifugation through a cesium chloride cushion. Northern blots and dot blots were hybridized with ^{32P}-dUTP labeled 0.55 kB EGF probe (from G.I. Bell) subcloned into pGEM7(z)-ribovector. After autoradiography the dot blots were scanned.

Northern blot probed with EGF anti-sense strand showed a 4.8kB band in intact jejunum. In the EGF stimulated cultures EGF-mRNA signal was increased 3-9 folds as compared to the control cultures.

To conclude, EGF is expressed in fetal gut during second trimester. Our preliminary results show that EGF upregulates its own mRNA levels in jejunum. This suggests an auto- or paracrine function for EGF in the intestinal epithelium.

O 236 TWO INDUCERS OF DIFFERENTIATION CAUSE A DOWN-REGULATION OF TGF-α AND HST-1/KFGF AND A LOSS OF MALIGNANT POTENTIAL IN A HUMAN TERATOCARCINOMA CELL LINE. Wilson H. Miller, Jr., J. Kurie, D. Moy, H. Masui, and E. Dmitrovsky. Dept. of Medicine, Memorial Sloan-Kettering Cancer Center, NY, NY 10021. The human teratocarcinoma cell NTera-2 cl. D1 (NT2/D1) is a cloned multipotential cell line that differentiates into a neuronal phenotype after retinoic acid (RA) treatment and a different morphologic phenotype after treatment with hexamethylene bisacetamide (HMBA). We find that differentiation of NT2/D1 cells by either agent leads to decreased clonability by limiting dilution or soft agar assays and diminished tumorigenicity after injection into nude mice. We also find that mRNA expression for both the human homeotic gene, Hox 2.1, and the nuclear RA receptor, RAR-ß, is induced by RA but not by HMBA, further indicating that each agent induces a distinct pathway. However, we find both RA and HMBA cause a loss of TGF-α and hst-1/kFGF mRNA expression after 1-2 days of treatment. The correlation between the loss of growth factor expression and proliferative potential is supported by results showing that the addition of exogenous growth factor protein will augment NT2/D1 cloning efficiency. Furthermore, in an embryonal cancer cell line that is resistant to RA-induced differentiation, RAR-ß is up-regulated while growth factor expression is not decreased. These data suggest that the growth factors TGF-α and hst-1/kFGF play an important role in the regulation or maintenance of the malignant growth and differentiation of NT2/D1 cells. To further explore this role, we are stably introducing into NT2/D1 cells SV40-driven cDNAs for human TGF-α and hst-1/kFGF.

O 237 RENAL CYST FLUID OF CPK MICE CONTAINS URINARY LEVELS OF EPIDERMAL GROWTH FACTOR (EGF). David W. Moskowitz, Sheri L. Bonar, and Vincent H. Gattone II, Depts. of Medicine and Physiology, St. Louis University School of Medicine, St. Louis, MO 63104, and Dept. of Anatomy and Cell Biology, University of Kansas Medical Center.

Mice with autosomal recessive congenital polycystic kidney disease (cpk) undergo rapid enlargement of renal cysts after birth and die of renal failure at age 3 to 4 weeks. Renal cyst fluid was found to contain elevated levels of receptor-reactive and immunoreactive (ir) EGF. EGF concentrations of cyst fluid were no higher by radioreceptor assay than by RIA, suggesting that EGF, rather than TGF-alpha, accounted for all of the receptor-reactive material. The ratio of ng ir-EGF/mg creatinine was similar in cyst fluid and urine of 2- and 3-week old cystic mice, although smaller than urinary EGF/creatinine in normal littermates. EGF/creatinine of cyst fluid was about 50-fold higher than that of plasma. Immunoprecipitation and Western blot analysis of cpk cyst fluid revealed multiple EGF-ir species of approximately 130, 110, 24, 16, and 6 kD, similar to the pattern of ir-EGF seen in urine of normal and cystic mice. We conclude that cpk renal cyst fluid contains species and quantities of ir-EGF similar to those of urine, suggesting that cyst fluid EGF, like urinary EGF, is derived mainly from endogenous renal synthesis. The accumulation of circulating EGF within cyst fluid, e.g. by glomerular filtration or receptor-mediated transcytosis, appears to be quantitatively less important than endogenous production. The role of elevated cyst fluid EGF concentrations in the pathogenesis of this disease is still unclear.

O 238 TRANSFORMING GROWTH FACTORS ARE POTENTIAL REGULATORS OF PLACENTAL DEVELOPMENT, Linda Munson, J. Erby Wilkinson, Valarie F. Boltz, Department of Pathology, National Zoological Park, Smithsonian Institution, Washington, DC 20008 (LM, VFB) and Department of Pathobiology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901 (JEW)

Maternal endometrial epithelium and fetal chorion undergo extensive parallel growth during pregnancy in species with epitheliochorial placentation. The specific factors regulating this growth are unknown. We have investigated the possibility that TGFs of placental origin are major regulators of placental development. TGF immunoreactivity could be demonstrated in mid-gestation bovine placentomes. TGF-α immunoreactivity was present in trophoblastic, endometrial epithelial, and endometrial stromal cells, but not fetal chorioallantoic stroma. TGF-β immunoreactivity was localized in trophoblastic, endometrial epithelial, and endometrial stromal cells. TGF-β immunoreactivity was stronger in endometrial epithelial cells than other cell types. Mitogenic effects of media conditioned by trophoblastic or endometrial epithelial cells were assessed on trophoblastic cell cultures. Both endometrial epithelial and trophoblastic cell-conditioned medium enhanced trophoblastic cell proliferation above levels in serum free medium. Trophoblastic cell conditioned medium had TGF-α and TGF-β activity in NRK cell soft agar assays. These findings suggest that TGFs may regulate placental development by autocrine and paracrine mechanisms.

O 239 EPIDERMAL GROWTH FACTOR (EGF) ADVANCES THE CLOCK REGULATING PROGRESSION OF FETAL LUNG DEVELOPMENT, Heber C. Nielsen and Jon Klein. Department of Pediatrics, Tufts School of Medicine and New England Medical Center, Boston, MA 02111.

The development of surfactant synthesis in late gestation fetal lung is regulated by fibroblast-epithelial cell communications. It is characterized by appearance of fibroblast pneumonocyte factor (FPF), a marked increase in surfactant synthesis, and then a final decrease in FPF production and surfactant synthesis. These events occur earlier in the female fetus. Our data indicate that EGF affects the timing of these events in vitro. EGF causes immature lung fibroblasts to make FPF earlier and more mature fibroblasts to down regulate FPF production earlier. Organ cultures of immature lung exposed to EGF show an earlier increase in surfactant synthesis; cultures of more mature lung exhibit an earlier decline in synthesis. EGF binding to fetal lung plasma membranes increases as FPF and surfactant synthesis increase, then decreases as the lung approaches maturation. These events all occur earlier in the female fetal lung. The outcome on fetal lung development observed after EGF exposure depends on the underlying stage of lung maturation. EGF causes a more rapid advancement through the stages of fetal lung maturation.

O 240 DISCORDANT REGULATION OF CARDIAC AND SKELETAL α-ACTIN TRANSCRIPTION IN CARDIAC MYOCYTES BY TGFβ1 AND FIBROBLAST GROWTH FACTORS. Thomas G. Parker, Robert J. Schwartz, and Michael D. Schneider. Departments of Medicine, Cell Biology and Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

We have recently demonstrated that TGFß1, basic FGF and acidic FGF each augment ß myosin heavy chain and, reciprocally, decrease αMHC mRNA in cultured neonatal cardiac myocytes. In contrast, TGFß1 and basic FGF increased α-skeletal actin expression 2-3 fold, with no effect on α-cardiac actin, whereas acidic FGF down-regulated both sarcomeric α-actin transcripts by >60%. Chick α-skeletal (-202/+1) and α-cardiac (-318/+1) actin promoters linked to chloramphenicol acetyltransferase were transfected by electroporation and, following 48 hours of serum withdrawal, produced 87% and 53% of the activity of pSV2CAT, respectively. TGFß1 and basic FGF increased α-skeletal actin promoter activity with little effect on the α-cardiac construct while acidic FGF suppressed both α-actin promoters. In all 6 permutations studied, modulation of the transfected promoters by growth factors paralleled the response of the corresponding endogenous gene. Thus, acidic FGF and basic FGF antithetically regulate α-skeletal actin mRNA, in large part at the level of transcription. Moreover, the impact of all 3 peptides extends beyond the contractile proteins, as they each induced atrial natriuretic factor and down-regulated the sarcoplasmic reticulum Ca^{2+} ATPase gene in cardiac myocytes. Therefore, these ligands may recapitulate a generalized "fetal" phenotype as seen in vivo with pressure-overload hypertrophy.

O 241 LOCALIZATION OF bFGF AS PARTICULATE INCLUSIONS IN DEVELOPING HEART CELLS OF THE EARLY EMBRYO. Mary H. Parlow, David L. Bolender and John Lough, Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI. 53226.

Recent findings regarding the role of growth factors in development have led us to hypothesize that the differentiation and maturation of the myocardium may be enhanced by endogenous growth factors, such as basic fibroblast growth factor (bFGF). bFGF has been identified in cardiac muscle cells of stage 12-29 chicken embryos (Joseph-Silverstein, et al., J. Cell Biol. 108:2459, '89). However, its presence between the time of gastrulation and early heart septation has not been investigated. In this study, we have mapped the appearance of bFGF between stages 4-16 of chicken embryogenesis. Tissue sections from cryopreserved embryos were reacted with polyclonal antibody prepared against recombinant human bFGF, followed by indirect localization using flouroscein-labeled secondary antibody. As early as stage 9, bFGF was observed to be restricted to developing myocardial cells, as evidenced by its co-localization with myosin heavy-chain. Within these cells, the bFGF staining pattern was punctate to globular, suggestive of packaging in secretory vesicles. In addition to its particulate intracellular localization, bFGF was also detected at stage 15 in the adjacent myocardial basement membrane; this staining declined in intensity toward the endothelium. These results suggest paracrine and/or autocrine roles for bFGF during cardiac morphogenesis. Supported by NIH grant HL 39829.

O 242 LOCAL APPLICATION OF BASIC FIBROBLAST GROWTH FACTOR INDUCES POSTSYNATIC DEVELOPMENT IN MUSCLE CELLS. H. Benjamin Peng, Lauren Baker and Qiming Chen. Department of Cell Biology and Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599. Skeletal muscle cells synthesize both basic fibroblast growth factor (bFGF) and its receptor. This factor plays an important role in myogenesis. Following myogenesis, the muscle fibers are innervated by motor axons. In this study, we tested the premise that bFGF can also serve as a factor for the induction of postsynaptic differentiation in muscle cells. Cultured muscle cells isolated from the myotomes of Xenopus embryos were used in this study. To mimic the local interaction between the nerve and muscle during synaptogenesis, we applied bovine bFGF locally via latex beads to muscle cultures. The growth factor was conjugated to beads by adsorption. After an incubation period of 12 to 24 hrs, the cells were labeled with fluorescent α-bungarotoxin to localize the acetylcholine receptor (AChR) clusters. We found that AChR clusters were discretely localized at the bead-muscle contacts. The number of bead-associated clusters was proportional to the number of bead-muscle contacts. Each cluster was often composed of smaller AChR aggregates. Suramin, at a concentration of 10μM, abolished the bead-induced AChR clustering. Uncoated beads or beads coated with acidic FGF were ineffective in inducing AChR clustering. These results show that the local application of bFGF via beads can mimic the action of motor axons in effecting the postsynaptic differentiation. Thus, synaptogenesis at the neuromuscular junction may be triggered by the activation of a growth factor receptor system in the muscle cell.

O 243 **IL-1ß INDUCED EXPRESSION OF PDGF-AA ISOFORM IS MODULATED BY TGFß IN RABBIT ARTICULAR CHONDROCYTES.**

Francesca Peracchia, Giorgio Ferrari and Domenico Rotilio. Consorzio Mario Negri Sud, via Nazionale, 66030 S. Maria Imbaro (Chieti), Italia.

Interleukin 1ß (IL-1ß) and Platelet derived growth factor (PDGF) induce proliferation in many cell types. These peptides are released by activated macrophages and other cells in response to injury and are thought to play a crucial role in a number of pathological processes. We found that IL-1ß stimulates proliferation of rabbit articular chondrocytes and induces synthesis and release of PDGF into their culture medium. This effect, is time and dose-dependent (0.05-5ng/ml)and is restricted to PDGF-AA, as indicated by Western blot using specific antibodies kindly provided by dr. C.H. Heldin, Uppsala and found restricted to PDGF-AA, one of the three PDGF isoforms; IL -1ß effect on PDGF is inhibited by actinomycin D, a RNA synthesis inhibitor, suggesting a transcriptional regulation of PDGF-A chain upon IL -1ß stimulation. To support this hypothesis we evaluated the expression of PDGF-A chain mRNA by Northern blot using a cDNA probe for mRNA PDGF A chain, kindly provided by dr. C. Betsholtz, Uppsala, Sweden: maximal expression appeared after 2h of IL-1ß treatment. Transforming growth factorß (TGFß), a dimeric polypeptide which displays multiple biological activities, inhibits in a dose dependent manner (1-10ng/ml) not only cell proliferation but also PDGF-AA production induced by IL-1ß. IL-1ß stimulates PDGF-AA synthesis also in the presence of indomethacin, a prostagladin synthesis inhibitor, suggesting that this IL-1ß effect is not mediated by prostaglandin production. These studies suggest that IL-1ß and TGFß may interact with both positive and negative effects on growth response.

O 244 Two Forms of the basic Fibroblast Growth Factor Receptor-like mRNA are Expressed in the Developing Mouse Brain, Hugh H Reid, Andrew F Wilks* and Ora Bernard, The Walter and Eliza Hall Institute of Medical Research, and *The Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Post Office, Royal Melbourne Hospital, Victoria 3050, Australia

The embryonic neuroepithelium [NE] gives rise to the components of the central nervous system in the mature animal. To study the early development of the murine central nervous system we have sought to isolate growth factor receptors from the neuroepithelium of the neural tube of 10 day old [E10] mouse embryos. Since many growth factor receptors are members of the protein tyrosine kinase [PTK] family we have employed the polymerase chain reaction to amplify mRNA sequences from E10 NE lying between the nucleotide sequences of two highly conserved amino acid motifs from the catalytic domain of PTKs. Using this technique we have isolated a clone encoding a receptor for murine basic fibroblast growth factor [bFGF-R], as well as a shorter form of this mRNA. This latter cDNA comprised 75% of the bFGF-R cDNA clones isolated from immortalized NE cell lines. RNAse protection assays indicate this variant mRNA, designated here as N-bFGF-R, is expressed at higher levels in neural cells at early stages of development. The bFGF-R is a member of a multigene family as demonstrated by Southern blot analysis and the cloning of two other members of this family.

O 245 ANALYSIS OF TRANSFORMING GROWTH FACTOR ALPHA (TGFα) EXPRESSION IN TRANSFORMED RAT TRACHAEL EPITHELIAL (RTE) CELLS, Alice T. Robertson, Patrice C. Ferriola, and Paul Nettesheim, NIEHS, RTP, NC 27709. TGFα is a small polypeptide growth factor (mature size of 6 kDa) which is mitogenic for many epithelial cells. Primary RTE cells express low levels of TGFα mRNA, but still require EGF for growth in culture. Conversely, transformed RTE cells (RTE-T) express high levels of TGFα and do not require EGF for optimal growth. As part of our ultimate goal of defining the mechanisms involved in the up-regulation of TGFα expression in the RTE-T cell lines, we have undertaken experiments aimed at: 1)quantifying (by RIA) and characterizing (by western blot) the TGFα protein species secreted by RTE-T cells , and: 2)determining the molecular level at which regulation of TGFα occurs. Quantification of TGFα protein secreted by several RTE-T cell lines overexpressing TGFα mRNA indicated that the amount of protein secreted (from 0.6 to 13.0 ng/10^6 cells) was directly proportional to steady-state message levels found in the cells. At least two protein forms (6 kDa and 18 kDa) were secreted by all cell lines tested. When TGFα mRNA and protein levels were measured as a function of growth state of the cell culture, both TGFα mRNA and protein (ng secreted/10^6 cells) levels remained constant (through log phase to confluence) in RTE-6T cells. However, in RTE-5T cells, both TGFα mRNA and protein were down-regulated in confluent cultures. In both cell lines, the amount of TGFα protein secreted as a function of cell density correlated directly with steady state mRNA levels. Thus, in this system, TGFα production appears to be differentially regulated at the RNA level and, at least in one case, may be dependent on growth state of the cells.

O 246 EXPRESSION, PURIFICATION AND PROPERTIES OF K-FGF. David T. Rogers and Neil Wolfman, Genetics Institute Inc. 87 Cambridgepark Drive, Cambridge MA 02140. Recently a large family of factors structurally related to basic and acidic FGF have been identified. Little is known about their biological roles and relationships although several laboratories have shown that their expression differs temporally during development. The gene for K-FGF (or HST), a recent addition to the FGF family, was originally isolated from both human Kaposi's Sarcoma tissue (Delli Bovi and Basilico (1987) Proc. Natl. Acad Sci. 84 5660-4) and independently from human stomach tumor tissue (Taira et al (1987) Proc. Natl. Acad. Sci. 84 2980-4) using a mouse oncogene assay. Our knowledge about the properties of K-FGF and its relationship to other FGFs has been limited since pure protein has not been available. In the work presented here the mature protein was expressed as a cytoplasmic product in E. coli and an unusual purification protocol developed that does not require protein refolding and only one column chromatography step to produce fully active homogeneous material. Studies investigating the biological properties of purified K-FGF have been initiated.

O 247 EXPRESSION OF BIOLOGICALLY ACTIVE RECOMBINANT KGF IN A PROKARYOTIC SYSTEM: STRUCTURE-FUNCTION ANALYSIS OF KGF MUTANTS. Dina Ron, Jeffrey Rubin, David Morris, Donald Bottaro, Paul Finch and Stuart A. Aaronson, LCMB, NCI, Bethesda, MD 20892

Keratinocyte growth factor (KGF) is a human mitogen that is specific for epithelial cells. The nucleotide sequence of KGF cDNA has demonstrated that it is a new member of the FGF gene family. The pattern of KGF expression *in vivo* and *in vitro* strongly suggests its role as a stromal mediator of epithelial cell proliferation. In order to explore the structural and functional properties of KGF, we utilized the T7 expression system to produce it in large quantities. Recombinant KGF was biologically active, and its specific mitogenic activity was even greater than that of the naturally occurring form. Using *in vitro* mutagenesis, we generated KGF deletion mutants in the domain which is the most divergent among known FGF family members. Analysis of mutant proteins produced in bacteria revealed a region of about 20 amino acids which were required for mitogenic activity. The interactions of KGF and these mutants with low and high affinity cell surface receptor(s) will be presented.

O 248 TGFα PROMOTES SELECTIVE ORGAN HYPERPLASIA AND ALTERED EPITHELIAL AND MESENCHYMAL CELL DEVELOPMENT IN TRANSGENIC MICE.

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Transforming growth factor alpha (TGFα) is a ligand for the epidermal growth factor receptor and is notable because of its elevated expression by transformed cells. To examine the influence of this factor upon both normal development and tumor formation in vivo, we have produced transgenic mice bearing fusion constructs linking either the mouse metallothionein-I (MT) or rat elastase (EI) promoter to a rat TGFα cDNA. MT-TGFα was expressed at high levels in multiple tissues, and caused a two to three fold increase in wet weight of adult liver, pancreas, gastrointestinal tract, and coagulation glands. However, total body weight and the weights of some organs that expressed high levels of growth factor were unchanged. There was also no visible effect upon fetal or early postnatal development. Although the livers of transgenic mice were enlarged, they appeared histologically normal. In contrast, TGFα induced fibroplasia and metaplasia of the pancreas and dramatic hyperplasia and dysplasia of coagulation gland secretory epithelium. The effect upon pancreas was reproduced in EI-TGFα transgenic mice. Some pancreatic changes were also visible in mice bearing an EI-mutant-TGFα construct encoding a proTGFα that cannot be cleaved to release mature growth factor, arguing that factor secretion is not critical for development of all observed tissue alterations. Our findings demonstrate that TGFα can influence both epithelial and mesenchymal tissue development, and underscore the complexity of the interactions between this growth factor and its targets in vivo.

O 249 EXPRESSION OF BASIC FIBROBLAST GROWTH FACTOR IN THE BOVINE RIB GROWTH PLATE.

Joachim Sasse, Georgeann Smale, Barry Haase, Anne Middleton and Ann Cory. Shriners Hospital for Crippled Children, Tampa Unit, and Department of Biochemistry and Molecular Biology, USF, Tampa FL 33612.

Little is known about the identity of the factors that attract blood vessels into the lowermost hypertrophic zone of the cartilage growth plate or that stimulate vessel ingrowth into the area of hypertrophic cartilage during the formation of the secondary center of ossification. Does the cartilage express a factor with chemotactic and mitogenic activity for endothelial cells, thus inducing capillaries to grow toward the hypertrophic tissue? In order to address these questions we have studied the expression of chemotactic and mitogenic growth factors that might be involved in attracting blood vessels into the lowermost hypertrophic zone of the cartilage growth plate. Fetal bovine rib growth plate was dissected into six different zones and each zone extracted with 2M NaCl. The extracts were then tested for their ability to stimulate the proliferation of 3T3 mouse fibroblasts. We also absorbed aliquots of the extracts to heparin-sepharose in order to assay what proportion of growth promoting activity would belong to the class of heparin-binding growth factors. We found growth promoting activity in all zones of the cartilage; however, the amount greatly increased at the interface between hypertrophic and proliferating cartilage. The majority of the growth-factor activity bound to heparin-sepharose. These results were confirmed by studies using anti-peptide antibodies and Western-blotting and also by radioimmunoassay specific for basic FGF. In order to study the level of basic FGF mRNA in bovine rib growth plate we used the messenger RNA phenotyping technique and the polymerase chain reaction. We found basic FGF mRNA in all zones of the cartilage growth plate. Paralleling the expression at the protein level, basic FGF mRNA is low in the hypertrophic zone and greatly increases in the zone of proliferating cartilage. These results that demonstrate the presence of a gradient of growth factor expression are compatible with the notion that angiogenic growth factors can act as attractants causing the ingrowth of blood vessel capillaries into the cartilage growth plate.

O 250 EXPRESSION OF ACIDIC AND BASIC FGF DURING DEVELOPMENT

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Acidic and basic FGF are heparin binding growth factors with diverse biological activities. This includes neurotrophic, mesoderm inducing and angiogenic properties. We have previously characterized FGF like molecules from embryonic brain and kidney. The presence of these factors during embryonic blood vessel formation in these organs suggested a role in angiogenesis. To further elucidate the biological functions of the FGFs we have isolated chick genomic and cDNA clones and used them in Northern analysis. Both genes are transcribed as early as 3-4 days in the embryonic chick brain. Expression increased throughout development and reached a maximum in the adult. In situ hybridization of an aFGF probe to adult and embryonic brain sections showed expression exclusively in neurons. These data suggest that aFGF may be important for neuronal functions.

O 251 CHARACTERIZATION OF HUMAN BASIC FIBROBLAST GROWTH FACTOR GENE

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In order to understand the regulation of basic fibroblast growth factor (bFGF) gene expression, we cloned and characterized the gene and its regulatory elements. A human lymphocyte genomic library was screened using the human bFGF cDNA as a probe. Our map is nearly identical to that previously published except that the orientation of exon 2 fits when the published maps are inverted 180°. Although there is a gap between exon 1 and exon 2, these clones span 56kb of nucleotide sequence. Intron 1 is at least 16kb long and intron 2 is 16kb long. The human bFGF gene is therefore at least 36kb long. To identify the DNA containing the bFGF gene promoter, we used a standard heterologous bacterial CAT gene expression system. A 2.0kb bFGF genomic DNA fragment from our genomic clone, HBF17 which extends from the first BamHI site in Exon 1, was subcloned into promoterless CAT expression vector. This DNA was transfected into the human cell line TEG6714, extracts were prepared and assays were performed. The low level of CAT activity was observed with transfection of this clone. Further analysis of this promoter region was performed by deletion analysis which suggested the presence of negative regulatory elements.

O 252 HUMAN TGFα AND EGF EXPRESSION IN THE MOUSE EYE, David W. Silversides, Marie-Louise Oosterloo, Paul A. Overbeek, Department of Cell Biology and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030. Minigenes were constructed using the mouse αA crystallin promotor linked to cDNAs encoding human transforming growth factor alpha (hTGFα) or human epidermal growth factor (hEGF). Minigenes were microinjected into mouse FVB/N zygotes to generate transgenic mice. Five transgenics were obtained, 2 with hTGFα and 3 with hEGF. A phenotype of corneal opacity and absence of the anterior chamber was seen. Transgenic lines were generated from both hTGFα and 2 hEGF founder animals. Histological examination of the transgenic eyes revealed thinning of the corneal epithelium, absence or thinning of the corneal endothelium, absence of Bowman's and Descemet's membranes, and iris cells adherent to the inner surface of the cornea. Adhesion of iris cells to the corneal endothelium is described in the human genetic condition of Peter's Anomaly. The transgenic lenses were histologically normal in appearance. Homozygous mice showed the same phenotype as heterozygous mice. In hTGFα lines the transgenic mice all showed the novel phenotype while eyes were often normal in hEGF lines. No gradation in the phenotype was observed. These transgenic mice provide a system where growth factor production is directed to one organ (lens) with phenotypic changes observed in adjacent tissues (cornea, iris). Our findings suggest that mouse EGF receptor is present and developmentally important in the iris and cornea but absent in the lens. The mouse EGF receptor will bind hEGF or hTGFα, resulting in a perturbation of normal development of the iris and cornea. The hTGFα/hEGF transgenic mouse lines described may represent a model system for Peter's Anomaly in humans.

O 253 bFGF STIMULATES MELANOGENESIS IN EMBRYONIC QUAIL DORSAL ROOT GANGLIA IN VITRO, Kate M. Stocker, Larry S. Sherman, Sean M. Rees, and Gary Ciment, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

Neural crest (NC) and some of the NC-derived cells of the dorsal root ganglia (DRG) of early avian embryos undergo pigmentation in culture. Dorsal root ganglia from older embryos (7 days or older), however, do not produce melanocytes under the same culture conditions. The loss of melanogenic capability by these older DRG cells can be reversed by culturing them in the presence of 12-0-tetradecanoylphorbol-13-acetate (TPA) (Ciment, et. al., Devel. Biol. 118:392-398, 1986).

This study demonstrates that basic fibroblast growth factor (bFGF) also stimulates melanogenesis in DRG and peripheral nerve (PN) cultures from 7-day quail embryos. bFGF (10ng/ml) acted additively with 10^{-8} or $3x10^{-9}$M TPA to cause pigmentation in 90-100% of DRG cultures. Furthermore, in the absence of TPA, bFGF caused pigmentation in 20-60% of DRG cultures. We obtained similar results with PN cultures, suggesting that the DRG cells that undergo melanogenesis in the presence of bFGF are of the Schwann cell lineage. Various other growth factors, including NGF, TGF-alpha, and EGF, had no effect on pigmentation in DRG cultured in the presence or absence of TPA. In contrast, TGF-beta (10ng/ml) significantly decreased pigmentation in DRG cultures in the presence of TPA or bFGF. These data suggest that bFGF and TGF-beta may play important roles in the survival and/or developmental decisions made by at least one subpopulation of NC cells.

O 254 cAMP DEPENDENT PHOSPHORYLATION OF BASIC FIBROBLAST GROWTH FACTOR BY AN ECTOKINASE ASSOCIATED WITH THE CELL SURFACE OF SK-HEP CELLS. **Isabelle VILGRAIN and Andrew BAIRD. The Whittier Institute for Diabetes and Endocrinology. Department of Molecular and Cellular Growth Biology. 9894 Genesee Avenue. La Jolla, CA 92037.**

Basic Fibroblast Growth Factor (bFGF) belongs to a family of polypeptide growth factors which have numerous mitogenic and non-mitogenic effects on a wide range of target cells. For this reason, it has been critical to understand what mechanisms exist in vivo to regulate its activity. Previous studies from our laboratory(P.N.A.S., 1989, 86, 3174) have shown that a phosphorylated form of bFGF is detected in human hepatoma cells in culture (SK-Hep cells). Here, we show that a cAMP dependent protein kinase capable of phosphorylating basic FGF is localized on the surface of these cells. The incubation of bFGF with SK-Hep cells and (γ^{32})ATP revealed significant basal phosphorylation of bFGF as determined by SDS-PAGE and autoradiography. The addition of cAMP resulted in a linear increase in the incorporation of radioactivity over 0.5 to 10 min with a maximal effective concentration of cAMP between 1 and 10 μM. The phorbol ester TPA had no effect. Similarly, no tyrosine phosphorylation could be detected using Glu-Ala-Tyr(6:3-1) as a substrate. When the cells were pretreated with heparinase, the incorporation of phosphate into bFGF was decreased by about 50%. In contrast, when the binding of bFGF to its receptor was blocked with 1 mM suramin, no phosphorylation of FGF was detected. Similarly, coincubation with Ala^{112}-FGF(106-146), a receptor binding antagonist of bFGF that has no PK-A phosphorylation site, prevented the phosphorylation of bFGF. The detection of cyclic AMP dependent kinase on the cell surface supports the proposed role of phosphorylation in the regulation of bFGF.

O 255 CELL-SPECIFIC CYCLIC AMP-MEDIATED INDUCTION OF THE PDGF RECEPTOR, Gerry Weinmaster and Greg Lemke, Molecular Neurobiology Laboratory, The Salk Institute for Biological Studies, San Diego, CA 92138.

Cyclic AMP (cAMP) cooperates with a wide variety of polypeptide growth factors to synergistically stimulate the proliferation of many vertebrate cell types. The cellular mechanisms underlying these cooperative interactions are for the most part unknown, however. We have identified one such mechanism by observing that (1) cultured rat Schwann cells proliferate in response to platelet-derived growth factor (PDGF) only if simultaneously cultured in the presence of agents that elevate intracellular cAMP, and (2) this unmasked PDGF response is accounted for by a dramatic cAMP-mediated induction of PDGF receptor mRNA and protein. cAMP-mediated induction of the PDGF receptor results in enhanced, ligand-dependent receptor autophosphorylation, and in enhanced PDGF activation of *c-fos* gene expression. In addition, this induction is unique to those cells, such as Schwann cells, for which cAMP is itself mitogenic. These results indicate that the synergistic proliferative effect obtained from the combination of cAMP and polypeptide growth factors may in large part result from the cAMP-mediated induction of growth factor receptors.

O 256 REGULATION OF PROLIFERATION AND DIFFERENTIATION IN THE FIBROBLAST STEM CELL SYSTEM. Jörg H. Weßendorf, Pal I. Francz, and Klaus Bayreuther, Institut für Genetik, Universität Hohenheim, D-7000 Stuttgart 70, FRG. Primary and secondary prenatal and postnatal skin and lung fibroblasts of Valo-chicken, C3H-mice, BN-rats and man differentiate spontaneously along a seven stage terminal cell lineage in three compartments of the fibroblast stem cell system. This system comprises the proliferating and differentiating progenitor compartment with mitotic fibroblasts MFI, MFII, MFIII, the maturing and terminally differentiating fibroblast compartment with postmitotic fibroblasts PMFIV, PMFV, PMFVI, and the postmitotic degenerating compartment with fibroblast PMFVII. The cell types are morphologically recognizable and exhibit various cell type specific biological and biochemical dissimilarities (e.g. ^{35}S-methionine polypeptide pattern). Methods have been worked out (Rodemann et al., Exp. Cell Res. 180 (1989), 84-93) to select pure populations of the three mitotic (MFI, MFII, MFIII) and the four postmitotic (PMFIV, PMFV, PMFVI, PMFVII) fibroblast cell types of human dermal fibroblasts HH-8. Experiments are in progress to elucidate the role of polypeptide growth factors like bFGF, PDGF, IGF-I, EGF and of components of the intercellular matrix, like heparin, hyaluronic acid and collagens and combinations of them. The effects of factors inducing competence and/or progression are studied in pure populations of the mitotic and postmitotic fibroblasts and in defined mixtures of them.

O 257 ENDOTHELIAL CELL GROWTH SUPPLEMENT (ECGS) STIMULATES UROKINASE TYPE PLASMINOGEN ACTIVATOR (u-PA) PRODUCTION BY HUMAN PULMONARY ARTERY SMOOTH MUSCLE CELLS (HPASMC), Johann Wojta, Alois Gessl, Günter Christ, Michael Holzer, Irene Lang, Bernd R. Binder, Lab. Clin. Exp. Physiol., Dept. Med. Physiol., Univ. Vienna, Austria

Vascular smooth muscle cells play a crucial role in the development of arteriosclerotic lesions by proliferation of this cell type in the intimate layer of the artery. It is also known that proliferation of vascular smooth muscle cells is stimulated by acidic as well as basic fibroblast growth factor (FGF). On the other hand, proliferation of cells is often correlated with an increase in the production of plasminogen activators (PAs). It was the aim of this study to investigate the influence of ECGS prepared by streptomycin and ammonium sulfate precipitation from bovine hypothalamus on the production of u-PA and tissue-type plasminogen activator (t-PA) by HPASMC. HPASMC were characterized by immunofluorescence staining with an anti-α-actin-antibody. Such characterized HPASMC were incubated for 48 hours in the absence and presence of 100µg/ml ECGS. u-PA antigen as determined in the conditioned media (CM) by a specific ELISA increased from 0.7 ± 0.08 in the control CM to 16.35 ± 0.4ng/10^5 cells in the CM of ECGS treated HPASMC. t-PA antigen determined by a specific ELISA in the respective CM increased from 0.7 ± 0.1 to 3.2 ± 0.05ng/10^5 cells. The expression of specific u-PA RNA increased significantly after ECGS treatment for 4 and 8 hours, respectively, as determined by Northern blotting. Purified a-FGF or b-FGF at concentrations of 100ng/ml, however, did not affect neither u-PA nor t-PA production of HPASMC. The stimulatory effect of ECGS on u-PA production could be abolished by boiling for 5' and by pretreatment at pH 3 for 30'. Heat treatment for 1 hours at 56°C or pH 5 for 30' resulted in a decrease of the stimulating activity to approximately 50% as compared to untreated ECGS. When ECGS was preincubated to heparin-Sepharose, no reduction in the stimulating effect could be observed.

Hematopoiesis; Neurogenesis; Pattern Formation

O 300 **INTERCELLULAR SIGNALLING IN THE DEVELOPING *DROSOPHILA* EYE**

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Competition and lateral inhibition are thought to be widespread mechanisms for pattern formation during development, and are probably used to generate an array of R8 photoreceptor cells that serve as a foundation for the rest of eye development in *Drosophila*. R8 precursor spacing can be altered in either direction by *Notch* mutations, and is reduced by loss-of-function at the *sca* locus. Genetic evidence suggests activity of *N* depends in part on the amount of *sca* product. Both genes function in R8 cells; the *sca* gene seems to control an inhibitory signal whereas *N* is involved in its reception. *N* is known to encode a transmembrane protein with EGF-repeats; *sca* encodes a putative secreted protein related to vertebrate fibrinogens. We suggest these the interaction of these proteins mediates an inhibitory signal used to space R8 cell development. It appears protein domains used in the blood coagulation cascade derived from genes used in intercellular communication in invertebrates.

O 301 BIMODAL EFFECTS OF PROTEIN KINASE C (PKC) ACTIVATION ON SURFACE IMMUNOGLOBULIN (sIg) MEDIATED MURINE B CELL ACTIVATION, Anil K. Balapure, Robert I. Glazer* and James J. Mond, Department of Medicine, USUHS, Bethesda, MD 20814 and *Georgetown University, 4 Research Court, Rockville, MD 20850.

PKC mediates growth and differentiation in a variety of cell types including B lymphocytes. We have previously demonstrated that while phorbol ester PDBU inhibits anti-Ig stimulated B cell activation, another PKC activator Indolactam (IL) enhances sIg mediated B cell activation. To clarify why these two PKC activators exert such different consequences on B cell activation, we examined their effect on PKC activity in B cells. Exposure of B cells to both PKC activators resulted in the translocation of PKC from the cytosol to the membrane at rates which were concentration dependent. On a molar basis, IL induced a much slower and less efficient translocation of PKC from the cytosol to the membrane. Additionally, on a molar basis, the depletion of PKC activity was significantly slower than that induced by PDBU. Thus, while PDBU at 0.1 and 1 µM induced an approximately 75% loss of PKC activity within 2 hours, IL at these concentrations induced on average a loss of only 35% of the activity. When taken together, this data suggests that the stimulatory effect of IL on sIg mediated B cell activation may reflect its ability to activate PKC without causing a rapid depletion of PKC as is induced by PDBU. Since anti-Ig induced crosslinking of sIg stimulates increases in diacylglycerol, the prolonged availability of its substrate (PKC) combined with the added stimulation of PKC by IL may result in enhanced anti-Ig mediated proliferation of B cells.

O 302 EVX 1, A MURINE *EVEN-SKIPPED* HOMOLOGUE, IS EXPRESSED IN A SUB-POPULATION OF CELLS IN THE DEVELOPING NEURAL TUBE, Helge Bastian and Peter Gruss, Max-Planck-Institute of Biophysical Chemistry, Department of Molecular Cell Biology, Göttingen, FRG. The approach to unravel molecular mechanisms of vertebrate development by analyzing homologues to *Drosophila* regulatory genes is supported by studies in *Xenopus* and mouse. With the exception of *bcd* and *eve,* murine homologues to all *Drosophila* homeobox classes known so far have been isolated. Using a *Drosophila eve* type homeobox probe we have isolated clones of two murine genes, *Evx 1* and *Evx 2*, from genomic and embryonic cDNA libraries. *Evx 1*, *Evx 2*, *eve* and the *Xenopus Xhox-3* constitute a family of related genes based on the similarity of their homeobox sequences. In addition *Evx 1*, *Evx 2* and *Xhox-3* share extended amino acid conservation outside of the homeobox. Both *Evx 1* and *Evx 2* map to the same region on mouse chromosome 2. *Evx 1* is expressed in undifferentiated F9 stem cells but not in cells differentiated with retinoic acid. A single 3.2 kb Evx 1 transcript accumulates from day 9.5 days p. c. to 12 during embryogenesis and decreases sharply thereafter. *Evx 1* RNA is restricted to specific cells along the entire lengths of neural tube and hindbrain. *Evx 1* expression coincides temporally and spatially, with maturation of early forming interneurons, possibly commissural interneurons. The transcription pattern is compatible with a role for *Evx 1* in specifying neuronal cell fates in the differentiating neural tube in analogy to *eve* in the embryonic CNS of *Drosophila.*

O 303 SPATIAL AND TEMPORAL EXPRESSION OF CARCINOEMBRYONIC ANTIGEN (CEA) FAMILY MEMBERS IN MOUSE EMBRYOS, Nicole Beauchemin, Jing Qi Huang and Claire Turbide, McGill Cancer Centre, McGill University, Montreal, Canada.
Carcinoembryonic antigen (CEA) is a membrane-associated glycoprotein abundantly expressed in many human tumors, particularly in the gastrointestinal tract, in breast and lung tumors. This protein as well as several other related family members have been characterized and belong to the Ig superfamily. CEA has also been shown to function as an intercellular Ca^{++}-independent cell adhesion molecule. We have recently cloned murine analogues of the CEA family and have been investigating their expression during murine embryonic development. Northern analyses on RNA prepared from complete embryos have revealed that several transcripts corresponding to various CEA family members are expressed as of 11.5 days post-coitum. These transcripts are particularly abundant in embryonic intestine as of day 16 and continue to be expressed into adulthood. We have used in situ hybridization and immunostaining with anti-human CEA antibodies to detect transcript/protein expression in these embryos. Our results indicate that the CEA family members are expressed in placenta at 10.5 days of development, in chondrocytes and perichondrium as of 13.5 days, in meninges and dermis as of 14.5 days and in intestine as of 15.5 days. Our results imply that members of the murine CEA family are involved in the growth of various cell types along specific differentiation pathways during development. Several members of the TGF-ß family are known to be expressed in the same tissues during murine embryogenesis: we are presently investigating the functional relationship between these growth factors and the murine CEA family members.
(Supported by the MRC of Canada and the Cancer Research Society).

O 304 *MOLECULAR ANALYSIS OF A TRANSGENIC MOUSE WITH A MOTOR DISORDER AND REDUCED MALE FERTILITY.* Nooshine Dayani, Paul E. Neumann, and Jon W. Gordon, Brookdale Center for Molecular Biology, Mt. Sinai MedicalCenter New York NY 10029 and Dept. of Neurology, Children's Hospital, Boston, MA 02115. We identified an insertional mutation in a line of transgenic mice which is allelic with the spontanous mutation hotfoot The mutation was discovered by noting a coarse motor ataxi in progeny of hemizygous crosses, which began at about day 10 of postnatal development. In addition, affected males were sterile. To clone DNA from the mutated locus, we constructed a genomic library in the bacteriophage EMBL3 , and 4 recombinant phages containing DNA flanking the transgene were recovered. Digestion of one of the phages with BamH1-PvuII generated a 1Kb fragment (BP1) which is closely approximated to the transgene integration site. This fragment was subcloned and used to evaluate possible DNA rearrangement associated with foreign gene integration. We digested transgenic and control DNA with BamH1-PvuII and found the expected 1Kb fragment in both samples, but we also noted additional restriction fragments in the transgenic sample that were homologous to BP1. Because the intensity of the expected 1Kb fragment was the same in both samples, we tentatively interpret the results as indicating a duplication of host DNA associated with integration of the foriegn plasmid. Positive hybridization of BP1 to DNA from a mouse-chinese hamster hybrid cell line, known to carry mouse chromosome 6 is consistent with the hypothesis that we have cloned genetic material from mouse chromosome 6, possibly very close to the hotfoot gene. We have screened a normal mouse DNA library, and several positive clones have been isolated. We conclude that with these clones we should be able to isolate and characterize the hotfoot gene at the molecular level.

O 305 LEUKEMIC CELLS CONSTITUTIVELY RELEASE A POLYPEPTIDE (APOLYSIN) WHICH MAY FACILITATE THEIR EGRESSION FROM BONE MARROW. Dittmann K.[1], Petrides P.E.[1,2], [1]Molecular Oncology Laboratory, University School of Medicine Munich, [2] Clin. Hematology (GSF) D-8000 Munich Germany. In leukemic bone marrow, there is a drastic decrease of the extent to which adventitial cells cover the sinus endothelium. Therefore we have investigated the interactions of transformed white blood cells - with enhanced capacity to egress from the bone marrow - with adventitial cells. The latter represent an important constituent of the bone marrow/blood barrier. We have identified a polypeptide produced by HL-60 cells, which is able to induce in vitro an alteration in cell shape of the adventiatial cell (retraction of extentions, rounding up and finally detachment). Experiments with radiolabeled proteins indicate that this biological effect is caused by modulation of extracellular matrix proteins. Although there is a strong degradation of collagen type I, Apolysin does not seem to be a collagenase. Experiments with various proteinase inhibitors reveal that the activity is a serine protease which cleaves peptides bonds between GLY and GLN. Partial biochemical purification utilizing Sephadex G-75 and DEAE-anion exchange chromatography yields a polypeptide which posesses a MW of 50-60 kd on SDS-gel-electrophoresis. Induction of differentiation of HL-60 cells causes a decrease of Apolysin secretion which indicates that the release is stage specific and may be important for marrow cell egress. Supported by DFG (SFB 324) and GSF (71967)

O 306 DESIGN AND DEVELOPMENT OF A NOVEL QUANTITATIVE ELISA FOR HUMAN mCSF, GM Fenton, PJ Szklut, MM Dunn, JD Aghajanian, ML Kolowsky, AM Paone, NA St.George, DH Wilson, and EM Alderman, Immunology Laboratory, Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140.

A panel of 6 murine monoclonal antibodies (mAbs) with specificity directed against human mCSF were assessed for the ability to bind soluble or immobilized antigen. Although three members of this panel were capable of precipitating soluble mCSF, when each MAb was directly immobilized on ELISA plates, only one (hm7/5) could consistently 'capture' mCSF from fluid samples. Each panel member was subsequently biotinylated and screened for compatiblity with hm7/5; a compatible mAb (hm7/4) was selected for use as the detector in a highly reproducible asymmetrical sandwich ELISA (sensitivity 10 - 500 ng mCSF/ml). Substitution of a biotinylated rabbit anti-mCSF polyclonal IgG for the detector mAb has enhanced this sensitivity range to 0.25 - 5 ng mCSF/ml, allowing detection of endogenous mCSF in human serum samples.

O 307 MAPPING OF A NOVEL REGION OF huEPO INVOLVED IN BIOLOGICAL ACTIVITY, Mathias R. Fibi, Werner Stüber, Petra Hintz-Obertreis, Dorothea Krumwieh, Bernhard Siebold, Gerd Zettlmeißl and Hans A. Küpper, Behringwerke AG, Department of Molecular Biology, Post Box 11 40, 3550 Marburg 1, F.R.G.

In order to identify biologically active domains of human erythropoietin (huEPO), we synthesized five different peptides (P1: 84-95; P2: 152-166; P3: 52-63; P4: 7-23; P5: 110-123) homologous to relatively hydrophilic regions of the hormone for antibody production. All peptides were able to induce high titers of peptide specific antibodies in rabbits. Antisera induced by huEPO contained a realtively high amount of antibodies preferentially directed against three peptides, including the amino-terminal region (P4). The same three peptides were able to induce huEPO specific antibodies. Only antisera induced by one of the peptides inhibited the biological function of huEPO in a cell proliferation assay. The peptide is coincident with a novel region most probably involved in biological activity of huEPO.

O 308 EXPRESSION AND FUNCTION OF THE C-KIT PROTO-ONCOGENE IN MOUSE ERYTHRO-LEUKEMIA CELL LINES, Roger A. Fleischman, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235.

The proto-oncogene c-kit, a membrane receptor with tyrosine kinase activity, has recently been shown to map to the mouse W locus. Mice homozygous for mutant alleles of W/kit exhibit a severe macrocytic anemia, as well as other defects in hematopoietic cells, melanocytes, and germ cells. In order to understand the role of the c-kit receptor in erythropoiesis, we have isolated an inducible erythroleukemia line that carries the severe W mutation, W^x. Although mice homozygous for this allele exhibit marked anemia, we have shown that the W^x cell line undergoes erythroid differentiation after exposure to the inducer dimethylsulfoxide with kinetics that are identical to wild-type lines. Southern analysis of the mutant line with a c-kit c-DNA probe confirmed the presence of the mutant allele, the absence of the wild-type gene, and further ruled out the possibility that additional re-arrangements or amplification of the mutant W^x allele had occurred during malignant transformation. Northern analysis of the mutant and wild-type control lines, demonstrated that the W^x mutation results in a complete absence of the normal 5.5 kb W/kit transcript, although a novel transcript at 3.2 kb was detected. While most of the wild-type lines expressed W/kit transcripts, one additional line, which exhibits normal differentiation in vitro, was found to express little, if any, c-kit m-RNA. The absence of a correlation between the expression of W/kit and the kinetics of erythropoiesis in vitro suggests that differentiation inducers, such as DMSO, may act by an independent pathway to bypass the tyrosine kinase activity of the normal W/kit receptor. Alternatively, the W/kit genetic defect may not be expressed at this stage of erythroid maturation. Additional experiments are in progress to determine if induction of differentiation in wild-type lines results in decreased levels of c-kit transcripts.

O 309 LEUKEMIA INHIBITORY FACTOR (LIF) AND IL-7 STIMULATE *IN VITRO* PROLIFERATION OF MURINE OF MURINE MULTILINEAGE HEMATOPOIETIC PROGENITORS, Frederick A. Fletcher, Douglas E. Williams, Charles Maliszewski, Dirk Anderson, Michelle Rives and John W. Belmont. Howard Hughes Medical Institute, Baylor College of Medicine, Houston, TX 77030 and the Departments of Experimental Hematology, Immunology, and Molecular Biology, Immunex Corp., Seattle, WA 98101.

We have evaluated the effect of LIF and IL-7 on the *in vitro* growth of murine hematopoietic precursors. LIF has previously been shown to support the undifferentiated growth of totipotent embryonic stem cells but direct effects on normal hematopoietic progenitors have not been observed. After 72h stimulation in liquid culture with either 10 or 1000 U/ml recombinant LIF an approximately two-fold increase in the number of day 13 CFU-S was observed. The introduction of unique genetic markers into individual CFU-S by retroviral vector infection provided further indication that LIF affected CFU-S and their precursors. The efficiency of vector infection of CFU-S increased from 13% to 84-91% during LIF stimulation. Southern analysis of individual CFU-S demonstrated five examples of identical pairs of clonotypic vector insertions indicating that CFU-S precursors had also been infected. Histologic analysis indicated no restriction of the developmental potential of these CFU-S. Il-7, which is known to support B cell progenitor growth, also supported multilineage CFU-S growth and increased CFU-S infection efficiency. Southern analysis performed on the retrovirus vector-infected CFU-S revealed a large increase in the number of integration events per clone (from an average of 2-4 integrations to 8-10). These experiments demonstrate novel effects of these growth factors on early myeloid lineage cells and raise the possibility that one or both play a role in the growth regulation of the most primitive hematolymphoid precursors.

O 310 TRANS-ACTIVATION OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR AND THE INTERLEUKIN-2 RECEPTOR IN TRANSGENIC MICE CARRYING THE HUMAN T-LYMPHOTROPIC VIRUS TYPE 1 TAX GENE, Jeffrey E. Green,[1] C. Glenn Begley,[2] David K. Wagner,[2,3] Thomas A. Waldmann,[2] and Gilbert Jay,[4] Laboratory of Molecular Virology[1] and Metabolism Branch[2], National Cancer Institute, Bethesda, Maryland 20892; Westside Veterans Administration Medical Center, Chicago, Illinois 60612[3], and Laboratory of Virology, Jerome H. Holland Laboratory, American Red Cross, Rockville, Maryland 20855[4]

Three lines of transgenic mice carrying the human T-cell lymphotropic virus type 1 tax gene have previously been reported to develop neurofibromas composed of perineural fibroblasts. Tumors from these mice and tumor cell lines derived from them expressed high levels of tax RNA and protein. They also expressed high levels of the granulocyte-macrophage colony-stimulating factor (GM-CSF) gene as measured by proliferative responses and by Northern (RNA) blot analysis. Although other tissues, in the transgenic mice also expressed high levels of tax, they did not express the gene for GM-CSF. This indicates that tissue-specific cellular factors, in addition to tax, are required for GM-CSF gene expression. Systemic effects of excessive GM-CSF production were demonstrated. The interleukin-2 (IL-2) receptor was also found to be expressed by the tumors and tumor cell lines as measured by IL-2-binding and cross-linking studies. This is the first demonstration that the IL-2 receptor can be activated by tax in a nonlymphoid cell type. These in vivo findings are consistent with other reports which have demonstrated in vitro cis-regulatory elements within the 5'-flanking regions of the genes for GM-CSF and the IL-2 receptor which are responsive to trans-activation by the tax gene.

O 311 EXPRESSION AND REGULATION OF THE RBC-8C5 CELL SURFACE ANTIGEN ON MURINE BONE MARROW CELLS, [1]K. Hestdal, [2]R. Coffman, [3]C. Dubois, [4]S. E. W. Jacobsen, [5]F. W. Ruscetti, [6]D. Longo, and [7]J.Keller, [1,7]Biological Carcinogenesis Development Program,PRI, [3,4,5]Laboratory of Molecular Immunoregulation.,[6] BRMP, NCI-FCRF, Frederick, MD, 21701 and [2]DNAX, Palo Alto,CA.
Murine bone marrow cells expressing the cell surface antigen RBC-8C5 were identified by FACS analysis using a rat monoclonal antibody. The fluorescent profile showed that there are two positive populations, 8C5 bright ($8C5^{hi}$) and 8C5 dull ($8C5^{lo}$) which represented 19% and 21% respectively of the bone marrow analysed. Morphological analysis of the sorted populations demonstrated that the $8C5^{hi}$ were enriched for end stage neutrophils (76%) and the $8C5^{lo}$ contained myeloblasts, promyelocytes and myelocytes (82%). Lymphocytes were <5% in any population, erythroid and other progenitors were 8C5 negative (8C5-). The 8C5- and $8C5^{lo}$ cell populations contained most of the CFU-c (>80%), and all the CFU-GEMM and BFU-E were in the 8C5- population. 8C5 expression can be induced on 8C5- cells in liquid culture by IL-3 and GM-CSF. Using radiolabeled ligands, we found that IL-1α- and GM-CSF-receptors were enriched in $8C5^{hi}$ cells. To study the regulation of 8C5 expression in vivo, IL-1α, 1 ug/animal was injected i.v.. There was a preferential loss of $8C5^{hi}$ cells (81%) after 12 hours. Thus, 8C5 antigen is expressed mainly on myeloid cells, it is increased during differentiation and is inducable by cytokines.

O 312 RETROVIRAL LINEAGE MARKING SHOWS THAT MYOBLASTS MIGRATE ACROSS BASAL LAMINA DURING MUSCLE DEVELOPMENT, Simon M. Hughes and Helen M. Blau, Department of Pharmacology, Stanford University Medical Center, Stanford, CA 94305.
Basal lamina separates cells into distinct groups during morphogenesis and could form a barrier to cell migration. Retroviral vectors that express β-galactosidase were injected into rat lower hindlimb muscle at a late stage when muscles have the adult number of multinucleate fibers, each fiber individually wrapped in a basal lamina sheath. Myoblasts within the sheath are known to proliferate and fuse into pre-existing fibers as the animal grows. The question we addressed is whether myoblasts cross the basal lamina and contribute to adjacent fibers or are they restricted to fuse only into the fiber with which they are associated. Retroviral vectors expressing β-gal targeted to either the nucleus or the cytoplasm were used to mark the clonal progeny of single myoblasts in vivo during postnatal weeks 2-3. Co-injection of both vectors demonstrated that localized clusters of marked fibers were clonally derived. A majority of such clones contain more than one marked fiber. This indicates that myoblasts migrate across basal lamina from fiber to fiber during normal development.

O 313 THE ROLE OF c-fms PROTO-ONCOGENE IN REGULATION OF HL-60 CELL DIFFERENTIATION, Wu Jiong, Jian Qin Zhu and Dexu Zhu, Department of Biochemistry, Nanjing University, Nanjing 210008, P. R. China
Human leukemia HL-60 cells were induced to undergo differentiation along two different directions. One gives rise to granulocytes induced by dimethyl sulfoxide (DMSO) and human recombinant granulocyte-macrophage colony stimulating factor (hrGM-CSF), and the other to macrophages (Mϕ) induced by phorbol esters (PMA), 1α,25-dihydroxyvitamine D_3 ($1,25-(OH)_2D_3$) and hrGM-CSF. By using a modified polymerase-chain reaction (PCR), we showed that the induced-macrophagic differentiation of HL-60 cells was accompanied with transcription of c-fms gene in the cells, but the induced-granulocytic differentiation was not. We then treated HL-60 cells with a c-fms antisense oligomer (10 μM) in presence of different inducers. The c-fms anti-sense oligomer strongly inhibited the expression of c-fms gene. It was shown that this treatment resulted in significant decrease of Mϕ formation induced by PMA and hrGM-CSF (42.8% and 51% of inhibition in comparison to controls respectively). In contrast, the Mϕ formation induced by $1,25-(OH)_2D_3$ was nearly same in presence or absence of the antisense oligomer. Furthermore, HL-60 cells were incubated with PMA, hrGM-CSF and $1,25-(OH)_2D_3$respectively in presence of another antisense oligomer, macrophage-CSF (M-CSF) antisense oligomer. Result showed that the PMA- and hrGM-CSF- but not $1,25-(OH)_2D_3$ -induced Mϕ formation was dramatically inhibited compared to controls. Moreover, M-CSF antisense oligomer acted synergically with c-fms antisense one in inhibition of PMA- and hrGM-CSF-induced Mϕ formation from HL-60 cells. It can be concluded that there may exist a autocrine circut of c-fms products (M-CSF receptors) and M-CSF in PMA- and hrGM-CSF-induced Mϕ formation from HL-60 cells.

O 314 THE MURINE HEMOPOIETIC DIFFERENTIATION ANTIGEN M1/69-J11d IS A VERY SMALL, GPI-LINKED PEPTIDE WITH MULTIPLE GLYCOFORMS. Robert Kay, Fumio Takei, Patricia Rosten and R. Keith Humphries, Terry Fox Laboratory, B.C. Cancer Research Centre, and Department of Medicine, University of British Columbia, Vancouver B.C. Canada.

Antigens detected by M1/69 and J11d MAbs are found in heterogeneous forms on the surfaces of many immature and differentiated murine hemopoietic cells. Loss of expression of M1/69-J11d is associated with the maturation of thymocytes and their exit from the thymus as circulating T cells. The induction or extinction of expression of these antigens occurs during several other developmental progressions in both lymphopoiesis and myelopoiesis. cDNAs encoding the M1/69-J11d peptide were cloned from a myeloid cell line by immunoselection of COS cells transfected with expression libraries. The cDNAs encode a surprisingly small peptide, predicted to contain only 30 amino acids after removal of a signal sequence and displacement of the C-terminal region by the glycosyl-phosphatidylinositol group that anchors the peptide to the cell surface. Despite their considerable structural diversity, most forms of M1/69-J11d antigens appear to be the translation products of the single mRNA species represented by the cloned cDNAs. Almost all of the mass of M1/69-J11d accumulates through extensive N- and O-linked glycosylation at multiple sites in the short peptide, with differential site utilization generating the observed heterogeneity of the antigens. The M1/69-J11d antigens may serve as ligands for lectin-like adhesion molecules, with the specificity of their interactions being determined by differential glycosylation. Several murine genes hybridize with the cDNA. Some of these may encode hemopoietic M1/69-J11d homologs that are not detectable with the available antibodies, while other M1/69-J11d homologs are expressed in non-hemopoietic cells, including neural tissue. Human homologs of the M1/69-J11d antigens have also been identified.

O 315 IN VITRO DIFFERENTIATION OF MURINE SPLENIC STEM CELLS. Vijaya Manohar, Elinor Brown, Jonathan Keller, Jeffrey Rossio, Michael Taplits, Thomas Hoffman and Thomas Chused. Laboratory of Cell Biology, Division of Blood and Blood Products, CBER, FDA. Bethesda, MD 20892

New Zealand Black Mice develop spontaneous splenomegaly with age. Using two color flow cytometry and cell sorting, the hypercellularity of the spleen is shown to be due to non-T and non-B null cells. A major subset of these null cells is characterized phenotypically and functionally as undifferentiated stem cells. In vivo, these purified stem cells form 12-day splenic colonies and confer radioprotecion lethally-irradiated, H-2 compatible recipients. In vitro these cells have been exposed to a panel of recombinant cytokines as well as conditioned media from a bone marrow-derived stromal cell line. Under these conditions, these cells differentiate into three distinct cell morphologies; lymphoid, myeloid and adherent macrophage-like cells. Furthermore, cell types belonging to the T, B and macrophage lineages have been identified phenotypically by their surface determinants.

O 316 EFFECTS OF IFNs ON FRIEND LEUKEMIA CELL DIFFERENTIATION

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The effect of Interferons (IFNs) on cell differentiation have been mainly studied in hematopoletic cells and particularly in Friend leukemia cells (FLC). FLC are erythroid precursors blocked in their differentiation pathway at the proerythroblastic stage. They are induced to differentiate by treatment with dimethysulfoxide (DMSO) and others inducers. Administration of pure murine IFNs β, α1, α4, α6 to DMSO induced FLC, exhibits a stimulatory effect on erythroid differentiation. Number of benzidine positive cells (B+) in cells treated with DMSO+IFN β and or IFN α double with respect to cells treated with DMSO alone. IFN β seems to increase the erythroid maturation more than the three species of IFNs α. The stymulatory effect of IFN β on Hb synthesis is also associated with a comparable increase of globins mRNA levels and chains synthesis as well as with a rise in the expression of transferrin receptors. A marked increase in heme content is also observed. It is well established that heme plays multiple role in mammalian cells at both structural and functional level. In fact heme represents a component of Hb and other molecules, it stimulates the rate of transcription of globin mRNAs and it is a necessary factor for optimized mRNAs translation in reticulocytes and FLC. An unifying hypothesis may be considered. In differentiating FLC, IFNs exert their primary stimulatory action on heme synthesis: this might lead to increase TfR expression, thus further stimulating heme synthesis and promoting transcription of globin genes.

O 317 LIPID MEDIATORS OF INTRACELLULAR SIGNALLING IN ERYTHROPOIESIS.

Meredith Mason-Garcia and Barbara S. Beckman, Department of Pharmacology, Tulane University School of Medicine, New Orleans, LA 70112.

Erythropoietin (Ep) is the peptide growth factor whose actions on the erythroid progenitor cell induce its terminal differentiation. Despite much past research, the intracellular signalling system which is activated by Ep in these cells is still poorly understood. We have identified two signal transduction pathways which are activated by Ep and which involve lipid molecules as mediators of the cellular response. In the first of these systems, there is an early (2-5 min) increase in levels of two lipoxygenase metabolites of arachidonic acid (AA): leukotriene B4 (3-5 fold) and 12-hydroxyeicosatetraenoic acid (12-HETE) (2 fold). These responses can be blocked by lipoxygenase inhbitors, by phospholipase inhibitors or by an inhbitor of diacylglycerol lipase, suggesting that diacylglycerol (DAG) may serve as an important source of AA; indeed, the DAG analog, dioctanoyl glycerol (DiC8), will induce significant elevation of these metabolites in the absence of Ep. We find no increase in levels of inositoltrisphosphate, however, indicating that phosphatidylinositol is probably not the source of DAG. DAG is also known to activate protein kinase C (PKC), and this is the second lipid-mediated signal we have identified in this system. We have found that an inhibitor of PKC (staurosporine) will inhibit Ep-induced erythroid colony formation and Ep-induced thymidine incorporation in erythroid progenitor cells, and that DiC8 will induce thymidine incorporation in the absence of Ep. The activation of a nuclear PKC has been reported recently for prolactin and nerve growth factors in nuclei of their target cells, suggesting that this may be a novel signalling pathway for a group of trophic factors. In nuclei isolated from erythroid progenitor cells, Ep will induce a time- and dose-dependent activation of PKC, providing further evidence that this DAG-mediated process is an important intracellular event in the actions of Ep.

O 318 RESTORATION OF IN VITRO HEMATOPOIESIS IN B-CHRONIC LYMPHOCYTIC LEUKEMIA BY ANTIBODIES TO TUMOR NECROSIS FACTOR. Rita Michalevicz, Reuven Porat, Michal Vechoropoulos, Shoshana Baron, Miriam Yanoov, Zvi Cycowitz and Shlomo Shibolet. Institute of Hematology and Department of Internal Medicine "D", Ichilov Hospital; Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel. Hematopoiesis was evaluated in 15 B-CLL patients using the mixed colony formation assay. It was found that the mean growth of all types of colonies in B- CLL peripheral blood was significantly lower than that of 10 normal controls ($p<0.05$). To investigate whether TNF is the cytokine involved in the reduced growth of hematopoietic progenitors in B-CLL, neutralizing anti- TNF antibodies (anti-TNF Abs) were added to the cultures. Anti-TNF Abs restored in vitro hematopoiesis in 11 out of 15 B-CLL patients. A significant increase in the growth of all types of colonies was noted in cultures with anti-TNF Abs as compared to baseline cultures ($p<0.05$). In patients with stage IV disease, the restoration of erythroid and multipotential progenitors growth was more prominent than in patients with earlier disease stages. Furthermore, high TNF levels were measured in conditioned media from CLL patients that suppressed normal bone marrow hematopoietic progenitor growth. It is concluded that TNF plays a role in the suppression of hematopoietic progenitor growth in B-CLL, and that anti-TNF Abs significantly restore this impaired growth. These findings may have clinical applicability.

O 319 HEMOPOIETIC GROWTH FACTOR EXPRESSION IN PRE- AND POST IMPLANTATION MOUSE EMBRYOS, Richard Murray, Choy-pik Chiu, and Frank Lee, DNAX Research Institiute of Molecular and Cellular Biology, Palo Alto, CA 94304. Cytokines play a vital and well documented role in maintaining hemopoiesis in the adult animal. We have addressed the expression of four hemopoietic growth factors (IL-3, IL-6, LIF, and GM-CSF), thought to be active on early hemopoietic cells, during mouse embryogenesis. IL-3 and IL-6 transcripts have been identified in preimplantation mouse blastocysts. IL-6 protein was found as a secreted product from cultured blastocysts. These same cultured embryos were still capable of forming viable offspring when implanted in surrogate animals. Preliminary results indicate that recombinant IL-3 and IL-6 mediate differentiation and proliferation of colonies of embryonic stem (ES) cells in a semi-solid matrix, implying that the inner cell mass may be a target for these products in the blastocyst. Additionally, cytokine expression has been analyzed during the onset of hemopoiesis in the day 8 and day 9 yolk sac, embryo, and decidua at the mRNA and protein level. The <u>in vivo</u> role of these molecules is currently being addressed by gene ablation experiments in ES cells, with the aim of generating animals deficient in a particular cytokine gene.

O 320 NG2 PROTEOGLYCAN IN THE DEVELOPING RAT LIMB, Akiko Nishiyama, Patricia A. Healy, Kimberlee J. Dahlin, William B. Stallcup, La Jolla Cancer Research Foundation, La Jolla, CA 92037

NG2 is a chondroitin sulfate proteoglycan which is expressed on the surface of glial progenitor cells capable of differentiating into oligodendrocytes or type 2 astrocytes (Stallcup and Beasley, J Neurosci. 7: 2737, 1987). The expression of NG2 in the developing rat limb was examined using rabbit antiserum and monoclonal antibodies against NG2 core protein. NG2 was first detected in the limb buds on embryonic day 14 (E14) on the surface of condensed mesenchymal cells. In the E16 limb intense NG2 immunoreactivity was seen in the mesenchymal core of the distal cartilage primordia and in the differentiating chondrocytes of the proximal cartilage. The number of NG2-immunoreactive cells decreased by E17. In the phalanges of the E17 limb NG2 immunoreactivity was stronger in the cells at the periphery of the cartilage and in the developing joint. NG2 was not detected in the cartilage of E17 femur or humerus which showed morphological characteristics of mature chondrocytes. No NG2 immunoreactivity could be seen in the cartilage of postnatal limbs. Thus, NG2 was found to be present on the surface of immature chondrogenic cells but was immunohistochemically undetectable in differentiated chondrocytes, suggesting a role of NG2 in the early events in chondrogenesis.

O 321 BIOCHEMICAL IDENTIFICATION OF TA1 ON SEVERAL CANCER CELL LINES BY RP-HPLC AND MOLECULAR SIZING, Karen K. Oates, Dawn Royall and Michael E. Erdos, Department of Biology, George Mason University, Fairfax, VA 22030

Many tumor cells have been shown to produce and release growth regulatory factors in an autocrine manner. Attempts to study the effect of growth factors on cancer cells led to the surprising discovery of the thymosin alpha-one (TA1) like polypeptide in the cell supernatants and cytosolic preparation of MCF-7 human breast cancer cell lines as well as SCLC (varient), bronchi, bladder and lymphoma cell lines. The MCF-7 cells secreted TA1-like polypeptides as early as six hours from time of seeding into TA1 free media. Preparative reverse phase HPLC analysis followed by RIA identified immunoreactive TA1. A molecular sizing HPLC procedure found that the immune-reactive secreted form was shorter than native 28 amino acid TA1 and co-migrates with both N14 and C14 terminal of TA1 by molecular sizing. We have used indirect immune fluorescence antibody staining to localize TA1 on the MCF cell line, as well as several other cell lines.
This data, along with our previously published results suggest that TA1 is secreted from the tumor cell lines and may be acting as an autocrine factor for the growth of these lines. Current experiments now in progress are aimed at identifying the biological effects and the identification of TA1 receptors on the cell lines.

O 322 REGULATION OF IL-6 AND ITS RECEPTOR DURING *IN VITRO* DIFFERENTIATION OF MONOBLASTIC U-937 CELLS. F. Öberg, L-G. Larsson, N. Hult and K. Nilsson. Dept. of Pathology, University of Uppsala, S-75185, Sweden.

The human monoblastic cell line U-937 can be induced to differentiate by phorbol ester (PMA) and several physiological inducers. When induced to differentiate U-937 cells will undergo morphological and functional changes similar to those observed in normal monocytic differentiation and eventually become irreversibly growth arrested. The regulation of IL-6 and IL-6 receptor mRNA expression during this process was studied. In exponentially growing cells IL-6 transcripts where undetectable. The phorbol ester (TPA) induced a rapid peak of IL-6 mRNA expression within 12 hrs, decreasing but still detectable during the time studied (5 days). Differentiation induced by retinoic acid (RA) or 1,25(OH)2vitD3 did not display this transient IL-6 expression and transcripts were undetectable during 6 days of induction. IFN-γ was also shown to induce a transient expression of IL-6 mRNA of a lower magnitude than TPA. The IL-6R mRNA is expressed in both uninduced and differentiated U-937 cells and shows only a slight reduction in differentiated cells, independent of which protocol is used. Since IL-6 mRNA was rapidly induced with TPA and IFN-γ , the possible function of IL-6 was examined. Exposure to rhIL-6 resulted in a dose dependent growth inhibition. Furthermore MHC class I antigen expression was shown to increase but not a late marker of monocytic differentiation, CD14. This indicates a role for the endogenously produced IL-6 during monocytic differentiation.

O 323 NOVEL GENES EXPRESSED DURING GM-CSF-STIMULATED MYELOPOEISIS, Amos Orlofsky, Mark S. Berger, and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

As part of a study of the regulation of gene expression during myelopoiesis, we have identified approximately 10 previously uncharacterized tissue-specific mRNAs that are expressed in murine bone marrow (BM) differentiating in vitro in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF). These messages had been selected by differential hybridization to be absent in the T-lymphoma cell line, EL4. We show that this is an efficient means of isolating BM-specific cDNA clones, and that, in addition to novel sequences, known myeloid-specific products are represented as expected. Time-course studies of expression during BM culture indicate that the novel BM-specific messages can be grouped into two sets that show distinct patterns of modulation during culture. One set is expressed early in culture, is absent in macrophages, and is thus likely to contain mRNAs specific to the granulocytic lineage. The second set increases during culture and is expressed in macrophages. Interestingly, the expression of several of the latter is dramatically stimulated early after initiation of culture, when mature macrophages are still uncommon. These may represent a cohort of genes under coordinate control early in myelomonocytic development. The isolation of such cohorts should facilitate the identification of regulatory elements that mediate developmental stage-specific events in myelopoeisis.

O 324 AUTO- AND PARACRINE GROWTH REGULATION OF HL-60-CELLS. P.E.Petrides, M.Kellerer, K.Dittmann, H.J.Gross, U.Wallner, B.Obermeier and H.U.Häring. Dep. of Medicine, Univ.of Munich, Clin. Hematology (GSF) and Diabetes Research Unit, Munich

HL-60-cells produce growth factors for autostimulation of proliferation but are still dependent upon the external stimulation by insulin like growth factors and transferrin. Modification of the culture conditions abrogates the requirement for exogenous transferrin since the endogenous production of transferrin like molecules is sufficient for growth. Optimal proliferation, however, is only observed when IGF-I is present as a paracrine stimulator. This dependence upon of an external growth factor could be explained by altered signal transduction in the leukemic cell. We have indeed found that HL-60 cells produce an altered tyrosine kinase β-subunit of the IGF-I-receptor (pp105 instead of pp95). HPLC tryptic mapping as well as deglycosylation experiments suggest that differences in primary structure are the cause for this difference. Since a similar IGF-I-receptor is also present in fetal tissues the malignant phenotype may be conferred by reexpression of the fetal version of a receptor for a physiological growth factor in an adult tissue. Supported by DFG (SFB 324, Hä 1229) and GSF (71967).

O 325 USE OF PLATELET FACTOR 4 (PF4) AS A MEASURE OF IN VITRO MEGAKARYOCYTOPOIESIS IN RESPONSE TO INTERLEUKINS AND OTHER CYTOKINES, Bruce M. Pratt, Louise Palmer, Kristen Perrott, Sara Yankelev, Ray Withy, Recombinant Protein Development Dept., Genzyme Corp., Framingham, MA 01701. Megakaryocytes were grown in vitro using 4 day cultures of acetylcholinesterase-negative rat bone marrow cells fractionated on discontinuous Percoll density gradients. Cells were cultured in MEMα + l-glutamine, penicillin, streptomycin, β-mercaptoethanol and 5% horse serum. Megakaryocyte development was assayed by the measurement of PF4 spontaneously secreted into the culture medium during the 4 day culture period. PF4 was quantitated with a double antibody capture (sandwich) ELISA using polyclonal rabbit anti-human PF4. The ELISA was capable of measuring rat PF4 between 0.5 to 6.0 ηg/ml. Incubation of the bone marrow cultures with noradrenalin (15 min, 37°C) at day 4, prior to collection of conditioned medium did not enhance the detectable release of PF4. The effects of several individual cytokines, including rat IL3, human IL1α, IL6, erythropoietin and TNFα, on PF4 production were examined. All cytokines examined produced positive, titratable responses in PF4 production. Specifically, the following fold increases over control were observed: IL1α 1ηg/ml, 2.8x; IL3 5U/ml, 2.8x; IL6 25U/ml, 2.2x; EPO 1.8U/ml, 2.1x; and TNFα 1ηg/ml, 3.2x. Currently, the effects of these cytokines are being examined in cultures which have also been depleted of stromal cells and/or T lymphocytes. Thus far, IL1α, IL3, and TNFα have given positive results in stromal-free cultures. This assay allows for a relatively rapid assessment of PF4 in bone marrow cultures, thus permitting higher sample throughput than previous assay methods. Furthermore, when used in conjunction with neutralizing antibodies to known cytokines, this assay should prove useful for the detection and purification of factors from conditioned media, or other sources, that affect megakaryocyte differentiation and maturation.

O 326 **NEUTROPHIL COLONY-STIMULATING ACTIVITY RELEASED BY PERIPHERAL BLOOD LEUKOCYTES IN RESPONSE TO SERUM FROM APLASTIC ANEMIA PATIENTS - ATTEMPTS TO CHARACTERIZE THE STIMULUS AND THE PRODUCT**, Valerie F.J. Quesniaux, Susi Wehrli, Irma Ziegler, Hiroshi Ohtsu, Beatrice Legendre, William Wishart, Barbara Fagg and Catherine Nissen, Sandoz Preclinical Research and Department of Research, University Hospital, Basel, Switzerland.
The serum of aplastic anemia patients has been shown to induce human peripheral blood leukocytes (HuPBLs) to produce a neutrophil colony stimulating activity for human bone marrow. We started dissecting the different components of this system to ask whether the "releaser" activity present in aplastic anemia serum and the induced neutrophil colony stimulating activity represent new or known hematopoietic growth factors.
In order to identify and quantitate the known growth factors present in the aplastic anemia sera and in the medium conditioned by HuPBLs incubated for 2 days with or without aplastic anemia serum a variety of bioassays was used. The expression of growth factor messenger RNA by these stimulated or unstimulated HuPBLs was also analyzed. No IL-1, IL-2, IL-3, IL-4, or IL-6 could be detected in aplastic anemia sera, although low amounts of GM-CSF and G-CSF were present. Unstimulated PBLs alone produced low amounts of IL-1, IL-6, and G-CSF, and this production was enhanced 4-10 fold after incubation with aplastic anemia sera.
The individual involvement of these factors in the observed activities is being examined by means of 1) experiments with specific neutralizing antibodies and 2) trying to reproduce the activities by combinations of the factors. IL-1 seems to be a necessary intermediate since the induction of neutrophil stimulating activity is partially blocked by anti IL-1 neutralizing antibodies although IL-1 alone cannot reproduce the "releaser" activity.

O 327 CORRELATIONS BETWEEN SPONTANEOUS IN VITRO IL-1/IL-6 PRODUCTION AND HEMATOLOGIC CHARACTERISTICS IN DE NOVO AML PATIENTS, Salamon J., Urbanski A., Köller U., Lutz D. Ludwig Boltzmann Institute for Leukemia Research and Hematology, Hanusch Hospital, A-1140 Vienna, Austria.
Blasts derived from patients with acute myeloid leukemia can produce a variety of cytokines in vitro, including Interleukin 1 (IL-1) and Interleukin 6 (IL-6). We have undertaken an analysis correlating the in vitro IL-1 and IL-6 production with characteristics of AML blasts, haematologic data and the clinical outcome of the disease. 23 blast populations derived from blood or bone marrow were cultured in serum free medium and assayed for IL-production. 12/23 samples produced IL-1 spontaneously in vitro (group I) and 11 did not (group II). In group I there was a higher incidence of monoblastic leukemias (8/12 vs 5/11, resp.). Immunophenotyping revealed that blasts of group I had a higher surface expression of CD11b, CD13 and CD14, but HLA-DR, CD15 and CDw65 expression was identical. Comparing both group, the peripheral blast count was higher in group I patients (median 19.500 vs 9100/µl $p<.05$), as was serum LDH (median 955 vs 346 U/ml, $p<.05$); the median age was not different (41 vs 46, $p>.2$). The clinical outcome was poor in group I (5/11 treated patients reached a CR vs 10/10; $p<.05$). Regarding the in vitro IL-6 production, no such correlations were found. Our results indicate that in vitro IL-1 production by leukemic blasts may identify a subgroup of patients with unfavorable prognosis.

O 328 A BIFUNCTIONAL MNC-DERIVED FACTOR FOR TUMOR CELL GROWTH: ACTIVITY ON HUMAN COLONIC TUMOR CELL LINES, George Sandru[1], Peter Veraguth[1], Beda Stadler[2], Bernard Sordat[3], [1]Radiobiology Laboratory, [2]Institut of Immunology, University of Bern, [3]Cancer Research Institute, Epalinge, Switzerland.
A MNC-derived factor which stimulates growth of some human hematopoietic and nonhematopoietic tumor cell line cells has previously been reported (Cancer Res.,1988,48,5411). This growth stimulatory activity was detectable on human colonic tumor cell line cells such as SW 620, SW 480, Co 115, Co-Sut, and HT-29, either under serum-free conditions or in the presence of small concentrations of FCS, or of FCS inactivated for SH-containing growth factors by dithiothreitol, or of platelet-poor plasma-derived serum. The same conditioned media from MNC cultures or chromatographically purified fractions thereof inhibited growth induced by FCS in parallel identical cultures at either 24 h, 48 h, 72 h, or 96 h of incubation. Some known growth factors and cytokines such as bFGF, EGF, PDGF, TGF-beta, GM-CSF, IL1-alpha, IL1-beta, IL2, IL3 and IL6 seemed to be different from this activity based on biological characteristics and the use of specific antibodies. Purification is in progress.
This work was supported by grants from the Bernese Cancer Liga (# 2.02.1989), and the Swiss National Science Foundation (# 31-25707.88).

O 329 LYMPHOKINE REGULATION OF GROWTH AND FUNCTION IN AN IL-2-DEPENDENT HUMAN ACUTE T LYMPHOCYTIC LEUKEMIA (T-ALL) CELL LINE. Daniela Santoli, Rosemary O'Connor, Alessandra Cesano, Beverly Lange, Steven Clark and Giovanni Rovera, The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA 19104

The TALL-103/2 cell line was established from the marrow of a child with an immature T-ALL having the t(8;14) translocation and biphenotypic (T-myeloid) differentiating capacity. The leukemic cells first acquired a myeloid phenotype upon expansion in rh IL-3, and subsequently became lymphoid committed by adaptation to growth in rhIL-2. The myeloid to lymphoid lineage switch of the IL-2-maintained TALL-103/2 cells was documented by their exclusive surface expression of T cell-specific differentiation antigens (CD2, CD3, CD7, and CD8) and of the non-disulfide-linked form of the TCRγδ. The karyotype of the TALL-103/2 cell line is identical to that of the original malignant clone present in the bone marrow. In addition, genotypical analysis shows rearrangements of the TCR γ and δ loci. The TALL-103/2 cells are strictly and exclusively IL-2-dependent, and, as determined by chemical cross-linking studies, they express both low (p50) and intermediate (p70) affinity IL-2 R. The effects of various lymphokines on the IL-2-dependent growth of TALL-103/2 cells were investigated. IL-1α synergizes with IL-2 in supporting the short- and long-term growth of this cell line: these effects are, at least in part, due to the significantly increased expression of IL-2R (Tac protein) induced by IL-1α. IL-4, in contrast, displays a dose-dependent inhibition on the IL-2-dependent proliferation of TALL-103/2 cells. Functionally, the TALL-103/2 cells are endowed with MHC-nonrestricted cytotoxic activity which is significantly enhanced by addition of either IL-4, IL-6, or IFN-γ. Because of its properties and its stable requirement for IL-2 for continuous growth, this T-ALL-derived cell line represents an interesting model to analyze ontogeny of leukemic T cells as well as growth promoting and inhibiting effects of various cytokines.

O 330 NEURAL INDUCTION IN VITRO IS INHIBITED BY AMILORIDE-DEXTRAN, Amy K. Sater and Richard A. Steinhardt, Division of Cell and Developmental Biology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720. In *Xenopus*, neural induction arises by a combination of two signalling systems: early signals from the dorsal lip of the blastopore up through the plane of the tissue to the adjacent dorsal ectoderm, and later vertical signals that reach the dorsal ectoderm from the underlying chordamesoderm once it has involuted during gastrulation. We have found that culture of explants that include the dorsal lip and the dorsal ectoderm can permit the induction of neuronal differentiation via the early planar signals alone. We have used this explant culture system to examine the effects of amiloride-dextran (A-35), an extracellular inhibitor of Na^+/H^+ exchange, on the response to neural induction. Treatment of explant cultures with 20 μM A-35 early in gastrulation (st. 10-10.5) inhibits the response to neural induction: 23% of treated explants show neuronal differentiation, as compared with 85% of untreated controls. Addition of A-35 to explants prepared at the end of gastrulation has no apparent effect on neuronal differentiation. The convergent extension movements characteristic of both the chordamesoderm and the prospective neural ectoderm are not prevented by treatment of explant cultures with A-35. These results suggest that the regulation of intracellular pH and/or intracellular Na+ levels may participate in the initial response to neural induction.

O 331 AN INDUCIBLE NUCLEAR FACTOR BINDS TO AN NF-KB ELEMENT IN THE MACROPHAGE COLONY STIMULATING FACTOR (M-CSF) PROMOTER IN THE HL-60 PROMYELOCYTIC CELL LINE. Richard A. Sater[1] and James E. Niedel[2], [1]Dept. of Pharmacology, Duke University, Durham, NC, [2]Glaxo Inc., Research Triangle Park, NC.

Phorbol esters, calcium ionophore (A23187) and Tumor Necrosis Factor (TNF) induce M-CSF gene expression in HL-60 cells. Interaction of the M-CSF promoter with nuclear factors may mediate part of this induction. Gel retardation assays reveal multiple DNA-protein complexes between HL-60 nuclear extracts and the 5' flanking region of the M-CSF gene. Treatment of HL-60 cells with PMA (32 nM, 2 or 24 hours), A23187 (1 uM, 4 hrs) and TNF (100 U/ml, 4 hrs.) induce a protein that binds an 87 base pair DNA probe extending from -77 to +10 of the M-CSF promoter. This protein is also inducible by 4 hour treatment with 10 ug/ml cycloheximide, indicating a post-translational action. The protein can be partially purified by elution off a P11 phosphocellulose column with 0.3 M KCl. DNAse I and Copper/phenanthroline footprinting show that the partially purified protein protects the probe from -66 to -57 (AGGGTGATTT). This sequence overlaps a stretch of DNA with 10/11 identity to the NFKB/H2TF1 consensus element based on the sequences found in the kappa immunoglobulin light chain and the murine MHC Class I genes. A synthetic double stranded oligomer comprising the NFKB element competes more effectively against the M-CSF promoter probe than an H2TF1 oligomer. Although the K562 cell line expresses M-CSF, this factor is not found in K562 control or PMA treated extracts. The U937 cell line does not express M-CSF and does not contain this factor. Studies are underway to determine if this cell-specific nuclear factor is NF-KB, H2TF1 or another member of that family. Stable transfection of promoter/marker constructs into HL-60 cells will determine the functional significance of the DNA-protein interaction.

O 332 HORMONAL REGULATION OF GLUCOSIDASE I DURING THE ONTOGENY OF THE RAT MAMMARY GLAND, K. Shailubhai, E.S. Saxena, A.K. Balapure and I.K. Vijay, Department of Animal Sciences, University of Maryland, College Park, MD 20742.

Glucosidase I catalyzes the first step in the processing of N-linked glycoproteins. It is critically positioned between the dolichol-linked assembly of the tetradecasaccharide $Glc_3Man_9GlcNAc_2$ and its protein linked post-translational modifications. Thus, it could play an important role in the overall regulation of glycoprotein biosynthesis. Developmental changes in hormonally responsive tissue are usually associated with the enhanced synthesis of N-linked glycoproteins. The mammary gland serves as an excellent model system for studying the developmental regulation of N-glycosylation. Our results indicate that the synthesis of glucosidase I is modulated during the ontogeny of the gland. Upon lactogenesis, the levels of the enzyme increase seven-fold compared to those in the virgin tissue. Using rat mammary organ cultures, we demonstrate that the synthesis of glucosidase I is regulated by a multiple interaction of lactogenic hormones, viz., insulin (I), hydrocortisone (F), and prolactin (P). Insulin alone stimulated glucosidase I synthesis significantly and this stimulation was severely inhibited by F. P along with I further enhanced the levels of the enzyme and inclusion of F in combination with I and P resulted in maximum induction. The implications of the results with overall hormonal modulation of N-linked glycoprotein biosynthesis will be discussed. Supported by Maryland Agricultural Experiment Station and a grant DK-19682 from N.I.H.

O 333 DIFFERENTIAL RESPONSE OF HUMAN AND MOUSE GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTORS, Armen B. Shanafelt and Robert A. Kastelein, DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304. Human and mouse granulocyte-macrophage colony stimulating factors (GM-CSF) are extensively homologous proteins, yet no cross species reactivity is observed. Detailed mutagenesis of human and mouse GM-CSF revealed distinctly unique regions which are implicated in the biological response. Deletion analysis of the two homologues (1, 2) identified small individual stretches of amino acids putatively responsible for species specificity: amino acid residues 77 to 82 in human GM-CSF, and 34 to 41 in mouse GM-CSF. Response patterns of human/mouse GM-CSF hybrid proteins in *in vitro* assays also reflected characteristic differences between each species. These results suggest that the interaction of each cytokine with its respective receptor is not equivalent between these two species.

1. Shanafelt, A. B. and Kastelein, R. A., "Identification of critical regions in mouse granulocyte-macrophage colony-stimulating factor by scanning deletion analysis" (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4872-4876.

2. Shanafelt, A. B., Dang, W. and Kastelein, R. A., "Human and mouse granulocyte-macrophage colony stimulating factor: Identification of two unique regions involved in species specificity" (1989) *Submitted for publication.*

O 334 GENE EXPRESSION DURING MURINE FETAL THYMIC DEVELOPMENT, Pierette M. Shipman-Appasamy, and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104

A number of events common to developing systems take place in the fetal thymus, i.e., cell-cell interactions, proliferation, and differentiation. Processes specific to the thymus include development of: tolerance to self-antigens and MHC-restricted reactivity to foreign antigens, as well as T cell-specific helper and cytotoxic functions. An approach to identifying the molecular mechanisms underlying these processes is the identification of genes which are regulated and possibly regulatory during during fetal thymic development; cell biological and molecular approaches may then be utilized to determine the role and mechanisms of regulation of these molecules. Therefore, we have identified a number of genes whose expression is regulated at the RNA level in the murine fetal thymus. Using Northern blot analysis, we found that steady-state levels of *lck* RNA, which encodes a *src*-like lymphocyte-specific tyrosine kinase ($p56^{lck}$), increase 6-fold in CBA/J fetal thymic lobes from days 14 through 18 of gestation, and are expressed at low levels in the day 14 fetal liver (the source of pre-T stem cells). Levels of RNA encoding proliferating cell nuclear antigen (PCNA), an auxiliary protein for DNA polymerase delta, also increase (6-fold), reflecting rapid cell proliferation. In contrast, levels of RNA encoding lipocortin I, a phospholipase A_2 inhibitory protein, did not change significantly in the fetal thymus. Levels of RNA encoding the ß-chain of the T cell receptor increased 12-fold during development from day 14 to day 18; these data are consistent with the work of others demonstrating that rearrangement and expression of T cell receptor ß (and alpha) chain genes take place during differentiation of lymphoid cells within the fetal thymus.

O 335 TRANSGENIC MICE: A MODEL SYSTEM TO STUDY LYMPHOHEMATOPOIETIC GROWTH FACTORS IN VIVO, G. Sigounas[1], T.J. MacVittie[1], and R. Jaenisch[2], Armed Forces Radiobiology Res. Inst.[1], Bethesda, MD 20814, Whitehead Inst. Biomed. Res.[2], Cambridge, MA 02142. Efforts to understand hematopoietic stem cell (HSC) regulation in vivo have been greatly impeded by an inability of marker systems to repeatedly analyse several distinctly marked stem cells within a single animal. To monitor the proliferation and differentiation of stem cell progeny, a mixture of bone marrow cells from multiple strains of transgenic mice, derived from a common genetic background, was used as donor to reconstitute irradiated wild type mice. Using molecular detection techniques, we documented fluctuations of various transgenic donor cells in the blood of individual recipients over a 14 month period. Analysis of multiple donor origin cells from spleen, thymus, peritoneal exudates and bone marrow of the engrafted animals clearly show that the HSC compartment is extremely heterogenous and is characterized by a developmental hierarchy. At least four clones may be continuously activated and contribute to the reconstituted hematopoietic system. Two weeks post-transplantation, transgenic cells from most of the donors were present in the hematopoietic tissues of the recipients. Two months following the engraftment, the blood cells of recipient animals were chimeric for the recipient and donor cells. Transgenic marrow cells, either treated or untreated in vitro with growth factor (IL-3) produced spleen colonies (CFU-S) and co-participated in the reconstitution of the lymphohematopoietic system of lethally irradiated recipients. Therefore, the transgenic markers should facilitate the in vivo analysis of stem cell development and the influence of hormones/growth factors on these processes.

O 336 EFFECTS OF HUMAN C-FMS EXPRESSION ON MOUSE HEMATOPOIETIC STEM CELLS AND PROGENITORS, Lisa Spain and Richard Mulligan, Whitehead Institute and the Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142

The human c-fms gene encodes the CSF-1 (MCSF) receptor, a ligand dependent tyrosine kinase expressed on macrophages and macrophage progenitors. It is well established that such cells require MCSF for survival, proliferation, or function, but there is little known about the role, if any, of MCSF on the committment and differentiation of progenitors and stem cells. In order to address this question, we have used a retroviral vector to transduce and express the human c-fms gene in mouse hematopoietic stem cells and progenitors. The human gene was chosen since it does not bind or respond to mouse MCSF, and thus expressing cells may be assayed in vitro and in vivo.

c-fms expressed in the IL3 dependent hematopoietic cell lines BAF3 and 32D allows human MCSF to replace IL3 as a growth requirement. In addition, expression of the c-fms gene also completely abrogates factor dependence of a subset of infected cells, making them ligand independent. Expression of c-fms in primary IL3 dependent cells derived from infected mouse bone marrow does not abrogate factor dependence, and allows MCSF to substitute, at least partially, for IL3 as a growth requirement for these cells.

Bone marrow cells infected with the c-fms and control Tac retroviral vectors were selected by FACS for expression of the transgenes. Positive cells were injected into lethally irradiated mice in a spleen colony assay. The control cells formed spleen colonies with the same efficiency as unsorted cells, and the spleen colonies were infected, indicating that the vector expressed the gene in stem cells. In contrast, the c-fms expressing cells were totally depleted of colony forming activity in 2 of 3 experiments, indicating that c-fms may have a ligand independent differentiative or toxic effect on stem cells.

Positive cells were also assayed in vitro for colony formation and growth in response to ligand. The bone marrow cells expressing the c-fms receptor grew in response to MCSF and also maintained morphological complexity when compared to controls, suggesting that c-fms can function to support proliferation, and perhaps differentiation, in a variety of bone marrow cells.

O 337 RETROVIRUSES AS TOOLS TO STUDY THE GENES REGULATING NORMAL AND ABERRANT HEMATOPOIESIS, C. Stocking, C. Laker, C. Heberlein, M. Kawai*, G. Beck-Engeser, and W. Ostertag, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie, 2000 Hamburg 20, FRG, *Dept. of Microbiolgy, Aichi Medical University, Nagakute-cho, Aichi 480-11, Japan It has long been postulated that leukaemia develops from the accumulation of genetic lesions in a hemato-poietic stem cell which results in the abrogation of the normal controls that regulate the balance between proliferation, differentiation, and self-renewal. We have utilized retroviruses both as vectors and as insertional mutagens to identify and characterize genes whose aberrant expression leads to altered growth control of hematopoietic cells. In a first series of experiments, factor-independent mutants of a myeloytic cell line were isolated and characterized. In the majority of mutants, the acquistion of factor-independent growth could be contributed to activation of one of the known growth factor genes (GM-CSF or IL-3) by a transposable element. Introduction of the cDNA for one of the two growth factors by retroviral vector into either progenitor or stem cell lines lead to factor-independent growth but not true autonomous growth, although the frequency at which progenitor cells became autonomous was increased by four orders of magnitude as compared to cells which did not express a growth factor. Autonomous growth could be correlated with tumorigenic growth in progenitor but not multipotent cells. New mutants have been isolated from an IL3-dependent, progenitor cell line after retroviral infection. These mutants were selected on basis of their altered growth-factor response or factor-independent growth and were obtained at a frequency ten-fold higher than the spontaneous frequency. Thus the analysis of common retroviral integration sites should lead to the identification of other genes that play pivotal roles in normal and abnormal hematopoiesis.

O 338 EXPRESSION OF A MARROW STROMA AND THYMUS-ASSOCIATED ANTIGEN (ST3) IN THE RAT BRAIN: COMPARISON WITH THY-1. Arthur K. Sullivan, Ela Sharma, Alain Beaudet, and Linda L. Bambrick, McGill Cancer Centre, McGill University, the Division of Hematology, Royal Victoria Hospital, and the Neuroanatomy Laboratory, Montreal Neurological Institute, McGill University. Montreal, Canada H3G 1Y6.

Cells of the immunohemopoietic and nervous systems express certain molecules that generally are not found in other tissues. One example is the "ST3" antigen, which is present on the major population of fibroblastoid cells grown from rat bone marrow, but is not detected on adherent cells from most peripheral organs (e.g., lung). An immunohistological survey revealed ST3 also in the thymic cortex, the glomerular mesangial area, and the brain. Because this pattern of distribution is similar to that described for Thy-1, we compared the localization of the two antigens in the adult rat brain and found that there were areas where it was congruent and others where it was distinct. Staining for ST3 was absent from the white matter, but was especially notable in discrete layers of the frontal, orbital, parietal, and cingulate cortices, the substantia nigra, the inferior olivary nuclei, and the deep molecular layer of the cerebellum, as well as other scattered regions in the gray matter. This is in contrast to Thy-1, which stained more diffusely throughout the gray zones. In further experiments using primary brain cell cultures, ST3 was demonstrated on neurons, but not on oligodendrocytes or astrocytes. Similarly, it was found on the surface of cells of the PC12 neuronal line, but not on the C6 astrocytoma. This restricted distribution on a subpopulation of neurons raises the possibility that the ST3 epitope might be part of a cell interaction molecule of the marrow stroma, thymus, and brain.

O 339 HUMAN NEUROBLASTOMA: AN IN VITRO MODEL SYSTEM TO STUDY MOLECULAR MECHANISMS REGULATING NEURAL OR CHROMAFFIN CELL DIFFERENTIATION Carol J. Thiele, Carlo Gaetano, Kazue Matsumoto and Mark Israel, Pediatric Branch, National Cancer Institue, Bethesda, MD.

Human neuroblastoma (NB) are pediatric embryonal tumors derived from the neural crest. The neural crest gives rise to a wide variety of cell lineages including the neurons and glia of the peripheral nervous system, chromaffin cells of the adrenal gland and melanocytes. Studies indicate that NB tumors may correspond to cells derived from the sympathoadrenal lineage arrested at different stages of differentiation. The ability of retinoic acid (RA) to induce NB cells to differentiate into neural cells provides an in vitro model system in which to study the molecular mechanisms specifying neural or chromaffin lineages. The SMS-KCNR NB cell line expresses tyrosine hydroxylase (TH), NCAM, and Chromogranin A (CgA) mRNA, compatible with a neuroendocrine phenotype. 2-4 days after culture in 5uM RA, cell growth is arrested in the G, phase of the cell cycle and is accompanied by a decrease in cyclin and ornithine decarboxylase mRNA. Expression of NCAM is unchanged, CgA is transiently decreased and TH increases at this time while the expression of neuronal genes, GAP43, neurofilament (NF) and synaptophysin is markedly increased. These increases in neural gene expression coincide with signs of morphologic differentiation as evidenced by neurite extension and formation of neuritic bundles. Subtractive cDNA cloning is being utilized to isolate genes expressed in neuronally differentiated NB cells in order to identify genes which may be important in regulating differentiation along chromaffin or neural cell lineages.

O 340 POSITION SPECIFIC GROWTH OF 3T3 CELLS IN THE DEVELOPING MOUSE LIMB. Chris Trevino, Anne Calof[1] and Ken Muneoka, Dept. of Biol., Tulane Univ., New Orleans, LA 70118, [1]Neuroscience Program, Tufts Univ. School of Medicine, Boston, MA 02111.

Vertebrate limb development is characterized by a highly reproducible spatial pattern of cell proliferation. To investigate the relationship between position and growth *in vivo*, 3T3 cells were used as probes to monitor the growth regulatory influences that exist during limb development. 3T3 cells were genetically labeled by infection with a replication defective retrovirus that causes the expression of the human surface glycoprotein CD8 (CD83T3). Using exo-utero surgical techniques CD83T3 cells were injected into different positions of the developing mouse hindlimb bud. To investigate position specific growth ^{3}H-thymidine was introduced into the embryos 24 and 48 hours after cell injections. CD83T3 cells were identified immunohistochemically (MAb OKT8) and tissue sections were processed for autoradiography. Our results reveal that the growth of the injected CD83T3 cells correlates positively with that of surrounding limb cells over a labeling index range of 7% to 36%. The observed growth difference of CD83T3 cells *in vivo* is not related to contact inhibition since a comparative analysis of regions with high and low labeling indices showed identical cell densities. These results suggest that growth regulation during limb development is not cell autonomous but involves spatially localized growth regulatory cues present in the limb environment. In addition, these data demonstrate that cells that have been maintained *in vitro* for an extended period of time are still competent to respond to developmental signals present in the *in vivo* environment. This latter point has important implications for the validity of the use of established cell lines to probe developmental signals present in the *in vivo* environment. Supported by HD20662, HD23921, LEQSF(86-89)RDA01 and a gift from Monsanto Company.

O 341 LOCALIZATION OF THE MORPHOGEN DIF IN DEVELOPING *DICTYOSTELIUM.*

Gerald Weeks, Linda Kwong and Jennifer Xie, Department of Microbiology, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

The pseudoplasmodia of developing *Dictyostelium* is made up of two cell types, prestalk cells and prespore cells, in a distinct spatial pattern. We have utilized monolayers of isolated prestalk and prespore cells to determine which of the two cell types accumulates the potential morphogen, DIF. Unfortunately, monolayers of the wild type strain, V12-M2, rapidly dedifferentiated. Despite this dedifferentiation, prestalk cells accumulated DIF earlier than prespore cells, suggesting the possibility that prestalk cells generate DIF.

Prestalk and prespore cells of the sporogenous mutants HM18 and HM29 isolated on percoll gradients rapidly differentiated into stalk and spore cells respectively, suggesting minimal dedifferentiation in the monolayers. The low density (presumptive prestalk cell) populations of these strains accumulated 6-10 times more DIF than did the high density (presumptive prespore cell) populations. The low density cells contained the prestalk cell specific mRNAs (pDd63, pDd56, pDd26 and D11) and did not contain any prespore specific mRNA (D19). No dedifferentiation occurred during monolayer incubation. The high density populations contained the expected D19 mRNA, but in addition large amounts of pDd63 and D11 mRNA. They did not, however, express pDd56 and pDd26. These results suggest that DIF is generated by a subpopulation of prestalk cells that express pDd56 and pDd26.

O 342 A $p21^{ras}$ AND SERUM-INDUCED GENE PRODUCT WITH SIMILARITY TO THE HUMAN TUMOR MARKER CARCINO EMBRYONIC ANTIGEN (CEA)

A.K.Werenskiold, Gesellschaft für Strahlen-und Umweltforschung, Dept. Cellular Chemistry, Ingolst. Landstr.1, 8042 Neuherberg, FRG.

A cDNA clone, T1, has been isolated whose corresponding mRNA is transiently expressed at highly elevated levels after the conditional expression of the Ha-*ras*(EJ) and v-*mos* oncogenes and after mitogenic activation of quiescent NIH3T3 cells. Expression of T1 or T1-related transcripts was detected in primary fibroblasts of rodent and human origin, indicating an evolutionary conservation of this gene. Furthermore, an elevated expression of T1-related mRNA was observed in various human tumor cell lines. The derived primary structure of the T1 gene product predicts a 38kDa protein with features of a secreted or membrane associated glycoprotein. Its sequence bears similarity to the carcino embryonic antigen (CEA), an established tumor marker. The identification and biochemical characterization of the T1 gene product is in progress. Implications of the data on expression and function of this first oncogene inducible CEA-related molecule for the role of other members of this family of tumor markers will be discussed.

O 343 EFFECTS OF NITROGEN MUSTARD ON CELLULAR MORPHOLOGY AND PROLIFERATION,

June M. Whaun[1]. Nesbitt D. Brown[2], Division of Pathology[1], Division of Biochemistry[2]. Walter Reed Army Institute of Research, Washington, DC 20307 and John H. Darden, Elsa A. Chock, and Joe L. Parker, Dept. of Experimental Hematology, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Nitrogen mustard, an alkylating antiproliferative agent, is used in the treatment of neoplastic disease. We investigated the effects of nitrogen mustard on the proliferation of Friend erythroleukemia cells. This cell line is a virally transformed poorly differentiated proerythrocytic line. These cells can be induced to differentiate when exposed to a number of agents. Cells of clone DS19 were cultured in the presence of 10^{-7}M nitrogen mustard for up to five days. Samples were removed at appropriate times for preparation of extracts for polyamine analyses as well as adenosine and S-adenosylmethionine (AdoMet) determinations. Other aliquots were prepared for cytologic examination. Within 1 minute of exposure, mustard-treated cells showed decreased putrescine but elevated spermidine and spermine levels. By 10 minutes, all 3 polyamine levels rose, then fell at 30 min. Adenosine levels rose at 2 hr. but subsequently fell at 5 and 24 hr. AdoMet levels remained elevated at 2, 5, and 24 hr. In the presence of DMSO, the antiproliferative and morphological changes induced by mustard were attenuated. DMSO-induced differentiation in this erythroleukemia cell line can occur in the presence of nitrogen mustard. These studies underline the usefulness of culture systems to help define structural changes and mechanisms of actions of drugs and agents.

O 344 ECTOPIC EXPRESSION OF A PARTIAL LENGTH HOMEO BOX CONTAINING FRAGMENT CAUSES NEURAL DEFECTS IN THE MEXICAN AXOLOTL.

Mary Whiteley and John B. Armstrong, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

A homeo box containing DNA fragment was isolated from the Mexican axolotl. This clone was obtained from a partial genomic library enriched for sequences which cross-hybridized with the Drosophila Antp homeo box under low stringency hybridization conditions. DNA sequence analysis revealed that this sequence (Ahox1) was most closely related to the mouse Hox-1.6 homeo box (84% identity) and to the Drosophila labial (79% identity) homeo box sequence. Transcripts hybridizing to the Ahox1 homeo box probe were detected in a specific tissues in neurula and tail bud embryos. Expression of exogenous sequences, using a lacZ fusion construct (hsp68 promoter of mouse) revealed that exogenous sequences were expressed in high copy number, and in a large proportion of cells. To see if this system could be used to perturb development, a 560 bp KpnI fragment of Ahox1 containing the homeo box, cloned to be expressed under control of the mouse hsp68 promoter was microinjected. Defects in embryos injected with this construct were first observed in neurulation, with an abnormal neural plate being formed. These embryos developed with severely reduced heads. As a control a construct with the DNA binding helix of the homeo box removed was prepared. No defects were observed with this construct.

O 345 EXPRESSION AND ALTERNATIVE SPLICING OF TRANSCRIPTS ENCODING THE MURINE IL-4 RECEPTOR. Nick Wrighton, Atsushi Miyajima, Nobuyuki Harada, Dan Gorman, Maureen Howard and Frank Lee. DNAX Research Institute, 901 California Avenue, Palo Alto, CA 94304. Recently, cDNAs encoding the murine IL-4 receptor have been cloned (1, 2). However, in addition to molecules encoding the full-length receptor, cDNAs encoding alternative forms of the protein have also been found. One class of these contain a stop codon 5'- to the transmembrane domain and thus could give rise to a soluble, truncated form of the receptor molecule (1). Additional cDNAs have also been isolated that potentially encode yet more variant forms of the protein (3). In order to study the origin, the relative abundance, and potential biological significance of the different forms of the IL-4 receptor, S1 probes specific for the various mRNA species were generated and applied to the analysis of IL-4 receptor expression in different cell lines and tissues. In addition, a genomic clone of the receptor was isolated. Southern blot analysis using probes specific for the variant regions of the cDNAs and DNA sequencing were then used to investigate the possibility that expression of the receptor gene is regulated, in part, by alternative splicing.

1. Mosley *et al.*, (1989). Cell 59, 335-348.
2. Harada *et al.*, in press.
3. Harada *et al.*, unpublished results.

O 346 CLONED ATRIAL SPECIFIC MYOSIN HEAVY CHAIN CDNAS FROM THE DEVELOPING CHICKEN HEART, Steven R. Wylie and David Bader, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021. In order to characterize the developmental expression of avian cardiac MHCs, atrial MHC cDNA clones were isolated from a lambda GT10 library prepared from 14 day old embryonic atrial tissues. 4 clones ranging in size from 2.0 to 2.5 kilobases, were used for analysis of MHC expression. Northern blot analysis of total RNAs derived from atrial and ventricular tissues of 9 and 15 day old embryos, 2 days posthatched and adults, indicated that 3 of these clones are atrial-specific in the heart throughout development. In addition, these cDNAs also hybridized to adult Pectoralis total RNA, but not to smooth muscle or brain total RNAs, under similar conditions. Furthermore, the other clone showed partial hybridization to ventricular RNAs after age 9 days in the embryo in addition to strong hybridization to atrial RNA. These preliminary data suggest the occurence of an atrial specific MHC transcript, as previously indicated using protein analysis (1,2) and the presence of a similar MHC, with a more general distribution in developing cardiac tissue, as proposed (3). Restriction and sequence analysis are underway to determine the identity of these clones.

1.Gonzalez-Sanchez A, Bader D: Dev. Biol. 103: 151-158, 1984.
2.Sweeney LJ, Zak R, Manasek FJ: Circ. Res. 61: 287-295, 1987.
3.Evans D, Miller JB, Stockdale FE: Dev. Biol. 127: 376-383, 1988.

O 347 FAILURE TO DOWN-REGULATE RB IN DIFFERENTIATION DEFECTIVE HL-60 CELLS, A. Yen, R. Pearson, Y.K. Fung, S. Chandler and M.E. Forbes, Department of Pathology, Cornell University, Ithaca, NY 14853 and Department of Hematology/Oncology, Children's Hospital of Los Angeles, Los Angeles, CA 90027

HL-60 human promyelocytic cells normally undergo terminal differentiation along either the myeloid or monocytic pathways in response to various chemically defined inducers. A differentiation defective variant HL-60 cell fails to undergo terminal differentiation in response to sodium butyrate which is normally an inducer of monocytic differentiation. While wild type HL-60 cells down regulate the Rb gene product early during the metabolic cascade leading to either myeloid or monocytic differentiation, the variant cells fail to down regulate expression of the Rb gene product in the case of this inducer. The variant cells retain the capability to undergo myeloid differentiation in response to retinoic acid and monocytic differentiation in response to 1,25-dihydroxy vitamin D3. In these cases differentiation is anteceded by down regulation of Rb. Thus in this variant cell the ability or inability to differentiate correlate with an earlier ability or inability to down regulate Rb, the retinoblastoma anti-oncogene.

Steroid Hormones; Retinoids; Nuclear Factors

O 400 EXPRESSION OF ERA-1/HOX 1.6 PROTEINS DURING EMBRYONIC DIFFERENTIATION. Allison J. Ait-Aouane, Lan Hu, Lorraine J. Gudas. Dana Farber Cancer Institute, Boston, MA 02115. Retinoic Acid (RA) can induce some cell types, including murine F9 teratocarcinoma stem cells, to differentiate in culture. The F9 model differentiation system can be used to study the molecular mechanisms of differentiation and early embryonic development. We previously isolated two cDNA clones for a gene, ERA-1, which has the characteristics of an early, direct target of RA action. ERA-1-993 encodes a protein with predicted mass of 36 kilodaltons (kDa) that possesses the Hox 1.6 homeobox domain. ERA-1-399 lacks 203 bp within the ERA-1-993 open reading frame due to an alternate splice site and encodes a 15 kDa protein that lacks the homeobox domain and differs in its carboxy terminus. (LaRosa and Gudas. Mol.Cell Biol. 8:3906-3917, 1988). In order to study the proteins further, polyclonal antibodies have been generated using both synthetic peptides coupled to KLH and β-galactosidase -ERA-1 fusion proteins. The synthetic peptides and the fusion proteins were designed to generate antibodies recognizing either the homeobox containing protein, the truncated protein, or both. By Western analysis, using antisera directed against the homeobox containing protein, a protein of approximately 48 kDa is detected; this protein is present in extracts of F9 cells that have been treated with RA and absent in untreated cell extracts. This same antisera recognizes a doublet present in ERA-1-993 transfected Cos-1 cell extracts on a Western blot. Experiments are in progress to detect the ERA-1-399 encoded protein, to analyze the possible post-translational modifications of the proteins, and to study their expression during embryonic development.

O 401 MOLECULAR ANALYSIS of PAX1 in UNDULATED MICE.

Rudi Balling, Urban Deutsch and Peter Gruss, Max Planck Institute of Biophysical Chemistry, Department of Molecular Cell Biology, 3400 Göttingen, FRG. Pax1, a mouse paired box containing gene, was identified on the basis of sequence similarity to Drosophila segmentation and segment-polarity genes (Deutsch et al. 1988). Pax1 is expressed during mouse embryogenesis in the anlagen of the intervertebral discs, the sternum and the thymus. *Undulated(un)*, a mouse mutation affecting the development of the vertebral column, is located in the chromosomal region where Pax1 maps and was found to have a point mutation in the paired box of Pax1(Balling et al.1988). We have analyzed two additional alleles of *un* for alterations in the genomic structure and/or transcription of the Pax1 gene. A comparative molecular and embryological analysis of the *un*-alleles will be presented.

O 402 ISOLATION OF GENES REQUIRED FOR AFP INDUCTION DURING PC13 CELL DIFFERENTIATION. *Marisa S. Bartolomei and Shirley M. Tilghman, Department of* Biology, Princeton University, Princeton, NJ 08544. The alpha-fetoprotein (AFP) gene encodes a fetal serum protein that demonstrates tissue-specificity and temporal regulation during development in the mouse. The PC13 cell line is a mouse embryonal carcinoma cell line that can be used to study the induction of AFP gene expression. PC13 stem cells do not express AFP under normal cell culture conditions but when stimulated with retinoic acid to differentiate into cells of the visceral endoderm, the AFP gene is expressed. It is our aim to isolate genes that encode products that directly or indirectly activate the AFP gene. PC13 cell lines were constructed that contain two fusion genes: the first with the AFP control region linked to the selectable marker neomycin, and the second with the AFP control region linked to the immortalizing gene T antigen. To mimic the events that take place during retinoic acid-stimulated differentiation, human genomic DNA and/or mouse cDNA libraries will be transfected into the test cells, and the expression of the fusion genes assayed. Transfection and expression of a gene essential for AFP gene expression will result in G418 resistant cells and T antigen expression.

O 403 Molecular characterization of a Syrian hamster muscle cell line, PC44. T.L. Beaty, K.G. Cook, S.A. Bruce. The Johns Hopkins University, School of Hygiene and Public Health, Baltimore MD 21205.
We have isolated a clonal diploid myogenic continuous cell line, PC44, derived from 9 day gestation Syrian hamster embryos. To investigate the effect of prolonged culture on myogenic regulatory mechanisms, PC44 cells at PDL 75 and 300 were analyzed. PDL 75 cells are >98% diploid, have a 20 hr doubling time and exhibit >90% differentiation in a clonal assay. In contrast, PDL 300 cells have increased aneuploidy (60% diploid), grow faster (14 hr doubling time) and exhibit <10% differentiation. To determine whether the differentiation defective PDL 300 cells express muscle regulatory genes, total RNA was probed for MyoD1 and myogenin. Similar expression of these two regulatory genes was observed in both PDL 75 and 300 cells (MyoD1 expressed at 0 hrs in low (0.5%) serum and downregulated by 30 hrs in low serum; myogenin expressed at low levels at 0 hrs and upregulated by 30 hrs). To determine whether PDL 300 cells also express muscle structural genes, total RNA was probed for myosin heavy chain (MHC), myosin light chain (MLC) and actin. Again, similar expression of these genes was observed in both PDL 75 and 300 cells (MHC, MLC, and α-actin not expressed at 0 hrs, expressed by 30 hrs; ß- and gamma-actins downregulated within same period). The similar levels of muscle marker expression in PDL 75 and 300 cells suggests that the differentiation block in PDL 300 cells may be at the level of cell fusion. (DE-FG02-88ER60636)

O 404 HUMAN HOX2 HOMEOBOX GENES ARE SEQUENTIALLY ACTIVATED BY RETINOIC ACID, Edoardo Boncinelli, Antonio Simeone, Dario Acampora and Fulvio Mavilio, International Institute of Genetics and Biophysics, Via Marconi 10, Napoli, and Istituto Scientifico H.S. Raffaele, Via Olgettina 60, Milano, Italy. We previously reported that retinoic acid (RA) specifically induces human homeobox gene expression in the embryonal carcinoma cell line NT2/D1, providing an in vitro model to study the molecular mechanisms underlying their developmental regulation. We now show that the 9 genes of the HOX2 locus are differentially activated in NT2/D1 cells exposed to RA concentrations ranging from 10 nM to 10 µM. Genes located in the 3' half of the locus are induced at peak levels by 10 nM RA, whereas a concentration of 1 µM to 10 µM is required to fully activate 5' gene. In both mouse and human embryos the anterior border of expression of HOX2 genes in CNS is in the hindbrain for 3' genes and in the spinal cord for the 5' ones. Upon exposure to RA, HOX2 genes are sequentially activated in the 3' to 5' direction along the locus. Activation of early responding genes does not require protein synthesis and is not primarily due to stabilization of preexisting mRNA. These data show that RA activates the expression of human HOX2 genes in vitro by a concentration-dependent mechanism in a sequential order which is colinear with both their 3' to 5' order in the cluster and the spatially-restricted expression pattern along the embryonic anteroposterior body axis.

O 405 THE REGULATORY GENE HAIRY IS INVOLVED IN DEVELOPMENTAL DECISIONS AT SEVERAL STAGES OF DROSOPHILA DEVELOPMENT, Nadean L. Brown, Bruce Thalley, Stephanie Vavra, Jill Whyte, and Sean Carroll, Cell and Molecular Biology, University of Wisconsin, Madison, WI 53706
Embryonic segmentation in Drosophila requires several classes of regulatory genes. One of these genes, hairy, encodes a nuclear protein that appears to transcriptionally regulate other genes. Sequence analysis (by Rushlow et al) indicates the presence of a helix-loop-helix DNA binding motif that may allow the protein to directly interact with the regulatory regions of its targets. In addition to its striped pattern during early embryogenesis, hairy is also expressed in striking spatial patterns during larval and pupal development in several tissues, including eyes, legs, wings, and antennae. Its role during these stages of development may include both the overall patterning of tissues as well as the control of individual cell fates.

O 406 Nucleosome positioning modulates accessibility of regulatory proteins to the MMTV promoter, Ulf Brüggemeier, Benjamin Piña and Miguel Beato, Institut für Molekularbiologie und Tumorforschung, E.-Mannkopff-Str. 2, D3550 Marburg, F.R.G.

Minichromosomes containing the long terminal repeat region (LTR) of mouse mammary tumour virus (MMTV) exhibit precisely positioned nucleosomes over the hormone responsive element (HRE) (Richard-Foy and Hager, 1987). Chromatin reconstitution experiments with short DNA fragments derived from this region of the LTR also result in a precise positioning of the DNA double helix as revealed by DNase I, exonuclease III and hydroxyl radical footprinting. These results suggest that the information required for nucleosome phasing is contained within this short DNA sequence. These in vitro reconstituted nucleosomes bind the glucocorticoid and the progesterone receptors with affinities only 3-5 fold lower than naked DNA. The transcription factor nuclear factor I (NFI), which is essential for efficient utilization of the MMTV promoter, binds very tightly to naked DNA but fails to recognize its cognate sequence in the nucleosomally organized promoter. Binding of the hormone receptors to the MMTV nucleosome in vitro does not dissociate the nucleosome, but changes its structure leading to a higher accessibility of the promoter proximal nucleosome end to exonuclease III digestion. Our data suggest that precise positioning of one nucleosome over the MMTV promoter could repress transcription by selectively preventing binding of NFI in the absence of hormonal stimulus while still allowing activated steroid hormone receptor to interact with the HRE.

O 407 RETINOL COMPLEXED WITH SPECIFIC CARRIER PROTEINS IS REQUIRED FOR LYMPHOCYTE PROLIFERATION, Jochen Buck, Annette Garbe, Sharon Abish and Ulrich Hammerling, Department of Immunology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021. Sixty years ago Wolbach and Howe (1929) described, among other effects, a severe lymphadenopathy as a consequence of dietary vitamin A withdrawal. We have discovered what might be the in-vitro correlate by showing that activated lymphocytes maintained in vitamin A-deficient medium fail to proliferate. This profound inhibition applies to both activated B and T lymphocytes of at least two species, mouse and man. The growth-promoting effect is not provoked by free retinol in the medium but is dependcent on the presence of specific proteins, i.e., prealbumin and retinol-binding protein. Among the retinoids that can form an active growth factor with the carrier are several derivatives of the alcohol and aldehyde forms of retinol, whereas retinoic acid, a morphogen and differentiation-inducing agent for a variety of cells, exhibited no growth factor activity for lymphocytes.

O 408 SYNTHESIS OF PLATELET ACTIVATING FACTOR (PAF) AND METABOLISM OF RELATED LIPIDS IN EMBRYONIC CELLS, K. P. Chepenik, R.L. Wykle, Department of Anatomy, Jefferson Medical College of Thomas Jefferson University, Philadelphia, PA 19107 and Department of Biochemistry, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103. Primary cultures of mouse embryo palate mesenchyme (MEPM) cells incubated with 1-O-[^{3}H]alkyl-2-lyso-sn-glycero-3-phosphocholine ([^{3}H]lyso-PAF) incorporated radiolabel into 1-radyl-2-acyl-sn-glycero-3-phosphocholine (PC) and -phosphoethanolamine (PE). Chemical analysis indicated the PC was 1-O-alkyl lipid whereas the PE contained predominantly 1-O-alk-1'-enyl species with smaller amounts of 1-O-alkyl species.

Stimulation of MEPM cells with the ionophore A23187 in the presence of calcium and [^{3}H]acetate resulted in the production of [^{3}H]PAF, identified by its migration with authentic PAF and its conversion to [^{3}H]1-O-alkyl-2,3-diacetylglycerol upon treatment with phospholipase C and acetic anhydride.

These studies demonstrate that (1) MEPM cells are able to incorporate [^{3}H]lyso-PAF into the storage form of PAF, and into PE plasmalogen, and (2) MEPM cells have the ability to synthesize PAF, thus raising the possibility this compound may play a role in modulating growth and/or differentiation of these embryonic cells.

O 409 GROWTH EFFECT AND RECEPTORS OF STEROID HORMONES ON CULTURED CELLS FROM MASTOPATHIES AND NORMAL BREAST.
Crépin, M., Hamelin, R., Salle, V., Gamby, Ch. and Israel L.. I.0.C.M.H.- Université de Paris XIII - 129 route de Stalingrad, 93000 - Bobigny, France.
The culture of human breast epithelial cells is well adapted for the study of hormone and anti-hormone actions on cell proliferation. We have developped a culture system of breast cells from normal tissues, benign mastopathies and carcinoma. These primary cultures allow us to transfert SV 40-T-oncogene for immortalizing cloned cells. Thus we have established three cell lines from normal breast and proliferative mastopathies.
In primary cultures and established cell lines, the presence and the functionality of steroid receptors was demonstrated by scatchard analysis with a whole cell assay and by transactivation of Vitellogenin and MMTV promotor with CAT indicator gene. Our results show that oestradiol and progesterone receptor synthesis are modulated in normal and pathologic tissu before and after immortalization with SV 40-T-oncogene. Growth effects of these two hormones is directly related to the functionality of their receptors.

O 410 RETINOIC ACID INHIBITS THE CELL GROWTH OF HUMAN RHABDOMYOSARCOMA CELL LINES IN A STEREO-SPECIFIC MANNER, Gary D. Crouch and Lee J. Helman, Molecular Genetics Section, Pediatric Branch, NCI, National Institutes of Health, Bethesda, MD 20892
All-trans-retinoic acid (RA) has been demonstrated to have striking effects on the developing chick limb bud, and the exposure of a rat rhabdomyosarcoma cell line to RA resulted in terminal differentiation and growth inhibition. We have therefore been evaluating the effect of these compounds on the growth and differentiation of human rhabdomyosarcoma cell lines. Treatment of RD, an embryonal rhabdomyosarcoma cell line, with all-trans-RA in serum-free conditions resulted in a dose-dependent inhibition of cell growth with a maximal inhibition of 92% at a 5uM concentration as assayed by both ^{3}H thymidine incorporation and cell number determined by MTT assay. When 13-cis-RA was used under identical experimental conditions maximal growth inhibition was 41%. Treatment of RH30, an alveolar rhabdomyosarcoma cell line, with all-trans-RA in serum-free conditions also resulted in a dose-dependent inhibition of cell growth with a maximal inhibition of 66% at 5uM concentration compared with a 37% maximal inhibition when 13-cis-RA was used under identical conditions. Evaluation by Northern analysis of RD cells treated with all-trans-RA revealed no differences compared to controls in the expression of MyoD1, alpha-actin, myosin heavy chain, or creatinine kinase M. We conclude that RA inhibits cell growth in a stereo-specific manner with no evidence of morphologic or biochemical differentiation. The mechanism by which this stereo-specific inhibition of the cell growth occurs in human rhabdomyosarcoma cell lines is unknown and requires further investigation.

O 411 MODULATION BY RETINOIC ACID OF THE PROMOTER ACTIVITY OF THE HUMAN PAPILLOMAVIRUS TYPE-18 LONG CONTROL REGION (HPV18-LCR).
Michel Darmon, Chantal Delescluse, Catherine Gerst, and Bruno A. Bernard. Cell Biology Department, Centre International de Recherches Dermatologiques (CIRD), Sophia Antipolis, 06565 Valbonne, France.
In previous work (B.A.Bernard et al., in "Papillomaviruses", UCLA Symp. Mol. Cell. Biol., P. Howley and T. Broker, eds, Alan. R. Liss, Inc., New York, NY, 1989, in press), we had shown that addition of retinoic acid (RA) to human keratinocytes grown in medium containing delipidized serum resulted in the activation of transcription from the construct pH18CAT, containing the HPV18 long control region (HPV18-LCR) cloned upstream of the CAT reporter gene. In the present work, we performed footprinting experiments with keratinocyte nuclear extracts, to investigate whether addition of RA would result in modifications of the subset of factors able to interact with the HPV18-LCR. Moreover, since it is known that biological activity of retinoids is mediated by and correlates with affinity for specific nuclear receptors (M. Petkovich et al., 1987, Nature 330:444; M. Darmon et al., 1988, Skin Pharmacol. 1:161), we investigated whether retinoic acid receptors (RARs) could modulate, in the the presence of RA, the transcription from the HPV18-LCR. After cotransfecting into HeLa cells (known to be depleted in RARs) the plasmid pH18CAT together with RARa or RARb expression vectors, we found that this is indeed the case. Moreover, deletion of subfragments of HPV18-LCR suggest that this RAR-mediated modulation directly affects the promoter of the HPV18-E6 open reading frame.

O 412 IDENTIFICATION OF A RETINOIC ACID RESPONSIVE ELEMENT IN THE RETINOIC ACID RECEPTOR B GENE. Hugues de Thé, Maria Vivanco-Ruiz*, Pierre Tiollais, Henk Stunnenberg*, Anne Dejean. Institut Pasteur, Paris, France, * EMBL, Heidelberg, FRG.
Retinoic acid (RA), the first morphogen described so far in vertebrates, is a vitamin A derivative that exerts striking effects on development and differentiation. The identification of three retinoic acid receptors (RAR) as members of the nuclear receptor superfamily provides a molecular basis for the action of morphogenes on gene expression. Functional analysis of the RARs requires the identification of target genes and of their *cis*-acting RA-responsive elements (RARE). We have previously shown that the RAR β gene was transcriptionally up-regulated by RA. We now report the characterization of a functional retinoic acid responsive element in the RAR β gene that mediates *trans*-activation by RA. By deletion mapping we identified a 27 base pairs (bp) fragment that confers RA responsiveness to the herpes virus thymidine kinase (TK) promoter. This sequence contains a perfect direct repeat of a motif reminiscent of the 5' half palindrome of thyroid and oestrogen hormone responsive elements. Specific binding of the RAR β protein to the RARE was demonstrated, irrespective of the presence of RA. Both RAR α and β enhance RA response in CV1 cells, indicating that the two receptors can act through the same DNA sequence.

O 413 EFFECT OF THE ANTIPROGESTINS RU 486 AND ZK 98 299 ON mRNA OF ESTROGEN RECEPTOR, IGF-I AND IGF-I RECEPTOR IN THE RABBIT UTERUS, Karl-Heinrich Fritzemeier, Krzysztof Chwalisz, Walter Elger, David Henderson, Ekkehard Schillinger, Research Laboratories of Schering AG, Müllerstr. 170-178, 1000 Berlin 65, West Germany
The antiprogestins (AGs) RU 486 and ZK 98 299 interfere with certain estrogen effects in the uterus whithout binding to the estrogen receptor (ER). Treatment of ovariectomized (ovex) estradiol (E2)-substituted rabbits with AGs resulted in inhibition of proliferation and differentiation of the uterine epithelium. There was, however, stimulation of stromal tissue indicating that AGs affect growth regulating processes in the two compartments differently. The aim of the present study was to investigate the antiproliferative effect of AGs at the level of 1) ER/ER mRNA and 2) IGF-Iand IGF-I receptor mRNAs.
E2 substitution (3 µg/animal/day) caused a decrease in ER mRNA by a factor of 2-2,5 whereas treatment with E2 + 10 - 30 mg RU 486 or ZK 98 299, respectively, returned the ER mRNA level to that seen in the ovex controls. This effect was reversed by progesterone. Immunocytochemical studies revealed no change in ER-immunostaining of the uterine epithelium.
AG treatment decreased the concentration of IGF-I receptor mRNA. At the same time, we found a 4-fold increase in IGF-I mRNA concentration as compared to E2-substituted controls. These effects were reversed by progesterone treatment. The results of our study suggest that 1) the antiproliferative effect of AGs is not due to interference with ER biosynthesis, 2) the suppression of IGF-I receptor in epithelial cells after AG treatment could be one important factor for the antiproliferative action in the rabbit endometrium.

O 414 IDENTIFICATION OF A NOVEL ISOFORM OF THE RETINOIC ACID RECEPTOR-γ EXPRESSED DURING MOUSE EMBRYOGENESIS, Vincent Giguère[1,2], Janet Rossant[2] and Sue Varmuza[3], [1]Division of Endocrinology, Hospital for Sick Children, Toronto, Canada M5G 1X8 and [2]Department of Medical Genetics and [3]Zoology, University of Toronto, Canada. Retinoic acid is known to exert profound effects on developmental processes. It has been implicated as a putative morphogen in the developing chick limb bud and regenerating amphibian limb blastema, and has been demonstrated to have powerful teratogenic effects in mammals, including humans. Recently, three specific receptors for retinoic acid, RARα, β and γ, were identified and shown to be members of the steroid receptor superfamily. Here we report the identification of a novel RARγ isoform, mRARγB, which differs from the previously described mouse RARγ (RARγA) at its amino terminus. Transfection of COS-7 cells with an RARγB expression vector together with the reporter plasmid ΔMTV-TREp-luciferase shows that increasing concentration of RA lead to a 20-fold stimulation of luciferase activity with an ED50 of 0.5 to 1.0 nM. Both RARγA and RARγB are expressed maximally at midgestation and their expression appeared to be concentrated in the limbs, jaw and carcass which contains skeleton and skin. These results show that multiple RAR isoforms are expressed in the mouse embryo, each of which may play a unique or combinatorial role as a regulator of mammalian development.

O 415 RETINOIC ACID INDUCES EXPRESSION OF THE LIVER-ENRICHED HOMEOBOX TRANSCRIPTION FACTOR HNF-1 BY A POST-TRANSCRIPTIONAL MECHANISM. **Calvin J. Kuo, Dirk B. Mendel, Pamela B. Conley and Gerald R. Crabtree, Howard Hughes Medical Institute, Unit in Molecular and Genetic Medicine, Stanford University School of Medicine, Stanford, CA 94305-5425.** The homeodomain-containing transcription factor Hepatocyte Nuclear Factor-1 (HNF-1, also LF-B1, APF) represents the major DNA-binding protein of the hepatocyte lineage by virtue of its transcriptional activation of over 20 hepatic genes via a conserved 13 bp motif present in these promoters. The F9 teratocarcinoma cell line, in suspension culture, is inducible by retinoic acid (RA) to form visceral yolk sac endoderm, an embryonal tissue expressing several HNF-1-regulated liver markers, including alpha-fetoprotein (aFP) and albumin. Consistent with this liver-specific gene expression we have found that RA treatment of F9 cells evokes a pronounced, 20-fold increase in HNF-1 DNA-binding activity which is first apparent 24-36 h post-stimulation. By footprint, methylation interference, binding specificity and partial proteolysis, we have documented this induced activity to be identical to purified preparations of HNF-1. Moreover, in order to delineate the temporal pattern of HNF-1 mRNA during RA-induced differentiation, we have isolated murine cDNA clones encoding HNF-1. Surprisingly, by RNase protection analysis we found that HNF-1 mRNA was already present in untreated F9 cells, and that this initial level did not vary significantly over an 11 day RA stimulation during which HNF-1 DNA-binding activity exhibited an over 20-fold induction. These results suggest that RA exerts its marked stimulation of HNF-1 DNA-binding activity via a post-transcriptional mechanism, and that the F9 cell line will prove useful in defining the molecular basis for this regulation.

O 416 DIFFERENTIAL EXPRESSION OF mRNAS ENCODING EXTENDED AND TRUNCATED PL1 HOMEOBOX PROTEINS FROM HUMAN MYELOMONOCYTIC CELL LINES. C. Largman J. Corral, K. Detmer, H.J. Lawrence, and P. Lowney VA Medical Center, Martinez CA and Dept of Internal Medicine, UC Davis Medical Center, Sacramento, CA.

Expression of homeo-box containing proteins has been shown to play a role in the determination of cell type or lineage. We have previously reported the differential expression of some Hox 2 homeo-box genes in erythroid cell lines and of the novel homeo-box gene, PL1 in myelomoncytic cell lines. The PL1 gene, located on chromosome 7, encodes two transcripts. PL1 transcripts were cloned from two myelomonocytic cDNA libraries, U937 and ML3. The larger 3 kb transcript encodes a protein with a proposed protein binding region and the homeo-box DNA binding region. In contrast, the smaller 2.2 kb transcript encodes a protein which contains only the homeo-box DNA binding region. While the 3 kb transcript predominates in ML3 and PBL985 cells, the 2.2 kb transcript predominates in KG1 and U937 cells. We hypothesize that these proteins may compete for DNA binding and that their variable concentration may influence the determination of cell phenotype within the monocytic lineage.

O 417 REGULATION OF GENE EXPRESSION IN CULTURED EPIDERMAL CELLS AND IN EPIDERMIS OF TRANSGENIC MICE. A. Leask, M. Rosenberg, R. Lersch, R. Vassar and E. Fuchs. Dept. of Molecular Genetics and Cell Biology and Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637. The major structural proteins of the epidermis are the keratins, a complex family of proteins forming 10nm intermediate filaments in all epithelial cells. These proteins can be divided into two classes: type I keratins are smaller (40-56.5 kDa) and more acidic; whereas type II keratins are larger (53-67 kDa) and more basic. Keratin filaments are obligatory heteropolymers comprised of a specific pair of type I and type II keratins: each pair is expressed in a tissue-specific and differentiation-specific manner. The expression of the K5 (type II)-K14 (type I) pair is restricted to the basal layer of all stratified squamous epithelia, and is especially abundant in epidermis, comprising ~30% of the total protein. We have previously cloned, sequenced and characterized the human K14 and K5 genes (Marchuk et al., Cell 39, 491. 1984; Lersch et al., MCB 9, 3685. 1989). Using transient transfections and chloramphenicol acetyltransferase (CAT) assays in vitro and transgenic mice in vivo, we have begun to examine the transcriptional mechanisms controlling the expression and the pairwise regulation of K14 and K5.

O 418 **A SEQUENCE IN THE MMTV LTR IS ACTIVATED DURING PROVIRAL INSERTION AT THE INT-2 LOCUS.** P. Lefebvre, M.G. Cordingley and G.L. Hager, Lab. of Experimental Carcinogenesis, National Cancer Institute, N.I.H., Bethesda, Md 20892.
The Mouse Mammary Tumor Virus (MMTV) has been shown to activate a series of cellular genes, the int genes, during viral induced carcinogenesis in mice. The newly acquired provirus do not confer hormonal regulation to the int gene, it has thus been assumed that the viral DNA must harbor an enhancer, distinct from its promoter, responsible for this activation. As a model system, we used the RAC mouse mammary cell line that have integrated a provirus upstream the int-2 gene and is able to grow under two differentiation states (5E and 11P). Comparative studies of transcripts confirmed a direct correlation between MTV and int-2 expression (high level in 5E cells, low level in 11P cells). Primer extension analysis of the int-2 transcripts revealed a precise pattern of transcription with respect to the three promoters of this gene. The inability of glucocorticoids to stimulate MMTV transcription in 11P cells is not due to a loss or alteration of receptor or NF-1, an essential transcription factor. Chromatin structure studies of the proviral LTR showed an alteration of the phased array of nucleosomes in the upstream region of the LTR. Transient transfection experiments using LTR-Luciferase constructs were able to reproduce this differential level of expression. They provided evidence for the requirement for upstream regions of the LTR to obtain optimal regulation of trancriptional activity. Factors specifically interacting with these LTR regions distinct from the HRE have been characterized by gel retardation and footprinting experiments. An upstream region of the LTR thus contains an enhancer that may be responsible for int gene activation.

O 419 **RETINOIC ACID RECEPTOR ISOFORMS EXPRESSED DURING DIFFERENTIATION HAVE DISTINCT GENE ACTIVATION CAPACITIES,** Juergen M. Lehmann and Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037
Retinoic acid (RA) exerts profound effects on tissue development, differentiation, and morphogenesis. The cloning and characterization by us and colleagues of nuclear retinoic acid receptors (RAR) which belong to the steroid/thyroid receptor superfamily suggests that all RA dependent transcriptional regulation is mediated by the interaction of RARs with specific DNA sequences. To delineate molecular mechanisms underlying differentiation we have analyzed the RAR isoforms expressed in the human promyeloblastic leukemia cell line HL60 which can be differentiated into granulocytes by exposure to RA. Polymerase chain reaction was used to determine which of the RAR isoforms is expressed before and during differentiation. The finding that several isoforms are expressed simultaneously suggests that the RAR mediated response to RA is very complex. This is supported by our observation that the various RARs respond with different sensitivity to RA or a number of synthetic retinoids in transient transfection assays. The observed high basal activity of RARβ/ϵ suggests that this receptor has constitutive transcriptional activation capacity whereas RARα is highly dependent on RA for activation of transcription. A model for positive and negative regulation of gene transcription by RARs is proposed.

O 420 REGULATION OF MUSCLE GLYCOGEN PHOSPHORYLASE GENE EXPRESSION Jean M. Lockyer, Human Genetics Program, Tulane University School of Medicine, New Orleans, LA 70112.
Glycogen phosphorylase plays a key role in glycogen metabolism, initiating the breakdown of glycogen to glucose-1-phosphate. Three isozymes (muscle, liver, and brain) have been described, each of which exhibits a unique pattern of tissue-specific expression and is encoded by a separate gene. The muscle phosphorylase isozyme is differentially regulated during muscle development. During the final stages of muscle differentiation and terminal commitment to myotube formation, a battery of muscle-specific genes is expressed and fetal isoforms are replaced by adult isoforms. The mechanisms controlling this developmental switch have not yet been identified. We have detected the presence of regulatory sequences within 700 base pairs of the transcription initiation start site which appear to be important for the adult, muscle-specfic gene expression of glycogen phosphorylase. Deletion analysis was performed using pBLCAT3 constructs made with various regions of 5' flanking sequences of the human muscle phosphorylase gene. The plasmids were transfected into C2C12 mouse myoblasts and the resultant CAT activity was analyzed in proliferating and fused muscle cells. Maximal CAT activity in fused cells was obtained with a construct containing approximately 670 base pairs of 5' flanking sequence. Deletion of 190 base pairs decreased activity to 2% of the values obtained with the 670 base pair construct. Deletion of an additional 180 base pairs resulted in 7% of maximal CAT activity. The 50-fold increase in CAT activity observed in fused cells when comparing the 480 base pair construct to the 670 base pair construct was not observed in proliferating myoblasts, although a low level of CAT activity driven by sequences within the 480 base pair phosphorylase fragment was present in proliferating cells. A similar pattern of expression was obtained in the rat L6A1 muscle cell line. We are currently identifying protein(s) which bind to specific sites between -480 and -670 of the muscle phosphorylase gene. We are also investigating the influence of extracellular factors or growth conditions on the ability of these factors to bind to their specific site(s).

O 421 ANALYSIS OF THE FUNCTION OF THE MUSCLE DETERMINATION GENE MYO D1 USING HOMOLOGOUS RECOMBINATION IN MOUSE EMBRYONIC STEM CELLS.
Lesley A. Michalowsky,[1,2] Randall S. Johnson,[1] Virginia E. Papaioannou,[3] Stephen Tapscott, [4] Harold Weintraub,[4] and Bruce M. Spiegelman[1,2], Dana-Farber Cancer Institute,[1] Department of Biological Chemistry and Molecular Pharmacology,[2] Harvard Medical School, the Department of Pathology, [3] Tufts University, Boston, MA 02115, and Fred Hutchinson Cancer Research Center [4], Seattle, WA 98104.
Genetic manipulation of murine embryonic stem (ES) cells has been used to assess the role of the muscle determination gene MyoD1 in mouse embryological development. We have engineered a gene construct containing 7.7kb of the mouse MyoD1 locus interrupted by a neomycin resistance cassette, specifically designed to render the endogenous gene dysfunctional after homologous recombination. The construct also contains the HSV1-TK gene, allowing enrichment for homologous recombinants after transfection of the construct into ES cells. Homologous recombinant ES clones have been derived and are being used to make chimeric mice, and these mice will be assayed for germ line contribution. Germ line transmission of the altered MyoD1 allele will allow propagation of mice homozygous for that allele. These mice will allow us to study the function of MyoD1 in cell differentiation during murine development.

O 422 HORMONAL REGULATION OF GENE EXPRESSION DURING METAMORPHOSIS, Leo Miller and Peter Mathisen, Laboratory for Molecular Biology, University of Illinois, Chicago, IL 60680
We are studying the role of thyroid hormone (T_3), retinoic acid (RA), and glucocorticoids (DEX) in the induction of keratin gene expression during amphibian metamorphosis. In *Xenopus* the epidermis is an unkeratinized, two-cell layered epithelium from gastrulation until metamorphosis. The bilayered larval epidermis is then converted into a stratified, keratinized epithelium under hormonal control and is characterized by the appearance of adult specific 63kd keratins. A long latent period of two to three days exists between the addition of T_3 to skin cultures and an increase in concentration of 63kd keratin mRNAs. The induction of 63kd keratin genes by T_3 is inhibited by RA, whereas DEX acts synergistically with T_3. Induction can also be produced by a short exposure to T_3 at the beginning of the culture period. These results indicate that T_3 causes a stable change in epidermal cells, which can be expressed much later, after extensive cell proliferation has occurred, in the absence of T_3. Once the 63kd keratin genes are induced, however, they are stably expressed, and by the end of metamorphosis T_3 has no further effect on their expression. The results suggest that the mode of action of T_3 during metamorphosis is similar in many respects to the action of embryonic inducers.

Growth and Differentiation Factors in Development

O 423 ANALYSIS OF RETINOIC ACID RECEPTORS AND CRABP EXPRESSION IN EMBRYONAL CARCINOMA PCC4.aza1R CELLS, Clara Nervi, Thomas M. Vollberg, Joseph F. Grippo*, Margaret D. George, Michael I. Sherman* and Anton M. Jetten, NIEHS, RTP, NC 27709; ROCHE Research Center., Nutley, NY 07110. PCC4.aza1R were used as a model system to investigate the mechanism of action of retinoids and the respective roles of nuclear retinoic acid receptors (RARs) and the cytosolic retinoic acid binding protein (CRABP). Embryonal carcinoma PCC4.aza1R cells can be induced to differentiate into mesenchymal cells by treatment with nanomolar concentrations of retinoic acid (RA). Analysis of specific RA binding activity in nuclear and cytosolic extracts prepared from these cells showed the presence of RAR and CRABP activity. The RAR activity was solely associated with the nuclear fraction. In PCC4.aza1R cells biologically active RA analogs of the Ch-series strongly compete with ^{3}H-RA for binding to RAR but they do not bind to CRABP. Northern blot analyses demonstrated the mRNA expression of RARα, RARγ and very low levels of RARβ. RA treatment induced the expression of RARβ mRNA 10-30 fold in a time- (within 2 hr, and maximally at 24 hrs) and concentration- (EC50 ≈ 10^{-9} M RA) dependent manner. This induction was not dependent on protein synthesis. We propose that RARs in PCC4.aza1R cells mediate the mechanism of action of retinoids. RARs expression in RA-nonresponsive PCC4.aza1R mutant cell lines appears to indicate that these cells are not defective in the ligand binding-domain but may be defective at the level of transcriptional activation.

O 424 TRANSCRIPTIONAL ACTIVATION OF A CONSERVED SEQUENCE ELEMENT BY *ras* DOES NOT REQUIRE *fos* or *jun*. Michael C. Ostrowski and Russell D. Owen. Department of Microbiology & Immunology, Duke University Medical Ct., Durham, NC 27710. The expression of transforming growth factor β1 mRNA was increased by conditional expression of activated *ras*. A 31 base pair sequence found approximately 420 base pairs upstream of the human transforming growth factor β1 gene acted as a *ras*-responsive enhancer element in transient transfection assays. The human sequence contains the element TGACTCT that is also found in a murine *ras*-responsive enhancer. Analysis of nuclear factors present in cells stably transformed by *ras* indicated that both human and murine sequences were recognized by the same nuclear factor. The role of *fos* and *jun* in *ras* transcriptional activation was analyzed in transfection assays using murine elements that contained either the sequence TGACTCT or TGAGTAA. These experiments showed that while both elements are activated by *fos/jun* to nearly the same extent, only the former element responded to *ras*. In addition, *ras* activated the element TGACTCT 8-10 fold more efficiently than *fos/jun*. Gel retention experiments revealed that the nuclear factor present in cells transformed by *ras* exhibited the same sequence preference as demonstrated in the transient transfection assays. UV-crosslinking experiments indicate that a single protein that is 120 kD recognizes the *ras*-enhancer. This work identifies a persistent signal transduction pathway that links *ras* to nuclear transcription, and indicates that c-*fos* and c-*jun* are not nuclear targets of this pathway.

O 425 ***SKI* PROTO-ONCOGENE EXPRESSION DURING MUSCLE DEVELOPMENT,** Sonia H. Pearson-White, Department of Biology, University of Virginia, Charlottesville, Virginia 22901.

A recently described proto-oncogene family, the *ski* family, may have important functions in early embryonic development. The chicken v-*ski* oncogene has been shown to transform quail embryo cells to tumorigenicity, and can also convert these non-myogenic cells to the myogenic (muscle) pathway. Members of the *ski* family of genes, *ski* and *sno*, are expressed during skeletal muscle development in determined myoblasts and in differentiated myofibers, and at lower levels in adult muscle. The *ski* family genes, like many genes involved in embryonic development, are highly conserved between species. Several regulatory genes have been identified that convert fibroblasts to the myogenic pathway: MyoD1, myogenin, myf5, all of which share a helix-turn-helix *myc* homology region crucial for the conversion function. *Ski* has myogenic conversion activity like the other myogenic regulatory genes, but lacks the *myc* homology domain and thus represents a different class of muscle regulatory gene. Furthermore, unlike the MyoD1 family of muscle regulatory genes, *ski* is expressed during cardiac development and in adult cardiac cells, suggesting a potential role for *ski* or related genes in the formation of cardiac muscle. To investigate the role of *ski* family genes in muscle development, I have isolated two novel *ski*-related cDNAs from primary human myoblasts. Sequence, expression and transfection data will be presented as part of the preliminary characterization of the role of the *ski* family genes in muscle development.

O 426 SPATIAL AND TEMPORAL PATTERN OF EXPRESSION OF THE CELLULAR RETINOL AND RETINOIC ACID BINDING PROTEINS DURING MOUSE EMBRYOGENESIS, Ana V. Perez Castro*, Leslie E. Toth-Rogler, Li-Na Wei and M. Chi Nguyen-Huu*, Departments of Microbiology and Urology, Columbia University College of Physicians and Surgeons, New York, NY 10032. Retinol (vitamin A) and retinoic acid are potent teratogens, and also represent good candidates for normal morphogens during development. Their actions may be mediated by the cellular retinoic acid-binding protein (CRABP) and the cellular retinol-binding protein (CRBP). As a step towards understanding the possible function for CRABP and CRBP in morphogenesis, we have used in situ hybridization to analyze their expression during mouse development. Both CRABP and CRBP transcripts were detected at embryonic days 9.5 to 14.5. 1) In the nervous system, CRABP transcripts were found in the mantle layer of the dorsal spinal cord and hindbrain and in the marginal layer of the midbrain; CRBP transcripts were found in the ependymal and mantle layer of the ventral spinal cord and of the forebrain, as well as in the spinal nerves and the roof plate of the spinal cord. 2) In the eye, CRABP is expressed in the retinal layer and CRBP in both retinal and pigmented layers. 3) In the cranio-facial region, CRABP transcripts were found in the mesenchyme of the fronto-nasal mass and mandible, while CRBP transcripts were found in the mesenchyme of the naso-lachrymal duct and surrounding the auditory vesicle. Two general conclusions can be made. First, all the tissues that are known teratogenic targets of retinoic acid and retinol also express CRABP and CRBP transcripts. Second, the specific expression of CRABP and CRBP in numerous developing tissues indicates that these proteins may perform specific functions during morphogenesis of a broad variety of embryonic structures.
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O 427 **ACTIVATOR AND REPRESSOR FUNCTIONS OF THE HUMAN ESTROGEN RECEPTOR**, Magnus Pfahl, Maty Tzukerman, Xiao-kun Zhang, Thomas Hermann, Ken Wills, and Gerhart Graupner, La Jolla Cancer Research Foundation, La Jolla, CA 92037.
The conventional model for steroid hormone action has assumed that steroid hormone receptors act as transcriptional regulators only when complexed with their ligands. It has, however, become evident that most types of steroid receptors are present in the cell nucleus even in the absence of ligand. The presence of these regulatory proteins in the nucleus even in the absence of ligand suggest possible additional regulatory functions for these proteins. We have recently found that other members of the nuclear receptor family - the thyroid hormone receptors - have a dual regulatory role: in the presence of hormone they function as transcriptional activators whereas in the absence of hormone they are response element specific transcriptional repressors. Here we have investigated the transcriptional regulatory functions of wild type and mutant forms of the human estrogen receptors and their interaction with a specific DNA sequence the symmetric estrogen responsive element (ERE). We find that the mutant val-400 receptor behaves like a classical steroid hormone receptor. In the presence of ligand its affinity for the ERE is increased, and its transcriptional activator function is ligand dependent. The wild type gly-400 receptor is a constitutive transcriptional activator that functions as a transcriptional activator and repressor. In the presence of agonist its activator activity is increased. The presence of antagonist increases its repressor function. Possible consequences of changes in the regulatory properties of ER for pathological stages are discussed.

O 428 GENOMIC CLONING OF COUP TRANSCRIPTION FACTOR-A MEMBER OF THE STEROID/THYROID HORMONE RECEPTOR SUPERFAMILY, Helena H. Ritchie, Lee-Ho Wang, Sophia Y. Tsai, Ming-Jer Tsai and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030, The COUP Transcription Factor (Chicken Ovalbumin Upstream Promoter Transcription Factor; COUP TF) promotes the transcription of the chicken ovalbumin gene and belongs to the steroid/thyroid hormone receptor superfamily. COUP TF shows a striking homology to the C_4 + C_5 class of Zn-finger proteins. In order to understand the evolution of the COUP TF in this superfamily and to facilitate analysis of its transcriptional regulation, we cloned its genomic DNA. Six positive clones were identified from a human genomic cosmid library by using nick translated COUP cDNA's. The six clones could be classified into two genomic structures. Three overlapping cosmid clones (Cos HC-1, Cos HC-3, and Cos HC-5) together covered the whole COUP TF genomic gene. The remaining clones (Cos HC-2, Cos HC-6, and Cos HC-8) represented a second COUP TF gene. High stringency Southern blots analysis of human genomic DNA with cDNA probes identified three Eco Rl fragments of 12 kb, 2.4 kb, and 9.0 kb which are also found in cosmid clones Cos HC-1, Cos HC-3, and Cos HC-5. These clones should provide useful information on 1) structural organization of the genomic COUP-TF, 2) the exon-intron boundries and 3) the evolution of the steroid/thyroid receptor superfamily.

O 429 ERA-1/HOX 1.6 EXPRESSION IN MOUSE EMBRYOS AND F9 CELLS, Melissa Rogers, Allison Ait-Aouane, and Lorraine Gudas, Dana Farber Cancer Institute and Harvard Medical School, Boston, MA 02115. Retinoic acid (RA) modulates the expression of specific genes and induces some cell types, such as the murine F9 teratocarcinoma cell line, to differentiate *in vitro*. RA also dramatically influences pattern formation in both developing and regenerating vertebrates. As a step toward understanding the molecular mechanism(s) by which RA exerts these effects, we previously isolated cDNA clones for a murine gene, ERA-1/Hox 1.6, which may be an early, direct target for RA. Sequencing and S1 analyses indicated that 2 mRNAs are transcribed from this gene, one which encodes a protein with a predicted mass of ~36 kDa that possesses the Hox 1.6 homeodomain and another which encodes a protein of ~15 kDa that does not possess a homeodomain. Both transcripts are present in differentiated F9 cells and in gestational day 7.5-10.5 mouse embryos. Since homeodomain-containing proteins are often involved in pattern formation, the spatial and temporal patterns of ERA-1/Hox 1.6 message and protein are of interest. *In situ* hybridization has shown that the mRNA is present at very high levels in the primitive streak of gastrulating mouse embryos and in the somites of day 8 embryos. Experiments are in progress to detect these transcripts in earlier embryos, particularly in the extraembryonic cells which differentiated F9 cells resemble. Immunohistochemical studies are also being performed in order determine the embryonic protein pattern.

O 430 RETINOIC ACID RECEPTOR EXPRESSION IN THE EMBRYONIC CHICK. Susan M. Smith and Gregor Eichele, Department of Cellular and Molecular Physiology, Harvard Medical School, Boston, MA 02115. The processes by which embryonic cells form well-defined patterns remains unclear. In the developing chick limb, there is good evidence that a retinoic acid concentration gradient is used to establish the anteroposterior axis (thumb to little finger). Most likely the retinoic acid receptor (RAR) is used to interpret such a gradient. To that end, we identified 7 RAR clones from a stage 20 (3.5 day) embryonic chick cDNA library; all were RARβ isoforms. The gRARβ shares 95% overall amino acid identity with human RARβ, having strongest identity in the DNA- (98%) and ligand-binding (96%) domains. gRARβ hybridizes with 3 limb bud mRNAs, at 4.6, 3.4, and 3.2 kb, throughout limb bud development (stage 18-34). Interestingly, only the 4.6 kb message encodes a complete gRARβ; its expression diminishes as development progresses. The same expression pattern of these 3 mRNAs is also seen in embryonic head and body. We used in situ hybridization to study the spatial distribution of the 4.6 kb gRARβ message. At stage 20, gRARβ is highly abundant in neural tube, hindbrain, face, kidney, and heart. Also, gRARβ is unevenly distributed in the limb bud, having the highest concentration in the anterior domain, and opposing the RA ligand gradient. The presence of RAR in retinoid-sensitive tissues suggests that RA's morphogenetic activity may not be restricted to the limb bud.

O 431 PROTEIN-DNA INTERACTIONS IN SIGNAL TRANSDUCTION: GLUCOCORTICOID AND cAMP INDUCTION OF THE RAT TAT GENE, Stewart, A.F., Weih, F., Reik, A., Nichols, M. and Schütz G., Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG. The mechanism by which signals are transduced to cis-acting DNA elements can be divided into two classes. First, direct signals as exemplified by steroid hormones which activate their receptors to bind DNA elements and to regulate transcription. Second, signals that are transduced through the cell membrane to influence intracellular levels of second messenger which subsequently influences specific protein kinase activities. We have been studying a gene (rat tyrosineaminotransferase (TAT)) that demonstrates increased transcriptional activity in response to agents from both classes, namely glucocorticoids and glucagon via cAMP. The corresponding DNA response elements lie in enhancers either 2.5 kB (glucocorticoid) or 3.6 kB (cAMP) upstream of the start site of transcription. By in vivo and in vitro footprinting analyses and by transfection studies we have examined the alterations of protein-DNA interactions induced by signal transduction. We find that both glucocorticoids and cAMP induce changes in the DNA binding activity of the factors that bind to their respective response elements.

O 432 RETINOIC ACID PROMOTES TRANSCRIPTION OF THE PDGF α-RECEPTOR GENE IN F9 TERATOCARCINOMA CELLS Wang, C.[1], Kelley, J.[2], Bowen-Pope, D.[2], and Stiles, C.D[1]. Department of Cellular and Molecular Biology, Dana-Farber Cancer Institute[1] and Harvard Medical School[1], Boston, MA 02115, and the Department of Pathology, University of Washington[2], Seattle, WA 98195.

We isolated a full length cDNA clone of the murine PDGF α-receptor subunit gene. The clone was obtained from a cDNA library of mouse F9 embryonal carcinoma cells which had been treated with retinoic acid for 8 hours. A human PDGF α-receptor subunit cDNA was used to screen the F9 library. The mouse cDNA clone we obtained contains an open reading frame of 1089 amino acids. The predicted reading frame displays 41% overall amino acid positional identity with the mouse PDGF β-receptor subunit, and greater than 90% with the human PDGF α-receptor subunit. Undifferentiated F9 cells express very low levels of α-and β-PDGF receptor genes. Following exposure to retinoic acid and cyclic AMP, the differentiated F9 cell cultures express the α-receptor subunit gene predominently if not quite exclusively. The induction of α-receptor subunit message is sensitive to cycloheximide suggesting that newly synthesized protein(s) may be required for the expression of PDGF α-receptor subunit message. Nuclear run-off analysis shows that the retinoic acid induces accumulation of PDGF α-receptor subunit message by stimulating transcription of the gene. Collectively, these data account for earlier observations showing that differentiated, but not undifferentiated F9 cell cultures are capable of binding radiolabelled PDGF. The lack of PDGF binding in undifferentiated F9 teratocarcinoma cells is due to the lack of receptor mRNA and protein rather than down-regulation of the receptor. In differentiated F9 cells, the α-receptor is the major receptor type responsible for PDGF binding.

This work is supported by NIH grants to C.D. Stiles, Medical Research Council of Canada, and Alberta Heritage Foundation for Medical Research to C. Wang.

O 433 THE GROWTH FACTOR INDUCIBLE ZINC FINGER GENE NGFI-A IS CONTROLLED BY POSITIVE AND NEGATIVE REGULATORY ELEMENTS, K. Weck, P. Changelian, and J. Milbrandt, Department of Pathology, Washington University, St.Louis, MO 63110. The NGFI-A gene encodes a "zinc-finger" protein that is rapidly induced by nerve growth factor (NGF) in PC12 rat pheochromocytoma cells. The 5′ flanking region of the NGFI-A gene, from nucleotides -532 to -53, has been shown to confer NGF-inducibility to the chloramphenicol acetyltransferase (CAT) gene when placed in front of the CAT gene and transfected into PC12 cells. Deletion analysis of the 5′ flanking region of NGFI-A has revealed areas that are important for NGF inducibility, as well as an area that functions as a repressor region. The NGFI-A 5′ flanking region contains five "CArG" boxes, elements that are homologous to the core serum response element (SRE) of the c-fos and actin genes. At least one of the "CArG" boxes is able to confer NGF-inducibility in PC12 cells when placed alone in front of the CAT gene. Gel shift assays demonstrate that all five "CArG" boxes bind with similar affinity to protein(s) from PC12 nuclear extracts. Studies are currently underway to determine whether all five "CArG" boxes are functional, whether they act in concert as inducible elements, and exactly which sequences are important for NGF inducibility.

O 434 SEGMENTAL GENE EXPRESSION IN THE DEVELOPING MOUSE HINDBRAIN David Wilkinson, Robb Krumlauf, Patrick Charnay, Angela Nieto and Leila Bradley, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK.

Anatomical studies of the vertebrate hindbrain have shown that it is organised in a segmental pattern: rhombomeres (r) are bounded by sulci that restrict specific populations of developing motor neurons, and neurogenesis is first initiated in alternate segments. We have found that the expression of certain genes is restricted to specific hindbrain segments in the mouse embryo. Expression of the zinc-finger gene Krox-20 occurs in two domains of the early neural plate, and is subsequently found restricted to r3 and r5. This "pair-rule"-type expression pattern suggests that this growth factor-responsive gene could be involved in segmenting the mouse hindbrain. Four members of the Hox-2 homeobox gene cluster have boundaries of expression at rhombomeric sulci; Hox-2.6, -2.7 and 2.8 have anterior limits at the r6/7, r4/5 and r2/3 boundaries, respectively, while Hox-2.9 expression is restricted to r4. These data suggest that Hox-2 genes may specify segment phenotype, a role analogous to that of their counterparts in Drosophila. Finally, expression of the FGF-related proto-oncogene int-2 is restricted to r5 and r6 in the hindbrain, a pattern consistent with it being regulated by Hox-2 homeobox genes. Overall, we envisage that these genes are components in a cascade of interactions that organise the segmental pattern of the vertebrate hindbrain. We are currently undertaking manipulative approaches towards analysing the function of Krox-20, and are screening for further genes with segment-restricted expression.

O 435 LIGAND BINDING DOMAIN CONTROLS DNA BINDING CHARACTERISITICS : BIFUNCTIONAL ROLES OF THYROID HORMONE AND OTHER NUCLEAR RECEPTORS, Ken N. Wills, Xiaokun Zhang, Gerhart Graupner, Thomas Hermann, Maty Tzukerman, and Magnus Pfahl. La Jolla Cancer Research Foundation, La Jolla, CA 92037.

We have recently reported that thyroid hormone receptors (TR) can function as both positive and negative regulators of gene expression through specific thyroid hormone response elements (TRE). We now investigate the role of the ligand binding domain of receptors in mediating this bifunctional role. Transient transfection and gel retardation analysis led us to propose that in the absence of ligand, TRs can occupy a specific TRE and thereby prevent or compete other activated receptors from acting as transcriptional activators of the TRE. Our present studies show that different receptors bind to specific DNA sequences with varying affinities due to differences in their DNA binding domains. This affinity is further controlled by the intrinsic properties of their hormone binding domains. Hybrid receptors were constructed to create TRs with foreign hormone binding domains. The *in vitro* translated products of these constructs were used in gel retardation assays to determine the changes in affinity and binding pattern to specific DNA sequences resulting from the alternate hormone binding domains. We observed that each receptor could bind the specific TRE in both the absence and presence of their ligands, and that the addition of ligand could lead to changes in binding affinity different from that of the parental TRs. Transient transfection studies with the hybrid receptors also showed consistent and specific changes in their repression and activation of gene transcription. These changes corresponded with those observed for DNA binding affinity in the gel retardation experiments. Our results therefore suggest that the ligand binding domain plays an essential role in determining the functional role of thyroid hormone receptors through control of the receptor-DNA interaction.

O 436 EXPRESSION OF THE C-JUN AND C-FOS ONCOGENES IN HUMAN PRIMARY MELANOCYTES AND MELANOMAS, Douglas T. Yamanishi, Julie A. Buckmeier, Mark Graham, and Frank L. Meyskens Jr., College of Med, Dept of Hem/Onc, UC Irvine, Irvine, CA 92717.

The incidence of cutaneous malignant melanoma (CMM) has been increasing at an average rate of about 4% per year in the United States. Recently, it has been possible to culture human primary melanocytes in vitro. The media requirements for in vitro melanocyte growth are phorbol esters (12-O-tetradecanoylphorbol 13-acetate, TPA), agents that raise intracellular cAMP levels (3-isobutyl-1-methyl-xanthine, IBMX), hormones (insulin), and growth factors (basic Fibroblast Growth Factor, bFGF). In order to detect the oncogenes involved in the transformation of primary melanocytes, we examined the expression levels of two transcription factors (c-fos and c-jun) in primary melanocytes and melanomas. These oncogenes are known to be affected by the various growth promoting agents in melanocyte media. Cellular fos and c-jun mRNA levels were detected at an elevated level in melanomas. Depending on the growth promoting agents present in the media, the expression of c-jun and c-fos mRNAs could be induced in primary melanocytes. Thus we demonstrate the involvement of these two oncogenes in melanocyte growth and potential involvement in the transformation of melanocytes.

O 437 **THE STRUCTURE AND EXPRESSION OF THYROID HORMONE RECEPTOR GENES IN METAMORPHOSIS OF XENOPUS LAEVIS,** **Yoshio Yaoita and Donald D. Brown, Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210**

During amphibian metamorphosis, a complex and coordinated set of biological events that influence probably every tissue of tadpoles is initiated by thyroid hormone tri-iodothyronine (T_3). We have begun to study the molecular mechanism of metamorphosis, by cloning thyroid receptor (TR) genes. Xenopus has two genomic TR genes of the α family (α and α') that share more than 97% amino acid sequence homology. The ß-TR amino acid sequence is 94% homologous to the human ß-TR in the carboxyl two-thirds of the molecule that includes the DNA and ligand binding domains. The 5' untranslated region and the amino terminal end of the ß-TR is heterogenous. A rat pituitary-specific variant of the ß receptor is known to diverge from the ubiquitous form of ß-TR at its amino terminus (1). This point of divergence occurs at exactly the same amino acid residue as does divergence of the multiple Xenopus ß receptors. The expression of ß-TR mRNA correlates with metamorphosis and is markedly upregulated by T_3, while expression of the α family of TR genes is not influenced by T_3.

1. R.A. Hodin et al. Science 244, 76 (1989).

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O 500 CLONING OF POU DOMAIN GENES IN XENOPUS LAEVIS, Veena R. Agarwal and Sheryl M. Sato, Clinical Endocrinology Branch, NIDDK, NIH, Bethesda, MD 20892. Transcription factors containing the POU domain sequence (Pit-1, Oct-1 and 2, and unc-86) may act as regulators of tissue-specific gene expression during embryonic development. Little is known about the early specification of pituitary and neural tissue. To study these developmental processes, we have cloned at least two brain- and pituitary-specific POU domain genes from Xenopus laevis. Degenerate oligonucleotides representing all possible codons for two conserved 7 amino acid regions (IKLGYTQ and WFCNRRQ) of the POU domain were used as primers in the polymerase chain reaction (PCR). The cDNA derived from adult pituitary and brain poly A^+ mRNA served as a template for the PCR. The PCR-generated cDNA from Xenopus brain and pituitary hybridized to the rat Pit-1 cDNA probe (Cell 55:505-518, 1989) under moderately stringent conditions. Sequence analysis of one gene cloned from Xenopus brain by PCR showed >70% homology to the nucleotide sequence of the Pit-1 POU domain. The PCR cDNA products were gel-purified and used to probe a late gastrula-neural plate Xenopus cDNA library and several clones have been isolated. Currently, we are characterizing these cDNA clones to determine their developmental pattern of expression and their localization in Xenopus embryos.

O 501 *MYOSIN HEAVY CHAIN GENE EXPRESSION DURING EARLY CARDIAC DEVELOPMENT. Joseph G. Bisaha and David Bader. Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021.*

The processes involved in cardiac myocyte differentiation are poorly understood. The expression of myosin heavy chain , a major component of the contractile apparatus and a hallmark of the myogenic cell lineage , was studied to investigate cardiac development. A 3.0 kb chicken myosin heavy chain clone was isolated from a day 14 embryonic ventricular cDNA library. Northern blot analysis revealed this RNA to be expressed in the day 14 ventricle and not in the atrium or pectoralis. Further RNA analysis determined that this ventricular - specific myosin heavy chain was expressed as early as 5 days after fertilization. This expression was again confined to the ventricle. DNA sequence analysis showed significant similarity to the coding regions of a reported chick embryonic skeletal muscle clone (63.6%) as well as to the rat B cardiac myosin heavy chain (67.5%). However the 3' untranslated region was unique. Preliminary data indicate that this clone recognizes an RNA in the stage 8 chick heart which is the stage when the myosin heavy chain protein is first detected. We are using this cDNA clone to identify the earliest myosin heavy chain transcripts in the developing heart as well as to investigate tissue - and developmental stage - specific expression of myosin heavy chain genes.

O 502 A MUTATION IN COMPOUND EYE DEVELOPMENT OF *DROSOPHILA* THAT RESULTS IN CELL DEATH RATHER THAN DIFFERENTIATION, Nancy Bonini, William Leiserson, and Seymour Benzer, Division of Biology 156-29, Caltech, Pasadena, CA 91125.

Flies with the *eyes absent* (*eya*) mutation lack entirely the adult compound eye, while all other external structures appear normal. In wild type, the eye begins overt differentiation in the eye imaginal disc during the third larval stage. At this time, a wave of differentiation is marked by the advance of a "morphogenetic furrow", the appearance of differentiation antigens, and the formation of clusters of pre-photoreceptor neurons. In the *eya* mutant, there is an arrest in the normal expansion in size of the eye disc, and the usual morphological and antigenic signs of differentiation fail to occur. Cell division studies with bromodeoxyuridine indicate that precursor cells are being generated, but electron microscopic and acridine orange staining show that the cells undergo cell death rather than differentiation. The *eya* gene may be involved in the decision of precursor cells to take a differentiation pathway rather than a cell death pathway at a critical stage in their morphogenesis. We are currently cloning the locus to determine the molecular function of the gene.

O 503 PULSATILE PRESSURE AND STRETCH OF PULMONARY ARTERY ENDOTHELIAL CELLS INDUCES SMOOTH MUSCLE GROWTH INHIBITION, Toni Bothwell and Marlene Rabinovitch, Division of Cardiovascular Research, The Hospital for Sick Children, Toronto, Ontario, Canada. M5G 1X8. Pulmonary artery (PA) smooth muscle (SM) cells are growth inhibited in medium conditioned by stretching PA endothelial cells at high pulsatile pressure (100/60 mmHg, 60 Hz. for 24 h). Inhibition of PASM cell DNA synthesis was assessed by decreased ^{3}H-thymidine incorporation (liquid scintillation spectroscopy and autoradiography) and was confirmed by flow cytometry. This response was not observed when cells from the aorta were used instead of PA. The PASM growth inhibition was reversed by the addition of PDGF but not TGFß; both are positive growth regulators for confluent PASM cells. Lack of PDGF, however, did not explain the PASM growth inhibition observed as judged by radioimmunoassay (RIA) measurements or by the effect of PDGF neutralizing antibodies. RIA also failed to show an increase in pulsated conditioned medium (PCM) of SM growth inhibitors, i.e., PGE_2, PGI_2, α or γ interferon. Nor was there evidence that PASM growth inhibition was the result of increased heparin or heparan sulfate (HS). Although pre-incubation of all media with heparatinase abolished the inhibition in PCM relative to non-PCM and addition of HS reduced PASM thymidine incorporation in nonPCM, mimicking the inhibition, direct measurement of HS and fragments using ion exchange and size exclusion HPLC revealed low levels in PCM. Physical characteristics suggest that the inhibition in PCM is trypsin stable but may be heat and acid sensitive and is present in two molecular weight fractions, 30-100kD and <10kD. Reduced inhibitory activity resulted when protease inhibitors were added during pulsation suggesting that a protease may be involved in release or activation. We speculate that the inhibition may reflect release and loss from the sub-endothelial matrix of a novel compound that normally acts to control PASM proliferation. Alternatively, neosynthesis of an inhibitor by the endothelial cells during pulsation may limit the magnitude of the SM proliferative response apparent *in vivo* that may be related to another compound, e.g. platelet PDGF.

O 504 THE PROTO-ONCOGENE INT-1 ENCODES A SECRETED PROTEIN ASSOCIATED WITH THE EXTRACELLULAR MATRIX, Roger S. Bradley and Anthony M.C. Brown, Department of Cell Biology and Anatomy, Cornell University Medical College, New York, NY 10021.

The proto-oncogene *int*-1 plays an important role in mammary tumorigenesis when activated by proviral insertions of the mouse mammary tumor virus (MMTV). The gene is normally expressed only in spermatids of adult mice and in the embryonic neural tube, suggesting a developmental role for int-1 protein. Consistent with this notion is the evidence that the *Drosophila int*-1 homolog, *wingless*, is involved in embryonic pattern formation. In order to obtain a cell line expressing high levels of int-1 protein we have infected fibroblasts with multiple copies of a retroviral vector carrying *int*-1 cDNA. By immunoprecipitation and Western blot analysis we have identified a 44,000 M_r form of int-1 protein that is secreted from the cells. A small quantity of this protein is detectable in the conditioned culture medium. However, a much larger proportion can be extracted from extracellular material remaining on the culture dish after the cells are removed. This indicates that the majority of the secreted int-1 protein is associated with components of the extracellular matrix (ECM). The protein is more abundant in conditioned medium when cells are grown in the presence of soluble heparin and it binds heparin-sepharose *in vitro*, eluting at 0.65M NaCl. These data suggest that the protein may bind heparan sulfate or other glycosaminoglycans of the ECM. We are currently investigating the biological significance of these findings in terms of the mechanism of action of *int*-1.

O 505 *EXPRESSION OF A MUTATED BOVINE GROWTH HORMONE GENE RESULTS IN* SMALL TRANSGENIC MICE, Wen Y. Chen, David Wight, Thomas Wagner, and John J. Kopchick, Department of Zoology, Molecular and Cellular Biology Program, and Edison Animal Biotechnology Center, Ohio University, Athens, Ohio 45701

To determine the importance of an amphiphilic alpha helix (helix III) of bovine growth hormone (bGH), the following approaches were used: (1) Mutagenesis of the target region of bGH such as to generate a "perfect" amphiphilic helix. (2) In vitro expression analysis of the mutated bGH gene in cultured mouse L cells. (3) Mouse liver membrane binding studies of wild type and mutated bGH. (4) Transgenic mouse production using the mutated bGH gene. Oligonucleotide-directed mutagenesis protocol was used to generate an altered bGH (bGH-M8), which encode the following changes: glutamate 117 to leucine, glycine 119 to arginine and alanine 122 to aspartate. This bGH mutation was shown to be expressed in and secreted by mouse L cells and possessed the same binding affinity to mouse liver membrane preparations as wild type bGH. Transgenic mice containing the mutated bGH gene, however, showed significant growth retardation phenotype. The magnitude to growth retardation is directly related to serum levels of the altered bGH molecule.

O 506 cis REGULATORY SEQUENCES OF THE HUMAN MIP GENE. Ana B. Chepelinsky and M. Michele Pisano. Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health. Bethesda, MD 20892.
The MIP (Major Intrinsic Protein) gene product is specifically located in the lens fiber membrane of vertebrates. Gene transcription can be observed during development in the primary fibers as early as the lens vesicle stage. This protein appears to be phosphorylated by a cAMP-dependent protein kinase and may play a role in lens fiber cell-cell communication. We are presently studying the sequences involved in the tissue specific expression of this gene. To this end, we have cloned the human MIP gene by screening a human leukocyte genomic library with a bovine MIP cDNA (Gorin et al. Cell 39: 49, 1984). Sequencing data indicates that the gene is 3.6 Kbp long and contains four exons. The 5' flanking region of the human MIP gene contains a TATA box and two CCAAT boxes. 5' Flanking DNA fragments of different lengths, synthesized by PCR (polymerase chain reaction), were introduced upstream of the chloramphenicol acetyltransferase (CAT) reporter gene. Transient gene expression assays in explanted chicken lens epithelia indicated that a DNA fragment containing 300 bp of 5' flanking sequence contains an active promoter. This promoter functions with approximately the same efficiency as the promoter of the mouse Gamma 2 crystallin gene (which is also specifically expressed in lens fibers). We are presently mapping the cis regulatory elements responsible for the lens specific expression of the MIP gene.

O 507 THE PURIFICATION OF MESODERM-INDUCING PROTEIN FROM BOVINE AMNIOTIC FLUID. Oleg Yu. Chertov, Alexey L. Krasnoselsky, Mikhail E. Bogdanov*, Olga Hoperskaya*, Shemyakin Institute of Bioorganic Chemistry, 117871 GSP, Moscow, * - Institute of General Genetics, Moscow, USSR.
We found that bovine amniotic fluid contains mesoderm-inducing activity. We isolated a protein, which induces mesoderm from isolated animal poles of early gastrula embryos of *Xenopus laevis*. Purification scheme includes ion-exchange chromatography, chromatography on hydroxylapatite and two successive reverse phase HPLC on a C3 resin. Employing the described procedure we obtained a highly purified preparation of mesoderm-inducing factor, that was then subjected to SDS-PAGE in absence of reducing agent with subsequent transfer onto Immobilon membrane. The determination of mesoderm-inducing activity of the eluted proteins and comparison with the stained bands allowed us to estimate M of mesoderm-inducing protein to be 25000. This protein is stable in acidic conditions, but treatment with reducing agent and protease digestion comletely destroyed the activity. In low picomolar concentrations the protein induces muscle and notochord, as well as blood cells, in *X. laevis* test system. Some properties of the isolated protein (mol. mass, sensitivity to reducing agents, acid stability) and ability to induce mesoderm resemble that of TGF beta2. Identification study of the purified protein is currently in progress.

O 508 DEVELOPMENTAL EXPRESSION OF LEUKAEMIA INHIBITORY FACTOR (LIF) GENE IN PREIMPLANTATION BLASTOCYST AND IN EXTRAEMBRYONIC TISSUE OF THE MOUSE EMBRYO. François Conquet and Philippe Brûlet. Unité de Génétique Cellulaire. Institut Pasteur. 25, rue du Dr. Roux, 75015 Paris. FRANCE. Murine LIF protein is a growth factor which has the capability of maintaining the developmental potential of pluripotent embryonic stem (ES) cells through a specific receptor. We have examined the expression pattern of the LIF gene from the preimplantation stage (3.5 days post coitum) to the midgestation stage (12.5 days p. c.) of the mouse embryo. LIF transcripts were detected at the preimplantation blastocyst stage whereas no transcripts were detectable in ES cells. LIF gene transcription continues in the extraembryonic tissue of the 7.5-day p. c. and in the embryonic part of the placenta of 9.5-, 10.5- and 12.5-day p.c. embryos. No transcripts were detected in embryo proper of the corresponding stages. Our results suggest that this growth factor is synthesized in the extraembryonic part of the embryo and act upon the embryonic tissues during early mouse development.

O 509 INTEGRIN EXPRESSION IN EMBRYONIC STEM CELLS.
Helen Cooper and Vito Quaranta, Research Institute of Scripps Clinic, La Jolla, California

Integrins are transmembrane receptors for matrix components, and mediate both cellular adhesion and migration. In addition, integrins have been shown to play a critical role in Drosophila development. However, little is known about integrin expression in the early embryonic stages of mammalian development. Here we demonstrate that embryonic stem cells and F9 embryonic carcinoma cells, before and after differentiation into parietal endoderm, express integrins belonging to the β_1 integrin subfamily. The predominant integrin expressed in these cell lines was observed to be $\alpha_6\beta_1$ which has previously been demonstrated to be a laminin receptor. Since laminin is also expressed by embryonic stem cells and differentiated F9 cells, it is possible that interaction between $\alpha_6\beta_1$ and laminin may contribute to the early topographic organization of the mouse embryo.

O 510 DYNAMICS OF KERATIN FILAMENT ASSEMBLY: EXPRESSION OF MUTANT EPIDERMAL KERATIN CDNAS IN VIVO AND IN VITRO. Pierre Coulombe, Kathryn Albers, Anthony Letai, Yiumo Chan and Elaine Fuchs. Department of Molecular Genetics and Cell Biology, Howard Hughes Medical Institute, The University of Chicago, Chicago, IL 60637. There are ~30 different keratin proteins which constitute the intermediate filaments found in most epithelial cells. There are two distinct sequence classes: acidic type I (40-56.5K) and basic type II (53-67K), both of which are essential for filament formation. Each type has a 310 central α-helical domain which is 55-99% homologous within members of the same type, flanked by non-helical end domains of variable length and sequence. The helical sequences contain heptad repeats of hydrophobic residues, indicative of their ability to form coiled-coil dimers. The process of filament assembly involves ~20-30,000 polypeptides, which organize into a hierarchy of higher ordered structures in the apparent absence of auxiliary proteins and factors. To begin to elucidate (a) the nature of the molecular interactions that lead to filament formation and (b) the dynamics of this process in epithelial cells, we have introduced deletions and point mutations in the coding segments of epidermal keratin cDNAs, tagged these cDNAs with a C-terminal sequence encoding the antigenic portion of the neuropeptide substance P, and added a promoter/enhancer to drive the expression of these mutant constructs in various epithelial and non-epithelial cells. Using a monoclonal antibody against substance P, we have conducted both immunofluorescence and immunoelectron microscopy to examine the behavior of these mutant keratins in the assembly process. Our results reveal interesting insights into the dynamics and mechanisms of filament assembly.

O 511 EXPRESSION OF A MYOGENIC REGULATORY GENE, QMF1, IN DEVELOPING AVIAN EMBRYOS, Fabienne Charles de la Brousse, David J. Goldhamer and Charles P. Emerson, Jr., Department of Biology, University of Virginia, Charlottesville, VA 22901.
Qmf1 is a quail myogenic regulatory gene that is transcribed in skeletal myoblasts and differentiated muscle and shows sequence homology to MyoD1 and myf5. As shown for mouse MyoD1, the qmf1 cDNA, under the control of an SV40 promoter, also converts C3H10T1/2 cells to stable myoblast colonies. Using an *in situ* hybridization approach, we have examined qmf1 and contractile protein mRNA expression in the developing quail embryo. Our results show that qmf1 gene activation is coincident with cardiac actin mRNA expression and follows the rostral/caudal gradient of somite compartmentalization in early staged embryos. Qmf1 mRNA and contractile protein mRNAs localize in the myotome of more mature, compartmentalized somites. Furthermore, qmf1 mRNAs selectively localize over cells of the dorsal medial lip of the dermatome, suggesting that this region is a site of myogenic lineage determination. Qmf1 is also an early marker for limb myogenesis. In stage 24 hind limb buds, qmf1 mRNAs localize over dorsal and ventral regions that extend along the proximo-distal axis of pre-muscle mass formation. In contrast to the somite, dorsal and ventral localization of qmf1 expression preceeds the initiation of differentiation. To study qmf1 protein distribution in the embryo, we raised qmf1-specific anti-peptide antibodies. Preliminary immunocytochemistry results support the *in situ* analyses showing localization in the myotomal compartment of the somite.

O 512 PREIMPLANTATION EMBRYO DEVELOPMENT: COOPERATIVE INTERACTIONS AMONG EMBRYOS AND ROLE OF GROWTH FACTORS, S.K. Dey and B.C. Paria, Departments of OB/GYN and Physiology, University of Kansas Medical Center, Kansas City, KS 66103. There has never been a report that clearly shows the effects of a growth factor on preimplantation embryo development. Growth factors produced by the embryo or the reproductive tract may affect embryonic development and function in an autocrine/paracrine manner. If it is true that growth factors produced by embryos act on them, then it could be hypothesized that preimplantation embryos cultured individually in a defined volume of culture medium will show inferior development. This could reflect a greater dilution of the growth factors secreted from individually cultured embryos into the medium as compared to those co-cultured in groups. This inferior development should then be corrected by the addition of growth factors to the culture medium. To test this hypothesis, 2-cell mouse embryos were cultured in groups of 5 or 10, or individually in microdrops (25 μl) under silicon oil in Whitten's medium for 72h in the absence or presence of EGF or TGF-α (2-10 ng/ml). Embryos cultured individually showed inferior development to blastocysts as compared to those cultured in groups (48% vs 80%). The inferior development to blastocysts of embryos cultured individually was markedly improved (79%) by the addition of EGF (4ng/ml) to the culture medium. Furthermore, embryos cultured individually had fewer number of cells (34.1±1.9) at the blastocyst stage as compared to those cultured in the presence of EGF (62.7±5.7), or in groups (58.0±4.0). The addition of TGF-α (4 ng/ml) although increased the number of individually cultured embryos to blastocysts (78%), number of cells/blastocyst showed only a moderate increase (41.8±3.3). The stage of embryonic development that was primarily affected by these treatments appeared to be between 8-cell and blastocyst. Embryos (8-cell) cultured in groups, or individually in the presence of EGF also showed higher incidence of zona-hatching. The effects of EGF or TGF-α were coupled with the appearence of EGF receptors on the embryonic cell surface. The results suggest that growth factors of embryonic and/or reproductive tract origin participate in preimplantation embryo development and blastocyst functions in an autocrine/paracrine manner.

O 513 **Tissue interactions during gastrulation that induce expression of the EN protein in neural ectoderm of *Xenopus laevis*.** Tabitha Doniach, Ali Hemmati-Brivanlou and Richard Harland. Department of Molecular and Cell Biology, UC Berkeley, CA 94720

We have been studying the tissue interactions required for neural induction in *Xenopus laevis*. Specifically, we are studying the interactions required for induction of an anterior neural marker, the EN protein. This nuclear protein is expressed in a narrow band of cells in the midbrain region of the neurectoderm starting at stage 14 (early neurula). It is detected with a monoclonal antibody that recognizes the homeodomain of proteins in the *engrailed* family. Tissue interactions were studied by combining various portions of presumptive mesoderm from mid gastrulae with competent ectoderm from late blastulae or early gastrulae. In addition, presumptive ectoderm was explanted at different stages during gastrulation to determine when it has been specified to make EN. Recombinates and explants were cultured until neurula stages and processed to detect EN.

We found that in the majority of recombinates, neither anterior nor posterior involuted dorsal mesoderm nor dorsal blastopore lip was sufficient on its own to induce expression in competent ectoderm. In contrast, EN expression was detected in a majority of the recombinates when anterior mesoderm and dorsal lip were combined with ectoderm in the same recombinate. Posterior mesoderm showed only weak effect when combined with dorsal lip and ectoderm.

We conclude that dorsal lip and anterior mesoderm act together to induce EN in neural ectoderm during gastrulation. Some of this inductive information has already been passed to the ectoderm by mid-gastrula, since we have also found that ectoderm is specified to make EN protein if explanted at around stage 11.5 or later, but no earlier. Experiments are in progress to determine whether the information from the dorsal lip is passed through the mesoderm or directly to the ectoderm.

O 514 *THE ESTABLISHMENT AND PARTIAL CHARACTERIZATION OF EMBRYONIC QUAIL CELL LINES DERIVED FROM SPLANCHNIC MESODERM. Carol A. Eisenberg and David Bader. Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021.*

Six cell lines have been established from methylcholanthrene-treated embryonic tissue of Japanese quail (Coturnix coturnix japonica). Each line was derived from splanchnic mesoderm taken from stage 4 to stage 6 embryos and cultured in growth media on tissue culture plates precoated with fibronectin (10μg/ml). Primary cell cultures were cloned by the fourth passage with selective serial passages at clonal densities and have been maintained through 16 passages. The resulting cultures all exhibit mononucleated, binucleated, and multinucleated cells which differ in size and morphology. Cell division varies among the cell lines with doubling times ranging from one to two weeks. Preliminary immunofluorescence assays have demonstrated that all cells react positively with antibodies against muscle-specific actin and keratin, but negatively with antibodies against factor VIII, desmoplakin I and II, desmin and sarcomeric muscle myosin heavy chain (MF20). However, when exposed to DMSO or retinoic acid for over a week, myosin expression was induced in these cell lines as visualized by their positive reaction to MF20. Further studies will be necessary to determine the phenotypes of these cell lines and their developmental potential.

O 515 INFLUENCE OF WHITE FAT ON THE GROWTH, DIFFERENTIATION, AND METASTASIS OF A MURINE MAMMARY CARCINOMA. Elliott, B.E., Tam, S-P., Dexter, D. and Chen, Z-Q. Cancer Research Laboratories, Department of Pathology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Previously we have shown that a murine mammary carcinoma cell line, SP1, grows and metastasizes more efficiently when transplanted into the mammary gland compared to the subcutaneous site (Cancer Res. 48: 7237-7245, 1988). In this report, we have further examined the tissue specificity of this phenomenon. Our results showed that 3 X 10^3 SP1 cells grew more efficiently in the mesentery and ovarian fat pads compared to the mammary gland; virtually no growth occurred from the same cell inoculum in the subcutis or peritoneum. In addition, massive dissemination of tumors from the ovarian and mesentery sites to the liver, spleen, and diaphragm was also observed. In contrast, metastases from the mammary site occurred primarily in the lung. Co-transplant of 3 x10^3 SP1 cells with ovarian white fat fragments into the subcutis resulted in tumor formation compared to no tumors in sham controls. Explanted white fat fragments were capable of promoting the growth of SP1 cells on defined medium in vitro. Removal of the ovaries significantly reduced the incidence of tumor take in the ovarian fat pad but not in the mammary fat pad or the subcutis, suggesting that ovarian-derived growth promoting substances in white fat can affect local tumor growth. Increased expression of estrogen receptor (ER), progesterone receptor (PR), and class I major histocompatibility (MHC) antigens was demonstrable on SP1 cells growing in the ovarian fat pad compared to the subcutis. ER, PR, and MHC levels were reduced to background following transplant of tumors onto plastic. These results are consistent with the notion that white fat exerts a positive regulatory effect on SP1 mitogenesis and differentiation, including expression of steroid hormone receptors and class I MHC antigens. The SP1 system has therefore proven in our hands to be a useful model for studying the effect of tissue microenvironment on tumor growth and metastasis (This work was supported by grants from the National Cancer Institute of Canada and the Medical Research Council of Canada).

O 516 **INSULIN-LIKE GROWTH FACTOR II AND MOUSE DEVELOPMENT**

Carolyn J. Elliss, Heather Marshall, Lynne J. Richardson, Andrew Ward and Christopher F. Graham. Department of Zoology, University of Oxford, South Parks Road, Oxford, OX1 3PS, U.K.

IGF-II transcription can be detected throughout mouse embryonic development by RT-PCR and Northern blotting. However, transcription in most adult tissues is low, having declined rapidly in the neonate. What are the functions of IGF-II in development?

Embryonic stem (ES) cells, derived from the blastocyst, provide an *in vitro* model for primitive ectoderm function during embryonic development. An analysis of IGF involvement in ES cell growth and differentiation will be presented. Synthetic human IGFs can promote the multiplication of ES cells in serum free media, and differentiation of ES cells induced by withdrawal of DIA is accompanied by marked changes in IGF ligand and receptor expression.

Further insight into the actions of IGF-II *in vivo* might be provided by ectopic and elevated expression of the gene in transgenic mice. Preliminary data describing the phonotype of mice transgenic for human IGF-II will be discussed.

O 517 LEVELS OF SPARC EXPRESSION ARE CORRELATED WITH ALTERED GROWTH AND DIFFERENTIATION CHARACTERISTICS FOR F9 EMBRYONAL CARCINOMA STEM CELLS STABLY TRANSFECTED WITH SPARC cDNA. Elizabeth A. Everitt and Helene Sage, Department of Biological Structure, University of Washington, Seattle, WA 98195

SPARC is a secreted glycoprotein, present in embryonic and adult tissues undergoing proliferation, migration and differentiation. It is an abundant product (0.5% of mRNA) of murine parietal endoderm (PE), and is upregulated 20-fold in F9 teratocarcinoma cells treated with retinoic acid (RA) and cyclic AMP (cAMP); these chemicals induce F9 cells to differentiate to a PE cell phenotype. To determine whether premature overexpression or underexpression of SPARC in F9 cells alters the F9 cell phenotype, as well as the sequential expression of discrete proteins during differentiation with RA and cAMP, F9 cells were co-transfected with pKO neo and either sense or antisense SPARC expression vectors. Southern blot analysis of DNA from clonal F9 lines revealed that exogenous SPARC copy number varied among the lines, and Northern analysis indicated that the lines were expressing a SPARC transcript of the correct size predicted for the expression vectors. Radioimmunoprecipitations of culture medium showed the over - or underexpression of SPARC protein that corroborated the Southern and Northern data. Lines overexpressing SPARC protein had a delayed temporal response to inducers of differentiation and altered adhesion properties. These data suggest that stable incorporation of SPARC cDNA into the F9 cell genome modifies the cellular differentiation response and diminishes adhesion properties. Cell lines exhibit variations in several parameters, including 1) maintenance of stem cell characteristics 2) cell cycle times 3) response to exogenous SPARC, and 4) cytoskeletal modifications.

O 518 EXPRESSION AND ALTERNATE SPLICING OF THE β-AMYLOID PRECURSOR PROTEIN IN EARLY MOUSE EMBRYOGENESIS, Shannon Fisher, John D. Gearhart, and Mary L. Oster-Granite, Developmental Genetics Lab, Department of Physiology, Johns Hopkins School of Medicine, Baltimore, MD 21205

The β-amyloid peptide, the main component of the amyloid plaques of Alzheimer's disease and Down syndrome, is part of a larger precursor molecule, the amyloid precursor protein (APP). The APP gene has been mapped to human chromosome 21 and to mouse chromosome 16. The human gene demonstrates tissue specific alternate splicing, and the splicing regulation is similar in the mouse. As a step toward understanding the role of APP in development, we have examined its expression in mice from ovulated oocytes to the late embryonic stage, using combined reverse transcription and the polymerase chain reaction. While the three major splicing forms are present at all stages, the ratios of the three are not constant; the relative amount of the shortest splicing form increases from the egg cylinder to the neurula stage. Screens of cDNA libraries from the same two stages indicate that abundance increases from 0.001% to 0.01% over this time period. Cellular localization of APP at these stages will help in understanding the role APP is playing in development.

O 519 FIVE NOVEL INT-1 RELATED GENES ARE EXPRESSED DURING MOUSE DEVELOPMENT, Brian J. Gavin, Jill A. G. McMahon and Andrew P. McMahon, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Hoffman-La Roche Inc., Nutley, N.J. 07110

Int-1 is a mammalian proto-oncogene implicated in mammary tumorigenesis whose normal expression is restricted to the developing fetus and mature testis. Extensive in situ hybridization analysis has localized fetal expression to a small subset of cells located at the dorsal mid-line of the developing neural tube. As the Drosophila homolog of mammalian int-1 (wingless) is involved in positional specification in the developing embryo, an attractive hypothesis is that mammalian int-1 functions to specify cell position in the developing nervous system. To date, conventional screening methods have not revealed any genes homologous to int-1 (McMahon, unpublished data). However, recently an int-1 homolog was isolated by chance in a chromosomal walk (Wainwright et al., 1988). Like int-1 from all species examined, the predicted protein contains a signal peptide, a large number of absolutely conserved cysteine residues and small regions of highly conserved sequence. We have used a modification of the polymerase chain reaction (Kamb et al., 1989; Libert et al., 1989) to isolate five novel int-1 related genes directly from 9.5 day embryo cDNA. Expression of these genes is largely restricted to fetal development and is localized to specific regions of the fetus. We believe these genes, together with int-1 and irp, constitute a new gene family which may play a role in cell to cell communication in the developing fetus.

O 520 *IDENTIFICATION OF CRITICAL EARLY STAGES IN AVIAN CARDIAC MYOGENESIS AND PRODUCTION OF EMBRYONIC SPLANCHNIC MESODERMAL cDNA LIBRARIES REPRESENTATIVE OF THESE STAGES,* *Arlene Gonzalez-Sanchez, Michael Montgomery, Judith Litvin and David Bader, Dept. of Cell Biology and Anatomy, Cornell Medical College, NYC, N.Y. 10021.*

Identification of a particular cell lineage is crucial to investigating the cellular and molecular mechanisms which govern the determination and differentiation of the cardiac myoblast. Splanchnic mesodermal cells from stage 4-8 chick embryos were isolated, dissociated and grown in vitro at clonal or at high density for 2 days. The cultures were exposed to antibodies against: 1) common sarcomeric myosin heavy chain (MHC)(MF20); 2) atrial MHC (B1); 3) ventricular MHC (A19) and 4) conduction system MHC (ALD58). Cells grown at clonal density react to MF20 but not to the other antibodies whereas those grown at high density react to MF20, B1 and A19. Cardiogenic mesoderm from stages 4-8 was exposed to the co-carcinogen TPA, and grown as organ cultures. TPA irreversibly blocks the expression of muscle specific proteins in stages 4-7 but not in stage 8 as measured by reactivity to the MHC antibodies and 2-D PAGE of radiolabeled proteins. Having demonstrated early commitment of splanchnic mesoderm to the cardiac lineage and a chemically interruptible event(s) between stages 4 and 7 we have proceeded to isolate presumptive cardiac mesoderm from stages 4-8 of the chick embryo. Using both conventional and PCR techniques we have established unidirectional, transcriptionally and translationally competent cDNA libraries for each of these stages. We are currently attempting to isolate cDNAs for transcripts involved in the developmental regulation of cardiac commitment.

O 521 CELL SURFACE GALACTOSYLTRANSFERASE FUNCTION DURING AVIAN EMBRYOGENESIS IN VIVO, Helen J. Hathaway and Barry D. Shur, Department of Biochemistry and Molecular Biology, The University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030

Cell surface receptors allow cells to interact selectively with the extracellular environment, as well as initiate differentiative events by transducing appropriate signals across the plasma membrane. Cell surface β1,4 galactosyltransferase (GalTase) serves as a receptor during a variety of cellular interactions by binding its specific glycoconjugate substrate on adjacent cell surfaces or in the extracellular matrix. Such interactions include growth control of cultured cells, sperm:egg interactions, and mesenchyme migration (Hathaway and Shur, BioEssays, 9, 153-158). We are studying surface GalTase function during embryonic development in vivo, using the avian embryo as a model system. Anti-GalTase polyclonal antibodies raised against purified chicken serum GalTase were shown to react with avian GalTase by immunoprecipitation and Western immunoblotting. Furthermore, anti-GalTase IgG inhibits migration of avian neural crest cells on laminin substrates in vitro. To study the function of surface GalTase in vivo, anti-avian GalTase IgG was microinjected into the migratory pathway of cranial neural crest cells of stage 8-10 chicken embryos. Anti-GalTase IgG significantly inhibited the migration of neural crest cells on the injected side of the embryo, as compared to control embryos injected with preimmune IgG. These studies support the hypothesis that surface GalTase is an important component of the migratory process and suggest a role for cell surface GalTase during normal embryonic development.

O 522 HOMOLOGOUS RECOMBINATION IN TISSUE-SPECIFIC GENES IN ES CELLS; MODELS FOR PHYSIOLOGICAL INVESTIGATIONS INTO GENE FUNCTION, Randall S. Johnson,[1,3] Morgan Sheng,[2] Michael E. Greenberg,[2] Richard D. Kolodner,[1,3] Virginia E. Papaioannou,[4] and Bruce M. Spiegelman[1,3], Departments of Biological Chemistry and Molecular Pharmacology,[1] and Microbiology,[2] Harvard Medical School, the Dana-Farber Cancer Institute,[3] and the Department of Pathology, Tufts University[4], Boston, MA 02115

We have used murine embryonic stem cells (ES cells) in an attempt to study the function of certain genes which mayplay important roles in tissue-specific cellular and systemic physiology. We have made constructions using the sequences encoding the c-fos, adipsin, aP2, and alpha-tumor necrosis factor genes and used these constructions to alter their endogenous counterparts via homologous recombination. The constructs were designed to make the genes dysfunctional, by disrupting either transcription or translation. Homologous recombinants in these genes were isolated in ES cells using a variation of the double selection method of Mansour, Thomas, and Capecchi. While we saw rates of homologous recombination over random integration (neomycin resistance) of between 10^{-3} and 10^{-5} in these transfections, which were consistent from construct to construct, we saw no correlation between these rates and the levels of expression of the genes in ES cells. c-fos, which is expressed at a low level in ES cells (as assayed by Northern blot) shows recombination at a rate of 2×10^{-4}; aP2 and adipsin, adipocyte-specific genes, have no detectable message on Northerns and show homologous recombination rates of 5×10^{-3} and 5×10^{-4}, respectively. We have used these ES cell lines to make chimeric mice in both outbred (CD-1) and inbred (C57/Bl6) strains. We have generated chimeras with the c-fos, adipsin, and aP2 recombinant ES cell lines, and are attempting at present to determine whether there has been a germ line contribution in any of the chimeras.

O 523 THE INDUCTION OF ENDODERM IN GROWTH FACTOR INDUCED ECTODERMAL EXPLANTS, Elizabeth A. Jones, Hugh R. Woodland and Margaret H. Abel, Department of Biological Sciences, University of Warwick, Coventry, West Midlands, CV4 7AL, U.K.

A monoclonal antibody which is highly specific for Xenopus endoderm has been obtained by immunisation with isolated endoderm from stage 42 embryos. This antibody identifies an antigen that first becomes expressed throughout the endoderm of the late neurula. As development proceeds, the distribution of the antigen becomes more restricted to the cells lining the lumen of the gut, and persists in the adult gut in a subset of luminal cells. This is the first example of an entirely endoderm specific marker.

This reagent, 4G6, has been used in conjunction with other monoclonal markers to study the differentiation of tissues within animal cap explants from blastula stage embryos after treatment with XTC-MIF and other growth factors. These explants clearly form endodermal tissue in addition to the normal range of mesodermal tissues. Dose response experiments will be described and the role of mesodermal inducers in the formation of endoderm discussed.

O 524 **THE DEVELOPMENTAL PLASTICITY OF THE SEA URCHIN MESOMERES:**

O. Khaner and F. Wilt, Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley Ca 94720.

The developmental potential of the sea urchin were re-examined. We have employed a new method to hand isolate substantial numbers of pure types of blastomeres from 16 cell stage embryos, and we have used newly available molecular markers to analyze possible vegetal differentiation. We have found that while isolated mesomere pairs behave according to the classical expectations, and develop into ectodermal vesicals, there is a clear effect of reaggregating two or more mesomeres. They survive better in long term culture, and after prolonged periods, they display an astonishing ability to express vegetal differentiation. We also combined micromeres or macromeres from the vegetal hemisphere, that had been stained with lineage tracer, and the ratio of the numbers of these vegetal cells to the number of mesomeres was varied. Though induction of guts and spicules was observed there was little if any effect of varying the ratio of cells on the kinds of differentiation obtained. Hence, the earlier findings of Horstadius on gradients of developmental potential are probably best interpreted as a result of morphogenetics rather than the ability to differentiate various cell types.

O 525 DEVELOPMENTALLY REGULATED EXPRESSION OF THE *XENOPUS LAEVIS c-MYC* GENE RESTRICTS, FROM THE OOCYTE AND EARLY EMBRYO, ONE FORM OF *c-MYC* mRNA WHICH POSSESSES TRANSLATIONAL INHIBITORY SIGNALS. MICHAEL W. KING[1], ROBERT N. EISENMAN[2], PHILIP LAZARUS[3], NAHUM SONENBERG[3]. [1]INDIANA UNIVERSITY SCHOOL OF MEDICINE, TERRE HAUTE CENTER FOR MED. ED. TERRE HAUTE, IN. 47809 [2]THE FRED HUTCHINSON CANCER CENTER, SEATTLE, WA. 98104 [3]DEPARTMENT OF BIOCHEMISTRY, McGILL UNIVERSITY, MONTREAL, CANADA H3G 1Y6. The *Xenopus c-myc* gene is structurally similar to the mammalian *c-myc* genes. There are 3 exons and the major open reading frame is located in exons 2 and 3. The *Xenopus c-myc* gene exhibits a restricted pattern of expression during oogenesis and early development such that one of two major *c-myc* transcripts detected by RNase protection is present in the embryo only after zygotic transcriptional activation at the mid-blastula transition (MBT). We have designated these transcripts as being initiated at two distinct sites termed P_1 and P_2 where the P_1 transcript exhibits the restricted pattern of expression. Chimeras were constructed between the region of transcription initiation in the *Xenopus c-myc* gene and the CAT reporter gene to assay for the presence of translational inhibitory signals in the 5' ends of the corresponding 2 transcripts. The *in vitro* synthesized mRNAs were injected into oocytes or early embryos and extracts were assayed for the level of CAT activity. Synthetic mRNAs containing the region of both P_1 and P_2 together or P_1 alone, inhibited the synthesis of CAT activity in oocytes. However, RNAs containing only the region of P_2 did not inhibit synthesis of CAT activity indicating that translational inhibition is conferred by mRNA sequences 5' to the P_2 site. Translational inhibition by the P_1 region was significantly diminished in *Xenopus* embryos by 10hrs after injection. The pattern of expression of *Xenopus c-myc* mRNAs in oocytes (and consequently pre-MBT embryos) is restricted to the use of the P_2 site. This may be a mechanism that allows unrestricted *c-myc* protein synthesis in pre-MBT embryos through restricted synthesis of *c-myc* mRNAs.

O 526 EXPRESSION AND INTRACELLULAR ACCUMULATION OF A SIGNAL PEPTIDE-LESS BOVINE GROWTH HORMONE IN TRANSGENIC MICE MAY INTERFERE WITH RECEPTOR TURNOVER, Stephen J. McAndrew, June Yun, Thomas Wagner, and John J. Kopchick, Department of Molecular and Cellular Biology, Edison Animal Biotechnology Center, Ohio University, Wilson Hall, Athens, OH 45701

Export of bovine growth hormone (bGH) from cultured fibroblasts or anterior pituitary cells requires an NH_2-terminal sequence known as a signal peptide. DNA sequences coding for this signal sequence were deleted and the signal-peptide-less bGH gene joined downstream of the mouse metallothionein I (MET) regulatory sequences. This plasmid, pMET-(ΔSP-bGHID), was shown to direct expression of the bGH gene in mouse L-cells albeit the protein was detected exclusively in cell lysates. Production of transgenic mice bearing and expressing this transgene showed marked reduction in the growth phenotype. Transgenic to non-transgenic growth ratios were between 0.79 and 0.90 at week 12. BGH was not detected in the serum of these transgenic mice but after zinc induction and partial hepatectomies, could be found within liver cells. Saturation binding studies are underway to characterize the total number (βmax) of growth hormone receptors present on liver cell membranes compared to non-transgenic littermates.

O 527 EXPRESSION OF THE *int-1* GENE FAMILY DURING MOUSE DEVELOPMENT. Jill A. McMahon, Brian J. Gavin, Andrew P. McMahon, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, NJ 07110. In the abstract by Gavin *et al.*, we report the isolation of a number of mouse genes related to the proto-oncogene *int-1*. Here we describe expression of members of this gene family during fetal and adult life.

O 528 ACTH-IMMUNOREACTIVE PEPTIDES ARE PRESENT IN MOUSE EMBRYO MESODERM AND STIMULATE MYOBLAST PROLIFERATION. M. Molinaro, G. Cusella-De Angelis, L. De Angelis, E. Vivarelli, C. Boitani, M. Bouchè and G. Cossu Institute of Histology, University of Rome, Via A. Scarpa, 14 -00161 Rome, Italy. ACTH and related POMC-derived peptides are potent and selective mitogens for fetal myoblasts and satellite cells (Cossu et al Dev Biol 131, 331, 1989). In as much as embryonic myoblasts arrest proliferation earlier than other myogenic cells, we investigated whether this might depend upon differential sensitivity to various growth factors. The results obtained showed that at concentrations of 1 nM, ACTH stimulates embryonic myoblast proliferation, with a maximal effect at about 20 nM. These results raised the problem of the physiological significance of the mitogenic effect of ACTH during an embryonic period which preceeds adenohypofhisis development. However, recent evidence of POMC transcripts and ACTH-immunoreactive peptides in non-neural tissues, prompted us to investigate the possible presence of such molecules in post-implantation mouse embryo. By R.I.A. the highest level of ACTH immuno-reactive material was found in the limb bud of 10 d.p.c. embryos, a period when the ACTH present in the placenta (a known ACTH producing organ) is still very low. Immunofluoresce analysis revealed the presence of ACTH-immunoreactive material in the basal lamina of primary myotubes. Indeed ACTH weakly binds to heparin, suggesting a basal lamina localization, common to many growth factors. Moreover both spinal cord neurons and myotubes in vitro contain significant amount of ACTH-immunoreactive material and are stained in the peri-nuclear region of the cytoplasm by an -ACTH antibody. Preliminary evidence show the presence of POMC transcripts, though at very low level, in mouse limb buds. We suggest that locally produced ACTH-like peptides might regulate myoblast proliferation in a paracrine fashion.

O 529 CULTURED DERMAL PAPILLA CELLS PARTICIPATE IN HAIR FOLLICLE DEVELOPMENT FOLLOWING TRANSPLANTATION INTO FOETAL SKIN, G. Philip M. Moore and Pilas Pisansarakit, CSIRO Division of Animal Production, P.O. Box 239 Blacktown, NSW 2148 Australia. The dermal papilla of the hair follicle is generally believed to have a central function in follicle development and fibre specification. Transplantation of isolated papillae into inactive follicles or skin has resulted either in the reactivation of fibre production or formation of new follicles (Pisansarakit *et al.* J. Embryol. exp. Morph. 94, 113-119, 1986). These observations suggest that papillae, which are composed of a unique population of mesenchymal cells, have specific inductive functions. In order to investigate this further, we dissected dermal papillae from individual sheep hair follicles and examined the activities of the component cells *in vitro*. In early passage cultures, the cells proliferated and as density increased, displayed aggregative behaviour reminiscent of that shown by mesenchymal cells during follicle initiation. An intrinsic capacity of these cells to form condensations in the absence of an epidermis is consistent with the hypothesis that foetal mesenchyme has an instructive role in follicular development. The viability and inductive activities of cultured papillae were also examined following transplantation of tritiated thymidine labelled cells between the epidermis and mesenchyme of midside skin from 14 day old foetal mice. When the skin recombinants were grafted onto *nude* mice, labelled cells were detected, by autoradiography, in the dermal papillae of the follicles that developed. The apparent retention of some differentiated functions by papilla cells following a period in culture suggest that they may be used to elucidate the nature of inductive processes *de novo* in the skin.

O 530 DEVELOPMENT EXPRESSION OF TWO TYROSINE KINASE RECEPTORS IN THE MOUSE EMBRYO, Avi Orr-Urtreger, David Givol, Yosef Yarden and Peter Lonai, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel

The developmental expression of c-kit, a receptor with an unknown ligand, encoded by the W locus of the mouse, and murine flg, a receptor for FGF were investigated. W mutants are defective in three stem cell systems: hemopoietic stem cells, primordial germ cells, and migrating melanoblasts. Transcripts of c-kit were detected in the yolk sac and the embryonic liver, in primordial germ cells in the germinal ridge in the branchial pouches and later in various craniofacial and cervical derivatives of the neural crest and also in migrating pigment cells. Thus c-kit is expressed in most of the organs and lineages which are defective in W mutants. In addition the gene is active in the developing brain and also in the adult hippocampus and cerebellum. c-kit is expressed during oogenesis in young and mature oocytes, but not in the mature male germline. c-kit expression in gametogenesis, oogenesis and early embryogenesis will be discussed. We have isolated and characterized the murine flg gene. flg is a receptor tyrosine kinase with unique cytoplasmic tyrosine kinase domains. flg binds aFGF and bFGF and is expressed in tissues of mesodermal and neuroectodermal origin. In midgestation embryos flg is expressed in the somites and their sclerotomic derivatives: in prevertebrae and later in invertebral discs. flg expression in presomitic embryos will be discussed.

O 531 CLONING AND ANALYSIS OF AN ORGAN-SPECIFIC CDNA EXPRESSED IN BOTH THE ECTODERMAL ADRENAL MEDULLA AND MESODERMAL ADRENAL CORTEX, Sharon E. Plon, Nancy Sack, Lee J. Helman, Mark A. Israel, Molecular Genetics Section, Pediatric Branch, NCI, National Institutes of Health, Bethesda, MD 20892

The adrenal gland is comprised of two different tissues, the medulla and cortex. The parenchymal cells of these tissues are derived from the ectodermal and mesodermal layers, respectively. Migrating neural crest cells invade the primitive adrenal cortex at 7 weeks in human development and there differentiate into functioning chromaffin cells. We have previously described a 200 bp cDNA, PG2, isolated from a pheochromocytoma library which is expressed in both the primitive adrenal cortex and in the medullary cells as they differentiate into mature chromaffin cells. Northern analysis of total RNA suggested that the message is 1.6 kb in length and is only expressed in the adrenal gland. To learn more about this adrenal-specific mRNA we have constructed a plasmid cDNA library from mRNA purified from a normal adult human adrenal gland. A 1550 basepair cDNA which overlaps the original PG2 sequence was isolated from this library. Analysis of the sequence reveals a 287 amino acid open reading frame with three methionines clustered at the amino terminal end. This ORF encodes a very acidic protein with an estimated molecular weight of 30.7 KD and a pKa of 2.40. Interestingly, this putative protein contains an N-glycosylation site and RGD tripeptide at the carboxy terminus suggesting that it may be a membrane-associated or extracellular protein, with a potential role in cell adhesion or organ morphogenesis. Ongoing studies include the production of anti-peptide antibodies to determine the cellular location and possible functions of the protein encoded by this novel organ-specific message.

O 532 **REGULATION OF GENE EXPRESSION DURING THE GROWTH AND DEVELOPMENT OF HAIR.** T.J. Rea[1], A. McNab[3], A.M. Flenniken[4], A.E. Buhl[2], T. Kawabe[2], G. Vogeli[3], B.R.G. Williams[4] and V. Groppi[1]. 1. Cell Biology, 2. Hairgrowth, 3. Molecular Biology, The Upjohn Co., Kalamazoo, MI 49001 and 4. Hospital for Sick Children, Toronto, Canada. The expression of several genes that are specific for different layers of the hair follicle were analyzed during growth and development of this organ. Previous studies indicated that the expression of an ultra-high sulfur keratin (UHSK) gene correlated with active hair growth in new born mice. Using in situ hybridization we established that UHSK was only expressed in the cortex of the keratogenous zone of growing vibrissa and coat hair follicles. Analysis of the expression of UHSK in embryos showed that this gene was not active until day 18 of development which correlates with the formation of keratinized hair. In the adult, the expression of UHSK was perfectly synchronized with the cycle of active hair growth. When vibrissa follicles were dissected and placed in culture, the expression of UHSK rapidly declined. Minoxidil (MNX), an agent that promotes the growth of hair, induced and sustained the expression of UHSK for up to 3 days in culture. In parallel experiments, the expression of c-fos, histone H3 and TIMP which are localized in the differentiating epithelium, germinal matrix and outer root sheath respectively, were also analyzed. Taken together, these data support the conclusion that changes in gene expression can be used to identify the molecular events associated with the growth and differentiation of hair. Finally, it appears that MNX is acting like a hair-specific growth factor by promoting the in vivo and in vitro growth and differentiation of this organ.

O 533 THE CULTURE MICROENVIRONMENT AFFECTS ACTIN EXPRESSION DURING BC3H1 MYOGENIC CELL DIFFERENTIATION. J.C. Reeser and A.R. Strauch, Department of Anatomy and the Molec., Cell. and Develop. Biol. Program, College of Medicine, The Ohio State University, Columbus, OH 43210-1239.
The expression of multiple actin isoform genes can be modulated in murine BC3H1 myogenic cells by altering culture conditions. Confluent BC3H1 myoblasts differentiate in serum-deficient medium and express two muscle-specific alpha-actins. To more completely characterize the pathway(s) leading to cytodifferentiation, we investigated the effect of modifying culture media formulation and substrate composition on the induction of the differentiated phenotype. Total RNA was isolated at various times after exposure to serum-supplemented (DME/0.5% FBS) or serum-free (N2) differentiation media and analyzed on northern blots using actin gene-specific probes. Post-confluent myoblasts permitted to differentiate in N2 showed a marked early increase in the level of alpha-actin mRNA compared to cells exposed to DME/0.5% FBS. Incorporation of radiolabeled thymidine by myoblasts was similarly low in both N2 and DME/0.5% FBS indicating that the observed differences in alpha-actin expression were not a consequence of unequal populations of cycling versus non-cycling cells. Myoblasts exposed to either media were equally competent to differentiate since both treatments promoted a similar level of myogenin mRNA expression which peaked within 48 hrs. Taken together the results suggest that alpha-actin mRNA accumulation in BC3H1 cells may be more closely linked to extrinsic factors than to intrinsic genetically-programmed developmental signals. The coupling of actin mRNA expression to extracellular signals was further indicated by the finding that sub-confluent myoblasts allowed to maturate in N2 on a myogenesis-permissive extracellular matrix prepared from fully-differentiated myocytes exhibited proportionally higher levels of alpha-actin mRNA expression than post-confluent myoblasts induced with this medium. DME/0.5% FBS was ineffective in promoting differentiation on this substrate. We propose that a hormone-supplemented, defined serum-free medium formulation in combination with proximity to a cytodifferentiation-permissive substrate stimulates optimum expression of alpha-actin mRNA in BC3H1 myogenic cells. Supported by AHA/Ohio (C-88-42) and the NIH (HL 43370).

O 534 UNIQUE DOMAINS OF THE DEVELOPMENTALLY REGULATED, Ca^{+2}-BINDING PROTEIN SPARC HAVE DIVERSE EFFECTS ON ENDOTHELIAL CELL BEHAVIOR.
H. Sage, T. F. Lane, and M. L. Iruela-Arispe, Dept. of Biological Structure, Univ. of Washington, Seattle, WA 98195.

SPARC (Osteonectin, BM-40) is a secreted Ca^{+2}-binding protein differentially synthesized in embryonic tissues, in certain adult tissues undergoing remodeling, or in response to proliferative cues or injury. SPARC was upregulated during the formation of endothelial capillary-like tubes *in vitro* and was induced by the angiogenic protein, basic fibroblast growth factor. mRNA levels of SPARC were highest in proliferating cells directly involved in tubular morphogenesis. To identify functional domains in the native protein, we synthesized polypeptides representing subdomains of SPARC and produced anti-peptide polyclonal antibodies. A peptide corresponding to the C-terminal Ca^{+2}-binding domain (an E-F hand) exhibited Ca^{+2}-dependent binding to extracellular matrix proteins, with specificities similar to those of native SPARC. In addition, peptides corresponding to both N- and C-terminal Ca^{+2}-binding domains inhibited cell spreading when added to endothelial cells in culture. Anti-spreading activity occured at millimolar concentrations (0.1-1.0mM), was blocked by specific anti-peptide antisera, and was enhanced by inhibitors of protein synthesis. We propose that SPARC plays a specific role in endothelial tube formation that involves overt changes in cell shape and interaction with the extracellular matrix.

O 535 A LINEAGE-SPECIFIC EFFECT OF LEUKEMIA INHIBITORY FACTOR (LIF) ON DIFFERENTIATION OF F9 EMBRYONAL CARCINOMA CELLS. Michael M. Shen and Philip Leder, Department of Genetics and HHMI, Harvard Medical School, Boston, MA 02115. We are studying the expression of endogenous LIF in F9 cells and in visceral or parietal endoderm derived after retinoic acid-induced differentiation. We have found that LIF expression is elevated in parietal endoderm monolayers derived from F9 cells, whereas uninduced F9 cells or visceral endoderm-containing embryoid bodies have a low basal level. Furthermore, we have asked whether exogenous LIF might affect F9 differentiation. For this purpose, we have overexpressed LIF in the murine plasmacytoma J558L, and have differentiated F9 cells in the presence of control vs. LIF-containing J558L-conditioned media. The addition of LIF appears to eliminate visceral endoderm formation on F9 embryoid bodies, as confirmed by their failure to produce alpha-fetoprotein. In contrast, parietal endoderm differentiation is essentially unaffected in the presence of LIF, as determined by cell morphology and by increased expression of laminin B1. This differential effect suggests that LIF specifically inhibits the pathway of visceral endoderm formation. Our results on the effects of LIF on the differentiation of other EC lines and ES embryoid bodies will also be presented.

O 536 TWO C-MYC GENES FROM XENOPUS LAEVIS, Georges Spohr and Etienne Principaud, laboratoire d'Embryologie moléculaire de l'Université, CH-1211 Genève 4. The c-myc proto-oncogene is well conserved in vertebrate evolution. Two homologous c-myc genes myc 1 and myc 2 have been isolated from a genomic X.laevis library. The degree of conservation between the two clones myc 1 and myc 2 in the coding area suggests that the X.laevis c-myc gene exists in two copies. We have isolated and identified the two putative promoter regions and determined their sequences. Comparison with those of mouse and human reveals common features. Among them, the negative regulatory element that affects the expression from both P_1 and P_2 human promoters. Construction of chimeric genes where the CAT coding sequence is under the control of the myc promoters and putative regulatory elements will now allow us to clarify the mechanisms controlling transcription in X.laevis oocytes and embryos.

O 537 THE LOCATION OF LEUKEMIA INHIBITORY FACTOR (LIF) EXPRESSION DURING MOUSE EMBRYOGENESIS. Colin L. Stewart, Lisa Brunet, Harshida Bhatt, Peter Kaspar and Susan Abbondanzo, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

LIF (Leukemia Inhibition Factor) is a protein secreted by a variety of cell lines, which has the property of inhibiting the differentiation of embryonic stem (ES) cells *in vitro*. Little, however, is known about its function *in vivo* during normal embryogenesis. We find that LIF is expressed at very low levels throughout all stages of embryogenesis. However, increased levels are observed in some extra embryonic tissues and in the uterus, which suggests that LIF may have a tissue distribution similar to other myeloid growth factors. To gain further insight into its function, we are altering the levels and location of LIF synthesis by expressing LIF from the phosphoglycerate kinase (PGK) promoter which can act constitutively. Transfection of ES cells with such a construct renders the ES cells feeder independent and also inhibits their differentiation. We are currently investigating how constitutive expression of LIF in ES cells affects their ability to participate in normal embryogenesis by introducing these clones into embryos.

O 538 STUDY OF ALPHA-SATELLITE DNA MAINTENANCE IN HUMAN CELLS DURING INDIVIDUAL DEVELOPMENT, G.D.Telegeev, K.N.Kiyanitsa, E.I.Terestchenko, S.S.Maliuta,Institute of Molecular Biology and Genetics, Acad.Sci. of the Ukrainian SSR,Kiev,252143,USSR he DNA repeated sequences number varies during individual development of an organism. We detected that the number both of 340 bp(dimeric) and 680 bp(tetrameric) alpha-satellite DNA in embryonic sample presented by chorionic villus of 17-19 days old human embryos decreased significantly as judged by quantitating the Eco RI restriction fragments of human DNA studied in comparison with the DNA samples for blood white cells taken from adult individuals. We showed that this number decrease was individual specific for DNA samples studied.

O 539 SYNDECAN EXPRESSION IS INDUCED AS AN EARLY RESPONSE TO EPITHELIAL-MESENCHYMAL INTERACTIONS IN THE DIFFERENTIATING KIDNEY AND TOOTH MESENCHYME, Seppo Vainio[1,2], Markku Jalkanen[3], Lauri Saxén[1], Eero Lehtonen[1], Scott Saunders[4], Merton Bernfield[4] and Irma Thesleff[2]; [1]Department of Pathology, University of Helsinki, Helsinki, Finland, [2]Institute of Dentistry, University of Helsinki, Helsinki, Finland, [3]Department of Medical Biochemistry, University of Turku, Turku, Finland, [4] Department of Pediatrics, University of Stanford, Stanford, USA. The development of the kidney and tooth is regulated by signals transmitted between epithelial and mesenchymal tissues. The molecular mechanisms of these interactions are poorly understood partly because of the lack of early markers for differentiation. We have shown earlier that syndecan expression in the kidney and tooth is transient and associates to the morphogenetic period. The tissue recombination experiments between rat and mouse epithelial and mesenchymal tissues showed that syndecan expression in the kidney as well as in the tooth mesenchyme is induced by the invaginating epithelial bud. These results were confirmed by using metabolic labeling and immunoisolation of syndecan synthesized by the induced and uninduced mesenchymes. The amount of radioactivity eluted from the immunoaffinity column was more than twice as high in the induced kidney and tooth mesenchymes as compared with the amount of radioactivity eluted from uninduced samples. Northern-blot analysis of the mRNA molecules isolated from uninduced and induced mesenchymes indicated a similar kind of activation of the mRNA (2.6 kb) for syndecan core protein. The results demonstrate that syndecan expression is activated early during kidney and tooth organogenesis and that syndecan can be used as a marker to identify factors involved in mediation of inductive signalling in these tissue interactions. We propose a role for syndecan in the kidney and tooth organogenesis, particularly in mediation of cell-matrix interactions during cell condensation, which is seen as an early morphological response to induction.

O 540 INDUCTION AND REGULATION OF MESODERMAL AND NEURAL DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS.

A.J.M. van den Eijnden-van Raaij, T. van Achterberg, A.H. Piersma and C.L. Mummery.
Hubrecht laboratory, 3584 CT UTRECHT, The Netherlands.

In mammalian embryogenesis mesoderm is formed from embryonic ectoderm in an area adjacent to visceral endoderm. An in vitro model for this process has been established using cocultures of P19 embryonal carcinoma (EC) cells and END-2 cells, a visceral endoderm-like cell line derived from P19. Coculture with END-2 cells causes P19 EC cells to aggregate and to form mesoderm-derived beating muscle cells (1). We have now shown that aggregation of EC cells in hanging drops in medium conditioned by END-2 cells, followed by plating of the aggregates onto tissue culture plastic (hanging drop assay) also resulted in the formation of beating muscle as well as neurons. Spontaneous differentiation of EC cell aggregates was eliminated by the use of media containing serum which was treated with dextran-coated charcoal (DCC). Using the hanging drop bioassay the differentiation-inducing activity secreted by END-2 appeared to be trypsin-, heat- and acid-labile while reducing agents had no effect, indicating that an FGF-like factor might be involved in the differentiation process. Activin A, a member of the transforming growth factor β family appeared to be a potent inhibitor of the END-2 induced differentiation. This factor had no mesoderm-inducing activity in P19 EC cell aggregates, in contrast to its effect on Xenopus ectoderm cells. These results suggest a possible role for the differentiation-inducing END-2 activity and for activin A in the induction and regulation, respectively, of mesoderm and neuron formation during normal murine embryonic development.

(1) A.H. Piersma et al. (submitted).

O 541 THE HIV tat GENE INDIRECTLY INDUCES BOTH DERMAL LESIONS RESEMBLING KAPOSI'S SARCOMA AND HEPATIC TUMORS IN TRANSGENIC MICE, Vogel, J.[1], Hinrichs, S.[2], Napolitano, L.[1] and Jay, G.[1], [1]Laboratory of Virology, Holland Laboratories, American Red Cross, Rockville, MD 20855, [2]Department of Human Pathology, University of California School of Medicine, Davis, CA 95616.

The HIV tat gene is critical for both viral gene expression and viral replication. When placed in transgenic mice under its own regulatory region, both skin lesions that resemble Kaposi's sarcoma (KS) and liver tumors are induced in male mice. These tumors are also present in patients infected with HIV. Expression of the tat gene is only present in the epidermal portion of the skin, and expression is not seen in the dermal tumors or in the hepatic tumors. This would suggest that the KS-like skin lesions and the hepatic tumors are indirectly induced by the tat gene, possibly by the induction of growth factors. These data imply that the HIV tat gene plays a role in the development of malignancies associated with HIV infection.

O 542 PRODUCTION OF TRANSGENIC MICE CONTAINING A cDNA CODING FOR AN ALKALINE PHOSPHATASE UNDER HEAT-SHOCK PROMOTOR CONTROL, Saul L. Zackson, Ralph J. Greenspan, Joel Berger, and Maureen C. O'Brien, Department of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110

Previous studies on amphibian embryos have implicated alkaline phosphatase as being involved in the guidance of embryonic cell migrations (*Dev. Biol* **127**, 435-442, *Development* **105**, 1-7). A cloned full-length cDNA coding for the human placental form of alkaline phosphatase (PLAP) was placed under the control of the human hsp 70 promotor. This construct was demonstrated to be heat-inducible in a mouse cell line. Attempts were made to produce transgenic mice using this construct cut out "cleanly" from the vector sequences. A large number of mice (>50) were born from injected eggs and screened for transformation by a polymerase chain reaction (PCR) assay. No transformants were recovered. However, when plasmid DNA was linearized so as to leave the vector sequences intact 5' to the promotor, 3 transformed mice were identified by PCR. We propose that when the construct is injected "cleanly," PLAP is expressed and is lethal to the embryo, but when injected with vector sequences intact, the background level of expression is reduced sufficiently to enable survival. Studies are underway to examine morphogenetic cell behavior in embryos induced for PLAP expression.

O 543 EFFECT OF THE EXUDATE OF TOWEL GOURD(LUFFA CYLINDRICA ROEM) ON RICE ANTHER CULTURE,Zhang Chengmei and Zhang Zhenhua,Shanghai Academy of Agricultural Sciences, Shanghai 201106,P.R. of China.

This paper reported that rice anther culture ability and natural diploid frequency of pollen-derived plants were considerably increased due to the addendum of 15%-20% exudate of towel gourd into the induction medium. It was found that the induction medium with 15%-20% exudate of towel gourd was optimum to form calli and the calli formed in such medium had high green plant differentiation frequency as well. The differentiation frequency of Japonica calli reached more than 95% and that of Indica calli reached more than 70%,which was almost twice as much as the CK. Natural diploid frequency of pollen plants was higher than the CK with a significant difference at 0.01 level. The experiment also revealed that after the subculture of the calli, except for the calli growing in the medium with the addendum of 25% exudate of towel gourd the calli growing in the media with 10%,15% and 20% exudate of towel gourd were 5.7-18.9g/10 clumps heavier in weight than their CK. The fresh weight and the dry weight of the green plants increased respectively 2.0-89.0mg/10 plants and 13.0-30.0mg/10 plants in the comparison with the CK. Consequently, in the range of 10-20% concentration, the exudate of towel gourd could promote plant cell division and growth and 15%(v/v) exudate was shown to be the best. The green plants derived from this medium were strong. It was believed through UV spectral analysis that the exudate of towel gourd contained some growth-promoting substances such as organic and inorganic components and cytokinin which were similar to those in the N_6 medium