GROWTH AND DIFFERENTIATION FACTORS IN VERTEBRATE DEVELOPMENT

Organizers: Rik Derynck and Zena Werb

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Plenary Sessions Page

Poster Sessions

Keynote Address

W 001 HOW DOES EXTRACELLULAR MATRIX REGULATE GENE EXPRESSION?: VIA INTEGRINS AND TRANSCRIPTIONALLY.
Mina J. Bissell, Lawrence Berkeley Laboratory, Berkeley, 94720

Along with the spectacular advances in our understanding of the structure of genes and their regulatory sequences, the pains-taking work of developmental and cellular biologists has determined that the microenvironment in which a cell finds itself decisively and specifically regulates the expression of tissue-specific genes. In the last decade, my laboratory has used two versatile model systems to define the important regulatory elements of such microenvironments: 1)the interaction of the extracellular matrix (ECM) with the mouse mammary epithelial cells in culture and 2)the interaction of Rous sarcoma virus with the embryonic limb *in ovo*.

While I will concentrate on the first system, the conclusions from both systems are that the microenvironment is dominant in allowing the expression of both normal and malignant phenotypes, and that extracellular matrix and the three dimensional structure of the tissues are crucial determinants of such regulations.

Our evidence indicates that the basement membrane in general, and laminin in particular, regulate the expression of β-casein gene, that the regulation is via interaction with integrins, that such regulation is transcriptional and that there is a unique ECM and prolactin response element (an enhancer) in the 5' region of the β-casein gene that requires both ECM and hormones for induction of gene expression. I will present a working model for how such elements may play a role in expression of tissue-specific genes in general.

Furthermore, the ECM selectively suppresses the expression of a number of other genes including growth factors and the ECM molecules themselves. I will discuss the importance of ECM *in vivo* and the possible relevance of these findings to malignancy and metastasis.

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Growth Factors and Gene Expression-I

W 002 SIGNAL TRANSDUCITON BY PDGF RECEPTORS, FGF RECEPTORS, AND BY A RECENTLY IDENTIFIED RECEPTOR FOR VASCULAR ENDOTHELIAL GROWTH FACTOR/PERMEABILITY FACTOR, L.T. Williams[1,2,3], J.A. Escobedo[1,2,3], W.J. Fantl[2,3], A. Klippel[1,2,3], C. de Vries[2,3], and N. Ferrara[4] Howard Hughes Medical Institute[1], Cardiovascular Research Institute[2], University of California, San Francisco[3], and Genentech Inc., South San Francisco, California[4].

The first step in signal transduction by tyrosine kinase growth factor receptors is ligand-induced formation of receptor oligomers, a process that facilitates receptor autophosphorylation. When phosphorylated on tyrosines, the receptors form non-covalent complexes with several cytoplasmic proteins that have been implicated in the regulation of cell proliferation. We identified the sites on the platelet-derived growth factor (PDGF) receptor and fibroblast growth factor (FGF) receptor that mediate binding of the receptors to signaling molecules. Each of these sites consists of a tyrosine in the context of a short sequence that determines which signaling molecule binds to the site. The interactions of each signaling molecule with the receptor can be selectively disrupted by either mutating the sites on the receptor or by adding short tyrosine-phosphorylated peptides that mimick the sites and compete for binding to the signaling molecules. In this way, we have been able to selectively block binding of PDGF receptors to phosphatidylinositol 3-kinase and GTPase activating protein, and binding of the FGF receptor to phospholipase C-γ. Selective disruption of each of these pathways has distinct functional consequences on the ability of the receptor to stimulate cellular response associated with growth factor action. We have also studied the sites on the signaling molecules that bind the phosphotyrosine-containing sequences on the receptors. These sites lie within SH2 domains of each signaling molecule. The interaction of each SH2 domain with its corresponding phosphotyrosine sequence is of high affinity (K_D equals 0.1 to 5 nM) and is extremely specific.

In a different set of experiments, we have identified a cDNA that encodes a receptor for vascular endothelial growth factor/vascular permeability factor (VEGF/VPF). This factor is a highly specific endothelial cell mitogen that induces an increase in capillary permeability. VEGF/VPF has some sequence similarity (20-25% identity) to PDGF. We cloned the cDNA that encodes a receptor for VEGF/VPF. This receptor has multiple immunoglobulin domains and a kinase insert region, establishing it as a member of the PDGF receptor family. The receptor binds VEGF/VPF with a K_D of approximately 20 pM. Some of the signal transduction properties of the VEGF/VPF receptor differ from those of the PDGF receptor.

Cell- Matrix Interactions in Development (Joint)

W 003 SYNDECAN, THE PROTOTYPE OF A FAMILY OF INTEGRAL MEMBRANE PROTEOGLYCANS, ACTS AS A HIGHLY REGULATED "RECEPTOR PARTNER", Merton Bernfield, Harvard Medical School, Boston, MA 02115

All adherent vertebrate cells have heparan sulfate, a heparin-like glycosaminoglycan (GAG), at their surfaces. These GAG can bind a wide variety of components in a cell's microenvironment, including extracellular matrix, growth factors, degradative enzymes and proteinase inhibitors. These components change during embryonic development and tumor invasion and can influence the adhesion, shape, growth, and differentiation of cells. Indeed, adding heparin to model systems developing in culture modifies their morphogenesis. Therefore, we hypothesized that changes in the amount and type of cell surface heparan sulfate could control the influence of these components during morphogenesis and tumorigenesis. A major source of cell surface heparan sulfate is an integral membrane heparan sulfate-containing proteoglycan, syndecan (from the Greek, *syndein*, to bind together), that is the prototype of a proteoglycan gene family. The expression of syndecan in embryos and neoplastically transformed cells is highly regulated. Syndecan appears soon after fertilization and localizes to the cells that will form the embryo. It is lost from the anterior mesenchyme following gastrulation and its subsequent expression follows morphogenetic rather than histologic patterns and is dictated by epithelial-mesenchymal interactions. It also shows cell-specific expression: syndecan is predominantly at the cell surface on epithelia, where it has smaller heparan sulfate chains, but is predominantly intracellular in mesenchymal cells, where its heparan sulfate chains are larger. Indeed, these heparan sulfate chains from epithelial and mesenchymal cells differ in the number and size of N-sulfated, iduronic acid-rich domains. Syndecan on epithelia associates with the actin cytoskeleton via its cytoplasmic domain and inducing syndecan deficiency in cultured epithelia alters their shape, ability to migrate within matrix and response to growth factors. Syndecan expression is reduced upon neoplastic transformation. Thus, because syndecan may interact with a variety of extracellular effectors, we propose that syndecan is a "receptor partner", acting in combination with more highly specific receptors, which mediates the actions of matrix components and growth factors on cells. Thus, change in expression of the syndecan family of proteoglycans at distinct times or sites may regulate the effects of the microenvironment on cells.

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W 004 GENETIC ANALYSES OF CELL ADHESION IN FLIES AND MICE. Richard O. Hynes,[1,2] Elizabeth L. George[1,2], Elisabeth N. Georges,[2] Yevgenya Grinblat,[3] Fotis Kafatos,[3] Stephenie Paine-Saunders,[1,2] Helen B. Rayburn[3], Joy T. Yang,[1,2] Gene Yee[2] and Susan Zusman,[1,2] [1]Howard Hughes Medical Institute and [2]Center for Cancer Research, Department of Biology, M.I.T., Cambridge, MA and [3]Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA.

Cell-matrix adhesion plays crucial roles in embryonic development, in normal physiological processes such as hemostasis and wound healing and in pathological processes such as thrombosis, inflammation and cancer. The molecules involved in these processes have been studied extensively *in vitro* but the functions *in vivo* have been much less analyzed.

In order to analyze the functions of the adhesive extracellular matrix protein, fibronectin, and of cell surface integrin adhesion receptors, we are using genetic methods to investigate their roles in intact organisms, namely flies and mice.

Fibronectin is being analyzed in mice using techniques of homologous recombination in embryonic stem (ES) cells to derive strains of mice which contain defective fibronectin genes. The fibronectin transcript is alternatively spliced to produce multiple different fibronectin isotypes. These alternatively spliced forms are expressed in tissue and developmentally specific fashion but their differences in function are not yet understood. We have generated both null mutants of fibronectin and alleles altered in their pattern of alternative splicing. The results so far demonstrate that FN-null is a recessive early embryonic lethal mutation. Mice heterozygous for fibronectin are viable but express reduced levels of the protein in their blood. They will be examined for more subtle defects in functions thought to involve fibronectin. The mutations altered in pattern of splicing will provide information on the functions of the alternatively spliced segments of fibronectin. Work in progress to generate transgenic mice expressing different alternatively spliced forms of fibronectin should provide complementary information. We are also analyzing the functions of certain integrin subunits which participate in fibronectin receptor function by similar approaches in mice.

We have been unable to detect fibronectin in *Drosophila* but integrins are present. Using preexisting mutations in the gene encoding the β subunit of the position-specific (PS) integrins we have been analyzing the functions of this family of integrins in the development of *Drosophila* embryos, muscles, wings and eyes. Using various genetic combinations and somatic clones, we have shown the involvement of these receptors in all four situations. Using P-element transposons, we have reintroduced wild type and mutant integrin subunits under the control of either their own promoter or a heat-inducible promoter. This allows definition of the times of requirement for integrins in the various processes and the requirement for specific structural elements within the protein.

We have also detected alternative splicing in the extracellular domain of the PS integrin β subunit and again using P-element rescue, have shown that some systems can use either spliced form but that proper embryonic development requires both forms. Further work should reveal the significance of the alternative splice for the structure and functions of this family of integrins.

Many processes involving cell adhesion and movement and axonal outgrowth proceed almost normally in flies in the complete absence of these integrins, suggesting that alternative adhesion molecules can suffice. Accordingly we have used PCR to search for other integrins in *Drosophila* and have discovered a novel β subunit which shows a highly restricted pattern of expression in the midgut of the developing embryo. The gene for this subunit has been mapped and small deficiencies obtained. These deficiencies should allow isolation of mutants in the gene encoding this novel β subunit.

Growth Factors in Gene Expression-II

W 005 POST-TRANSCRIPTIONAL AND TRANSCRIPTIONAL CONTROL OF AP-1 (JUN/FOS) ACTIVITY. Michael Karin, Tod Smeal, Bernard Binetruy, Hsin-Fang Yang-Yen, Jean-Claude Chambard, Tiliang Deng and Adriana Radler-Pohl, Department of Pharmacology - 0636, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0636.

AP-1 is a transcriptional activator composed of homo-and heterodimeric Jun and Jun/Fos complexes. It is involved in the activation of various target genes, such as: collagenase, stromelysin, IL2 and TGFβ1, by tumor promoters, growth factors and cytokines. In addition AP-1 activity is elevated in response to expression of transforming oncogenes including *H-ras*, *v-src*, and *v-raf* and is required for cell proliferation. AP-1 activity is subject to complex regulation both transcriptionally and post-transcriptionally. Transcriptional control determines which of the *jun* and *fos* genes is expressed at any given time in any given cell type. Therefore, transcriptional control determines the amount and composition of the AP-1 complex. Transcription of the *jun* and *fos* genes is subject to both positive and negative autoregulation and is highly inducible in response to various stimuli including those associated with cell proliferation.

AP-1 activity is also regulated at the post-transcriptional level. Both cJun and cFos are phosphoproteins that are subject to regulated phosphorylation. In the case of cJun, phosphorylation of sites near the DNA-binding domain inhibits its DNA-binding activity while dephosphorylation reverses this inhibition. It is also possible that phosphorylation of cJun on other sites increases its ability to activate transcription without affecting its DNA binding activity.

Another mechanism that modulates AP-1 activity is transcriptional interference by members of the nuclear receptor family. For example, the glucocorticoid receptor was recently shown to interact with cJun and cFos to form a protein complex that is no longer capable of binding to either the AP-1 recognition site or hormone response elements.

W 006 SH2 DOMAINS CONTROL INTERACTIONS OF TYROSINE KINASES WITH THEIR TARGETS. Tony Pawson, Xingquan Liu, Jane McGlade, Michael Reedijk, Luc Marengere, and Gerry Gish. Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, 600 University Avenue, Toronto, Ont. M5G 1X5, Canada.

Src homology (SH) regions 2 and 3 are noncatalytic domains that are conserved among a series of cytoplasmic signalling proteins, including Ras GTPase activating protein, phospholipase C (PLC)- , phosphatidylinositol (PI) 3'-kinase, and Src. The SH2 domains of these signalling proteins bind tyrosine phosphorylated polypeptides implicated in normal signalling and cellular transformation. Tyrosine phosphorylation acts as a switch to induce the binding of SH2 domains, thereby mediating the formation of heteromeric complexes at or near the plasma membrane. Autophosphorylation of growth factor receptors induces high affinity binding of several SH2-containing proteins, which are likely targets of receptor tyrosine kinase activity. Different receptors show distinct binding specificities for SH2-containing signalling molecules such as PLC- 1 and GAP. In some cases, the isolated SH2 domains of these proteins bind to activated receptors in vitro with the same specificity as do the intact native proteins in vivo. Hence, there is some specificity in the interactions of SH2 domains with receptors. The affinities with which different SH2 domains and autophosphorylated receptors associate may therefore control the activation of specific signalling pathways by growth factors.

The association of SH2 domains with tyrosine phosphorylated sites is also apparently involved in a network of cytoplasmic protein-protein interactions that follow growth factor stimulation. For example, the SH2 domains of GAP bind at least two tyrosine phosphorylated proteins, p62 and p190, that are candidates for regulators and targets of GAP. In addition, the c-Src SH2 domain can apparently interact with its own tyrosine phosphorylated C-terminal tail, and may thereby inhibit Src tyrosine kinase activity. Following Src activation, a number of tyrosine phosphorylated proteins associate with the Src SH2 domain. The biological consequences of these SH2-mediated interactions are under investigation.

Cell-Cell Interactions

W 007 ROLE OF THE *DROSOPHILA NOTCH* PROTEIN IN CELLULAR INTERACTIONS DURING DEVELOPMENT, Richard G. Fehon, Ilaria Rebay, Robert J. Fleming, and Spyros Artavanis-Tsakonas, Departments of Cell Biology and Biology, Howard Hughes Medical Institute, Yale University, New Haven, CT 06536.

The precise definition of cellular fate in most animals appears to depend, at some stage of development, upon local interactions between cells, all of which are potentially capable of adopting a particular fate. In *Drosophila*, genetic studies have shown that many of these decisions, and particularly those that specify the precursors of the embryonic nervous system, depend on proper function at *Notch* and at least five other loci, that are collectively called the neurogenic loci. *Notch* encodes a single transmembrane protein of over 2700 aa in length that contains an array of 36 EGF-like repeats in its extracellular domain, and a repeated motif in its intracellular domain that is similar to repeats found in the vertebrate proteins ankyrin, NF-kB, and others. To understand the developmental role of this structurally complex protein, our recent studies with *Notch* have concentrated on determining its cellular function. We first examined interactions between Notch and the protein product of another neurogenic locus, *Delta*, which also encodes a transmembrane protein with EGF-like repeats in its extracellular domain. Using inducible expression of each protein in *Drosophila* cultured cell lines, we showed that Notch and Delta interact via their extracellular domains sufficiently strongly to mediate cell-cell aggregation. More recently, we have used deletion mutagenesis and the in vitro aggregation assay to map the region of Notch that interacts with Delta. These experiments have shown that just two of the 36 EGF-like repeats in Notch are both necessary and sufficient to mediate Notch-Delta interactions in vitro, indicating a remarkable degree of functional modularity in the extracellular domain of Notch. Our cellular and subcellular analysis of Notch expression throughout development, together with genetic studies of interactions between mutant and normal cells (Heitzler and Simpson. 1991. Cell 64, 1083) strongly suggest that the biochemical interaction between Notch and Delta functions to mediate cell to cell signalling via a receptor-ligand interaction rather than a primarily adhesive function. In addition, the same two EGF-like repeats that mediate Notch-Delta interactions in vitro also appear to mediate interactions between Notch and another *Drosophila* transmembrane protein, Serrate. Taken together, these results suggest that Notch may function as a multivalent receptor for at least two and quite possibly more ligands that function during development to mediate signals necessary for the proper specification of cell fate. Our current studies are directed toward testing this hypothesis and examining the in vivo functions of particular regions of the *Notch* gene product.

W 008 CATENINS AND CADHERIN FUNCTION, Rolf Kemler[1], Jörg Stappert[1], Stefan Butz[1], Masayuki Ozawa[2], [1]Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, Germany, [2]Kagoshima University, Dept. of Biochemistry, Faculty of Medicine, 8-35-1 Sakuragaoka, Kagoshima, Japan

Cadherins are a family of transmembrane glycoproteins which mediate Ca^{++}-dependent cell-cell adhesion (2). The cytoplasmic region of the epithelial cadherin, uvomorulin, associates with distinct cytoplasmic proteins termed catenin α, β and γ (1). This association links uvomorulin to the actin filament network which seems to regulate strength of cell adhesiveness (2,3). In addition catenins connect uvomorulin via a cyto-cortical network with other membrane and cytoskeletal proteins (4). Using a combination of biochemical analyses we show that a single complex is composed of one molecule of uvomorulin, one or two molecules of β catenin, and one molecule of α catenin. Furthermore, β catenin seems to interact more directly with uvomorulin. In pulse-chase experiments β catenin is already associated with the 135 kD uvomorulin precursor (5) molecule but the assembly of the newly synthesized α catenin into the complex is only detected around the time of endoproteolytic processing (6). Antibodies against α catenin immunoprecipitate complexes which contain mouse P- or N-cadherin, chicken A-CAM, or Xenopus U-cadherin demonstrating that catenins serve as cytoplasmic anchorage for other cadherins (7). To learn more about the structure of α catenin and its involvement in transmembrane connection between cell adhesion molecules and the cytoskeleton we have cloned and sequenced murine α catenin. The deduced amino acid sequence reveals a significant homology to vinculin in the respective amino- and carboxy-terminal regions. The three internal repeats and the proline-rich region characteristic of vinculin are, however, not found in α catenin. We have also cloned and sequenced murine β catenin. This and the generation of antibodies specific for β catenin enables us to study in detail the molecular organization of the uvomorulin catenin complex. Our results suggest the possibility that each α and β catenin are members of protein families involved in the cytoplasmic anchorage of cell-cell and cell-substrate adhesion molecules.

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W 009 REGULATION OF CELL-CELL INTERACTIONS BY CADHERINS AND CADHREIN-ASSOCIATED PROTEINS, 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. The function of these molecules is regulated by cytoplasmic proteins which are associated with the intracellular domain. Recently, cDNAs of two of these cadherin-associated proteins were cloned and their primary structures were determined. One, called α-catenin or CAP102, was found to be a homologue of vinculin, and the other was similar to plakoglobin or Drosophila *armadillo* protein. With these proteins, cadherins form a molecular complex, and are concentrated in the specialized cell-cell junctions, called zonula adherentes or adherens junctions. In these junctions, other proteins, including vinculin, α-actinin, plakoglobin, radixin and actin, are clustered forming cytoplasmic plaques. Recent studies demonstrated that some of *src* family tyrosine kinases were also concentrated in these junctions. Thus, the cadherin-containing cell-cell junctions assume complex structures, suggesting that their functions themselves are complex.

We recently found a characteristic local expression of E-cadherin in the mouse embryonic brain. This molecule was expressed in the primordia of cerebellum, parts of mesencephalon, and the roof plate of mesencephalon and diencephalon at certain embryonic stages. In order to understand the role of this E-cadherin expression, we isolated the E-cadherin-containing regions of embryonic brains and organ-cultured them in the absence or presence of antibodies which blocked E-cadherin function. The results showed that the overall growth pattern of the explants was affected by the antibodies, suggesting that the E-cadherin expression in local parts of brain is necessary for this tissue to retain normal growth. Thus, cadherin-mediated cell-cell adhesion seems to involve some mechanism for regulating tissue growth.

We also have been investigating how cadherin-associated proteins control cadherin function, and found the following phenomena. We examined the expression and activity of cadherins in v-*src* transformed cells of various origins, and found that cadherin expression itself was not reduced by v-*src* transformation, but the activity of these molecules was partly impaired. Concomitantly, some of cadherin-associated proteins were phosphorylated at tyrosine residues after v-*src* transformation. Moreover, vanadate, a tyrosine phosphatase inhibitor, further inhibited strongly the residual cadherin activity in v-src transformed cells, but this reagent showed only a small effect on normal cell cadhenn activity. These results suggest that tyrosine phosphorylation may be involved in the regulation of cadherin function. It should also be noted that tyrosine phosphorylation of the cadherin system was correlated with metastatic potentials of the v-*src* transformed cells used. The regulation of cadherin function, thus, seems to be involved in multiple biological phenomena including growth control of tissues and tumor metastasis.

Neurogenesis

W 010 NEURAL CREST CELL LINEAGE. Marianne Bronner-Fraser, Developmental Biology Center, University of California, Irvine, CA 92717.

A major unanswered question concerning the neural crest is when and how the neural crest cells become determined. Migrating neural crest cells at all axial levels appear morphologically similar but differentiate into widely varied cell types, as diverse as pigment cells, neurons and cartilage. How is diversity generated within this apparently homogeneous population? One possibility is that the neural crest is a homogeneous population of multipotent cells, each with identical developmental potential and able to give rise to a large range of phenotypes. They may differentiate according to instructive cues encountered along their migratory pathway or at their final sites of localization. A second possibility is that the neural crest is a heterogeneous mixture of "predetermined" (unipotent) cells, each fated to become a given derivative. A third possibility is hat neural crest cells may represent a mixture of multipotent and predetermined cells.

To analyze the developmental potential of individual neural crest cells or their precursors, we have microinjected a vital dye, lysinated rhodamine dextran (LRD), intracellularly into the dorsal portion of the neural tube, containing premigratory neural crest cells (Bronner-Fraser and Fraser, 1988; 1989) or into the somitic sclerotome containing migrating neural crest cells. Our results show that: (1) premigratory, emigrating and migrating trunk neural crest cells *in situ* have the ability to assume multiple fates in neural crest derivatives; and (2) the neural crest is not a segregated population in the neural tube, but shares a common lineage with some neural tube cells. Because many early migrating neural crest cells are multipotent, our results suggest that restriction in the developmental potential of neural crest cells may result from interactions with surrounding tissues either during migration or at final sites of localization.

In support of a role for tissue interactions, we have found that either the notochord and/or the ventral neural tube are necessary for the expression of adrenergic traits in neural crest-derived cells. However, populations of neural crest cells do appear to become progressively restricted in developmental potential as a function of time. For example, the last neural crest cells that leave the neural tube can give rise to pigment cells and neurons, but not adrenergic, whereas early emigrating neural crest cells give rise to pigment cells, neurons, and adrenergic cells. Thus, late emigrating neural crest cells have a more limited repertoire of derivatives than early emigrating neural crest cells. In addition, the local environment may restrict the opportunities open to neural crest cells, since they fill their derivatives in a ventral to dorsal order. These experiments sum to suggest that initially multipotent neural crest cells become progressively restricted by virtue of cell interactions and/or intrinsic limitations on the range of possible cell fates.

W 011 THE EMERGING NEUROPOIETIC CYTOKINE FAMILY, Paul H. Patterson, Biology Division, California Institute of Technology Pasadena, CA 91125.

As in the hematopoietic system, the enormous variety of phenotypes in the nervous system arises, in part, through the action of instructive differentiation signals. Neuronal culture assays have allowed the identification and cloning of several proteins that control the expression of phenotype-specific genes in developing neurons. The cholinergic differentiation factor (CDF; also known as leukemia inhibitory factor; LIF) induces and suppresses the expression of a number of neurotransmitter synthetic enzymes and neuropeptides in several types of postmitotic neurons. Many of these effects are reversible, and the particular genes affected depend on the type of neuron under study. Ciliary neurotrophic factor (CNTF) alters the expression of the same set of genes in sympathetic neurons as CDF/LIF. The predicted tertiary structures of these two proteins suggest they belong to a family of cytokines that includes IL-6, ONC, MGF and G-CSF. This association is strengthened by the finding that the receptors for CDF/LIF and CNTF are homologous to the transducing and ligand binding subunits, respectively, of the IL-6-R. Moreover, CDF/LIF is a polyfunctional cytokine that shares a remarkable overlap with IL-6 in its actions on non-neural tissues.

To begin to study the role of CDF/LIF *in vivo*, we have localized its mRNA using RT-PCR and RNase protection methods. CDF/LIF mRNA levels are developmentally modulated, and substantial differences are observed between tissues. Particularly high expression is found in the target tissue of cholinergic sympathetic neurons, the footpads that contain sweat glands. Sweat glands were previously shown to induce noradrenergic sympathetic neurons to become cholinergic *in vivo*. Biochemical and immunological analysis indicates that a protein resembling CNTF is also present at the appropriate time in the footpad to mediate the phenotypic conversion that sympathetic neurons undergo during normal development. In addition, CDF/LIF mRNA is selectively expressed in discrete regions of the postnatal rat brain, and in liver and spleen at particular ages. These results support the possibility that CDF/LIF plays a role in hematopoiesis and in the mature CNS.

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W 012 CONTROL OF GLIAL CELL SURVIVAL IN VERTEBRATE NEURAL DEVELOPMENT, Martin Raff[1], Ian Hart[2] and Barbara Barres[1], [1]Medical Research Council Developmental Neurobiology Program, Biology Department, Medawar Building, University College London, UK, [2].....

A general feature of vertebrate neural development is that many neurons die soon after they have extended axons to their synaptic targets. From studies of nerve growth factor (NGF), it is thought that one mechanism that controls this normal cell death is that developing neurons compete for limiting amounts of signalling molecules (neurotrophic factors) released by their target cells, so that only a proportion of the neurons produced receive enough signal to survive. This mechanism is believed to play a role in matching pre- and post-synaptic cell numbers and in eliminating errors in the initial pattern of synaptic connections.

We have found that newly formed oligodendrocytes, the cells that make myelin in the CNS, also die during normal development and that, in culture at least, these glial cells depend on signalling molecules secreted by other cells in order to survive. Like neurons, these glial cells seem to die by active programmed cell death when deprived of survival factors, suggesting that such factors act by inhibiting cell suicide. We propose that the neurotrophic mechanism for controlling neuronal numbers is a specific example of a general mechanism that might help to control cell numbers in all developing tissues and organs, and that suicide is the default pathway for many, and perhaps all, developing animal cells.

W 013 MECHANISMS OF AXON GUIDANCE IN THE DEVELOPING VERTEBRATE SPINAL CORD, Marc Tessier-Lavigne, University of California, San Francisco.

To understand how the specific neural connections that underlie the functioning of the nervous system are generated during development, we have focused on the development of the vertebrate spinal cord, asking how spinal axons are guided to their target fields. This talk will focus on one mechanism of axon guidance, axonal chemotropism. Although chemotactic mechanisms are central to the directed motion of a variety of cell types, and have long been suggested to guide axons to their targets, there has been little direct evidence for axonal chemotropism in vivo. Evidence for chemotropic axon guidance has, however, been obtained in embryological experiments in which explanted neurons confronted with their targets in vitro have been shown to grow toward those targets in a directed manner. We have, in this way, obtained evidence for the operation of chemotropic guidance mechanisms in the spinal cord. One set of spinal neurons, commissural neurons, extend axons to the brain via an intermediate target called the floor plate[1], located at the ventral midline of the spinal cord. We have shown that the floor plate secretes a diffusible factor that can selectively influence the direction of growth of commissural axons in vitro[2,3]. Consistent with a role in guidance in vivo, the factor is secreted only by floor plate cells and is an effective chemoattractant: it can diffuse considerable distances through the neural epithelium, setting up a concentration gradient capable of reorienting all responsive axons within its range of action. I will discuss our current efforts to identify the floor plate-derived chemoattractant, to determine how axons orient in gradients of diffusible factors, and to determine the contribution of chemoattractants to axon guidance during development *in vivo*.

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W 014 **TRANSMITTER AND HORMONE-MEDIATED REGULATION OF NEUROTROPHIN SYNTHESIS,** Hans Thoenen, Francisco Zafra, Eero Castrén and Dan Lindholm, Max-Planck-Institute for Psychiatry, Department of Neurochemistry, Am Klopferspitz 18A, 8033 Planegg-Martinsried, FRG

The detection of new members of the NGF gene family (referred to as neurotrophins) resulted in a rapid accumulation of information on the spectrum of their biological actions, their regional distribution, their cellular localization and developmental expression (see Barde 1991; Thoenen 1991; Zafra et al. 1991). Besides the completion of the spectrum of biological actions of the neurotrophins and the identification and characterization of their receptors, the elucidation of the mechanisms of their regulation of synthesis represents the most essential aspect towards a comprehensive understanding of the physiological functions of the neurotrophins. NGF, BDNF and NT-3 are expressed in distinct regions of the rodent brain, predominantly in neurons, under physiological conditions the extent of expression in glial cells is below the detection limit. However, the expression of neurotrophins by glial cells may come into play under pathophysiological conditions, so far only demonstrated for NGF. The levels of neuronal NGF and BDNF mRNAs are regulated by neuronal activity in vitro and in vivo. The up-regulation is predominantly mediated by glutamate (NMDA and non-NMDA) receptors, whereas the down-regulation is mediated by the GABA system. The up-regulation depends on calcium influx and is further potentiated by cAMP, suggesting potential modulatory roles of neuropeptides and transmitter substances acting via cAMP as second messenger. The regulation of NGF and BDNF via the glutamate and GABA system could not only be demonstrated in primary cultures of hippocampal neurons, but also became apparent in vivo after administration of agonists and antagonists of glutamate and GABA receptors. Although the NGF and BDNF mRNA are regulated very similarily by neuronal activity, glucocorticoids increase the levels of NGF mRNA in hippocampal neurons in vitro and in vivo, whereas BDNF mRNA remains unchanged. NT-3 mRNA is neither regulated by the GABA and glutamate system, nor by glucocorticoids. An additional difference between NGF and BDNF is, at least in rodents, the basic levels of both the mRNA and protein levels, which are 20-30 times higher for BDNF than NGF.

NGF mRNA and protein levels in cultured astrocytes are up-regulated by a great variety of cytokines. This increase is abolished by glucocorticoids at concentrations reached under physiological conditions, which provides a possible explanation for the absence of detectable levels of NGF mRNA in glial cells in vivo. In contrast, the levels of BDNF mRNA in astrocyte cultures are extremely low and are not increased by any of these cytokines which markedly increase NGF mRNA and protein levels. In contrast to NGF mRNA, BDNF mRNA in astrocyte cultures is substantially increased by norpinephrine and forskolin. These effects are potentiated by calcium ionophores and glutamate.

The activity-dependent regulation of the synthesis of NGF and BDNF is discussed in the context of the potential function of these neurotrophins as mediators of neuronal plasticity.

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Thoenen H. 1991. Trends in Neurosciences, 14:165-170.
Zafra F., Castrén E., Thoenen H. and Lindholm D. 1991. Proc. Natl. Acad. Sci. USA, 88:10037-10041.

Induction Phenomena-I (Joint)

W 015 REGULATORY FACTORS IN ADIPOCYTE DEVELOPMENT, Bruce M. Spiegelman, Reed A. Graves, Peter Tontonoz, and Lisa Choy, Dana-Farber Cancer Institute, Division of Cellular and Molecular Biology and Harvard Medical School, Department of Biological Chemistry and Molecular Pharmacology, Boston, MA

We have been studying the regulation of adipocyte gene expression during cell differentiation and tissue development. The intracellular factors that activate adipocyte-specific gene regulation are not well understood. Toward this end, we have recently discovered an adipocyte-specific enhancer, and are performing a molecular dissection to identify key transcription factors binding to this DNA. This enhancer, from the adipocyte P2 gene, functions in cultured cells and transgenic animals. It binds several nuclear proteins including a member of the NF-1 family and mutational analysis indicates that binding at 5 separate sites are necessary for full activity. One factor, termed ARF6, appears to be developmentally regulated and may be the switch that activates this enhancer only in fat cells. The ARF6 factor itself is observed only in nuclear extracts from adipocytes. Multimers of the ARF6 binding site are sufficient to activate gene expression from a minimal promoter in adipose cells. C/EBP, a transcription factor that is induced during adipocyte differentiation, does not bind to this enhancer and an expression vector for C/EBP cannot transactivate this enhancer in preadipocytes. Current experiments address the general importance of the ARF6 factor in the activation program of differentiation in adipocytes. We have also been examining adipocytes for production of extracellular factors that may influence systemic energy balance. Our recent data indicates that adipocytes make and secrete several complement factors including factor D/adipsin. They also activate part of the alternative pathway of complement and generate several complement peptides with known biological effects. The role of this pathway in systemic energy metabolism is being investigated.

W 016 GROWTH FACTOR-MEDIATED INDUCTIVE SIGNALLING DURING *C. elegans* DEVELOPMENT, Paul W. Sternberg, Russell Hill, Phoebe Tzou, Jing Liu, Helen Chamberlin, Gregg Jongeward, Junho Lee, Linda Huang, and Raffi Aroian, Howard Hughes Medical Institute, Division of Biology 156-29, California Institute of Technology, Pasadena, CA.

An intercellular signalling pathway consisting of a growth factor, transmembrane receptor tyrosine kinase and a ras protein acts in several defined inductive events during nematode development. During vulval induction in the hermaphrodite, a single cell in the gonad, the anchor cell, induces three of six multipotent vulval precursor cells [VPCs] to undergo three rounds of mitosis and generate vulval cells. The three uninduced VPCs undergo a single mitosis and generate nonspecialized epidermis. Among genes necesary for this induction are *lin-3*, *let-23* and *let-60*. Defects in any of these three genes leads to a failure of vulval induction. Transgenic nematodes carrying high copy *lin-3* or *let-60* transgenes have excessive vulval differentiation. Using these dominant transgenes, we have ordered the action of these three genes: *lin-3* acts via *let-23*, which acts via *let-60*. *let-60* encodes a ras protein. *let-23* encodes a *C. elegans* homolog of the human EGF receptor and related receptor tyrosine kinases. *lin-3* encodes a putative growth factor precursor with the architecture of TGFα. Predicted *lin-3* proteins have an N-terminal leader sequence, a single EGF growth factor repeat, a membrane-spanning domain. Two alternatively spliced forms of *lin-3* differ in the presence of 15 amino acids between the EGF repeat and the transmembrane domain. *lin-3* is expressed in the anchor cell at the time of vulval induction. We propose that *lin-3* encodes the signal that induces vulval development. During male spicule development, we have shown that the F and U cells use this *lin-3-let-23-let-60* pathway to induce particular neurectoblasts fates including Bα and Bγ, which generate particular subsets of spicule cells. Ablation of F and U has the same result as mutation of *lin-3*, *let-23* or *let-60*: defects in the Bα and Bγ cell lineages. We are examining the negative regulation of this pathway by examining mutations that result in excessive vulval differentiation. Some of these mutations are in the *let-23* gene, others are in unlinked loci, *lin-15*, *unc-101*, and *rok-1*.

W 017 ADHESION AND MOTILITY OF EMBRYONIC AND CANCER CELLS. Jean Paul Thiery, Jean Claude Boucaut, Brigitte Boyer, Florence Broders, Thierry Darribere, Annie Delouvée, Sylvie Dufour, Jeanne Marie Girault, Jacqueline Jouanneau, Victor Koteliansky, Giovanni Levi, Ginette Moens, Jean Pierre Saint-Jeannet, Pierre Savagner, Lionel Simonneau and Ana Maria Valles. Laboratoire de Physiopathologie du Développement, CNRS-Ecole Normale Superieure 46, rue d'Ulm, 75230 Paris Cedex 05 FRANCE.

We have analysed in detail the program of expression of several adhesion molecules during morphogenesis focusing primarily on epithelial-mesenchymal cell interconversions and on migratory events. The neural crest, a transient embryonic structure of the neural epithelium undergoes a conversion to a mesenchymal state; these cells subsequently migrate thoughout the embryo to give rise to many derivatives including most of the peripheral nervous system and melanocytes. The pattern of expression and modulations of the cell adhesion molecules (CAMs) and the substrate adhesion molecules (SAMs) correlate with the different morphogenetic steps in the neural crest. During migration crest cells do not express functional CAMs but interact specifically with fibronectins in the extracellular matrix. Several distinct cell binding domains on the fibronectin molecules have been mapped and their relative contribution to adhesion, spreading and motility will be described. The role of cell adhesion is also investigated in amphibian embryos particularly during gastrulation.

A rat bladder carcinoma has been used as a model system to study the conversion of an epithelial to a migratory fibroblast-like state. This morphological transformation is triggered by collagens but not by fibronectins or laminin. A similar conversion is induced by acidic Fibroblast Growth Factor (aFGF) in subconfluent cultures while this multifunctional growth factor acts as a mitogen on high density cultures. In low density cultures, aFGF and several other growth factors acting through tyrosine kinase receptors induce a rapid internalization of desmosomes, a major adhesive structure of epithelia. The newly formed fibroblasts progressively lose their cytokeratins which are replaced by vimentin intermediate filaments. The transformation is fully reversible upon removal of the growth factor. Acidic FGF also triggers cell motility and production of gelatinases. On collagen substrates, the speed of locomotion is enhanced in the presence of aFGF and under these conditions the bladder carcinoma cells readily invade 3D collagen gels. The bladder carcinoma line can also become fibroblastic after transfection with an expression vector coding for aFGF, most likely through an autocrine mechanism. Thus, this model system may offer a unique opportunity to evaluate the role of the different adhesion modes and signalling factors in morphogenetic processes.

Induction Phenomena-II

W 018 IDENTIFICATION OF A RETINOIC ACID RESPONSIVE ENHANCER 3' OF THE MURINE HOMEOBOX GENE HOX-1.6, Alexander W. Langston[1], and Lorraine J. Gudas[2], [1]Cell and Developmental Biology Program and Biological Chemistry and Molecular Pharmacology Department, Harvard Medical School, Boston, [2]Department of Pharmacology, Cornell University Medical College, New York.

The putative vertebrate morphogen retinoic acid (RA) has been shown to induce many mammalian homeobox genes in cell lines, suggesting that the expression of this gene family in developing vertebrate embryos may be controlled in part by RA. Using the embryonic teratocarcinoma cell line F9 as a model system, we have studied the RA-response of the murine homeobox gene Hox-1.6. The Hox 1.6 (ERA-1) gene was originally isolated in this lab as an mRNA that was rapidly induced 20-30 fold in response to RA, even in the presence of protein synthesis inhibitors; these characteristics strongly suggested that the gene was directly regulated by RA. RA treatment of F9 cells causes the appearance of a DNAse I hypersensitive site 3' of Hox-1.6, and this site has been shown to reflect the presence of an RA-responsive enhancer downstream of the gene. The RA-responsiveness of the enhancer is controlled by a retinoic acid responsive element (RARE) identical to the RARE of the retinoic acid receptor (RAR) β gene, but other sequences also affect the activity of the enhancer, suggesting the presence of binding sites for novel proteins which regulate Hox-1.6 expression. The Hox 1.6 RARE differs from that in the promoter of the laminin B1 gene, a gene that is induced at later times after RA addition. Identification of the Hox-1.6 enhancer is a significant advance in our understanding of the molecular mechanisms by which pattern formation is controlled in vertebrate embryos.

W 019 THE DVR GENE FAMILY IN VERTEBRATE DEVELOPMENT, Brigid Hogan, Michael Jones, Nancy Wall, Christopher Wright, Karen Lyons and Manfred Blessing. Department of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232.

The DVR (Decapentaplegic-Vg-related) gene family is a subgroup of the larger TGF-β gene family. It constitutes at least 10 members in mammals and encodes secreted, dimeric molecules with a highly conserved C-terminal region held together by 7 di-sulphide bonds. These include the proteins originally named Bone Morphogenetic Proteins 2 through 7. We are particularly interested in the role of DVR-4 (BMP-4) and -6 (BMP-6, Vgr-1) in embryonic development. DVR-4 transcripts are first detected in the early somites stage mouse embryo, in the posterior, ventral mesoderm. Subsequently, transcripts are widely distributed in the embryo, particularly at sites of inductive tissue interactions. The initial localization raised the possibility that DVR-4 is involved in the specification of newly formed mesoderm and this has been tested using *Xenopus* embryos. Injection of RNA for human DVR-4 into oocytes blocks development at early gastrulation and no mesoderm is formed. However, animal caps dissected from injected embryos and cultured *in vitro* develop into vesicles containing ventral mesodermal cell types. The same animal caps transplanted into the blastocoel of host embryos induce the formation of secondary tails. These observations, combined with studies on gene expression and the effect of purified protein on animal caps, support the hypothesis that DVR-4 is involved in the specification of posterior/ventral mesoderm in the *Xenopus* embryo. Studies on DVR-4 function in the mammalian embryo will be discussed. DVR-6 transcripts are not detected in mouse embryos until a later stage than DVR-4, and are first seen in dorsal and ventral regions of the nervous system at around 9 days p.c.. A polyclonal rabbit antiserum specific for DVR-6 has been used to study the biosynthesis and localization of protein in the developing CNS and in a variety of other cell types.

W 020 INVOLVEMENT OF WNT PATHWAYS IN MEDIATING CELLULAR RESPONSES TO MESODERM — INDUCING GROWTH FACTORS, Randall T. Moon, D.J. Olson, and J.L. Christian, Department of Pharmacology, University of Washington School of Medicine, Seattle.

In *Xenopus*, growth factors of the TGF-β, FGF and Wnt oncogene families have been proposed to play a role in generating embryonic pattern. We have examined potential interactions between the bFGF and Xwnt-8 signalling pathways in the induction and dorsal-ventral patterning of mesoderm[1]. Injection of Xwnt-8 mRNA into 2-cell *Xenopus* embryos does not induce mesoderm formation in animal cap ectoderm isolated from these embryos at the blastula stage, but alters the response of this tissue to mesoderm induction by bFGF. Our results support a model whereby dorsoventral mesodermal patterning can be attained by a single mesoderm inducing agent, possibly bFGF, which is uniformly distributed across the prospective dorsal-ventral axis, and which acts in concert with a dorsally localized signal, possible a Wnt protein, which either alters the response of ectoderm to induction or modifies the character of mesoderm after its induction.

1. Christian, J.L., Olson, D.J., and Moon, R.T. (1992). *EMBO J.*, in press.

Growth Factors and Gene Expression-III

W 021 RECEPTORS FOR TUMOR NECROSIS FACTORS, Louis A. Tartaglia, Diane Pennica, Richard Weber, Van Thai Lam and David V. Goeddel., Molecular Biology Department, Genentech, Inc., South San Francisco, CA 94080.

Tumor Necrosis Factors-α and -β are pleiotrophic cytokines that have a wide range of biological properties. TNFs recognize two distinct receptors (TNF-R1 (55 kDa) and TNF-R2 (75 kDa)), both of which are present on most cell types. Studies of the cloned TNF receptors and agonistic antibodies indicate that signaling occurs through ligand-induced aggregation of the receptors. The two TNF receptors transmit distinct signals to the cell, with TNF-R1 being responsible for the majority of known TNF actions.

W 022 COLONY STIMULATING FACTOR-1 IN THE REGULATION OF PLACENTAL DEVELOPMENT, Jeffrey W. Pollard*,Robert J. Arceci+, Orin Chisholm*, Eric Daiter*, Serge Pampfer*, E. Richard Stanley* and Wieslaw Wiktor-Jedrzejczak#, *Dept. Developmental Biology and Cancer, Albert Einstein College of Medicine, Bronx, NY 10461, +Dept. Pediatric Hematology and Oncology, Dana Farber Res. Institute, Boston, MA, #Post-graduate Medical Center, CSK WAM, Warsaw, Poland.

Colony stimulating factor-1 although originally described as a humoral regulator of the mononuclear phagocytic lineage now appears to play a local role during pregnancy. In the mouse CSF-1 is synthesized by the uterine epithelium throughout gestation under the regulation of the female sex steroids. The CSF-1 receptor mRNA is expressed in oocytes, to be degraded at the two cell stage followed by re-expression predominantly in cells of the trophectodermal lineage. After implantation trophoblasts, decidual cells and macrophages express CSF-1 receptor mRNA. A rather similar pattern of expression for both CSF-1 and its receptor is found in humans with the exception that CSF-1 is synthesized by first trimester ctyo- and intermediate trophoblasts.

Studies on the biology of CSF-1 have been dramatically enhanced by the observation that the osteopetrotic (*op/op*) mouse posses an inactivating mutation in the CSF-1 gene. These mutant mice are entirely deficient in CSF-1. They are toothless, have increased bone mass, reduced femoral cellularity, dramatically reduced numbers of osteoclasts and are severely depleted in peripheral blood monocytes and in macrophages in locations such as the pleural and peritoneal cavity. In addition, matings between homozygous *op/op* mutants fail to yield progeny. Male *op/op* mice however, display almost normal fertility with *+/op* females. Somewhat surprisingly, *op/op* females when mated with *+/op* males did bear progeny but at only 40% of the level of heterozygote matings. The nature of this correction by *+/op* males is currently under investigation. Restoration of circulating levels of CSF-1 in *op/op* mice from day 3 of age corrected the osteopetrosis and toothless phenotype but failed to restore female fertility. These data therefore, indicate an obligatory role for locally synthesized CSF-1 during pregnancy.

Angiogenesis and Endothelial Cell Biology

W 023 REGULATION OF CAPILLARY MORPHOGENESIS IN VITRO. Roberto Montesano, Michael Pepper, Jean-Dominique Vassalli, and Lelio Orci. Institute of Histology and Embryology, Department of Morphology, University Medical Center, Geneva, Switzerland.

During angiogenesis, endothelial cells (EC) which line existing microvessels invade the perivascular matrix, where they form a new network of capillaries. To investigate the factors that might induce normally quiescent EC to become invasive, microvascular EC were grown on collagen gels and treated with phorbol myristate acetate (PMA), which stimulates their secretion of matrix-degrading proteases, such as collagenase and plasminogen activators. Whereas control EC were confined to the surface of the gels, PMA-treated EC invaded the collagen matrix, where they formed capillary-like tubules. The invasive process was accompanied by collagen degradation and was prevented by collagenase inhibitors[1]. Similar phenomena were observed in response to the angiogenic factor, basic fibroblast growth factor (bFGF)[2] and to sodium orthovanadate, an agent that increases tyrosine phosphorylation of cellular proteins by inhibiting phosphotyrosine phosphatases[3]. PMA, bFGF and vanadate also induced EC to form tubules within fibrin gels, provided that substrate dissolution was limited by the addition of fibrinolytic inhibitors, suggesting that physiological protease inhibitors play an important permissive role in angiogenesis by preventing excessive matrix degradation[4]. This concept is further supported by experiments with EC isolated from hemangiomas that develop in mice expressing the polyoma virus middle T oncogene (mT). Whereas normal EC formed branching tubules when suspended within fibrin gels, mT-expressing EC formed large hemangioma-like cysts. Neutralization of the excessive proteolytic activity of the hemangioma cells by addition of serine protease inhibitors corrected their aberrant behavior and restored normal angiogenesis[5]. Taken together these results suggest that a tightly regulated balance between proteases and protease inhibitors plays an essential role in angiogenesis.

1. Cell 42, 469 (1985).
2. PNAS 83, 7297 (1986).
3. J. Cell. Physiol. 134, 460 (1988).
4. J. Cell. Physiol. 132, 509 (1987).
5. Cell 62, 435 (1990).

W 024 EXTRACELLULAR FORMATION OF TGF-ß, Daniel B. Rifkin, Department of Cell Biology, New York University Medical Center, New York, NY 10016.

Transforming growth factor-ß (TGF-ß) is a homodimeric cytokine that has a variety of activities including inhibition of mitosis, immune suppression, stimulation of matrix biosynthesis, inhibition of protease production, and stimulation of protease inhibitor expression among others. TGF-ß is found under most conditions as a high molecular weight, inactive complex consisting of the mature growth factor non-covalently bound to its two propeptides. In addition, a second protein, the latent TGF-ß binding protein (LTBP), is covalently bound to the latent TGF-ß complex. Although this complex can be dissociated under denaturing conditions to release TGF-ß, the in vivo mechanism for activating latent TGF-ß has been unclear.

We have found that latent TGF-ß is activated in cocultures of numerous cell types. This activation requires the interaction of two different cells, each of which procedures latent TGF-ß, cell-cell contact or close apposition, the proteases plasminogen activator and plasmin and binding proteins both on the cell surface and in the high molecular weight complex. These binding proteins include the cation-independent IGF II/Mannose 6 phosphate receptor and LTBP.

The activation system is self-regulating since TGF-ß that is formed in cocultures acts upon the cells that produce it to induce increased expression of an inhibitor of plasminogen activator that is required for generation of the mature cytokine. This activation system may also function to modulate the activity of another growth factor, basic fibroblast growth factor (bFGF). Treatment of cells with bFGF stimulates plasminogen activator production and the level of plasmin level. This increase in plasmin, in turn, activates latent TGF-ß. TGF-ß blocks many of the effects of bFGF in mesenchymal cells. Retinoids also increase the production of plasminogen activator in may cell types. When bovine endothelial cells are treated with retinal, active TGF-ß is formed. This activation reaction proceeds through a series of steps similar to those observed in cocultures.

The potential implications of these observations in terms of certain cell-cell interactions, transformation, and developmental processes will be discussed.

Growth Factors and Gene Expression-IV

W 025 REGULATION OF FIRST BRANCHIAL ARCH MORPHOGENESIS DURING MANDIBULAR DEVELOPMENT IN CHEMICALLY-DEFINED MEDIUM. H.C. Slavkin, *L. Shum, **Z. Werb, Y. Sakakura, Y. Chai & P. Bringas. Center For Craniofacial Molecular Biology, School of Dentistry. Univer. South. Calif., Los Angeles, Calif. 90033, and *Ctr. Growth & Development, School of Dentistry and **Lab. Radiobiol. & Environmental Health, School of Medicine, UCSF, San Francisco, Calif. 94143

A major question in skeletal morphogenesis is how temporal and positional instructions are translated into form. A simple model to pursue this question is early embryonic mouse (E10, 42-44 somite pairs) mandibular explants which produce mandibular morphogenesis including Meckel's cartilage, bone, tooth organs and tongue development within 9 days using serumless medium (Slavkin et al, J. Craniofac. Genet. & Develop. Biol. 9:185-205, 1989). Under these experimental conditions EGF, TGF-alpha, RAR-gamma, and TGF-beta transcripts were identified using immunohistochemical localizations. The present studies were designed to use antisense oligodeoxynucleotides to investigate endogenous regulatory molecular effects on mandibular morphogenesis. Whole mount staining was used to evaluate normal versus dysmorphology of Meckel's cartilage. Histological serial sections of replicate specimens from different treatment groups were used for morphometric analyses. Antisense treatment (30 uM) designed to inhibit the translation of selected putative regulatory molecules (i.e. EGF, RAR-gamma, TGF-beta) produced several types of dysmorphogenesis. Sense and antisense AMEL (amelogenin) oligodeoxynucleotides were used as controls. In recovery experiments, where appropriate, exogenous growth factors added to antisense treated groups produced normal-like mandibular morphogenesis. Results were interpreted to suggest that endogenous regulatory molecules participate in the control of the timing and position of tissue boundaries during first branchial arch morphogenesis.

W 026 REGULATION OF CHONDROGENESIS AND OSTEOGENESIS BY THE BMP PROTEINS, John M. Wozney, Anthony J. Celeste, David Israel, and Vicki Rosen, Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140

We have previously identified and cloned a series of related human molecules, BMP-2 through BMP-7. These proteins were identified by their presence in purified extracts of bone which, when implanted subcutaneously in rats, induced *de novo* cartilage and bone formation. Based on their primary amino acid sequences, BMP-2 and BMP-4 are closely related molecules which are probably the human counterparts of the *Drosophila* decapentaplegic gene. BMP-5, BMP-6, and BMP-7 are also closely related to each other, as well as to the *Drosophila* 60A gene. Using human proteins derived from recombinant systems, it is now known that multiple individual proteins, including BMP-2, BMP-4, BMP-5, and BMP-7, each induce the formation of cartilage and bone in adult animals. However, they display distinct localization patterns during embryogenesis, suggesting differing roles for the individual BMP proteins. We have now identified and molecularly cloned several additional related molecules in the BMP family. The derived amino acid sequence of human cDNA clones for one of these proteins, BMP-8, indicates that it is most closely related to the BMP-5/6/7 subfamily. Heterodimeric forms of the BMPs have also been expressed in recombinant systems. The *in vitro* and *in vivo* activities of this large family of related homodimeric and heterodimeric proteins are being compared.

Wound Healing and Transformation

W 027 BIOLOGIC ACTIVITY OF FIBROBLAST GROWTH FACTORS REQUIRE LOW AND HIGH AFFINITY RECEPTORS, David M. Ornitz[1], Avner Yayon[2], John G. Flanagan[1], Carl M. Svahn[3], and Philip Leder[1], [1]Howard Hughes Medical Institute and Department of Genetics, Harvard Medical School, Boston, MA 02115, [2]Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, 76100 Israel, [3]R&D Cardiovascular, Kabi Pharmacia AB, Stockholm S-112 87, Sweden.

The heparin binding growth factors (HBGFs) include a family of seven structurally related proteins which interact with four known high affinity receptors. To understand the biochemical basis for multiple receptors and ligands in this family we have begun to characterize the binding properties of fibroblast growth factor receptors 1 and 3 (FR1 and FR3) with respect to acidic and basic fibroblast growth factor (aFGF, bFGF) and their biologic activity with respect to aFGF, bFGF, FGF-4/K-FGF, and FGF-5. We demonstrate that FR3 preferentially binds acidic FGF over basic FGF. FR3 mediated mitogenicity is also specific to acidic FGF and FGF-4 with very little response to basic FGF and FGF-5.

Recently it has been demonstrated that heparin is required for bFGF high affinity receptor binding on cells deficient in cell surface heparan sulfate proteoglycan (HSPG). This observation suggests that HSPGs may be important regulators of FGF biologic activity acting directly at the level of the cell surface receptor. To evaluate this interaction in a cell-free system we have designed a simple binding assay based on genetically engineered soluble forms of FR1 and FR3 tagged with placental alkaline phosphatase. Using this assay, we show that FGF receptor binding has an absolute requirement for heparin involving the formation of a trimolecular complex. By using a cytokine-dependent lymphoid cell line engineered to express these receptors, we also demonstrate that FGF-induced mitogenic activity is heparin dependent. Furthermore, we have tested a series of small heparin oligosaccharides of defined length for their ability to support bFGF receptor binding and biologic activity. We find that a heparin with as few as eight sugar residues is sufficient to support these activities. We also demonstrate that heparin can facilitate FGF dimerization, a property that may be important for receptor activation.

At one level, FGF activity appears to be regulated by the specific interaction with one of several high affinity FGF receptors. This interaction may in turn be regulated by a second class of low affinity receptors comprised of HSPG. Certainly other factors such as tissue-specific expression and regulated release from cells will also effect the bioavailability and subsequent biologic activity of this family of HBGFs.

W 028 CYTOKINES AND MATRIX MACROMOLECULES AS MEDIATORS OF EPITHELIAL-MESENCHYMAL INTERACTIONS: THEIR ROLE IN CANCER PATHOGENESIS.

Seth L. Schor[1], Ian Ellis[1], Ann-Marie Grey[1] and Ana M. Schor[2]. [1]Department of Cell and Structural Biology, Coupland 3 Building, University of Manchester, Manchester, M13 9PL, England; [2]Department of Medical Oncology, Christie Hospital, Wilmslow Road, Manchester, M20 9BX, England.

We have previously reported that (a) fetal fibroblasts migrate into 3D collagen gels to a significantly greater extent than do their normal adult counterparts, (b) this difference in behaviour is due to the production by fetal fibroblasts of a "migration stimulating factor" (MSF) which is not made by adult cells, and (c) fibroblasts obtained from breast cancer patients resemble fetal cells both in terms of their migratory phenotype and continued production of MSF. We have recently reported that MSF activity is present in the serum of approximately 75% of breast cancer patients, both prior to and following surgical resection of the primary tumour [Picardo et al, Lancet 337:130-133, 1991]. The presence of detectable serum levels of MSF in post-surgical breast cancer patients *with no evidence of residual disease* clearly distinguishes MSF from other onco-fetal proteins (which are essentially markers of tumor burden) and supports our hypothesis that the systemic presence of persistent "fetal-like" fibroblasts may contribute directly to cancer progression [Schor et al, BioEssays 7:200-204, 1987]. Related studies concerned with the mechanism of action of MSF on target fibroblasts indicate that it appears to act by exerting a primary stimulatory effect upon the synthesis of hyaluronic acid (HA). In view of the well-documented effects of HA on the proliferation and differentiation of mammary epithelial cells, these observations suggest a possible mechanism by which the presence of MSF-secreting fibroblasts may contribute to cancer pathogenesis. We have recently found that TGF-β is a potent inhibitor of MSF, both in terms of the stimulation of cell migration and HA biosynthesis.

MSF has been characterized in terms of a number of biochemical criteria, including N-terminal sequence. These data indicate that MSF displays considerable amino acid sequence homology with the collagen binding domain of fibronectin. Preparations of bona fide collagen binding fragments of fibronectin also stimulate cell migration, but differ from MSF in terms of a number of parameters, including serum requirement for activity, effect on HA synthesis and neutralization by an anti-MSF polyclonal antibody. The migration stimulating activity of both MSF and the fibronectin fragment are dependent upon the use of a 3D collagen substratum. These results will be discussed in the context of the complex interaction between cytokines and matrix macromolecules in the mediation of epithelial-mesenchymal interactions.

Growth Differentiation Factors; Action; Regulation

W 100 GENOMIC ANALYSIS AND EXPRESSION PATTERNS OF *BEK* AND *FLG*. J. Ali, A. Mansukhani, and C. Basilico, Department of Microbiology, NYU Medical Center, New York, NY 10016.

Protein tyrosine kinases appear to be involved in control of cell growth and differentiation. *Bek* and *flg* are members of the FGF-R family. The two genes are highly homologous. In this work we give an extensive analysis of the expression and the characterization of the regulatory elements that control expression of the two genes. RNase protection and Northern analysis reveal similar expression patterns of the two genes although *flg* is almost always more highly expressed than *bek*. Also *bek* is regulated in F9 cells when differentiated into parietal endoderm whereas *flg* is not. The above two findings indicate that control elements may be responsible for differential expression of the two molecules. To this end we screened a mouse genomic library and isolated clones for *bek* and *flg*. Three overlapping clones encompassing 45 Kb of genomic DNA have been isolated for both genes. Sequencing of promoter elements and CAT assays for promoter activity will be presented.

W 101 IONIZING RADIATION INDUCED WOUNDING LEADS TO ALTERED STROMAL EXPRESSION OF TRANSFORMING GROWTH FACTOR-β IN VIVO, M.H. Barcellos-Hoff, Division of Cell & Molecular Biology, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Ionizing radiation elicits a fibrotic response in many tissues that is dose-limiting for radiotherapy of cancer and bone marrow transplantation. The anomalous extracellular matrix production by both stromal and epithelial cells has similarities to early phases of wound repair but the mechanisms by which such changes develop and persist remain obscure. The purpose of the present studies is to examine the extracellular matrix and factors regulating its deposition immediately following radiation and preceding the development of evident fibrosis. Collagens type I and III and transforming growth factor β (TGF-β) were localized using immunofluorescence in the mammary gland of Balb/c female mice that had been exposed to 5 Gy of whole body ^{60}Co γ radiation. Each of these antigens were localized to various degrees in the stromal sheaths surrounding ducts and vasculature and in the fibrous septa. Collagen IV localized to the basolateral surface of the epithelia and adipocytes. At 1 hr post irradiation, there was no change in the pattern of collagen deposition. However, TGF-β appeared increased in the fatty stroma and fibrous septa relative to the control. Since the tissue sections were treated with acid this probably represents altered synthesis, rather than activation of TGF-β. At 3 and 6 days post irradiation, collagen III was increased in a pattern coincident with that of the altered expression of TGF-β. Collagen type I and IV appeared unchanged. Thus an early response of stroma to radiation is the induction of a factor that mediates myriad events during tissue homeostasis, growth and repair.

W 102 STRUCTURE / FUNCTION ANALYSIS OF THE TGF-α PRECURSOR CYTOPLASMIC TAIL, Marcus Bosenberg, Atanasio Pandiella and Joan Massagué, Department of Cell Biology and Genetics, *Howard Hughes Medical Institute and Memorial Sloan-Kettering Cancer Center*, New York, NY 10021

Previous work has shown that proTGF-α is an integral membrane glycoprotein that can bind to EGF receptors on adjacent cells, thereby mediating cell adhesion and stimulation. The transmembrane and cytoplasmic domains of proTGF-α are nearly identical in rat and human proteins, with only one conservative amino acid replacement over their 64 amino acid length, despite 20 base pair changes in this region. In contrast, released TGF-α has 4 changes in its 50 amino acid length. The possible roles of the cytoplasmic tail of proTGF-α were examined by constructing a series of 8 deletion mutations and 3 point mutations. These constructs were stably transfected into CHO and NIH 3T3 cell lines. The possibility that proTGF-α may have receptor-like activity was examined, as were effects on proTGF-α targeting, and activated cleavage by phorbol 12-myristate 13-acetate. None of the mutations altered targeting to the plasma membrane and all of the constructs were glycosylated. Some of the mutations did affect the extent of activated cleavage by PMA. These results show that the cytoplasmic tail is required for activated cleavage at the extracellular side of the membrane.

W 103 SEARCH FOR A FOURTH MURINE TGF-β ISOFORM REVEALS A TGF-β-LIKE GENE.
M.C. Dickson, R.J. Akhurst. Duncan Guthrie Institute of Medical Genetics, University of Glasgow, Glasgow G3 8SJ UK.

Transforming growth factor-β is the prototype of a gene superfamily found within a diverse range of species, including mammals, amphibians, avians and insects. Currently the TGF-β gene family has at least eight members, though only three isoforms TGF-β 1[1], TGF-β 2[2] and TGF-β 3[3] have been identified in mammals. A fourth homologue TGF-β 4[4] has been identified in the chick by cDNA cloning.

In order to determine whether any further TGF-β homologoues or TGF-β-like genes exist in the mouse, two lines of investigation were followed;

1)Southern hybridisation analysis of mouse genomic DNA using probes for mouse TGF-β 1, 2, 3 and chick TGF-β 4.

2) mouse genomic DNA was amplified using PCR primers homologous to chick TGF-β 4 and the resulting fragments analysed by Sanger sequencing.

The southern blot hybridisation analysis suggests that there is no murine homologue for TGF-β 4. An identical hybridisation pattern was seen with the probes for murine TGF-β 1 and chick TGF-β 4 indicating that these genes have extremely close evolutionary origins. We have also shown unique hybridisations patterns to C. elegans genomic DNA with the murine TGF-β 1, 2, and 3 probes whereas the chick TGF-β 4 probe gives an identical pattern to TGF-β 1.

None of the amplified products sequenced revealed extensive homology to any of the known mammalian TGF-β homologoues. However, one product has 66% homology to TGF-β 1 and has distinct hybridisation patterns from the other genes within the family. Zoo blot hybridisation analysis indicates that this is a single copy gene in the mouse genome although it is has been duplicated in the C. elegans genome.

Northern analysis will reveal whether this gene is involved in development. If so, *in situ* hybridisation will be used to determine the exact areas of expression of this gene. This work is funded by the MRC.

1)Derenck et al. (1985) Nature 316:701-705
2)Madison st al. (1988) DNA 7:1-8
3)ten Dijke et al. (1988) PNAS 85:4715-4719
4)Jakowlew et al. (1988) Mol. Endo 2:1186-1195

W 104 IDENTIFICATION AND PURIFICATION OF GLIAL GROWTH FACTOR-II. Andrew D.J. Goodearl*, John B. Davis*, Michael D. Waterfield†, Masayuki Otsu†, Luisa Minghetti*, Ian Hiles† and Paul Stroobant*, *Growth Factor and †Receptor Studies Laboratories, Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, UK.

Glial Growth Factor (GGF) is a potent polypeptide mitogen for rat Schwann cells and cortical astrocytes, with a molecular weight of ca 31 kDa. GGF activity is expressed in a nerve-dependent fashion during limb regeneration in the newt, implying its potential role in a developmental context. GGF activity has also been observed in certain human Schwann cell tumours (Brockes J.P. (1987) Meth. Enz. 147 217-225 and refs. therein).

Protein purification of GGF from whole bovine pituitary glands was undertaken with the primary aim of obtaining partial amino-acid sequence and further to enable cDNA and antibody studies on the developmental role of this novel growth factor to be initiated. During these efforts, two activities that were positive in the Schwann cell DNA synthesis assay were identified. These have molecular weights of ca 34 kDa and ca 59 kDa and were named GGF-I and GGF-II respectively. Both of these mitogens have been purified to apparent homogeneity as judged by silver-stained SDS-PAGE. It is clear that they are structurally distinct as judged by SDS-PAGE under denaturing conditions and also by chromatography on gel filtration and reversed phase matrices. However, the biological specificity and potency of GGF-II is indistinguishable from that of GGF-I. Structural studies on these novel mitogens promise to illuminate their relationship to each other and to other growth factor families.

W 105 MULTIPLE FORMS OF LEUKAEMIA INHIBITORY FACTOR: CHARACTERISATION BY ANTIBODY RECOGNITION, Laura M. Grey, and John K. Heath, Department of Biochemistry, Oxford University, Oxford, OX1 3QU. Leukaemia Inhibitory Factor (LIF) is a glycoprotein which has been observed to have a regulatory role in several embryonic and adult systems (LIF induces the differentiation of certain leukaemic cells and proliferation of hematopoietic stem cells, is involved in bone remodelling, and regulation of nerve differentiation, and is known to suppress differentiation of Embryonic Stem Cells).

Recent evidence has revealed that murine LIF protein exists in two forms with differential localisation. One form is freely diffusible while the other remains tightly associated with the extra-cellular matrix, the two forms resulting from the use of alternate transcriptional start sites and differing only in their N-terminal amino acid sequences.

Our investigation has led to the production of polyclonal antibodies to LIF which have been used to identify, by means of immunoprecipitation, several proteins that associate with LIF.

Comparison of human and murine LIF reveals a shared amino acid sequence identity of 78% with no insertions or deletions. A range of LIF mutants (and Human/Mouse chimeras) is currently being constructed to study the structure and differential localization of the protein.

W 106 CHARACTERIZATION OF A CELL-ASSOCIATED IGFBP IN BHK CELLS: REGULATION OF THIS PROTEIN BY IGF-I AND ITS ROLE IN MODULATING IGF-I ACTION, David Hsu and Jerrold M. Olefsky, Department of Medicine, University of California, San Diego, CA 92093.
The insulin-like growth factor binding proteins (IGFBP) are a family of low molecular weight proteins (18-50kd) that bind to IGF-I or IGF-II with high affinity. They are important factors which modulate IGF-I and IGF-II actions. So far six distinct classes of IGFBPs have been identified from both human and rat. These IGFBPs are secreted from a variety of cells into body fluids or tissue culture medium. In addition to the secreted form, IGFBP-1 and IGFBP-3 are associated with cell membranes in human fibroblasts. In BHK cells, we have observed that a large portion (55%) of the total IGF-I binding to cells represents binding to non-receptor sites. A more detailed analysis showed that, in addition to a large number of secreted IGFBPs in BHK cells, there are two cell-associated IGFBPs that appeared to have unique characteristics. These cell-associated IGFBPs have molecular weights of 30kd and 25kd (IGFBP-30kd and IGFBP-25kd) respectively. The IGFBP-30kd is associated with the cell membrane and can be readily labeled by affinity crosslinking using ^{125}I-IGF-I. The level of the IGFBP-30kd is stimulated 5-fold with serum starvation. The induction of this protein is evident after 4 hours of serum starvation (2.4-fold increase) and reached a plateau between 12-24 hours. This induction can be completely inhibited by cycloheximide, suggesting that the increased level of IGFBP-30kd on the cell surface is due to new synthesis of IGFBP-30kd, rather than a redistribution of the protein. The induction of this protein is inhibited by IGF-I (76%) and by des-IGF-I, an analog of IGF-I with very low affinity for IGFBPs, albeit to a lesser extent (51%). Insulin has a smaller effect to inhibit starvation mediated IGFBP induction (38%). The data suggest that expression of IGFBP-30kd is down-regulated by IGF-I and that the induction by serum starvation might be due to deprivation of IGF-I in the serum. These observations also show that down-regulation of IGFBP-30kd by IGF-I was mediated by IGF-I receptors, as well as by non-receptor mechanisms (possibly via IGFBPs).

W 107 INHIBITION OF FAT AND MUSCLE DEVELOPMENT BY TRANSFORMING GROWTH FACTOR ALPHA

Noreen C. Luetteke[1], Eric P. Sandgren[2], Ralph L. Brinster[2], Richard D. Palmiter[3], and David C. Lee[1]. [1]Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC 27599; [2]Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104; [3]Howard Hughes Medical Institute and Department of Biochemistry, University of Washington, Seattle, WA 98195.

Transgenic mice overexpressing transforming growth factor alpha (TGFα) via the metallothionein promoter had 20% lower body and carcass weight compared to nontransgenic littermates due to significant decreases in bone, fat and muscle mass. The greatest difference was observed in the fat compartment. Transgenic epididymal fat pad weights were reduced to between 20 and 80 percent and total body fat content was reduced to about 50 percent of control values. Cranial distal hindlimb muscle weights were 20% below normal, and other skeletal muscles also appeared smaller in size. These weight reductions were accompanied by an average of 25-45% fewer nuclei in the transgenic fat pads and muscles, as estimated by total DNA content. In addition, morphometry indicated that the number and cross-sectional area of striated muscle fibers were decreased in transgenic relative to control animals. These findings could not be attributed to differences in metabolic rates, as transgenic and control mice displayed comparable levels of energy expenditure per unit lean body mass as measured by calorimetry. Well characterized tissue culture models of differentiation were used to study direct effects of TGFα on fat and muscle cells. TGFα (10-100 ng/ml), added concurrently with inducing medium, suppressed the morphological conversion of 3T3-F442A preadipocytes into adipocytes. Northern analyses showed that TGFα also inhibited the expression of the known adipocyte differentiation markers glycerophosphate dehydrogenase, adipsin, and AP-2, in a reversible, dose-dependent manner. In contrast, in cultures of C2C12 mouse skeletal myoblasts, TGFα affected neither the expression of muscle-specific actin nor cell fusion and formation of myotubes. These results demonstrate that TGFα, a potent mitogen for most epithelial cells, can antagonize the differentiation and development of some mesodermal cells in vitro and/or in vivo.

W 108 THE INTERACTION OF FGF GROWTH FACTORS AND THEIR RECEPTORS: THE USE OF MUTANT RECEPTORS AS PROBES. A. Mansukhani, J. Ali, and C. Basilico, Department of Microbiology, NYU Medical Center, New York, NY 10016.

The fibroblast growth factors (FGFs) interact with a family of membrane spanning tyrosine kinase receptors. The FGF receptors flg and bek can be activated by three members of the FGF family aFGF, bFGF and K-FGF when expressed in receptor-negative cells such as CHO or 32D myeloid cells. However, these receptors are often coexpressed in many cell types and the reasons for this apparent redundancy remain unclear.

We have attempted to dissect the physiological specificity of growth factor-receptor interaction by introducing dominant receptor mutants (bek and flg) into FGF responsive cells. We have constructed bek and flg receptors that retain ligand binding capacity but lack tyrosine kinase activity and expressed them in 32D myeloid cells transfected with the normal bek or flg receptor. We show that the mutant receptors function by blocking normal bek and flg signalling in 32D myeloid cells presumably by blocking transphosphorylation and subsequent receptor activation. Using such an approach, we should be able to identify the relevant types of receptors (homodimers or heterodimers) that mediate FGF functions such as cell proliferation, fibroblast transformation or myoblast differentiation. Preliminary results indicate that a mutant bek receptor is capable of reverting the phenotype of NIH3T3 cells transformed by K-FGF. The basis for this reversion is being investigated.

W 109 INTERACTION OF LEUKEMIA INHIBITORY FACTOR WITH AT LEAST THREE DISTINCT BINDING PROTEINS

Agnes MEREAU, Maud THIO and John K HEATH, Biochemistry department, University of OXFORD, South Parks Road, OXFORD OX1 3QU (UK).

Maintenance of embryonic (ES) cells pluripotentiality is dependant upon the continuous presence of the multipotent regulatory factor Differentiation Inhibiting Activity/Leukemia Inhibitory Factor (LIF). In mammalian cells LIF is expressed as a 43 kDa glycoprotein, derived by extensive glycosylation of a 19 kDa core polypeptide. Molecular cloning and functional studies have revealed that LIF is expressed in two forms, differing in amino acid sequence at the N-terminus of the secretory signal sequence, which differ in their extracellular delivery properties. D-LIF is secreted into the cuture media of expressing cells and M-LIF is physically associated with the extracellular matrix (ECM).

The interaction of LIF with cellular and extracellular binding sites has been studied using UMR osteosarcoma cells and ^{125}I-labelled recombinant (unglycosylated) murine LIF. UMR cells exhibit relatively high numbers of specific high affinity plasma membrane receptors (2-5 000 s/cell; apparent Kd of 50-200pM) which are presumably involved in LIF signal transduction. In addition, it has been shown that LIF is also able to interact with specific bindings sites in the extracellular matrix secreted by these cells. This second binding site may be involved in the localisation and presentation of LIF to responding cells, thereby providing a mechanism for spatial control of LIF bioactivity in vivo. Additional studies have also demonstrated that soluble LIF also binds to the plasma protease-binding protein $\alpha_2$macroglobulin, like certain other secreted cytokines as IL1 and IL6. This interaction of soluble LIF with plasma proteins may provide a method for controlling both the delivery and plasma clearance of circulating LIF.

Together these findings demonstrate that the biological action of LIF could be regulated by interaction with at least three functionally distinct classes of binding protein and provides an outline molecular mechanism for the control of LIF action *in vivo*.

W 110 THE GENES FOR MIDKINE (MK) AND PLEIOTROPHIN (PTN) SHARE MANY COMMON STRUCTURAL FEATURES

Peter G. Milner and Dulari Shah, The Jewish Hospital of St. Louis at Washington University, 216 S. Kingshighway, St. Louis, MO 63110, USA.

Pleiotrophin (PTN), midkine (MK) and retinoic acid-induced heparin-binding (RI-HB) protein are members of a recently discovered family of developmentally regulated proteins with both neurotrophic and mitogenic activities. In order to better understand the relationship of these proteins we cloned the human PTN gene from a lambda phage human genomic library. We then determined the nucleotide sequence of the human PTN gene, and compared its sequence and organization to that of the mouse MK gene (Matsubara et al., 1990, J. Biol. Chem. 265:9441-9443). By this analysis we have identified many structural similarities; in particular both genes are encoded by 5 exons. Exon 1 of both the MK and PTN gene is not translated. The ATG sequence coding for the initiator methionine, and the start of the amino acid sequence, is found at the beginning of exon 2. The signal peptide cleavage site of both genes lies towards the 3' end of exon 2. For both the PTN and MK genes 6 of the 10 cysteine residues are encoded in exon 3, while the remaining 4 cysteines are in exon 4. The full length amino acid sequence determined from the gene sequence shares 51% absolute and 64% functional identity. The intron-exon splice junctions occur through the same amino acid residues; exons 2-3 are joined through a GLU (#4 for MK, #7 for PTN), exons 3-4 through ALA (#57 for MK, #65 for PTN), and exons 4-5 through ALA (#111 for MK, #119 for PTN). The two genes are most closely related in exons 2-4, while exon 5, which encodes the highly basic tail of both proteins and their translation termination codons (TAA), has little homology. In summary the mouse MK and human PTN gene appear to be closely related, in particular in exons 1-4. In view of their striking similarity of both sequence and organization, they most likely represent 2 members of a new family of developmentally regulated cytokines.

W 111 MIDKINE (MK) WHICH IS A RETINOIC ACID RESPONSIVE, HEPARIN BINDING GROWTH DIFFERENTIATION FACTOR. BIOLOGICAL ACTIVITIES, EXPRESSION IN HUMAN CANCERS AND ITS CONTROL ELEMENT.

Takashi Muramatsu, Tadashi Kaname, Hisako Muramatsu, *Seung Kim, Junichiro Tsutsui, Shuichiro Matsubara, Kenji Kadomatsu, **Ken-ich Yamamura and ***Akira Nakagawara, Department of Biochemistry, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima, Japan, *Department of Medicine, University of British Columbia, Vancouver, Canada, **Institute of Medical Genetics, *Kumamoto University, Medical School, Kumamoto, Japan and* ***Department of Pediatric Surgery, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

Midkine (MK), which was found as a product of a retinoic acid responsive gene is a heparin binding growth differentiation factor. It is rich in cysteine and basic amino acids and has molecular weight of 14 KDa. Pleiotrophin /HB-GAM, another growth differentiation factor, has been recently found to have strong homology with MK. MK promoted neurite extension of not only embryonic brain cells, but also embryonic dorsal root ganglion cells. Furthermore, MK promoted survival of dissociated embryonic brain cells. These results indicate important roles of MK in development of the nervous system. MK was detected in various human cancer cells. Especially, intense expression of MK was observed in all the 7 cases of Wilms tumor. The 2 kb 5' upstream sequence of MK was sufficient for retinoic acid induced gene expression in vitro and developmentally regulated gene expression in vivo. The latter conclusion was obtained by using transgenic mice with a fusion gene constructed from the 5' upstream region and a β-galactosidase gene. Localization of β-galactosidase also gave information about possible MK expression in discrete regions: the enzyme was found in specific regions of the brain of the late embryos and in migrating neural crest cells.

W 112 PATOLOGICAL CHANGES IN DIFFERENT ORGANS INDUCED BY TGF-ß1-OVERPRODUCING TUMOR INOCULATION IN VIVO
Noboru Ueki, Toshihisa Ohkawa, Yuji Yokoyama, *Tastuhiko Ikeda, Yoshiki Amuro, Toshikazu Hada and Kazuya Higashino
The Third Department of Internal Medicine, Hyogo College of Medicine, Hyogo 663, *King Brewing Co.,LTD. Central Institute, Hyogo 675-01, Japan

TGF-ß is a pleiotropic growth factor that is presumed to play a role in the regulation of many physiological and pathological processes. We have previously shown in TGF-ß1 transfected cells (TIA) xenograft in a nude mouse model that levels of plasma TGF-ß is substantially elevated 2 wk after TIA-inoculation in contrast to the absence in plasma of control mice. The present studies were carried out to investigate whether TIA-inoculation to nude mice over a 2-wk period affects a variety of pathological changes in different organs. Histologically, TIA-inoculation caused fibrotic changes, being more pronounced in the liver and the lungs, but no significant fibrotic changes occurred in th kidneys, the heart and other organs. In addition, the red pulp of the spleen almost disappeared in company with extensive granulopoiesis in the region. It is not clear from previous studies, whose intravenous or subcutaneous administration of high doses of TGF-ß1 to mice was performed, whether TGF-ß1 induces fibrotic changes in the liver and the lungs. Our results suggest that excessive doses of TGF-ß1 in plasma of TIA-inoculated mice can systemically involve certain pathological processes, particularly fibrotic changes, in different organs. More detailed studies are required to fully delineate the exact sequence and nature of the events. However, the finding that excessive and inappropriate actions of TGF-ß1 were associated with fibrosis in different organs in vivo confirms and indicates the specific involvement of TGF-ß1 in the process that occurs in chronic liver disease and in pulmonary fibrosis.

W 113 IDENTIFICATION OF A CRE/ATF-LIKE ELEMENT IN THE HUMAN TRANSFORMING GROWTH FACTOR-β2 GENE THAT IS ESSENTIAL FOR PROMOTER ACTIVITY
Michael A. O'Reilly, Andrew G. Geiser, Leslie A. Bruggeman, Anh X. Luu, Anita B. Roberts and Michael B. Sporn, Laboratory of Chemoprevention, NCI and Laboratory of Developmental Biology, NIDR, NIH, Bethesda, MD 20892

TGF-β2 is encoded by multiple mRNA transcripts of 5.8, 5.1, 4.0, 3.8 and 2.8 kb that are expressed in various human and monkey cells. Northern blot analysis using genomic fragments of DNA was used to demonstrate that some of this size heterogeneity is due to differences in the length of the 5'-untranslated region. Probes that were colinear with the first 600 nucleotides of the 5'-untranslated region detected only the 5.8, 4.0 and 3.8 kb transcripts. In order to identify DNA elements that regulate the transcription of these mRNA transcripts, deletion constructs of 5'-flanking DNA were ligated to the coding region for chloramphenicol acetyltransferase (CAT) and analyzed for promoter activity in several cell lines. Sequences responsible for putative enhancer and silencer regions were identified between -778 and -40 relative to the transcription initiation site. The presence of an upstream CRE/ATF-like element at -74 resulted in a 5-10 fold increase in CAT activity over a minimal construct extending to -62. DNA sequences between -257 and -187 acted to silence this stimulation, while additional sequences between -778 and -257 stimulated CAT activity. The basal level CAT activity observed with the CRE/ATF element at -74 was abolished when this DNA element was mutated. Gel mobility shift analysis demonstrated specific binding of nuclear factors to the CRE/ATF sequence. Competition studies with the mutant CRE/ATF sequence revealed that it had a weaker affinity for the nuclear factors. These studies identify multiple transcription initiation sites for TGF-β2; transcription from one of these promoters is dependent upon a CRE/ATF-like element located 5' of the TATA box.

W 114 ORGAN SPECIFIC EXPRESSION OF SELECTED TGF-ß SUPERFAMILY MEMBERS, Engin Özkaynak, Donald Jin, Frederick Warren and Hermann Oppermann, Creative BioMolecules, 35 South Street, Hopkinton, MA 01748.

We have analyzed the organ specific expression of several morphogenetic proteins (MGPs) in mice by Northern hybridization. Some of these TGF-ß related MGPs are capable of inducing new bone formation under appropriate conditions and were termed osteogenic protein (OP) or bone morphogenetic protein (BMP).

Highly specific probes were derived from murine cDNA clones for each of the following genes: OP-1 (osteogenic protein-1), Vgr-1 (Vg-1 related protein), bone morphogenetic proteins BMP-2, BMP-3, BMP-4 and BMP-5, as well as GDF-1 (growth and differentiation factor-1). mRNA was isolated from brain, spleen, lung, heart, liver, and kidney of young and adult mice and analyzed on agarose gels.

OP-1 mRNA was found to be expressed mainly in the kidneys; Vgr-1, BMP-3, -4 mRNAs in the lungs; BMP-5 mRNA in lungs and liver; and GDF-1 mRNA, as reported by others, in the brain. OP-1 mRNA was found to be more abundant in the kidneys of growing animals than in adults. BMP-5 mRNA was found mostly found in lungs and liver of growing animals. In contrast, Vgr-1 and BMP-4 mRNAs were found to be more abundant in the lungs of adult animals.

Different MGPs with osteogenic potential are found to be expressed in distinct organs. OP-1 stands out in that it is the only one primarily synthesized in the kidneys. The relatively high levels of expression of these MGPs in adult animals indicate the they have biological roles beyond embryonic development, presumably for regenerative or adaptive growth either in paracrine or endocrine mode.

W 115 SECRETION AND CELL SURFACE ASSOCIATION OF WNT-1 (INT-1) PROTEIN FROM TRANSFECTED CELL LINES AND DIFFERENTIATING P19 EMBRYONAL CARCINOMA CELLS,
Jackie Papkoff, Lindsay Hinck and Brian Schryver. Syntex Research, S3-1, 3401 Hillview Ave., Palo Alto, California, 94304

The Int-1 proto-oncogene, re-named Wnt-1, was originally identified as a target for transcriptional activation by insertion of MMTV proviral DNA. Aside from a role in mammary epithelial transformation, Wnt-1 functions normally during neural development. Since the specific role of Wnt-1 in growth and differentiation is unknown, we have characterized the biochemical properties of Wnt-1 protein. We previously showed, using transfected CHO and AtT-20 cells, that the cysteine rich Wnt-1 protein enters the constitutive secretory pathway where it is modified by N-linked glycosylation and subsequently secreted. Most of the secreted Wnt-1 protein remains tightly associated with the cell surface or extracellular matrix where it can participate in cell to cell signalling. Experiments are in progress to identify the surface/matrix structures that interact with Wnt-1 protein. We now provide the first identification and characterization of native Wnt-1 protein. When treated with retinoic acid, the P19 embryonal carcinoma cell line will differentiate along a neuroectodermal lineage and when treated with DMSO these cells will differentiate into mesodermal derivatives. We have identified, by immunoprecipitation with anti-peptide antibodies, the Wnt-1 protein in P19 cells differentiated along the neuroectodermal pathway. No Wnt-1 protein can be detected in undifferentiated or DMSO differentiated P19 cells. The biochemical properties of native Wnt-1 protein are similar to Wnt-1 protein from transfected cell lines i.e. it is glycosylated, secreted and associated with the cell surface. Further experiments showed that Wnt-1 protein is secreted as a monomer. Ectopic expression of Wnt-1 in P19 cells does not appear to alter the course of differentiation. We favor the hypothesis that secreted Wnt-1 protein participates in cell to cell signalling by modulating the function of cell junctions. Experiments are in progress with a variety of cell lines to assess the effects of Wnt-1 on several junctional proteins including those of the plakoglobin/armadillo family.

W 116 Immunohistochemical localization of TGFβ1, TGFβ2 and TGFβ3 in the mouse embryo: expression patterns suggest multiple roles during embryonic development, Ron W. Pelton, Babita Saxena[2], Melissa Jones, Harold L. Moses and Leslie I. Gold[2], Department of Cell Biology Vanderbilt University Medical School, Nashville, TN 37232 and (2) Department of Pathology, New York University Medical Center New York, NY 10016. Isoform-specific antibodies to TGFβ1, TGFβ2 and TGFβ3 proteins were generated and have been used to examine the expression of these factors in the developing mouse embryo from 12.5 - 18.5 days *post coitum* (d.*p.c.*). These studies demonstrate the initial characterization of both TGFβ2 and β3 in mammalian embryogenesis and are compared with TGFβ1. Expression of one or all three TGFβ proteins was observed in many tissues: *e.g.* cartilage, bone, teeth, muscle, heart, blood vessels, lung, kidney, gut, liver, eye, ear, skin and nervous tissue. Furthermore, all three TGFβ proteins demonstrated discrete cell-specific patterns of expression at various stages of development and the wide variety of tissues expressing TGFβ proteins represent all three primary embryonic germ layers. For example, specific localization of TGFβ1 was observed in the lens fibers of the eye (ectoderm), TGFβ2 in the cortex of the adrenal gland (mesoderm) and TGFβ3 in the cochlear epithelium of the inner ear (endoderm). Compared to the expression of TGFβ mRNA transcripts in a given embryonic tissue, TGFβ proteins were frequently co-localized within the same cell type as the mRNA but in some cases were observed to localize to different cells than the mRNA, thereby indicating that a complex pattern of transcription, translation and secretion for TGFβs 1-3 exists in the mouse embryo. This also indicates that TGFβ1, β2 and β3 act through both paracrine and autocrine mechanisms during mammalian embryogenesis.

W 117 FUNCTIONAL ANALYSIS OF IL-6 AND THE IL-6 REGULATED TRANSCRIPTION FACTOR IL-6DBP BY GENE TARGETING

Valeria Poli and Frank Costantini, Department of Genetics and Development of Columbia University, New York, NY 10032

Interleukin 6 (IL-6) is a multifunctional cytokine produced by a variety of cell types in response to different kinds of stimuli. It has been shown to be able to induce B lymphocytes differentiation, T lymphocytes proliferation, mesangial cells and keratinocytes growth, and it is a potent plasmacytoma/hybridoma growth factor; it can induce hematopoietic stem cells to divide and it is the most potent inducer of liver protein synthesis during the acute phase response. However, while its effects are well studied and characterized in vitro, its real functions in vivo are very difficult to study, due to the intricated network of interacting cytokines and growth factors and to the overlapping functions of many of these molecules. IL-6 exerts its effects through the interaction with a membrane receptor; the complex activates another membrane protein, gp120, which is responsible for the transmission of the signal; however, little is known about the signal transmission pathway. Recently a nuclear target for IL-6 was characterized, which is involved in the induction of transcription of liver acute phase proteins by IL-6. This transcription factor, IL-6 Dependent DNA Binding Protein (IL-6DBP), is induced in its activity by treatment with IL-6, and it is likely to be the mediator of at least part of the cell responses triggered by IL-6. To study in vivo the functions of IL-6, and to identify which of the IL-6 functions are triggered by IL-6DBP, we decided to make use of the gene targeting techniques to create mice deficient for these two proteins. Mouse embryonic cell lines were established carrying mutations inactivating the IL-6 or the IL-6DBP gene, respectively. The mutated cell lines were microinjected into mouse embryos, and chimeric animals were obtained. At the moment the chimeric animals are bred to transmit the mutations into the germ line.

W 118 THE MOLECULAR DIVERSITY OF THE FGF RECEPTOR FAMILY

Dina Ron[1], Orit-Aker Cohen[1], Ronit Reich[1], Carole Lengel[2], Toru Miki[2], Cheryl Smith[2], Andrew Chan[2] and Steven R. Tronick[2]. [1]Biology Department, Israel Institute of Technology, Haifa 32000, Israel and [2]Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892.

Fibroblast growth factors (FGFs) are polypeptide mitogens that induce the proliferation of a wide variety of cell types. Of the seven family members, the best characterized are basic and acidic FGF. In addition to their mitogenic activity, they participate in angiogenesis, differentiation and maintenance of survival of *neurons, cell migration and embryonal development.* The biological response of cells to FGFs is mediated through specific high affinity cell surface receptors that possess intrinsic tyrosine kinase activity and are phosphorylated upon ligand binding. We studied the complexity of the FGF receptor family and were able to *isolate five different variants of the FGF receptor from* human fibroblasts. These molecules were derived from a single locus but differed from each other in their extracellular domains. In addition we isolated a novel FGF receptor from normal mammary epithelial cells. Since studies of the FGF receptors have been complicated by the presence of several distinct FGF receptors in many cell types, we developed a system in which receptors could be expressed in a naive cell line. Thus, it was possible to study ligand-binding and activation of individual FGF receptors. These studies along with those concerned with the regulation of receptor expression *in vivo* will be discussed.

W 119 CRIPTO MULTIGENE FAMILY IN MOUSE AND MAN, Lorenzo Scalera, Rosanna Dono, Francesco Pacifico, Massimo Zollo, Nunzia Montuori and M. Graziella Persico. International Institute of Genetics and Biophysics (I.I.G.B.), CNR, 80125 Naples, Italy.

It has been shown that more than one form of each growth factor can exist due to alternative splicing or differential post-transcriptional modification. Another way to obtain different forms of a protein is to have a multigene family coding for modified proteins. We have described the isolation of a human cDNA encoding a protein of 188 amino acids sharing structural similarities with TGFα and EGF (Ciccodicola et al. 1989 EMBO J. 8:1987-1991). Over expression of CRIPTO leads to in vitro transformation of the normal mouse mammary epithelial cell line NOG-8 (Ciardiello et al. 1991 PNAS 51:1051-1054).

In human and mouse multiple highly-related genomic CRIPTO sequences are present (Dono et al. 1991 Am. J. Human. Genet. 49:555-565). One genomic sequence, mapping on human chromosome 3, is expressed in teratocarcinoma cells. A second genomic sequence is expressed in human hepatoma cell lines,HepG2 and HepB3. A third sequence represents a possible functional pseudogene and maps to the human X-chromosome. In mouse two different genomic sequences are expressed: one in teratocarcinoma cells and lung, the other in spleen and hearth of the adult animal.

This work was supported by a grant from AIRC.

W 120 EXPRESSION AND FUNCTION OF DIFFERENTIATION INHIBITING ACTIVITY (DIA/LIF) IN THE MOUSE EMBRYO, Austin Smith, Morag Robertson, Annette Dûwel and Jennifer Nichols, AFRC Centre for Genome Research, Universtiy of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JQ, UK

Differentiation Inhibiting Activity/Leukaemia Inhibitory Factor (DIA/LIF) is a cytokine which specifically suppresses the differentiation of pluripotential embryonic stem (ES) cells *in vitro*. Alternative transcription results in the production of the active protein in both a soluble form and as an immobilised factor associated with the extracellular matrix. DIA/LIF has a variety of other biological activities *in vitro* and *in vivo*. To investigate the potential role for DIA/LIF as a generalised stem cell factor, we have mapped the distribution of DIA/LIF mRNA throughout normal mouse development. A highly sensitive ribonuclease protection assay has been employed to discriminate between the alternative transcripts. This assay has enabled detection of very low levels of mRNA which are expressed in a tissue-specific and temporally regulated manner during development. DIA/LIF transcripts are detected at the egg cylinder stage, consistent with a role for DIA/LIF in regulation of the pluripotential primitive ectoderm. In later embryogenesis and the adult it is noteworthy that transcripts are found in the gut and skin. In both tissues maximal expression is observed during the perinatal period, a time of heightened stem cell activity. *In situ* hybridisation has been used to localise the sites of transcription within expressing tissues. A functional analysis of the role of DIA/LIF has been initiated by the isolation of ES cell transfectants in which stable and constitutive expression of DIA/LIF is directed by the housekeeping *pgk-1* promoter. These clones remain capable of differentiation but exhibit diminished dependency on exogenous DIA/LIF *in vitro*. Their introduction into blastocysts permits characterisation of the effects of deregulated DIA/LIF expression on early embryonic differentiation and gastrulation.

W 121 *Abstract Withdrawn*

W 122 A RETROVIRUS CARRYING THE K-*fgf* ONCOGENE INDUCES DIFFUSE MENINGEAL TUMORS AND SOFT TISSUE FIBROSARCOMAS. Daniela Talarico[1], Michael M. Ittmann[2], Roderick Bronson[3], and Claudio Basilico[1], Dept. of Microbiology[1] and Kaplan Cancer Center[1], Dept. of Pathology[2] and VA Medical Center[2], New York University School of Medicine, New York, NY 10016, Dept. of Pathology, Tufts University School of Medicine and Veterinary Medicine[3], Boston, MA 02111.

The K-*fgf*/*hst* oncogene encodes a growth factor of the FGF family and transforms cells through an autocrine mechanism The K-FGF protein is a powerful mitogen in vitro for a variety of cell types of mesodermal and neuroectodermal origin, including fibroblasts, endothelial cells and melanocytes. To identify the cell and tissue targets of K-*fgf* oncogenic potential in vivo, we constructed recombinant retroviruses carrying the human K-*fgf* cDNA and injected them, together with helper Moloney murine leukemia virus (MoMLV), into immunocompetent as well as nude mice. The original constructs were highly transforming in tissue culture, but produced no detectable pathologies in vivo, with the exception of a single fibrosarcoma which arose after a long latency. The virus produced by this tumor appears to have undergone a complex series of recombination events involving the helper MoMLV. It encodes an env/K-FGF fusion protein whose expression is under the control of an hybrid LTR. This virus (designated MFS) induces tumors in mice with high frequency and short latency. These neoplasms consist of aggressive fibrosarcomas of soft tissues, and diffuse meningeal tumors originating from the dura mater that surround the whole central nervous system and cause severe hydrocephalus. The env/K-FGF fusion protein expressed by the MFS virus is indistinguishable from native K-FGF in all of its biological properties, and thus the high frequency of meningeal tumors appears to result from an exquisite susceptibility of meningeal fibroblasts to transformation by the K-*fgf* oncogene.

W 123 **MUTATIONAL ANALYSIS OF THE *WNT*-1 PROTEIN,** Frank van Leeuwen and Roel Nusse, Department of Developmental Biology, Howard Hughes Medical Institute, Beckman Center , Stanford, CA 94305.

Wnt-1 is the prototype of a family of secreted polypeptides involved in cell-cell communication during development. All members of this family are thought to be related in structure and function. In mouse, these genes are predominantly expressed in the developing nervous system, in a spatially and temporally restricted fashion. Biochemical analysis of the mouse *Wnt*-1 protein reveals that the protein is glycosylated and secreted; subsequently it remains associated with the cell membrane or extracellular matrix.

The Drosophila homolog of *Wnt*-1 is *wingless*, a segment polarity gene required for proper pattern formation in each segment. Consistent with the biochemical data on the *Wnt*-1 protein, *wingless* appears to be secreted, traveling only over short distances to neighboring cells, suggesting that *wingless* is involved in cell-cell communication.

Our current research focuses on the mechanism of action of *Wnt* genes at the cellular level and the identification of targets for *Wnt* gene action. Expression of the *Wnt*-1 cDNA in mammalian cell culture systems may help in clarifying certain aspects of *Wnt*-1 function. Using oligonucleotide directed mutagenesis, we have introduced a number of mutations in the *Wnt*-1 cDNA, that are thought to affect *Wnt*-1 function. The effect of these mutations will be assessed on the cell biological and biochemical level using various cell lines, some of which are known to respond to expression of the *Wnt*-1 gene. Analysis of these mutants is currently underway.

W 124 A NEW FAMILY OF HEPARIN BINDING PROTEINS (RIHB, PLEIOTROPIN), Marc Vigny, Daniel Raulais, Catherine Guettet, Yves Courtois, Jean-Claude Jeanny and Delphine Duprez, INSERM U.118, CNRS, Association Claude-Bernard, 29, rue Wilhem, 75016 Paris, France

A 19 kDa heparin binding protein was previously purified from chicken embryos. Its synthesis was induced by retinoic acid (RA) in myoblast-myotubes in culture. We named it RIHB for retinoic acid heparin binding. The sequence of RIHB contains 121 aminoacids and is very rich in basic aminoacids and cysteines. Northern blot analysis indicates that RIHB mRNA is strongly expressed during early chicken embryogenesis (2-6 days) and it is induced by RA treatment in myoblast-myotubes and embryonic brain neurons in culture but not in fibroblasts which strongly express this messenger in absence of retinoid.

RIHB belongs to the same family as pleiotropin and MK protein two other heparin binding proteins developmentally regulated and exhibiting growth and/or neurotrophic activities. RIHB is largely expressed in almost all early embryonic tissues where it is essentially associated with basement membranes. Furthermore, there is clear relationship between the expression of the protein and the differentiation of the tissue. In contrast chicken pleiotropin is essentially expressed after birth in particular in the central nervous system. However we purified small amount of RIHB from adult chick brain and immunofluorescence studies indicates that it is specifically localized in a discret set of cell.

In conclusion we propose that RA could regulate some aspect of differentiation and development by inducing the synthesis of a new family of growth and neurotrophic factors.

W 125 THE PRESENCE OF A TGFα TRANSGENE RESULTS IN REPRODUCTIVE ABNORMALITIES IN FEMALE CD-1 MICE

David Walmer[1], Mark Wrona[1], Robert Feola[2], Karen Nelson[2], Nancy Bossert[1], Robert Dickson[3], Glenn Merlino[4] and John McLachlan[2], [1]Dept. of Ob/Gyn, Duke University Medical Center, Durham,NC, [2]Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, RTP, NC, [3] Lombardi Cancer Center, Georgetown University, [4] NCI Molecular Biology, NIH

To better define the role of transforming growth factor alpha (TGFα) in normal and pathological tissue physiology, transgenic mice (MT-42) containing a fusion gene consisting of the mouse metallothionein I promoter and a human TGFα cDNA were produced by Dr. Glenn Merlino (Cell 61:1137,1990). TGFα overproduction in the transgenic mice results in alterations of proliferation, organogenesis, and neoplastic transformations involving the liver, mammary gland and pancreas. Breeding problems in the form of a shortened breeding life span (3-5 mo) and reduced plugging rates (~1% vs 16%) were noted in these mice. The reduced plugging rate did not appear to be due to problems in cycling throughout estrus based on a random examination of vaginal histology. The reduced plugging rate was not improved by human chorionic gonadotropin (hCG) priming. However, superovulation with pregnant mare serum gonadotropin (PMSG) and hCG induced a normal plugging rate (9/17 vs 6/12 in controls). Despite the corrected plugging rate with superovulation and mating, fewer oocytes were flushed from the transgenic mouse oviducts on day 0 (6.75/ mouse, n = 54 embryos vs 15.2/ mouse, n = 76 embryos in controls). Although there was no apparent attrition of embryos in the first three days of pregnancy (7.3/ mouse, n = 44 embryos vs 12.1/ mouse, n = 169 embryos in controls) there was a greater number of embryos with delayed development in vitro (23/44 embryos, 52% vs 57/169, 33% in controls). An analysis of implantation sites is in progress. Examination of the MT-42 reproductive tracts at 6-8 weeks of age demonstrated histologic abnormalities of multiple cell types in the uterus. In addition, the ovaries and uteri of the transgenic mice were sensitive to tumor induction with DMBA. The oviducts and vagina were grossly and histologically normal. This study demonstrates that the presence of a TGFα transgene leads to abnormalities in mating behavior, ovulation and endometrial histology in female mice as well as an increased susceptibility to DMBA-induced tumors. TGFα therefore appears to have an important role in reproduction.

W 126 IFFERENTIAL SPLICING IN THE EXTRACELLULAR REGION OF FGF RECEPTOR 1 GENERATES RECEPTOR VARIANTS WITH DIFFERENT LIGAND BINDING SPECIFICITIES.

Sabine Werner, Dah-Shuhn R. Duan, Carlie de Vries, Kevin G. Peters and Lewis T. Williams.
Cardiovascular Research Institute, Department of Medicine, Howard Hughes Medical Institute, University of California, San Francisco, CA 94143-0724, USA

We have cloned a genomic region of the murine Fibroblast growth factor receptor 1 (FGFR1) gene that includes three alternative exons for the third immunoglobulin-like domain in the extracellular region of the receptor. The mRNA of one of these splice variants encodes a secreted receptor that lacks transmembrane and cytoplasmic sequences as well as a portion of the third immunoglobulin-like domain. Highest levels of mRNA encoding this variant were found in brain, skeletal muscle and skin. We expressed this form of FGFR1 in CHO cells and showed that the recombinant secreted protein binds acidic FGF. We also discovered a novel alternative exon in the third immunoglobulin-like domain that encodes part of a transmembrane FGFR1 mRNA. This exon is highly homologous to the corresponding region of the keratinocyte growth factor receptor. Transcripts including this exon were present at highest levels in the skin. We cloned an FGFR1 cDNA which includes this exon and expressed this receptor variant in L6 rat skeletal muscle myoblasts. The new receptor variant had a 50-fold lower affinity for basic FGF than the published FGFR1 variant, whereas both forms of receptor bound acidic FGF with high affinity. These results show that the third immunoglobulin-like domain plays an important role in determining the binding specificities for different FGFs. Our data provide the first evidence that differential splicing in the extracellular region of a receptor gene generates receptor variants with different ligand-binding specificities.

W 127 THE RAT INSULIN-LIKE GROWTH FACTOR II GENE IS POST-TRASCRIPTIONALLY REGULATED IN CULTURED LIVER CELLS.

Raffaele Zarrilli,[1] Stefano Casola,[1] Piernicola Boccuni,[1] Vittorio Colantuoni,[2] and Carmelo B. Bruni[1]. *Centro di Endocrinologia ed Oncologia Sperimentale del Consiglio Nazionale delle Ricerche, Dipartimento di Biologia e Patologia Cellulare e Molecolare "L. Califano",[1] and Dipartimento di Biochimica e Biotecnologie Mediche,[2] Università di Napoli, Via S. Pansini 5, 80131, Napoli, Italy.*

The rat insulin-like growth factor II (IGF-II) gene is expressed at high levels during embryonic and fetal life and at low levels in adult animals. The molecular mechanisms underlying the developmental regulation of IGF-II gene expression were studied in a system of cultured cells derived from the same embryonic lineage but from different developmental stages. The synthesis and localization of the IGF-II transcripts was analyzed in BRL3A cells, derived from the Buffalo rat liver and producing high levels of IGF-II, or in two other cell lines that do not produce detectable levels of IGF-II mRNA, the BRL30E cells, also derived from the Buffalo rat liver, and the FAO cells, a differentiated rat hepatoma expressing several markers of the adult liver. Our data show that the IGF-II gene is transcribed at a similar rate in producing and non-producing cells, whereas its nuclear and cytoplasmic RNA levels are diversely distributed in the cells. IGF-II RNA is more abundant in the cytoplasmic than in the nuclear RNA fraction of BRL3A cells and is present in the nucleus but not in the cytoplasm of the non-expressing cells. However, both precursor and mature IGF-II nuclear RNA levels are reduced in the latter cells.

The data we present indicate that the reduced abundance of IGF-II RNA in non-producing cells is due to post-transcriptional mechanisms altering the stability of both the nascent transcripts and the mature RNA.

W 128 *Abstract Withdrawn*

Signalling and Gene Expression in Development

W 200 THE ROS-PROTOOCOGENE IS EXPRESSED TRANSIENTLY AND IN A SPATIALLY RESTRICTED MANNER DURING MOUSE EMBRYOGENESIS.

Carmen Birchmeier, Eva Sonnenberg, Axel Gödecke and Barbara Walter, Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Carl-von-Linné-Weg 10, 5000 Köln 30.

The ros gene was originally identified by virtue of its transforming potential; the protooncogene encodes a receptor-type tyrosine specific protein kinase which is closely related to sevenless from Drosophila.

As a step towards the understanding of the normal physiological function of c-ros, we analyzed the expression of the gene in the mouse by a combination of RNase protection and in situ hybridisation experiments. We found a transient, developmentally controlled and limited spatial expression pattern of c-ros during embryogenesis in the kidney, intestine and lung. Expression coincides in time with mayor morphogenetic and differentiation events in these organs. This temporary restricted nature of expression is unusual for tyrosine kinase receptors and suggests a role of c-ros during development. Furthermore, in kidney development c-ros transcripts a confined to subgroups of ureter cells known to be involved directly in inductive interactions between ureter epithelium and metanephric mesenchyme. Thus, this study implies a tyrosine kinase receptor in mesenchymal epithelial interactions during development and suggests a molecular basis for these important inductive events.

W 201 DIFFERENTIAL EXPRESSION OF A NOVEL GROWTH FACTOR RECEPTOR DURING MAMMARY GLAND DEVELOPMENT

Blaschke, R.J., Andres, A-C., Zürcher, G.S., Ziemiecki, A.

University of Berne, Lab for Clinical and Experimental Cancer Research, Tiefenaustr. 120, 3004 Berne (Switzerland)

Protein tyrosine kinases (PTKs) play an indispensable role in signal processing and are excellent candidates for regulators of complex, externally-triggered differentiation events such as seen during mammary gland (MG) development. To identify novel PTKs involved in this process we employed a MG-derived cell culture system comprising two clonal cell lines that upon co-culture are able to differentiate, both morphologically and functionally *in vitro*. Using cDNA from these cells, and primers for PCR-amplification corresponding to highly conserved regions within the catalytic domain of PTK-genes, we have isolated several tyrosine kinase specific clones. One of these clones , A67, represents a novel growth factor receptor related to the *eck/elk/eph* subfamily of PTKs. A67 detects three transcripts (3.5, 4.5 and 8 kb) in a wide range of organs with the exception of testes where no expression is seen. In lung only the shortest mRNA is present. During MG-development, the highest expression is detected in virgin stages, declining during the course of pregnancy, and is absent in lactating glands. A67 specific mRNAs are again detectable within the first two days of involution, a process involving apoptotic regression of the MG-epithelium beginning immediately after weaning. Sequence analysis suggests that at least one of the three transcripts encodes a truncated receptor defective in the tyrosine kinase catalytic domain. The co-expression of intact and truncated forms of this receptor may imply a novel mode for the regulation of its biological activity.

W 202 SPATIAL AND TEMPORAL EXPRESSION OF AN EPITHELIAL MUCIN, Muc-1, DURING MOUSE DEVELOPMENT, Vania M.M. Braga, Lucy Pemberton, Trevor Duhig and Sandra J. Gendler, Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The Muc-1 mucin (called PEM for polymorphic epithelial mucin in the human) is found as a transmembrane protein in the apical surface of glandular epithelia. An association of the cytoplasmic tail with the actin cytoskeleton and the high homology (87%) of the transmembrane and cytoplasmic domains between the human and mouse homologues suggest that this region may be important to its function. One possible function is the stabilization of polarization in epithelial tissues. In order to better assess the possible participation of Muc-1 in epithelial polarity, we have performed studies on RNA and protein expression during mouse embryogenesis using three different techniques: RT-PCR, northern blots and immunohisto-chemistry. Our results indicate that Muc-1 expression correlates with epithelial differentiation in stomach, pancreas, lung, trachea, kidney, and salivary glands. Once started, Muc-1 synthesis continually increases with time, mainly due to epithelial area growth. Our data is suggestive that expression of Muc-1 gene is under spatial and temporal control during organogenesis. Although Muc-1 is present in different organs, its expression is not induced systemically, but according to the particular onset of epithelial polarization and branching morphogenesis of each individual organ. It is of particular interest that Muc-1 protein can be detected lining the apical surfaces of the lumens when the epithelium of the studied organs is still undergoing folding and branching, and secretory activity has not yet started, as, for instance, in the 12 day embryonic pancreas. The detection of Muc-1 expression very early during organogenesis and much before organs start to function properly, is indirect evidence that the presence of this mucin might be relevant to the proccess of maintenance of epithelial polarization during mouse embryogenesis.

W 203 ANALYSIS OF THE PROTO-ONCOGENE N-*MYC* FUNCTION DURING MOUSE DEVELOPMENT, Jean Charron[1,2], Peter E. Fisher[2], Barbara A. Malynn[3] and Frederick W. Alt[3], [1]Centre de recherche en cancérologie de l'Université Laval Québec City, P. Q. Canada G1R 2J6, [2]Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, [3]Howard Hughes Institute, Harvard Medical School, Boston, MA 02115.

The N-myc gene is thought to play a potential role in the processes of cell growth and differentiation, but its specific function remains to be elucidated. Analysis of gene function has often relied on isolation of mutants in which expression of the gene was inactivated. Therefore, we have used targeted mutagenesis by homologous recombination to disrupt the mouse N-*myc* gene in ES cells to assess its function during embryogenesis. ES cell clones containing one allele of the disrupted N-*myc* gene have been injected into blastocysts to produce chimeric mice and a mouse strain carrying one null allele of the N-*myc* gene. Heterozygous N-*myc* mutant mice are as healthy and fertile as their wild type littermates. Heterozygous animals for the N-*myc* null allele were intercrossed to assess the effect of loss of N-*myc* protein in homozygous offspring. Until now, no live born homozygous mice for the mutation have been found suggesting that the mutation results in embryonic lethality. To determine the time of death, heterozygous animals for the N-*myc* mutation were intercrossed, and pregnant females were sacrified at different times of gestation. At 9.5, 10.5 and 11.5 dpc, the ratio of wild type, heterozygous and homozygous animals for the mutation is 1:2:1 as expected if the mutation is transmitted in a Mendelian fashion. However, at 9.5 and 10.5 dpc, the embryos homozygous for the mutation look often underdeveloped when compared to their wild type or heterozygous littermates. In addition, at 11.5 dpc, 7 out of the 10 embryos genotyped as homozygous were dead and in the process of resorption. At 12.5 dpc, no live embryos homozygous for the mutation were found, but dead embryos and many sites of resorption were detected. This analysis indicates that absence of N-*myc* function results in embryonic lethality between 10.5 and 12.5 dpc.
Preliminary histological analyses of the embryos homozygous for the mutation suggest that some cell lineages might be affected in their proliferative potential. Requirement of the N-*myc* function in embryogenesis is presently under investigation and the resultsa will be discussed.

W 204 ***tek* a Novel Receptor Tyrosine Kinase Gene Located on Mouse Chromosome 4 is Expressed in Endothelial Cells and Their Presumptive Precursors.** **Daniel J. Dumont, Terry P. Yamaguchi, Ronald A. Conlon, Guo Hua Fang, Janet Rossant, and Martin L. Breitman. Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, CANADA.** A search by reverse transcription linked to the polymerase chain reaction (RT-PCR) for receptor tyrosine kinases (RTKs) expressed during murine cardiogenesis resulted in the isolation of a novel tyrosine kinase that we have designated *tek*. Sequence analysis of cDNA clones suggested that *tek* encodes at least two RTK isoforms that contain extracellular, transmembrane and catalytic domains, as well as a 21 amino acid kinase insert. The RTKs encoded by *tek* are most closely related in their kinase domain to FGFR1 and the product of the *ret* proto-oncogene. Using a strain dependent restriction site polymorphism, we have mapped the *tek* locus to mouse chromosome 4 between the *b* and *pmv-23* loci. *In situ* hybridization analysis of adult tissues, as well as sectioned and whole mount embryos, showed that *tek* is specifically expressed in the endocardium, the leptomeninges and the endothelial lining of the extra- and intraembryonic vasculature from the earliest stages of their development. Moreover, examination of the morphology of *tek*-expressing cells, and staging of *tek* expression relative to that of the endothelial cell marker von Willebrand factor revealed that *tek* expression precedes von Willebrand factor and appears to mark the embryonic progenitors of mature endothelial cells. Our results suggest that *tek* encodes a transmembrane tyrosine kinase that may be critically involved in the determination and/or maintenance of cells of the endothelial lineage.

W 205 CYCLOPAMINE CAUSES ABNORMAL DEVELOPMENT IN *XENOPUS LAEVIS*, Michael K. Dunn and David D. Moore, Departments of Genetics and Biological Chemistry and Molecular Pharmacology, Harvard University, and Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114.

We have used the amphibian *Xenopus laevis* as a model system to begin characterization of the defects caused by cyclopamine, a mammalian teratogen. Cyclopamine was initially identified as a teratogen when it was found to be a causal agent of cyclopia in sheep. Epidemiological studies showed that cyclopamine and other related molecules could lead to limb and cranio-facial defects in many livestock species, as well as chickens and some laboratory rodents. We have extended these observations to include *Xenopus*. Here we describe the pharmacological and physical characterization of the effect of cyclopamine on the developing embryo.

The initial defect observed in drug-treated *Xenopus* embryos is curvature of the tail, which is apparent at approximately 4 days post-fertilization (Nieuwkoop stage 37/38). The following day (stage 41), head defects become apparent. Prolonged exposure to the drug is not necessary, and tail defects can be induced if drug is applied at any time during tail growth.

Whole mount immunostaining was used to determine distribution of muscle-specific antigens. While drug-treated animals appear to contain muscle, this tissue is not properly organized in the chevron pattern of naive tail muscle. Whole mount staining with alcian blue, which stains cartilage-specific proteoglycan, shows a deficiency of staining in the trunk-tail junction area at day 4 post-fertilization in the drug-treated animals, and a much more dramatic deficiency in staining in the head region at day 5. These differences in alcian blue staining correlate with the physical abnormalities, both temporally and spatially. Histological staining with the Feulgen/light green/orange G protocol described by Cooke (1979) confirms disorganization of tissues in the drug-treated group. Histology also suggests that the physical manifestations of drug treatment may be a culmination of indirect effects brought about by cyclopamine's action on a single cell type, possibly an undifferentiated precursor cell.

W 206 HORMONAL REGULATION OF EXPRESSION OF THE CELL SURFACE MUCIN (MUC1) IN THE HUMAN AND MOUSE FEMALE REPRODUCTIVE TRACT, Sandra J. Gendler, Vania' M.M. Braga, Joyce Taylor-Papadimitriou and John O. White*, Imperial Cancer Research Fund, PO Box 123, Lincoln's Inn Fields, London WC2A 3PX and *Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN, U.K.

The MUC1 mucin (designated Muc-1 in the mouse) is a highly glycosylated integral membrane glycoprotein which is expressed at the apical surfaces of glandular epithelia. The mucin is developmentally regulated (see abstract from Braga et al., this meeting) and has been known as a tumor marker which is expressed in an aberrantly glycosylated form in >90% of carcinomas. We here report that the MUC1 mucin is expressed in the human and mouse female reproductive tract at the luminal and glandular surfaces of the uterus (simple columnar epithelium) as well as by the cervix and vagina (stratified squamous epithelium). Immunohistochemical staining was evaluated at different stages of the hormone cycle, and expression was found to vary in intensity at different times during the cycle. Expression of MUC1 by human endometrium was very low during the proliferative phase of the menstrual cycle. At day 15 expression in the endometrium was greatly increased and remained high throughout the secretory phase. In the mouse the uterine luminal epithelium and apical surfaces and secretions in the glands showed the most intense staining during the diestrous phase. Diestrous is comparable, hormonally, to the secretory phase in the human and is approximately the time when implantation of the blastocyst occurs. The level of staining was lowest in the mouse estrous stage, with intermediate intensities observed during proestrous and metestrous. In the cervix and vagina similar hormonal regulation was observed, although the level of expression appeared to be less than in the uterus. Quantitation of the mRNA levels and protein are in progress. An increase in expression of the mucin during the time of implantation in the uterus is intriguing, and the temporal correlation may suggest a possible involvement of this molecule in implantation.

W 207 STIMULATION OF $G_s\alpha$ EXPRESSION AND HORMONE-RESPONSIVE ADENYLATE CYCLASE ACCOMPANIES EMBRYONIC STEM CELL DIFFERENTIATION, Ruth K. Globus, Gordon J. Strewler and Robert A. Nissenson, Department of Medicine, VA Medical Center SF and University of California SF, San Francisco, CA 94121

The developmental potential of embryonic stem cells (ES) is modified by their ability to respond to extracellular signals. Differentiation of F9 embryonic carcinoma (EC) cells following retinoic acid treatment is accompanied by activation of adenylate cyclase in response to parathyroid hormone-related protein (PTHrP), a protein produced by both extraembryonic and embryonic tissues. Furthermore, PTHrP accelerates differentiation of retinoic acid-treated F9 cells. We determined whether increased expression of the stimulatory coupling protein for adenylate cyclase, $G_s\alpha$, and responsiveness to PTHrP are regulated as a function of pluripotent ES and EC cell differentiation. Differentiated ES cells (D3 and E14 cell lines) grown as aggregates to form embryoid bodies expressed 10-fold higher steady-state levels of $G_s\alpha$ mRNA than control cells, an increase that was detectible within 24 hrs of aggregation. $hPTHrP_{(1\text{-}34)}$ increased adenylate cyclase activity 4.1-fold above basal in membranes from differentiated cells but had little effect on control cells (1.4-fold increase). Activation of the PTHrP-responsive cAMP pathway and induction of $G_s\alpha$ expression in pluripotent EC cells (P19) occurred under conditions that favored the formation of cell-cell contacts (aggregates) or stimulated differentiation (retinoic acid-treated aggregates). In conclusion, amplification of the PTHrP-responsive cAMP pathway occurred during differentiation and aggregation of non-malignant and totipotent ES cells. Increased $G_s\alpha$ expression during differentiation of embryonic stem cells may function to sensitize the cAMP pathway and consequently regulate the cellular response to extracellular signals such as PTHrP.

W 208 EXPRESSION OF PKC IN F9 TERATOCARCINOMA CELLS, H.C. Kindregan, S. Rosenbaum, S. Ohno, and R. Niles, Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118

It is well established that treatment of F9 teratocarcinoma's with retinoic acid or retinoic acid plus cyclic AMP results in their differentiation to primitive or parietal endoderm respectively. We have found that three isoforms of Protein Kinase C (PKC) are differentially expressed when F9 cells are differentiated. When the cells are treated with retinoic acid or 8 bromo cyclic AMP plus retinoic acid for 120 hours PKCα protein levels increase whereas those of β and γ decrease. The steady state levels of RNA exhibit the same expression pattern. The PKCα protein levels begin to increase within 24 hours of drug treatment and reach maximum level by 120 hours. The level of PKCβ protein decreases significantly by 48 hours. In order to determine the significance of the induction of PKCα in differentiated F9 cells, we made two stable transfectants which overexpress PKCα between 4- and 7-fold when compared to control cells. The level of expression of the PKCβ protein in these clones is similar to the levels found in differentiated F9 cells. We have begun to further characterize these clones to determine whether PKCα overexpression drives differentiation by looking at the expression of type IV collagen and pST6-135 which serve as useful markers for differentiation.

W 209 IDENTIFICATION AND PARTIAL CHARACTERIZATION OF THE C-JUN ONCOGENE IN XENOPUS LAEVIS. P. Lazarus. American Health Foundation, One Dana Road, Valhalla, NY 10595.

In initial studies on the role of c-*jun* in signaling for egg maturation, differentiation and embryogenesis, preliminary data on the characterization of c-*jun* in Xenopus laevis A6 kidney cells are presented. Employing PCR methodology, a 275 nt cDNA fragment corresponding to the DNA-binding region at the carboxyl end of the Xenopus c-*jun* protein product was amplified. Sequencing analysis shows that this region exhibits 77-79% nucleotide homology and 93% amino acid homology with the DNA-binding region of mouse or human c-*jun* and contains an intact leucine zipper for dimerization and a basic region for DNA-binding. This 275 nt amplified cDNA was purified and P^{32}-labelled for Northern analysis of Xenopus c-*jun* gene expression. These studies showed that Xenopus c-*jun* mRNA is induced in quiescent A6 cells by TPA, serum, EGF and PDGF, and super-induced by cycloheximide, within 1hr post-treatment. In addition, two species of Xenopus c-*jun* mRNA appear to exist in the range of 2-2.3 kb. The "immediate-early" response of Xenopus c-*jun* is similar to that observed for its mammalian counterpart.

W 210 NEONATAL ESTROGEN EXPOSURE RESULTS IN THE PERSISTENT INDUCTION OF GROWTH REGULATORY PEPTIDES IN MOUSE VAGINA. Karen Gray Nelson, Yasuhiro Sakai, Ben Eitzman, and John Mclachlan, Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27514

Neonatal treatment of rodents with high doses of estrogen induces hormone-independent persistent vaginal cornification as well as neoplastic lesions of the cervix, vagina and uterus. The mechanism by which estrogens permanently alter the growth, differentiation and function of the developing reproductive tract is unknown. Recent evidence suggest that steroid hormones mediate their effects on target tissues by the regulation of a variety of multifunctional growth-regulatory peptide factors. We have investigated by in situ analysis the effects of estrogen on the expression of epidermal growth factor (EGF), lactoferrin and actin in mouse uterus and vagina at various times following diethylstilbestrol (DES) treatment (2μg/day/pup) of neonatal female CD-1 mice on Days 1 through 5 of life. In situ analysis clearly demonstrates that neonatal DES exposure results in a persistent elevation of mRNA expression for the estrogen-inducible growth factor, EGF, in the cervix and vagina. Similar to EGF, actin expression is maintained at high levels following neonatal DES exposure in the epithelium of the cervix and vagina but not in the uterus. However, neonatal DES treatment does cause a transient, cell specific alteration in the pattern of actin mRNA distribution in the uterus. The expression of lactoferrin, an iron-binding estrogen inducible protein, is also elevated in an ovarian hormone independent fashion in the cervix and vagina of neonatally treated females up to 54 days of age. Whereas, the induction of lactoferrin mRNA in the uterine epithelium is only transient, associated specifically with the initial neonatal estrogen exposure. Our data indicates that exposure to exogenous estrogen during critical stages of development results in defective expression of estrogen-regulated genes which then may contribute to abnormal organogenesis, tissue function and carcinogenesis.

W 211 EXPRESSION OF GROWTH HORMONE AND PROLACTIN RECEPTORS IN THE DEVELOPING MOUSE PANCREAS, Jens H. Nielsen and George Gittes, Hagedorn Research Laboratory, Gentofte, Denmark, and Hormone Research Institute, University of California San Francisco, CA, USA

The growth and differentiation of the pancreas depend on epithelial mesenchymal interactions. In order to identify factors involved in this interaction we analyzed the expression of various growth factors and receptors by the polymerase chain reaction of reverse transcribed RNA (RT-PCR) isolated from the pancreas anlage of mouse embryos on day 9 (E9) and 11 (E11) after conception. In an attempt to quantitate the relative abundance of the mRNA we analyzed the amplified products at different cycles of the PCR reaction after labelling with $\alpha[^{32}P]$-dATP by polyacrylamide electrophoresis. The radioactivity was quantitated on a Phospho-Imager and the intensities were plotted on a log scale versus the cycle number. A β-tubulin fragment was co-amplified in the PCR reaction and the growth factor or receptor signal was expressed relative to the β-tubulin signal in a range where the degree of amplification was the same. The results showed that the mRNA's of the growth factors IGF II, IGF I and $TGF\beta_2$ were highly abundant in decreasing order, whereas those of TGFα, aFGF and bFGF were less abundant. It was of particular interest, that mRNA's for the growth hormone (GH) and prolactin (PRL) receptors (R) are present already in the E9 pancreas anlage as GH and PRL are potent growth factors for the postnatal endocrine pancreas. When the epithelium and mesenchyme separated by microdissection of the E11 pancreas anlage were analyzed by RT-PCR both IGF I and II as well as GH-R and PRL-R mRNA seemed to be relatively more abundant in the mesenchyme. As GH and PRL only are expressed later in the pituitary and if biological active receptors are formed it is suggested that other fetal tissues and/or the placenta are sources of GH, PRL or related hormones, which may play a role in the development of the pancreas.

W 212 Xenopus Y-BOX BINDING PROTEINS : CHARACTERIZATION IN VIVO, Mukul Ranjan, Sherrie R. Tafuri and Alan P. Wolffe Laboratory of Molecular Embryology, NICHD, NIH, Bethesda, MD 20892 The sequence specific DNA binding proteins, FRG Y1 and FRG Y2 have previously been shown to stimulate transcription in vitro from promoters containing a Y-box (1). The FRG Y proteins have a distinct pattern of expression during embryogenesis and possibly play an important role in Xenopus development. These proteins are also interesting transcriptionally, since they appear to represent a distinct family of sequence specific DNA binding proteins, along with the human Y-box binding proteins. This work further characterizes the two proteins in a transient transfection system in Xenopus A6 cells. Both FRG Y1 and FRG Y2 cause an increase in transcription from a CAT reporter plasmid containing the hsv thymidine kinase promoter. This stimulation is seen only at low levels of FRG Y expression, while a marked inhibition of transcription is seen with higher levels of these proteins. These results have been correlated to the levels of expressed protein and mRNAs of both reporter and FRG Y proteins to determine the mechanism of inhibition. The cellular localization of the expressed proteins was also monitored for clues regarding their function.

1. Tafuri, S.R. and Wolffe, A.P. 1990 PNAS, USA 87, 9028-9032

W 213 THE DEVELOPMENTAL FATE OF ADRENOCORTICAL CELLS DEPENDS ON ALTERATIONS IN SIGNAL TRANSDUCTION AT THE LEVEL OF CALCIUM REGULATION. Calvin Roskelley and Nelly Auersperg, Department of Anatomy, University of British Columbia, Vancouver B.C. V6T 1Z3, Canada

The zones of the adrenal cortex contain distinct populations of cells which share a common developmental origin and steroidogenic template. In the rat, zona glomerulosa cells respond to Angiotensin II (Ang II) with increased steroidogenesis. As they differentiate further into zona fasciculata cells, this response is lost. In an effort to determine the mechanism responsible for this difference we have examined Ang II-mediated signal transduction in homogeneous sub-populations of glomerulosa (GLOM) and fasciculata (FASC) cells that were separated by density gradient centrifugation. In both cell types, Ang II treatment significantly increased the levels of 3[H] labelled inositol phosphates as well as the total mass of inositol 1,4,5 triphosphate. In contrast, the two cell types exhibited very different Ang II-mediated changes in free intracellular calcium ($[Ca^{2+}]i$). Ang II (10nM), induced $[Ca^{2+}]i$ increases of >50nM in 90% of individual GLOM cells (53/58), but in only 28% of FASC cells (11/39). These $[Ca^{2+}]i$ responses occurred after a transient Ang II stimulation (<1 min.), in the presence of verapamil and in the absence of extracellular calcium, indicating an intracellular release. In groups of 10-30 cells, stimulation with 1, 10 and 100 nM Ang II induced $[Ca^{2+}]i$ increases of 78, 178 and 215nM respectively in GLOM cultures compared to only 35, 64, and 65 nM in FASC cultures. However, thapsigargin treatment, which releases calcium from intracellular stores in an inositol phosphate independent manner, elicited comparable $[Ca^{2+}]i$ increases in both cell populations. Importantly, a calcium ionophore-induced elevation of $[Ca^{2+}]i$ increased steroidogenesis in both cell types. These results suggest that rat zona fasciculata cells lack a steroidogenic response to Ang II, at least in part, because of an interruption of the signalling pathway at the level of increased intracellular calcium. Therefore, in the rat adrenal cortex, divergent differentiation of related cell types may involve alterations within signal transduction pathways distal to initial receptor-mediated events (ie. inositol phosphate production) and proximal to downstream effector events (ie. steroidogenesis). Supported by N.C.I. Canada.

W 214 CREM: A TRANSCRIPTION FACTOR WITH AN AMAZING ORGANIZATION AND DEVELOPMENTALLY REGULATED EXPRESSION.

Paolo Sassone-Corsi, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Faculté de Médécine, 11, rue Humann, 67085 Strasbourg, France.

The CREM (cAMP-response element modulator) gene has a remarkable organization. It contains two DNA-binding domains (DBD) which are used alternatively by differential, cell-specific splicing. The two DBDs are constituted by slightly different leucine-zippers and basic regions, suggesting that various CREM proteins might have different promoter targets. We have recently described that the CREM gene generates various antagonists of the cAMP transcriptional response by the use of at least three transcript isoforms. CREM antagonists block the function of the activator CREB (1). We have also shown that the proto-oncogene c-*fos* is a target of the repression exerted by the CREM antagonists, that are involved in the down-regulation which follows the c-*fos* induction by activation of the cAMP pathway (2). More recently, we discovered that the CREM gene encodes also an activator of cAMP-induced transcription (3). Thus, the single gene CREM encodes both antagonists and activator of transcription. The CREM activator is generated in a developmentally regulated fashion. Mammalian spermatogenesis consists of a series of complex developmental processes which are under the control of the pituitary/hypothalamic axis. This flow of biochemical information is directly regulated by the adenylate cyclase signal transduction pathway. We documented the expression of a novel CREM isoform in adult testis. The testis CREM differs from the previously characterized CREM antagonists (1) by the coordinate insertion of two glutamine-rich domains which confer transcriptional activation function. During spermatogenesis we observed an abrupt switch in CREM expression (3). In premeiotic germ cells CREM is expressed at low levels in the antagonist form. Subsequently, from the pachytene spermatocyte stage onwards, a splicing event generates exclusively the CREM activator. This new transcript accumulates at extremely high levels. This splicing-dependent reversal in CREM function represents an important example of developmental modulation in gene expression. We will discuss the endocrine regulation of the splicing events which dictate the differential functionality of the CREM products.

References: (1) Foulkes, N.S., Borrelli, E. & Sassone-Corsi, P. (1991) *Cell* **64** 739-749; (2) Foulkes, N.S., Laoide, B.M., Schlotter, F. & Sassone-Corsi, P. (1991) *Proc. Natl. Acad. Sci. USA* **88** 5448-5452; (3) Foulkes, N.S., Mellstrom, B., Benusiglio, E. & Sassone-Corsi, P. (1991) *Nature*, in press.

W 215 AMYLIN EXPRESSION IS INCREASED DURING FETAL RAT DEVELOPMENT D.T. Stein, T.R. Pieber, D.W. Weaver, K.L. Luskey, Dept of Internal Medicine, University of Texas Southwestern, Dallas, TX 75235. Amylin (Am) is a 37 aa polypeptide that is coexpressed and co-secreted with insulin (I) from ß-cells in adult pancreas. Its precise physiologic role is unknown; however, a role in carbohydrate and calcium metabolism has been proposed. Recent studies indicate that in healthy adult nonobese rodents and humans, plasma Am levels are <10pmol/l, and percent Am:I molar ratios <2-3%. The aim of the present study was to investigate expression of Am and I during fetal rat development from embryo day 14 (E14) to after birth. We used a highly sensitive and specific RIA for rat amylin 1-37, and rat insulin.

RESULTS: Amylin immunoreactivity and insulin immunoreactivity were detectable in amniotic fluid (AF) from day E14 onwards and peaked at day E21 in both AF and plasma (P):AF-Am 54.2± 3.8pmol/L, P-Am 101.8±2.4, AF-I 693.8±91.8, P-I 1440.3±318.8 respectively (mean±SE, n= 3 litters). Percent Am:I molar ratio in AF was 21.8%±2.2 at E14, elevated >60% E15-E17 peaking at E16 and decreased near term, E21 8.02%±0.9. Percent plasma Am:I ratio during days E20-22 was similar to that in AF. By day 2 after birth, Am and I decreased to 25.6pmol/l±3.6 and 448.4± 93.0, respectively, percent Am:I 6.6%±3.1. In addition RNA blot hybridization analysis of tissue distribution in day E21 fetuses revealed abundant amylin signal in pancreas with trace amounts in bowel. Insulin message was found in pancreas and extraplacental fetal membranes only.

CONCLUSION: During gestation, amylin expression may be regulated independently of insulin (assuming clearance rates of both hormones are unchanged). Immunoreactive amylin, both total and relative to insulin, is increased during gestation. At its peak, before birth, it is 10 fold elevated over adult levels, and rapidly falls thereafter. These findings suggest a role for amylin in fetal development and in the transition to extrauterine life.

W 216 CELL TYPE SPECIFIC REPRESSION OF AFP TRANSCRIPTION IN THE ADULT MOUSE, Angela L. Tyner, Lisa Cirrillo, Jean Vacher,* Dept. of Genetics, University of Illinois College of Medicine, Chicago, Illinois, and Dept. of Molecular Biology, Princeton University, Princeton, New Jersey.* α-fetoprotein (AFP) is the major serum protein expressed in the mammalian fetus. During fetal development, transcription of the AFP gene is activated in three tissues, the yolk sac, liver and intestine, albeit at very different rates. In the liver, the level of AFP transcription peaks shortly before birth, after which transcription is repressed approximately 10,000 fold. In the intestine the highest levels of expression are also detected shortly before birth, and most epithelial cells lining the small intestine express AFP mRNA at this time. After birth, low level expression of AFP is maintained in a subset of enteroendocrine cells, one of the four major intestinal epithelial cell lineages. Recently it was determined that the cis-acting sequences that mediate repression of AFP transcription lie between -800 and -248 of the AFP gene. Transgenic animals carrying an AFP minigene including the three AFP enhancers and a deletion of the sequence between -800 and -248, no longer appropriately repress AFP transcription in the adult mouse liver or small intestine. AFP minigene mRNA is expressed at a level comparable to that of the fetal liver, in both of these adult tissues. We have investigated the nature of the cells that continue to express high levels of AFP minigene RNA in these transgenic animals using in situ hybridization. Although all fetal hepatocytes, and most fetal intestinal epithelial cells transcribe the AFP gene, only distinct cell types in the adult transgenic animals overexpress the AFP minigene. These include a layer of hepatocytes surrounding the central veins in the liver, and the enteroendocrine cells in the intestine. These data suggest that most adult hepatocytes and adult intestinal epithelial cells lack the positive factors required for AFP transcription and only a few specialized cells actively repress transcription of the AFP gene after birth.

W 217 MODULATION OF RETINOIC ACID SIGNALLING BY NUCLEAR HORMONE RECEPTORS

K. Umesono, S. A. Kliewer, R. A. Heyman*, D. J. Mangelsdorf, J. A. Dyck, and R. M. Evans
The Salk Institute for Biological Studies, La Jolla, California, *Ligand Pharmaceuticals, Inc. San Diego, California.

The vitamin A metabolite retinoic acid (RA) exerts profound biological effects in specifying body patterns during vertebrate development. At the cellular level, RA controls growth and differentiation through two families of intracellular receptors: the retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Both receptors belong to a superfamily of steroid/thyroid hormone receptors which act as ligand-dependent transcription factors to regulate gene expression by binding to regulatory DNA sequences termed hormone response elements (HREs). To understand the complex cascade of events underlying the hormonal effects of RA, we characterized modes of target gene recognition by RAR and RXRs. The HREs for the nuclear hormone receptors are composed of either a palindrome or a direct repeat (DR) of hexameric half-sites. We have shown recently that the spacing (5, 4, or 3 nucleotides) of the half-sites in the direct repeat HREs confers specificity of response for RARs, thyroid hormone receptors, and vitamin D3 receptor, respectively. This "3-4-5 rule" was further expanded to fill in a remaining spacing option of 1 nucleotide (DR-1) which creates a response element for the RXRs. We have found that the same DR-1 motif also stands as a high affinity binding site for another member of the receptor family, COUP-TF, of which ligand is not known yet. Such DR-1 motif is identified in the rat cellular retinol binding protein type II (CRBPII) gene promoter and this promoter is indeed upregulated by RA in the presence of the RXRs but not RARs. Interestingly, overexpression of the RAR or COUP-TF suppresses the RA- and RXR-dependent induction of the CRBPII gene. Analyses of this negative effect conferred by either RAR or COUP-TF have revealed that in vitro, a physical interaction exists to mediate heterodimer formation such as RAR/RXR and COUP-TF/RXR. These results indicate that an interplay among distinct members of the nuclear hormone receptors modulates RA signalling positively and negatively to generate diversity of the RA action during vertebrate development.

W 218 STUDIES OF THE MOUSE CELLULAR RETINOIC ACID-BINDING PROTEIN GENE REGULATION IN TRANSGENIC MOUSE EMBRYOS. Li-Na Wei, Y. Chu, J. Tsao and G. Chen. Department of Microbiology, Chang Gung Medical College, Tao Yuan, Taiwan. R.O.C.

Various sequences of the 5'-flanking region of the mouse cellular retinoic-acid-binding protein (CRABP) gene were isolated and fused into a LacZ reporter sequence and DNA fragments of these fusion genes were microinjected to generate transgenic mice. Embryos were obtained, and assayed for the LacZ activities. Only the transgenic mouse embryos of the fusion gene with a 3 kb DNA fragment upstream from the initiation of transcription fused to the reporter sequence showed spatially and temporally specific expression of LacZ activities, which agreed with the expression pattern of the endogenous CRABP mRNA shown by in situ hybridization. Expression of this fusion gene at the RNA level was further confirmed by RNAase protection assays. Neither non-transgenic embryos nor the transgenic mouse embryos of a shorter fusion gene containing only 583bp from the same upstream region of the mouse CRABP gene fused to the same reporter sequence showed specific LacZ activities. Thus, it is concluded that the 3 kb sequence, but not the 583bp sequence, of the mouse CRABP gene contains information for its temporally and spatially specific expression in mouse embryos.

W 219 DNA BINDING PROPERTIES OF ULTRASPIRACLE, THE DROSOPHILA RETINOID X RECEPTOR HOMOLOGUE

T.P. Yao, M. McKeown*, and R.M. Evans

Gene Expression Lab,
*Molecular Biology and Virology laboratory,
The Salk Institute, La Jolla, CA 92186-5800

Drosophila ultraspiracle locus (usp) encodes a member of the steroid hormone receptor family that is the fly homologue of the vertebrate Retinoid X receptor (RXR). usp has been shown to be a pleiotropic factor which is required in multiple tissues and developmental stages throughout Drosophila development. By expressing the recombinant usp protein in E.coli, we are able to show that usp can bind to DNA with a specificity toward a direct repeat of two AGGTCA half sites spaced by one nucleotide (DR1), which is similar to the specificity of RXR . A dramatic stimulation of usp binding to the DNA element can be achieved if the in vitro translated usp is mixed with certain members of the receptor family, or with the antibody against usp. This result indicates that multimerization or conformational change is necessary for usp to bind DNA with high affinity. A similar observation also has been made with RXR. The similarity in DNA binding specificity and the requirement for additional factors to achieve high affinity binding in vitro supports the idea that usp and RXR are not only structurally related but may also share a similar molecular mechanism to exert their biological function.The interaction between receptor members, which appears to be evolutionarily conserved, suggests an additional mechanism of how receptors may function in transcriptional regulation of their specific targets. The search for the factors which may genetically interact with usp in Drosophila and the potential significance of this interaction in the whole organism are currently underway.

Early Development; Pattern Formation; Homeobox Genes

W 300 XENOPUS REL/DORSAL ANTIGENS ARE DISTRIBUTED IN A CYTOPLASMIC GRADIENT IN THE EGG AND ENTER THE NUCLEUS AT STAGE 6 OF EMBRYOGENESIS. E.L. Bearer. Div. Biology and Medicine, Brown University, Providence, RI 02912.

The Drosophila maternal effect gene, dorsal, is 60 % homologous to the rel family of oncogenes in the amino terminus. The gene product is distributed uniformly in the egg cytoplasm and enters the nuclei in a ventral to dorsal gradient. Its presence in the nucleus is necessary for the proper formation of ventral structures such as the mesoderm and neural tube, and for the suppression of dorsal structures on the ventral side. I have used antibodies to the chick rel protein to identify rel-dorsal antigens in Xenopus embryos. The antibodies stain the egg cytoplasm in a gradient, with highest concentration over the animal cap. At Stage 6, staining is observed in the nuclei over the animal cap, and in the deep cells of the marginal zone. Staining persists through gastrulation. By Western blot these antibodies recognize a single 95 kD protein in eggs and early embryos, but stain several bands after stage 9 of embryogenesis. I obtained three different sized DNA oligomers by polymerase chain reaction using homologous domains between rel and dorsal as primers, and DNA from reverse-transcription of RNA from Stage 11 gastrulae as template. I conclude that at least one dorsal-rel homologue is present in Xenopus eggs and embryos.

This work is supported by Council for Tobacco Research Grant No. 3192.

W 301 SPEMANN'S ORGANIZER ACTIVITY AND *GOOSECOID* HOMEOBOX GENE, [1]Ken W. Y. Cho, [2]Bruce Blumberg, [2]Hebert Steinbeisser, and [2]Eddy M. De Robertis, (1) Department of Developmental and Cell Biology, University of California, Irvine, Ca 92717, (2)Department of Biological Chemistry, University of California, Los Angeles, CA 90024

The dorsal blastopore lip of the amphibian gastrula has potent inductive properties. When this region is transplanted to a host gastrula, it is able to organize a complete secondary axis, thus, it is an ideal system to study the axis-forming mechanisms. A search for genes potentially regulating organizer activity was initiated by constructing dorsal lip cDNA library and screening it for homeobox containing genes (Blumberg et al., 1991). These genes were initially chosen because they have been shown to mediate axis-formation in *Drosophila* and vertebrates. A novel homeobox containing gene, *goosecoid*, having the same DNA-binding specificity as *Drosophila bicoid* was isolated. The *goosecoid* gene is present in all vertebrates and expressed only during gastrulation. Microinjection of this gastrulation specific message into blastomeres of early *Xenopus* embryos induces secondary axes. The *goosecoid* gene is immediately induced by a growth factor, activin (without the requirement for synthesis of another protein factor) and is then specifically expressed in the mesoderm of the dorsal lip region. These properties suggest that the *goosecoid* gene product plays fundamental regulatory roles during gastrulation and Spemann's organizer phenomena.

W 302 MOLECULAR BASIS OF LIMB MORPHOGENESIS: HOMEOBOX GENES AND RETINOIC ACID RECEPTORS IN THE NEWT. L.L. Crews and J.P. Brockes. The Ludwig Institute for Cancer Research. 91 Riding House St. London. W1P 8BT. England.

We work on the molecular mechanisms that specify positional information in vertebrate limb morphogenesis. Our model system, which focuses on limb regeneration in the adult newt, provides a unique opportunity to examine this issue. Our studies are based largely on two findings: (1) that retinoic acid (RA) can respecify positional information along the proximal-distal axis of the limb in a dose-dependent manner and (2) that the expression of homeobox-containing genes is consistent with an important role in the specification of positional information. The effects of RA may be mediated through specific receptors (RARs). Two RARs have been found in the newt and are currently being manipulated to determine the role of specific RARs in axial specification. Coincidently, we have isolated three homeobox-containing genes: NvHBox-1, -2 and -7. The expression pattern of each gene is unique and does not seem to change in response to RA. Further, we have developed a functional assay wherein cells containing gene constructs are implanted in the regenerating limb. Subsequent examination of the tissue will reveal whether a given construct influences the fate of the cells as they proliferate, differentiate and assume their position. Many of the principles underlying axial specification are conserved. While regeneration may seem a biological curiosity, it provides an ideal setting in which to study the influence of potential morphogens and homeobox-containing genes in complex structures such as the limb.

W 303 HOX 7.1 EXPRESSION AND REGENERATION IN EMBRYONIC MOUSE LIMBS.

A. DiStefano[1], Y. Wang[2], D.A. Sassoon[2] and K. Muneoka[1]. Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118[1] and Dept. of Biochemistry, Boston University Medical School, Boston, MA 02118[2]

We have investigated the relationship between the expression of Hox7.1 and regenerative ability of embryonic mouse digits. Hox7.1 is normally expressed within the progress zone; a narrow band of mesenchymal cells underlying the apical ectoderm of stage 7/8 (12.5 days p.c.) mouse limb buds. By stage 11 (day 15.5 p.c.) transcription of Hox 7.1 is primarily restricted to the distal-most region of each developing digit, which will form the nail bed, and also in the inter digital regions undergoing cell death. Utilizing *exo utero* surgical techniques we have found that embryonic digits have the ability to regenerate distal structures, including phalangeal elements and the nail bed, and that regenerative ability declines with developmental age. Thus, there is an apparent relationship between Hox7.1 expression and regenerative ability; regeneration occurs from limb regions that express Hox7.1 and does not occur in Hox7.1 negative regions. To further investigate this relationship we have performed *in situ* hybridization studies to document the expression of Hox7.1 during digit regeneration. We find that Hox7.1 is expressed in the digit blastema 24 and 48 hours following amputation at levels that display a regenerative response. Hox7.1 is not expressed following amputations at proximal levels that do not display a regenerative response. Thus, the expression of Hox7.1 correlates with the inherent regenerative ability of embryonic mouse digits suggesting that Hox7.1 expression may play a key role in regulating regenerative responses in mammals.

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W 304 A NOVEL, ACTIVIN INDUCIBLE, BLASTOPORE LIP SPECIFIC GENE OF XENOPUS LAEVIS CONTAINS A FORK HEAD DNA BINDING DOMAIN, Marie Luise Dirksen and Milan Jamrich, Laboratory of Molecular Pharmacology, Division of Biochemistry and Biophysics, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

The organizer region, or dorsal blastopore lip, plays a central role in the initiation of gastrulation and the formation of the body axis during Xenopus development. A similar process can also be induced in ectodermal explants by activin or by injection of activin mRNA into embryos. We have searched early embryo specific cDNA libraries for genes expressed in the organizer region of blastula/gastrula stage embryos that are at the same time inducible by activin. We have isolated a gene, XFHK4, that fulfills both requirements. It is specifically expressed in the dorsal blastopore lip of early gastrulae, and is inducible by activin even in the absence of protein synthesis. XFKH4 contains a DNA binding domain similar to that of the Drosophila homeotic gene fork head and rat hepatocyte nuclear factor HFN3ß. Because of its spatial and temporal expression pattern, as well as its inducibility by activin, this gene is a good candidate to have a regulatory function in the initial processes of axis formation in Xenopus laevis embryos.

W 305 AROMATASE IS A DEVELOPMENTAL SWITCH IN SEX DETERMINATION OF CHICKENS, Alex Elbrecht and Roy G. Smith, Department of Animal Genetics and Molecular Biology, Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065.

The presence of the Y chromosome determines sex in mammals and predicts a pattern of male sex differentiation. A gene on the Y chromosome has been identified as a candidate for the testis-determining factor. Other factors that are involved in development of the male phenotype are anti-Mullerian hormone and the gonadal steroids. In contrast to mammals, the heterogametic sex in chickens is female and the factors involved in sex determination have not been identified. In chickens the differentiation process occurs between days six and eight of embryonic development. Thus, during the first week of egg incubation the embryonic gonads are considered to be bipotential. Treatment of chicken embryos with an aromatase inhibitor during this first week of incubation results in female to male sex reversal of the adults. These genetic females possess bilateral testes capable of sperm production and have the behavioral and physical characteristics of normal males. Aromatase is the enzyme that catalyses the final step in the biosynthesis of the gonadal steroids, i.e. the conversion of androgens to estrogens. These experiments identify regulation of aromatase enzyme activity as a crucial step in sex determination in chickens.

W 306 EXPRESSION OF HOMEOBOX GENE FUSIONS AND THE EFFECTS OF RETINOIC ACID IN TRANSGENIC MICE. T. Gridley, M. Zhang[1], M. Gendron-Maguire, H.-J. Kim[1], D.A. Lucas[1], A. Baron[2], and J.F. Grippo[1]. Dept. of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110; [1]Dept. of Toxicology and Pathology, Hoffmann-La Roche Inc.,Nutley, NJ 07110; [2]Basel Institute for Immunology, Basel, Switzerland

The murine homeobox (*Hox*) genes play important roles in pattern formation during embryonic development and may provide a molecular mechanism for understanding retinoid-induced teratogenesis. *In vitro* studies have shown that retinoic acid can dramatically alter *Hox* gene expression. We have been studying one of the *Hox* genes in the *Hox 1* complex, *Hox 1.6*. In order to characterize cis-acting regulatory elements of *Hox 1.6*, transgenic mice were generated using constructs containing the *lacZ* reporter gene under the control of *Hox 1.6* regulatory elements. The initial construct contained 6.3 kb of *Hox 1.6* genomic sequence upstream of the transcription initiation site, 0.7 kb of the first exon fused in-frame with *lacZ* and a SV40 polyadenylation signal. This transgene is prominently expressed in a restricted region of the developing mouse hindbrain (rhombomere 2) at days 7.5-10.5 of gestation, thus exhibiting a more anterior boundary of expression than endogenous *Hox 1.6*. During this time a lower level of expression is seen in the somites. When the embryos are exposed to retinoic acid at 7.5 days of gestation, expression of the transgene in the hindbrain is repressed, while that in the somites is not affected. The inappropriate anterior boundary of expression of the transgene may be due to the presence of regulatory elements of a new homeobox gene in the *Hox 1* complex, *Hox 1.11*, on the transgene construct. Studies are underway to analyze the expression pattern of the endogenous *Hox 1.11* gene, and to further characterize the retinoic acid responsiveness of additional *Hox-lacZ* reporter gene fusion constructs.

W 307 THE ISOLATION AND CHARACTERIZATION OF THE MOUSE HOMEOBOX GENE, HOX 1.11, J.F. Grippo, D.A. Lucas, H.-J. Kim, M. Zhang, M.T. Gendron-Maguire[1], A. Baron[2], and T. Gridley[1]. Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ, 07110; [1]Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ, 07110;[2] Basel Institute for Immunology, Basel, Switzerland.

A murine homolog of the human HOX 1K homeobox gene has been identified that is located between the Hox 1.5 and Hox 1.6 gene coding regions on mouse chromosome 6. Sequence analysis of an 1800 bp clone obtained from an 8.5 day p.c. mouse cDNA library shows that this homeobox gene, Hox 1.11, exhibits a high level of amino acid identity within the homeobox domain with the human HOX 1K gene. Preliminary data suggest that the genomic organization of the Hox 1.11 gene is similar to that of other Hox 1 cluster genes. In situ hybridization analysis reveals that expression of Hox 1.11 is detectable between 8.0 and 8.5 days p.c., following the expression of Hox 1.6 which first appears at approximately 7.5 days p.c. Hox 1.11 exhibits an anterior boundary of hindbrain expression in rhombomere 2, with higher levels of expression seen in rhombomeres 3 and 5. Expression is detected in the neural crest of the VII/VIII ganglion complex and of the second and third branchial arches. We have been interested in the effects of retinoids on gene expression during hindbrain development and have found that expression of Hox 1.6, but not Hox 1.11 is dramatically altered following treatment of pregnant dams with retinoic acid between 6.5 and 8.5 days p.c.

W 308 *THE ORIGIN OF CELL DIVERSITY IN ZEBRAFISH*, Kathryn Helde and David Grunwald, Department of Human Genetics, University of Utah, Salt Lake City, Utah 84112

Cell lineage analysis of zebrafish (*Brachydanio rerio*) and frog (*Xenopus laevis*) embryos indicates striking differences in the early development of these two animals. In frog embryos, blastomeres from the animal hemisphere have fates different from those in the vegetal hemisphere. The differences in developmental potential are consistent with the presence of molecules in vegetal but not animal cells. Vg1 RNA is present only in vegetal cells, and may be involved in generating further cell differences. In zebrafish, all early blastomeres appear to be developmentally equivalent and their developmental fates are not yet restricted or predictable. In order to determine how zebrafish embryos generate cell diversity, we are studying the expression of the Vg1 homolog in zebrafish.

The Vg1 putative protein is a member of the Transforming Growth Factor-ß family of extracellular signalling molecules. The putative amino acid sequence of the TGF-ß region is 75% identical and 90% similar between fish and frog, suggesting that this molecule has a function essential to development.

In order to investigate whether Vg1 RNA marks initial differences between cells in zebrafish embryos, we are characterizing its expression pattern. We found that Vg1 RNA is abundant in ovaries and unfertilized eggs, and is present in embryos in decreasing amounts throughout embryogenesis. In adults, Vg1 RNA is expressed only in ovaries. The temporal profile of Vg1 RNA expression is consistent with the hypothesis that it has a role in early development. We are looking at its spatial expression in whole and sectioned embryos. Our preliminary results indicate that Vg1 RNA is expressed evenly throughout oocytes and early blastomeres. This lack of localization is different from the expression pattern seen in frogs, but is consistent with experiments in zebrafish which indicate that early blastomeres are developmentally equivalent.

Our current studies are examining Vg1 RNA expression at later stages, and describing Vg1 protein expression. These studies will help elucidate the molecular mechanisms underlying the initiation of cell differences.

W 309 CELL-EXTRACELLULAR MATRIX INTERACTIONS REGULATE THE BALANCE BETWEEN GROWTH AND DIFFERENTIATION IN MOUSE MYOBLASTS. D.J. Milasincic, J. Dhawan, and S.R. Farmer. Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118.

Growth and differentiation are generally considered to be mutually exclusive events. In mouse myoblasts, growth arrest by serum deprivation or growth to high density results in fusion of cells to form myotubes. We have shown that myoblasts can be growth arrested in high serum by suspension in methylcellulose-containing gel without commitment to the differentiated phenotype. Growth arrest of suspended myoblasts is accompanied by a down regulation of c-myc, myo D, and Id (a transcription factor that inhibits differentiation) and by negligible levels of muscle specific gene expression. Adhesion of these suspended myoblasts to a substratum (plastic or fibronectin) in high serum activates the immediate early growth response as well as myoD and Id. These cells progress into S phase and do not express the myogenic phenotype. Adhesion in low serum (differentiation medium), while capable of initiating the early growth response, does not activate Id expression. These cells do not progress into DNA synthesis but instead express myogenic factors and fuse into myotubes. In contrast, adhesion to fibronectin in low serum activates growth and Id expression and prevents fusion into myotubes. Present studies are aimed at understanding how adhesion to fibronectin influences these nuclear factors which regulate the switch between growth and differentiation in myoblasts.

W 310 Expression of murine Distal-less homeobox genes during embryonic development. Gertraud W. Robinson, Susan Wray and Kathleen A. Mahon. Laboratory of Mammalian Genes and Development, NICHD, NIH. Bethesda, MD 20892

The homeobox genes of Drosophila perform key functions in embryonic pattern formation, and their vertebrate counterparts may play similar developmental roles. Using polymerase chain reaction technology, we have identified four murine homologues of the Drosophila Distal-less homeobox gene that are expressed in midgestation embryos. The homeodomains encoded by these genes vary considerably from other known homeodomain sequences and represent a new family of vertebrate homeobox genes called Dlx. We isolated cDNAs for two of these genes and studied their expression by in situ hybridization. The Dlx-3 gene is strongly expressed in the developing branchial arches. In contrast, the Dlx-2 gene shows a restricted pattern of expression in the ventral forebrain, extending from the olfactory bulb to the ventral diencephalon. This domain of expression may delineate an ontogenetically defined subdivision within the forebrain. Thus Dlx-2 may provide positional cues in the developing forebrain in the way that the Hox genes are believed to act in more posterior regions of the central nervous system.

W 311 EXPRESSION CLONING OF A cDNA FOR A NOVEL POLYPEPTIDE WITH DORSAL AXIS INDUCING ACTIVITY FROM GASTRULA STAGE XENOPUS EMBRYOS. William C. Smith and Richard M. Harland, Department of Molecular and Cell Biology, Division of Biochemistry and Molecular Biology, 401 Barker Hall, University of California, Berkeley, CA 94720.

Dorsal/ventral patterning in Xenopus is initiated early in embryogenesis and is apparent at the time of gastrulation by differences in the behavior, and later in the fate, of marginal zone (presumptive mesodermal) cells. Dorsal marginal zone cells initiate gastrulation movements first, forming the dorsal lip (also known as the "Spemann Organizer"), and contribute directly to the dorsal axial mesoderm (e.g., notochord and muscle). We have observed that mRNA from hyperdorsalized (lithium ion treated) gastrula stage Xenopus embryos has the ability to rescue dorsal axis formation when injected into dorsal axis deficient (UV treated) Xenopus embryos. To identify mRNAs responsible for this activity, a plasmid cDNA library of approximately 100,000 independent clones was constructed from size-fractionated stage 11 lithium-treated Xenopus embryo mRNA. Sense RNA transcripts synthesized from the library using SP6 RNA polymerase contained the axis rescuing activity when assayed by injection into axis deficient embryos. Single clones with axis rescuing activity were isolated by injecting transcipts from progressively smaller pools of clones. Two clones have been isolated by this process, the wnt family member Xwnt-8, and a second clone which encodes a previously uncharacterized protein. This predicted protein has a calculated molecular weight of approximately 26,000 Da and a contains strongly hydrophobic amino-terminal sequence, suggesting it is secreted. RNA transcripts from this second clone have the ability to both rescue complete dorsal axis formation when injected into UV treated embryos and, at higher doses, to result in hyperdorsalized embryos. The activity of this clone in embryo injection experiments is thus similar to that of wnt family members, although no sequence homology is apparent between this new cDNA and any known growth factors, including the wnt family members. In northern blot analysis this cDNA hybridizes to two mRNAs of about 1.8 and 1.4 kb which are present at low levels in oocytes and eggs. These two mRNAs are upregulated in late-blastula and early gastrula stage embryos, reaching peak levels at stages 10-11. This upregulation is stronger in LiCl treated embryos and essentially absent in UV treated embryos. The role of this protein in dorsal mesoderm induction, and the relationship between it and other putative mesoderm inducers, including the FGFs and activins, remain to be determined.

W 312 EXPRESSION OF LEUKEMIA INHIBITORY FACTOR (LIF) IN THE UTERUS COINCIDES WITH THE ONSET OF IMPLANTATION OF THE BLASTOCYST, Colin L. Stewart, Susan Abbondanzo, Harshida Bhatt, Lisa Brunet, Petr Kaspar* and Frank Koentgen,** Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110. *Institute of Molecular Genetics, Prague, Czechoslovakia, **F. Hoffman-La Roche Ltd., Basle, Switzerland. LIF is a cytokine that has multiple functions, among which is the inhibition of differentiation of mouse embryonic stem (ES) cells. To gain further insights into its function *in vivo*, we analyzed its expression in mice. The principal site of expression is in the uterus with a peak of expression occurring on the 4th day of pregnancy (day 1 = day of plug). This maximal expression of LIF occurs in the uterus at the time blastocysts are present. *In situ* analysis revealed that expression was confined to the endometrial glands. Subsequent studies showed that LIF expression is under maternal control since expression occurs in pseudopregnant with the same kinetics, and in mice undergoing delayed implantation there is a burst of LIF expression which always precedes implantation of its blastocysts. These results suggest that one of LIF's principal functions *in vivo* is to regulate the growth of and to initiate implantation of the blastocyst. Attempts are in progress to manipulate LIF's expression *in vivo* in order to determine whether LIF is essential for preimplantation development and implantation of the mouse embryo.

W 313 EXPRESSION OF THE LIM CLASS HOMEOBOX GENE XLIM-1 IN EARLY XENOPUS EMBRYOS

Masanori Taira, Milan Jamrich, Peter J. Good, Hiroki Otani and Igor B. Dawid, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892

We isolated LIM domain-containing homeobox genes from Xenopus laevis, one of which is named Xlim-1. This gene is closely related to C. elegans genes, lin-11 and mec-3, in the LIM and homeobox domains. The zygotic Xlim-1 mRNA is first detectable at the early gastrula stage by Northern blot analysis. This Xlim-1 mRNA is mostly present in the dorsal half. In adult tissues the Xlim-1 gene is predominantly expressed in the brain and weakly in the eye and kidney. Whole-mount in situ hybridization showed that in gastrula embryos the Xlim-1 mRNA is localized in the dorsal lip and the dorsal mesoderm, i.e., in the region of Spemann's organizer. At the later stages the Xlim-1 mRNA was present in the notochord and the middle portion of the lateral plate. At the neural plate stage the staining of notochord decreased and a part of the neural plate started to be stained. At the neural tube stage the spinal cord was stained and the pronephros region was more pronounced in the lateral staining; Xlim-1 expression in this region precedes the morphogenesis of pronephros. At the early tadpole stages the midbrain and hindbrain as well as the spinal cord were stained and the pronephros was only stained in the lateral region. This expression pattern is consistent with the adult tissue distribution of the mRNA. We next examined the Xlim-1 expression in early development by using animal explants from blastula embryos. Xlim-1 mRNA was induced in animal explants by the mesoderm inducer activin A and by retinoic acid which is not mesoderm inducer but affect the patterning during Xenopus embryogenesis. Application of activin A and retinoic acid together resulted in synergistic induction. There have been no reports of gene activation by retinoic acid alone in Xenopus animal explants. Thus the Xlim-1 gene probably has a role in establishing body pattern during gastrulation and in morphogenesis of the central nervous system and kidney.

W 314 THE QUAIL HOMEOBOX GENE, QUOX 8, IS REGULATED BY DORSO-VENTRAL POLARITY AND TISSUE INTERACTIONS

Yoshiko Takahashi+ and Nicole Le Douarin, Institut d'Embryology du CNRS, Nogent, France. +:Present address, Institute of Neuroscience, University of Oregon, Oregon 97403

We have cloned a homolog of the mouse homeobox gene, Hox 8, from a quail cDNA library, and named it Quox 8. One of the early embryonic tissues expressing Quox 8 mRNA is the dorsal neural tube. This expression is later followed by coordinated expression in three different cell types; dorsal neural tube, its overlying superficial ectoderm and intervening mesodermal mesenchyme between them.

We have studied the developmental significance of this expression with respect to the establishment of dorso-ventral polarity in the nervous system and interactions with mesenchyme overlying the neural tube. The results are as follows: 1. Quox 8+ mesenchyme at the dorsal aspect of the neural tube formed dorsally located vertebral cartilage but not lateral cartilage. 2. Quox 8 expression was abolished in a dorso-ventrally inverted neural tube. In this case no expression was observed in dorsal mesenchyme and this resulted in a lack of dorsal cartilage. 3. An ectopically grafted neural tube induced Quox 8 expression in surrounding mesenchyme, which resulted in supernumerary cartilage.

These results suggest that the coordinated expression of Quox 8 in the dorsal portion of normal embryos is established in part by dorso-ventral polarity in the nervous system and also by tissue interactions. In addition the Quox 8 gene product seems to be involved in dorsal body patterning.

W 315 EXPRESSION OF THE FIBROBLAST GROWTH FACTOR RECEPTOR FGFR-1/flg DURING GASTRULATION AND SEGMENTATION IN THE MOUSE EMBRYO.

Terry P. Yamaguchi*, Ronald A. Conlon, and Janet Rossant*. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada, M5G 1X5. *Department of Molecular and Medical Genetics, University of Toronto.

Recent genetic evidence has demonstrated that growth factors and their receptor tyrosine kinases (RTKs) play essential roles in regulating both vertebrate and invertebrate development. We have used PCR to screen embryonic stem cells for RTKs that are expressed in a tissue or temporally-specific manner during early mouse embryogenesis. 125 clones consisting of both novel as well as previously-identified tyrosine kinases have been sequenced to date. The novel RTKs belong to the insulin, *ret*, *kit*, and FGF receptor families. We have used both standard and whole-mount *in situ* hybridization techniques to analyze the temporal and spatial expression patterns of RTKs isolated in this screen. We have concentrated on FGFR-1 expression in order to help define the role of FGFs in the processes of gastrulation and segmentation. FGFR-1 transcripts were expressed in the embryonic ectoderm during gastrulation as well as in the newly-formed embryonic, but not extraembryonic, mesoderm. Within the ectodermal lineage, FGFR-1 mRNA became localized to the neural ectoderm during its formation and continued to be expressed at high levels throughout neural development. In the mesodermal lineage, FGFR-1 transcripts became concentrated in the medial mesoderm of the embryo as it condensed to form paraxial mesoderm. The most striking expression patterns were observed before and during segmentation when FGFR-1 was strongly expressed in the presomitic mesoderm and the rostral half of the newly formed somites. Expression was subsequently detected in both sclerotome and dermatome. The patterns of expression are consistent with a role for FGFR-1 in the formation of neural ectoderm and posterior mesoderm as well as in the early events that establish compartments within the developing somites (supported by NCIC and Bristol-Myers/Squibb).

W 316 EXPRESSION OF ACTIVIN RECEPTORS IN THE CHICK HENSEN'S NODE

R.T. Yu, K. Umesono, A. Kakizuka, C. Kintner*, C. Stern+, L. Mathews**, W. Vale**, and R.M. Evans

Gene Expression Lab, *Molecular Neurobiology Lab, **Peptide Biology Lab, The Salk Institute, La Jolla, CA 92186-5800
+Dept. of Human Anatomy, University of Oxford, UK

Peptide growth factors serve as important biological signals to control important events which occur during vertebrate development such as gastrulation, establishment of the anteriorposterior axis, and limb development. Among them, activin is interesting because of its potent mesoderm-inducing activity in amphibian and avian embryos. A question is addressed as to whether receptor proteins are present in the Hensen's node where the activin has been implicated as playing a major role in inducing mesoderm in the chick embryo. As an initial step in understanding the action of activin during the chick embryonic development, we attempted to characterize expression pattern of the activin receptor (ActR) gene. Accordingly we prepared a Hensen's node cDNA library from stage 4 chick embryo. Screening of the library with a mouse ActR cDNA as a probe identified multiple independent chick cDNA clones. Structural analyses of these clones revealed that at least two distinct genes, which we named ActR-A and ActR-B, are expressed in Hensen's node. Genomic southern blot analyses confirm that these two ActR genes are conserved in Xenopus, mouse, and human. Using a COS cell transfection system, we expressed the two ActR gene products and were able to detect specific binding to ^{125}I-activin A with the same affinity. Whole mount in situ hybridizations are being done to describe the distribution patterns of the two receptors at different stages during chick development where activin may play a major role. Functional assays are also underway to further characterize these two activin receptors and to clarify the distinct roles that each receptor may play during embryogenesis.

Cell-Cell and Cell-Matrix Interactions

W 400 THE ROLE OF METALLOPROTEINASES IN HEART DEVELOPMENT, Thomas K. Borg, Masao Nakagawa, Kristofer Rubin and Louis Terracio, University of Uppsala, Uppsala, Sweden and University of South Carolina, Columbia,SC 29208

Growth factors (PDGF, TGF), including cytokines (IL-1), are expressed at critical stages during the development of the heart. These growth factors are believe to provide important signals that initiate the morphogentic events of cardiogenesis. The dramatic remodeling during cardiogenesis includes the modification of the components of the extracellular matrix (ECM) and the growth factors are thought to influence this process by regulation of metalloproteinases (MMP). Immunohistochemical localization of the 72 kD MMP and IL-1 shows a peak expression by 11.5 days of embryonic development which correlates with the extensive ECM modification associated with valve formation, trabeculation and septation. In vitro gel contraction assays with fibroblast from these stages indicates that MMP expression is affected by PDGF, TGF and IL-1 as well as by mechanical tension. Data from these assays show that mechanical tension alone could cause the activation of the latent 72 kD MMP to the 57 kD active form. The mechanical tension in the collagen gel is analagous to that tension involved in cardiac remodeling during embryogenesis. Although circumstantial, these data support the hypothesis that growth factors/cytokines are important regulators of ECM remodeling during heart development. Supported in part by HL- 37669 and HL-42249.

W 401 ALTERED EXPRESSION OF CELLULAR ADHESION-RELATED PROTEINS RESULTING FROM EXPRESSION OF WNT-1 IN PC12 CELLS, 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 Wnt-1 is required for normal fetal brain development in the mouse while its Drosophila homolog, wingless, plays an essential role in pattern formation within the fly embryo. We have previously shown that Wnt-1 encodes a secreted protein associated with extracellular matrix and which is strongly implicated in cell-cell communication. Phenotypic effects of Wnt-1 on cultured cells are limited to very few cell lines, one of which is the pheochromocytoma line PC12 (G.Shackleford and H.Varmus, in preparation). We have been characterizing changes in cellular adhesion resulting from expression of Wnt-1 in these and other cell lines. PC12 cells expressing Wnt-1 display increased cellular adhesion and become epithelioid in appearance. By immunoblot analysis we find increased expression of E-cadherin in these cells, with a concomitant decrease in N-CAM levels. In addition, Wnt-1/PC12 cells show elevated expression of plakoglobin (a protein associated with intercellular adhesive junctions), and increased membrane localization of this protein. Plakoglobin is homologous to the Drosophila protein armadillo, whose expression is known to be modulated by the action of wingless in Drosophila embryos. Our data suggest possible evolutionary conservation of elements in the wingless signaling pathway and raise the possibility that in some cell types Wnt-1 may exert its effects by modulating intercellular adhesion.

W 402 EXPRESSION OF N-CADHERIN IN XENOPUS EMBRYOGENESIS. Florence Broders, Lionel Simonneau, Giovanni Levi, Jean Pierre Saint Jeannet and Jean Paul Thiery, Laboratory of Physiopathology of Development CNRS Ecole Normale Superieure Paris France

Cadherins are calcium-dependent cell adhesion molecules which play a key role in morphogenesis and histogenesis (Takeichi, 1991; Science, 251: 1451). In *Xenopus*, several cadherins have recently been characterized. At least three of these, E/P-cadherin (Ginsberg et al., 1991; Development, 111: 315), XB-cadherin (Wedlich et al., 1991; Mechanisms of Development, 35: 33) and U-cadherin (Angres et al.,1991; Development ,111: 829) are already expressed before the midblastula transition, and can be detected in the egg and in all blastomeres. Further during development, these cadherins become restricted to some differentiating tissues (e.g. E/P-cadherin persists in muscle and epidermis) while a new group of cadherin genes start to be activated in cells already engaged in a differentiation pathway. The exact number and mode of regulation of *Xenopus* cadherins is not yet known and we are now attempting to isolate novel cadherins. We have already established the pattern of distribution of two "late" cadherins : E-cadherin which appears only during gastrulation in non-induced ectoderm (Levi et al., 1991; Development, 111: 159) and N-cadherin. The pattern of N-cadherin expression was determined at the mRNA level by *in situ* hybridization with a large riboprobe to the EC5 domain of *Xenopus* N-cadherin. This part of the sequence is the least conserved in the cadherin gene family minimizing the risk of cross-hybridization to other cadherins. High levels of N-cadherin are expressed in the central nervous system since its first differentiation at neural fold stage up to stage 50, after which the expression of the molecule declines rapidly. The expression of the molecule does not however appear to be uniform throughout the CNS; at stage 26, for example, discrete zones of high expression are present in the rhombencephalon. The lens placode as well as all other neurogenic placodes also express high levels of N-cadherin mRNA. Endodermal and non-neural ectodermal cells are always negative. Mesodermal derivatives express variable amounts of N-cadherin mRNA. High levels of transcripts are present in the notochord. The somites are stained weakly up to tailbud stage 40 when low levels of N-cadherin mRNA can be clearly detected in myotube-forming cells and in the apical region of the limb bud. At stage 26, the heart rudiment is particularly strongly labelled; a very intense expression is maintained throughout heart development and in the functional organ.

W 403 Possible Regulatory Functions of $TGF\beta_{1,2}$ in Mandibular Morphogenesis and Tooth Bud Formation. Y. Chai*, C. Crohin, S. Groff, Y. Sakakura, P. Bringas, Jr., V. Santos and H.C. Slavkin (Center for Craniofacial Molecular Biology School of Dentistry, University of Southern California, Los Angeles, CA 90033).

A number of studies indicated that multiple regulatory molecules are implicated in craniofacial morphogenesis. Growth factors sharing homology with members of the TGFß family may mediate epithelial-derived instructions for ectomesenchyme allocation, determination and differentiation to become chondrogenic and/or osteoprogenitor cells. This study was designed to investigate the functions of $TGF\beta_{1,2}$ in regulating mandibular morphogenesis and tooth formation during early embryonic mouse development. Swiss-Webster mouse embryos obtained from E8-E11 were used to verify the presence of TGFß transcripts employing reverse transcription-polymerase chain reaction (RT-PCR). A serumless, chemically-defined medium was used to culture E10 (42-44 somite pairs) mandibles. Antisense strategy was used to inhibit $TGF\beta_{1,2}$ translation. $TGF\beta_1$ transcripts were identified in E8, E9, E10, and E11 mandibular processes. The simple culture model resulted in the formation of Meckel's cartilage demonstrated using whole mount alcian blue staining. $TGF\beta_1$ antisense treatment (30µM) resulted in a significant decrease ($p < 0.05$) in chondrocyte cell number per 1000 μm^2 in Meckel's cartilage. Preliminary data showed $TGF\beta_2$ antisense treatment resulted in the significant enlargement of dental tooth bud within the cultured mandibles. We interpret these results to indicate that endogenous $TGF\beta_1$ may function to regulate the formation of Meckel's cartilage, and that $TGF\beta_2$ may regulate the size of dental tooth organs during tooth formation.

This study was supported by Center Grant P50 DE09165, NIDR, NIH, USPHS.

W 404 FUNCTIONAL ANALYSIS OF THE CHICK **limb deformity** GENE, José Luis de la Pompa, Andreas Trumpp, Patricia A. Blundell, Dominic James and Rolf Zeller. EMBL Differentiation Programme, Meyerhoftrasse 1, 6900 Heidelberg, FRG.

Mutations in the recently isolated mouse limb deformity (**ld**) gene alter morphogenesis of limbs and kidneys.

Molecular analysis of the chick **ld** homolog reveals a high degree of evolutionary conservation. Western blot analysis shows a 180 kd protein expressed in chick embryos and specific adult tissues. Immunofluorescence studies reveal that **ld** proteins are nuclear, showing a characteristic punctate pattern. In the developing chick limb bud, **ld** proteins are expressed in the newly differentiated AER and the mesenchymal compartment, where an unequal distribution along the antero/posterior and later on the dorso/ventral axes is observed. During early kidney morphogenesis, expression is initially restricted to the epithelial compartment of the pro- and mesonephros.

The function of the **ld** gene in pattern formation is being analyzed at two levels: Biochemical analysis suggest that the properties of the **ld** proteins, are compatible with those of DNA-binding proteins. The developmental role of the **ld** gene will be studied using a retroviral expression system that we have established for the chick embryo. Our aim is to introduce and express specific **ld** transcripts in defined positions in the developing chick embryo, and examine the developmental consequences of such ectopic expression.

W 405 SUPPRESSION OF COLLAGENASE TYPE I EXPRESSION IN PROGERIOD FIBROBLASTS

R.A. Grymes*, J. Altman, J. Chan, and E.A. Bauer, Department of Dermatology, Stanford University School of Medicine, Stanford, CA 95405; *Life Science Division, NASA-Ames Research Center, Moffett Field, CA 94035

Cellular processes relevant to aging and senescence have been examined in the model of Werner's syndrome (WS), an autosomal recessive progeriod syndrome exhibiting changes in skin and connective tissue *in vivo*. WS dermal fibroblasts over-produce collagenase I and are insensitive to the normal collagenase induction response and mitogenic effects of platelet-derived growth factor (PDGF). Through study of the induction and suppression of collagenase I, the basis of this dysregulation has been explored. Functional assays have failed to localize the WS mutation to collagenase I gene control elements. This report describes the effective utilization of the hydrocortisone (HC) suppression pathway, presumably mediated by the glucocorticoid receptor element (GRE). Cell strains derived from Werner's syndrome patients, a heterozygotic relative, and an aged control subject were studied. Suppression of collagenase expression was examined, as were the antagonistic inductive effects of PDGF and tetradecanoyl phorbol ester (TPA). HC suppressed collagenase I (by indirect ELISA) to 8-21% of control values in WS cells, while the heterozygote and aged control continued to secrete 42-44% of normal levels. Immunoreactive collagenase was induced an additional 73% (PDGF) and 54% (TPA) in the control cells. Less significant reponses to both PDGF and TPA were detected in the heterozygote (24% and 11%) and in one WS strain (30% and 8%), and TPA stimulated another WS strain (32%). PDGF and TPA enhanced enzyme synthesis following release from HC suppression in the control and the single marginally responsive WS strain. PDGF showed additive suppression (18-52%) in the other WS strains and the WS heterozygote. Thus, both the suppressive (GRE) control pathway and the previously studied inductive (AP-1) pathway demonstrate some unique responses in Werner's syndrome dermal fibroblasts, as measured by collagenase gene expression.

W 406 CELLULAR SPREADING ON FIBRONECTIN ISOFORMS,

Laurie B. Joseph and Joseph V. Lombardi, Department of Orthopaedic Surgery, Thomas Jefferson University, Philadelphia, PA 19107

Extracellular matrix components, such as fibronectin, appear to play a crucial role in the maintenance of cell function during a variety of events including wound healing. Plasma fibronectin (pFn) is important in the initial stabilization of the wound, while cellular fibronectin (cFn) appears to affect later events and differs from pFn as a result of alternative splicing of the transcript. We have utilized ethyl methyl sulfonate to develop a series of CHO cell mutants, which adhere to pFn but have impaired spreading properties, as compared to the wildtype cells (J. Cell Sci. 1990, 96 519-526). Using morphometric analysis, the degree of cellular spreading on pFn differs between wildtype and mutant cells ($p<0.05$). But, when the mutant and wildtype cells were plated on alternatively spliced cFn no differences in the degree of cellular spreading were apparent ($p<0.05$). Both cell types, exhibit a rounded more motile morphology, when visualized by SEM and rhodamine-linked phalloidin, as compared to the cells plated on the pFn. PKH2-GL, a highly hydrophilic molecule which embeds itself within the lipid bilayer, had a patchy distribution in both the wildtype and mutant cells plated on cFn as compared to a normal well dispersed presentation within the membrane of the wildtype cells plated on pFn. Furthermore, the fibronectin receptor showed a clumped distribution in both the wildtype and mutant cells when plated on cFn, as compared to the wildtype cells needle-like distribution of the receptor when plated on pFn. We suggest that the wildtype cells may interact with pFn in a different manner than cFn. Differences in the spreading patterns of the wildtype cells on cFn may be due to changes in the conformation of the cell binding domain due to alternative splicing of the transcript.

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W 407 ANTISENSE bFGF OLIGODEOXYNUCLEOTIDE INHIBITS CARDIOCYTE PROLIFERATION AND FUNCTION IN DIFFERENTIATING PRECARDIAC SPLANCHNIC MESODERM, John Lough and Yukiko Sugi, Department of Cell Biology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

This laboratory recently reported the occurrence of extensive deposits of bFGF-like proteins in the chicken myocardium, beginning at the onset of heart development (stage 9+; *Dev. Biol.* 146:139; 1991). Recent evidence using FGF isoform-specific antibodies suggests that these deposits contain aFGF and FGFK, in addition to bFGF. We have begun to assess the role of these growth factors in cardiogenesis by treating explanted stage 5/6 precardiac splanchnic mesoderm with antisense oligodeoxynucleotide (ODN) that is complementary to a sequence of 15 nucleotides spanning the exon1/exon2 boundary of chicken bFGF mRNA. Within 4 days, the multilayer of cells that normally develops into rhythmically contractile heart tissue displayed a 50% decline in cell cycle transit, as determined by bromodeoxyuridine incorporation, as well as loss of contractile function. Treatment with sense ODN or antisense ODN plus bFGF protein did not affect cardiogenesis in the explant. Similar determinations are in progress to assess the effects of antisense aFGF ODN on cardiogenesis; also, the effects of ODN treatment on the synthesis of FGF protein in the explants are being determined by immunoprecipitation analysis. Our findings to date suggest that bFGF is required for the normal proliferation and differentiation of cells in the developing heart. (Supported by NIH grant HL 39829)

W 408 A TARGETED MUTATION REVEALS A ROLE FOR N-*MYC* IN BRANCHING MORPHOGENESIS IN THE MOUSE LUNG, Cecilia Bernelot Moens, Alexandra Joyner, Anna Auerbach, Ronald Conlon, and Janet Rossant, Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute at Mt. Sinai Hospital, 600 University Ave., Toronto, Ont. CANADA, M5G 1X5, and the Department of Medical Genetics, University of Toronto. The N-*myc* proto-oncogene encodes a putative transcription factor which has been postulated to be involved in the control of differentiation in a number of lineages at various stages during mammalian embryogenesis. We have generated a leaky mutation in N-*myc* by gene targeting in embryonic stem cells. Mice homozygous for this mutation, called N-myc^{9a}, die immediately after birth due to an inability to oxygenate their blood, a phenotype which results from a defect in the epithelial-mesenchymal interactions that are the basis of lung morphogenesis. Analysis of N-*myc* expression in wild-type and homozygous mutant embryonic lungs suggests that N-*myc* is required for the proliferation of the lung epithelium in response to local inductive signals emanating from the lung mesenchyme. Homozygous mutant embryos are smaller than normal and also have a defect in the development of the spleen, but other tissues that normally express N-*myc* appear to be unaffected by the N-myc^{9a} mutation. These tissues express the normal N-*myc* transcript at higher levels than do the lungs. These results illustrate one way in which gene targeting can be used to generate partial loss of function mutations, and support the importance of generating a series of alleles at a given locus in order to elucidate the various different functions of the gene during development.

We are also studying the phenotype of embryos that are heterozygous for both N-myc^{9d} and a null mutation in N-*myc* which was generated in the lab of Dr. L. Parada. These embryos are more severely affected than N-$myc^{9a/9a}$ homozygous embryos, and we hope that they will elucidate earlier functions for N-*myc* in mouse development.

Funded by the National Cancer Institute of Canada.

W 409 SPLICING OF NEURAL CELL ADHESION MOLECULE (NCAM) IN HUMAN TUMOURS, Kalpana Patel, Elizabeth Phimister, Rosalind Rossell, Anne Culverwell and John T. Kemshead, The Imperial Cancer Research Fund, Paediatric & Neuro-Oncology Group, Frenchay Hospital, Bristol, Avon, BS16 1LE, UK

The behaviour of tumour cells is likely to be influenced by various molecules expressed on its cell surface. Amongst these, cell adhesion molecules are prime candidates whose absence/presence may have important consequences. The expression of the adhesion molecule, NCAM, has been investigated in human tumour cell lines. By Western blotting, neuroblastoma (NB), rhabdomyosarcoma and Small Cell Lung Carcinoma (SCLC) lines were found to express highly polysiaylated NCAMs. Removal of the polysialic acid indicated that all cell lines expressed the 140 and 120 kDa isoforms. In addition, some NB lines expressed the 180 kDa isoform while some SCLC lines also expressed a poorly characterised band of 95 kDa which was recognised by an anti-NCAM monoclonal antibody.

Further detailed characterisation by PCR and DNA sequencing revealed that all lines tested expressed NCAM which either lacked or contained the VASE mini-exon. The splicing of the mini-exon, MSD-1, proved to be complex. Southern blotting of PCR products, using mini-exon-specific oligonucleotides, suggest only MSD-1a and MSD-1b were present in NB and SCLC lines, whereas the whole of the MSD-1 was present in rhabdomyosarcoma lines.

Further studies will determine the contribution made by NCAM in tumour cell adhesion and the influence of polysialic acid on NCAM interaction and interaction between other adhesion molecules and their counterpart ligands.

W 410 **INDUCTION OF VASCULAR SMOOTH MUSCLE FROM NEURAL CREST IN VITRO.** Claire L. Raeuber & Thomas Rosenquist, Dep't. Cell Biology & Anatomy, Medical Coll. of Georgia, Augusta, GA 30912-2000. Proliferation of vascular smooth muscle cells [VSMC] is the basis of atherosclerosis [AS]. VSMC in the vessels that are most resistant to AS (eg. aortic root) share a unique lineage, derived from neural crest cells[NC/VSMC]. NC cells express the VSMC phenotype significantly later in embryonic development than *mesenchyme*-derived VSMC of the great vessels.Regional variation in AS expression thus may be based in VSMC heterogeneity; however there is little data on the regulation of VSMC differentiation, especially the NC/VSMC program. To test the hypothesis that NC/VSMC is regulated by extracellular matrix [ECM] factors and cytokines, rhombencephalic NC explants from stage 8-9 chicken embryos were grown on substrates of fibronectin [FN], laminin, chondroitin-6-SO_4,tenascin, collagens, and combinations of these, in 1% fetal bovine serum[FBS].Immunofluorescence of VSM alpha actin showed expression of the VSMC phenotype on all matrices that included FN,which was necessary and sufficient for NC migration, differentiation, and growth. Thus, NC was grown on a substrate of FN alone in serumless medium with cytokines known to be VSMC-effective: bFGF; TGF-b1 or 2; PDGF-AA, AB or BB. bFGF proved most effective. TGF-b1 or 2 and PDGF-AA did not support NC cell migration, proliferation, growth or differentiation. PDGF-BB and AB were intermediate. These results indicate that FN is necessary for proper cell attachment that leads to cytokine responsiveness; and that bFGF may play a major role in the differentiation of NC/VSMC. bFGF has been shown by others to be present in the embryonic outflow tract at a time that is appropriate for the regulation of the NC/VSMC differentiation program.
(Supported by: NIH HL-36059, HL-42164, HL-45337; and by the American Heart Association, GA Affil.)

W 411 IDENTIFICATION OF PROTEINS THAT MEDIATE AN EPITHELIAL-MESENCHYMAL TRANSFORMATION IN HEART DEVELOPMENT, Mehrdad Rezaee, Keitaro Isokawa, Edward Krug, and Roger Markwald, Department of Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226.
Embryonic cardiac endothelium must undergo a regionally restricted epithelial-mesenchymal transformation to generate the anlagen of valvular and septal tissues. Previous studies indicate the transformation of cardiac endothelium into mesenchyme is dependent upon a developmentally regulated signal expressed by its associated myocardium. This bioactivity can be extracted from the embryonic myocardial basement membrane (MBM) with EDTA and partially purified (8-10 proteins) by ultracentrifugation. Polyclonal antibodies (ES Abs) generated against this biologically active preparation have been used to show that ES antigens have a temporally relevant distribution in the MBM of the developing heart, as well as at other sites of epithelial-mesenchymal transformation, e.g. limb bud and neural crest. In addition, ES Abs reversibly block the transformation of endothelium into mesenchyme in culture. Culture assays indicate that this event is not elicited by basic or acidic FGF, TGF-beta, EGF, or fibronectin. Hence, we hypothesize that ES antigens are novel constituents within the MBM that elicit the formation or cardiac mesenchyme. To identify individual ES antigens and assess their function we have used ES Abs: 1) to screen an embryonic heart expression library, and 2) for immunoaffinity purification, in conjunction with relevant bioassays. Our library screening has yielded an interesting clone (r2.1a - 2700 bp) whose cDNA sequence exhibits no significant homology to those in data banks. Antibodies generated against r2.1a fusion protein recognize a 130 kDa protein. Using PCR, in situ hybridization, and immunohistochemistry, it was demonstrated that both message and protein for r2.1a are present in the developing chick heart prior to and during the formation of mesenchyme. Most significantly, antisense oligonucleotides to r2.1a block the epithelial-mesenchymal transformation in culture. Additional clones are in the process of being characterized similarly. In parallel studies we have immunopurified four ES proteins (27, 44, 63, and 70 kDa): N-terminal sequencing and co-migration upon SDS PAGE (+/- DTT) has identified the 70 kDa as transferrin. Although this protein has been shown by others to have myotrophic activity for skeletal muscle, its function in cardiogenesis remains unexplored. Experiments are in progress for determining its role in the epithelial-mesenchymal transformation of cardiac endothelium.
Supported by NIH HL44928, HL33756, HD20743 and Wisconsin AHA 90-FA-15.

W 412 ROLE OF RECEPTOR-PROTEIN TYROSINE KINASES DURING HEART DEVELOPMENT.
Joseph C. Ruiz and Elizabeth J. Robertson, Department of Genetics and Development, Columbia University, New York, NY 10032

In D. melanogaster and C. elegans receptor-protein tyrosine kinases (R-PTKs) play key roles during embryogenesis (e.g., cell fate specification). The analysis of heart formation in the mouse provides a unique model system to examine the role of R-PTKs during mammalian embryogenesis. The primitive heart is formed between 7.5 and 8.5 days post coitum (dpc). At 8.5 dpc, the heart consists of two mesodermal-derived cell types, myocardial and endocardial cells, organized as two concentric tubes. We have used the reverse transcriptase-polymerase chain reaction (RT-PCR) to amplify PTK specific products from cDNAs obtained from hearts dissected from 8.5 dpc mouse embryos. Sequence analysis of the PCR products has lead to the identification of 23 PTKs; a number of these kinases encode novel R-PTKs. In order to determine which of these R-PTKs play a role in heart development, the temporal and spatial expression pattern of these novel kinases during early mouse embryogensis was examined.

W 413 TRANSFORMING GROWTH FACTOR-α PRODUCED BY METANEPHROS IS REQUIRED FOR ORGANOGENESIS IN VITRO. Gabriella Ryan, Sharon A. Rogers and Marc R. Hammerman, Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110. The role of transforming growth factor-α (TGF-α) in metanephric development was examined. Metanephroi were removed from 13-day rat embryos. Total RNA was isolated from metanephroi and reverse transcribed. The mRNA for TGF-α was detected using the polymerase chain reaction, and confirmed by sequencing. Metanephroi were grown in organ culture for up to 6 days under serum-free conditions. Immunoreactive TGF-α was produced by metanephroi in vitro and released into the culture medium. Levels of TGF-α were relatively constant during 4 days in culture and averaged 10^{-10} M. During this time, metanphroi increased in size and morphologic complexity as evidenced by increased tubulogenesis. Upon addition of anti-TGF-α antibodies to the medium, growth of the anlage, arborization of the ureteric bud and tubulogenesis of the metanephric blastema were inhibited. These data demonstrate production of TGF-α by developing rat metanephroi in culture and suggest a necessary role for the peptide for growth and development in vitro.

W 414 REGULATION OF TYPE I COLLAGEN GENE EXPRESSION BY ras. James L. Slack, Victoria R. Robinson, and Paul Bornstein, Departments of Biochemistry and Medicine, University of Washington, Seattle, WA 98195. In this study, we have investigated the role of the ras oncogene in regulating expression of the genes encoding type I collagen. In Rat 1 fibroblasts containing an oncogenic ras gene driven by the dexamethasone-inducible MMTV-LTR, levels of α1(I) mRNA began to decline within 8 h of dexamethasone addition and reached 2-10% of control levels after 96 h. Similar results were seen with the α2(I) gene, whereas there was no change in mRNA levels of ß-actin. In control cells not containing an inducible ras gene, dexamethasone had little or no effect on α1(I) or α2(I) mRNA levels. The effect of ras was reversible, as removal of dexamethasone resulted in a gradual re-accumulation of α1(I) mRNA to pretreatment levels. Overexpression of normal ras had no effect on α1(I) mRNA levels, suggesting that the normal ras p21 protein is not involved in regulation of collagen gene expression. Both the transcription rate of the α1(I) gene, and the half-life of the α1(I) transcript, were significantly reduced in ras-transformed compared to normal cells. However, transfection studies utilizing a number of different constructs containing α1(I) 5'-flanking and intronic sequences demonstrated essentially equivalent promoter activity in both normal and ras-transformed cells. We conclude that ras affects α1(I) gene expression at both transcriptional and posttranscriptional levels, and that the effect on transcription is either mediated by sequences far removed from the basal promoter, or requires that the gene be in its native chromosomal context.

W 415 Regulation of First Branchial Arch Morphogenesis During Mandibular Development In Chemically-Defined Medium In Vitro. H.C. Slavkin, *L. Shum, **Z. Werb, Y. Sakakura, Y. Chai and P. Bringas. Center For Craniofacial Molecular Biology, School of Dentistry, Univer.South. Calif., Los Angeles, Calif. 90033, and *Ctr. Growth & Development, School of Dentistry and Lab. Radiobiol. & Environmental Health, School of Medicine, UCSF, San Francisco, Calif. 94143

A major question in skeletal morphogenesis is how temporal and positional instructions are translated into form. A simple model to pursue this question is early embryonic mouse (E10, 42-44 somite pairs) mandibular explants which produce mandibular morphogenesis including Meckel's cartilage, bone, tooth organs and tongue development within 9 days *in vitro* using serumless medium (Slavkin et al, *J Craniofac. Genet. & Develop. Biol.* 9:185-205, 1989). Under these experimental conditions EGF, TGF-alpha, RAR-gamma, and TGFbeta transcripts were identified using mRNA phenotyping (RT-PCR). Translation products were identified using immunohistochemical localizations. The present studies were designed to use antisense oligodeoxynucleotides to investigate endogenous regulatory molecular effects on mandibular morphogenesis. Whole mount staining was used to evaluate normal versus dysmorphology of Meckel's cartilage. Histological serial sections of replicate specimens from different treatment groups were used for morphometric analyses. Antisense treatment (30 uM) designed to inhibit the translation of selected putative regulatory molecules (i.e. EGF, RAR-gamma, TGFbetas) produced several types of dysmorphogenesis. Sense and antisense AMEL (amelogenin) oligodeoxynucleotides were used as controls. In recovery experiments, where appropriate, exogenous growth factors added to antisense treated groups produced normal-like mandibular morphogenesis. Results were interpreted to suggest that endogenous regulatory molecules participate in the control of the timing and position of tissue boundaries during first branchial arch morphogenesis.

W 416 TENASCIN: DISTRIBUTION, ORIGINS, AND REGULATION IN SITU AND IN VITRO, Richard P. Tucker, John A. Anstrom, Kristin K. Anstrom, James A. Hammarback, David Jenrath, Sharen E. McKay, Eleanor J. Mackie* and Yue Xu, Department of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27157-1010 and *Rheumatology Research Unit, Addenbrooke's Hospital, Cambridge, United Kingdom

Tenascin is a hexameric glycoprotein that is abundant in the developing central nervous system and along the pathways of neural crest cell migration. Using a cDNA probe generously provided by Dr. R. Chiquet-Ehrismann (Basel), we have localized tenascin mRNA in the developing chick by in situ hybridization. In the brain and spinal cord, tenascin transcripts are concentrated transiently in a subset of glia as they proliferate and migrate. Tenascin mRNA is also found in trunk and cranial neural crest cells both in situ and in vitro. Thus, the role of tenascin is not likely to be related to regulating the pathways of migrating cells, but may be related to promoting invasive cell behavior. The importance of this role is demonstrated by its phylogenetic conservation: we have identified a tenascin-like molecule on the surface of sea urchin primary mesenchyme cells. Tenascin is also present in mesenchyme underlying morphogenetically active epithelia. Using in situ hybridization, we have found that some epithelia are the source of mesenchymal tenascin, and that the expression of tenascin appears to "flip" from epithelia to mesenchyme over time. Finally, we have used cell lines representative of the major sources of tenascin, fibroblasts and glia, to study the effects of growth factors on tenascin expression in vitro. We have found that TGFβ1, bFGF, and PDGF all increase tenascin expression, and that the effects of bFGF and TGFβ1 are synergistic. Using western blot and northern blot analysis, we have found that different splice variants are expressed in cells treated with different factors, indicating that not only the amount but the form of tenascin present in the matrix is regulated by growth factors. This work was supported by grants from the NSF (BNS-9021124) and the North Carolina Biotechnology Center (ARIG 0402) to R.P.T.

W 417 A NOVEL ROLE FOR PLATELET-DERIVED GROWTH FACTOR IN ONCOGENESIS IS SUGGESTED BY A STIMULATORY EFFECT ON CONNECTIVE TISSUE STROMA FORMATION. Bengt Westermark[1], Karin Forsberg[1], Istvan Valyi-Nagy[2], Carl-Henrik Heldin[3] and Meenhard Herlyn[2], [1]Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden, [2]The Wistar Institute, Philadelphia, PA, [3]Ludwig Instutute for Cancer Research, S-751 23 Uppsala, Sweden

The ability of tumor cells to elicit a stroma reaction is well established and much attention has been focused on the vascular system in tumors. In comparison with the neovascular system, little attention has been paid to the connective tissue stroma component of malignant tumors. In analogy with what we know about the mechanism of tumor angiogenesis, one might assume that the development of a fibroblastic stroma constsitutes a host response to tumor-derived connective tissue growth factors. In the present study we asked the question whether PDGF-BB, a potent mitogen and chemoattractant for fibroblasts, is such a growth factor.

The minimal protein coding region of PDGF B-chain cDNA (0.7 kb) was cloned into the multiple cloning site of the pCDNA1/NEO vector, downstream of the CMV promoter and transfected into human WM 9 melanoma cells, that are devoid of endogenous PDGF production; mock transfected cells were used as controls. Northern blot analysis of mass selected cells using PDGF B-chain cDNA as a probe, showed a high expression of a 0.7 kb transcript in the PDGF-B transfected cells; there was no signal in the control cells. Synthesis and secretion of PDGF-BB was confirmed by immunoprecipitation and by determination of mitogenic activity in conditioned medium from PDGF-B transfected cells. For analysis of tumor growth in vivo, $2x10^6$ cells of each cell type were injected subcutaneously in the backs of 4-6 week old female Balb/c nu/nu mice, 5 animals per group. All mice developed tumors. Tumors from mock-transfected cells contained large necrotic areas; only few blood vessels, with narrow lumina could be observed. No connective tissue was present. Tumors from PDGF-B transfected cells contained nests of tumor cells divided by septa of connetive tissue. An abundance of blood vessels was observed in the connective tissue septa and also within the tumor cell nests. There was a complete absence of necrosis in these tumors.

The present results suggest that tumor-derived PDGF-BB is a potent mediator of connective tissue stroma formation. The connective tissue framework that is generated in response to PDGF-BB may form a solid support for newly formed vessels and thereby facilitate the formation of a functional vascular system in the tumor.

W 418 EFFECTS OF GROWTH FACTORS ON ORGANOTYPIC THYROID FOLLICLE REGENERATION IN COLLAGEN GEL CULTURE Kerstin Westermark[1], Mikael Nilsson[2] and Bengt Westermark[3], [1]Department of Internal Medicine and [3]Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden, [2]Department of Anatomy, University of Gothenburg, S-400 33 Gothenburg, Sweden.

The smallest anatomical unit of the thyroid gland is the follicle, which is built up as a sphere of thyrocytes surrounding a colloid lumen. In thyroid hyperplasia, such as seen in goitrous tissue, the number of follicles is increased. The mechanism by which the integrity of the gland is preserved during the hyperplastic process, has not been completely clarified. Using a collagen gel culture system of porcine thyroid follicles, we have previously shown that the addition of epidermal growth factor (EGF) leads to an increase in cell multiplication as well as cell migration. Cells migrate radially from the mother follicle, into the surrounding gel, where they form satellite follicles. This observation suggests that the formation of new follicles is the result of an active migration, and that this process is regulated by specific growth factors. In the present investigation, we have studied the effect of transforming growth factor-β (TGF-β) in this culture system.

Structurally and functionally intact thyroid follicles were obtained after infusion of a collagenase solution into the thyroid artery of porcine thyroid glands. Immediately after isolation, the follicles were embedded in collagen type I gels, prepared from rat tendon. After two hours of culture, EGF and TGF-β were added at various concentrations together with a single dose of ^{3}H-thymidine. EGF was found to stimulate follicle neogenesis and to increase the fraction of ^{3}H-thymidine labeled cells in a dose-dependent fashion; labeled cells were found both in mother and in satellite follicles, with a higher proportion in the latter. Cultures treated with TGF-β for 48 h showed no change in the level of ^{3}H-thymidine incorporation; only a small stimulatory effect on cell migration was found. When given simultaneously, TGF-β had no effect on EGF-stimulated cell migration but the proliferative response to EGF at 1 ng per ml was completely blocked by TGF-β. Moreover, the gross architecture of the follicles was markedly affected by TGF-β, and their epithelial lining was frequently disrupted. Our findings suggest that growth factors of the EGF family stimulate growth of the thyroid with perfect preservation of the structure. TGF-β is an inhibitor of the growth stimulatory activity of EGF, has no inhibitory effect on cell migration and negatively affects the structural integrity of the tissue.

Tissue Development: Epithelial-Angiogenesis, Bone and Cartilage - Myogenesis

W 500 TGFβ EXPRESSION AND FUNCTION DURING EPITHELIAL GROWTH, DIFFERENTIATION AND NEOPLASIA, R. J. Akhurst[1], A. Balmain[2], L. Duffie[1], D. R. FitzPatrick[1], D. J. Fowlis[1], S. Haddow[2], F.A. Millan[1]. [1]Duncan Guthrie Institute of Medical Research (University of Glasgow) G3 8SJ and [2]Beatson Institute for Cancer Research, G61 1BD, Glasgow, UK.

In vitro, TGFβs are potent negative growth regulators of all epithelial cell types, and can induce differentiation in a sub-set of these. Our objectives have been:

1) to examine whether TGFβs might be endogenous regulators of epithelial homeostasis

2) to assess how this regulation is perturbed during development of neoplasia

3) to examine the biological consequences of inappropriate epithelial expression of TGFβs *in vivo*

We have shown, by *in situ* hybridisation, that TGFβs are endogenously synthesised by epithelial cells *in vivo*. In adult mouse skin, the TGFβ1 gene is activated in differentiating keratinocytes when homeostasis is perturbed (either spontaneously or by chemical treatment)[1]. During mouse embryogenesis, all three TGFβ isoforms are expressed by subsets of epithelial cells. Expression of TGFβs 1 and 3 appear to be exclusively associated with morphogenetic activity involving epithelial/mesenchymal interactions. In contrast, TGFβ2 may play a role in maintanence of epithelial homeostasis in the fetus, since this gene is widely expressed in epithelia undergoing growth and differentiation[2,3].

During development of skin neoplasia, the negative growth response to TGFβ is lost. We have shown that resistance to TGFβ is acquired late in tumour progression, since kerationocytes from benign tumours show complete growth inhibition in response to TGFβ[4]. Immunolocalisation studies, in combination with *in situ* hybridisation, suggest that translational and/or post-translational mechanisms are of major importance in locally down-regulating TGFβ bioactivity within these benign skin tumours *in vivo*[5].

We are now using transgenic mouse technology to further our understanding of the involvement of TGFβs in maintenance of epithelial homeostasis. This work is funded by CRC, MRC and Wellcome Trust.

[1] Akhurst et al (1988) Nature 331: 363-365
[2] FitzPatrick et al (1990) Development 109: 585-595
[3] Millan et al (1991) Development 111: 131-144
[4] Haddow et al (1991) Oncogene 6: 1465-70
[5] Fowlis et al (1992) Cell Growth Diff. in press

W 501 EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR DURING EMBRYONIC ANGIOGENESIS AND ENDOTHELIAL CELL DIFFERENTIATION

Georg Breier, Ursula Albrecht, Sylvia Sterrer[1] and Werner Risau, Max-Planck-Institut für Psychiatrie, Am Klopferspitz 18A, 8033 Martinsried, Germany. [1]present address: Max-Planck-Institut für Biophysikalische Chemie, Am Fassberg, 3400 Göttingen, Germany

Vascular endothelial growth factor (VEGF) is a secreted angiogenic mitogen whose target cell specificity appears to be restricted to vascular endothelial cells. Such factors are likely candidates for regulatory molecules involved in endothelial growth control. We have characterized the murine VEGF gene and have analysed its expression pattern in embryogenesis, particularly during brain angiogenesis. Analysis of cDNA clones predicted the existence of three molecular forms of VEGF which differ in size due to heterogeneity at the carboxy terminus of the protein. The predicted mature proteins consist of 120, 164 or 188 amino acid residues. Homodimers of the two lower molecular weight forms, but not of the higher molecular weight form, were secreted by COS cells transfected with the corresponding cDNAs and were equally potent in stimulating the growth of endothelial cells. During brain development, VEGF transcript levels were abundant in the ventricular neuroectoderm of embryonic and postnatal brain when endothelial cells proliferate rapidly but were reduced in the adult when endothelial cell proliferation has ceased. The temporal and spatial expression of VEGF is consistent with the hypothesis that VEGF is synthesized and released by the ventricular neuroectoderm and may induce the ingrowth of capillaries from the perineural vascular plexus. In addition to the transient expression during brain development, a persistent expression of VEGF was observed in epithelial cells adjacent to fenestrated endothelium, e.g. in choroid plexus and in kidney glomeruli. The constitutive expression in these structures suggests that VEGF might be involved in the establishment or maintenance of fenestrated endothelium. The data are consistent with a role of VEGF as a multifunctional regulator of endothelial cell growth and differentiation.

W 502 **MOLECULAR CLONING OF BMP-8: A PROTEIN PRESENT IN BOVINE BONE WHICH IS HIGHLY RELATED TO THE BMP-5/6/7 SUBFAMILY OF OSTEOINDUCTIVE MOLECULES.** Anthony J. Celeste, R. Taylor, N. Yamaji, J. Wang, J. Ross and J. Wozney. Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140.

We have previously reported the identification of molecular clones encoding of a family of TGF-β-like proteins found in osteogenic extracts of bovine bone, BMP-2 through BMP-7. We describe here the molecular cloning of a protein we call BMP-8, a new member of the BMP family which is most closely related to the BMP-5/6/7 subfamily. BMP-related tryptic peptide sequences identified in bovine bone extracts were utilized to design degenerate oligonucleotide primers. These primers were employed to perform PCR on a bovine genomic DNA template, resulting in the identification of an amplified DNA product which encoded a portion of the BMP-8 protein. DNA sequence information derived from this PCR product has enabled the isolation of bovine and human BMP-8 genomic and cDNA clones.

W 503 THE EFFECTS OF FIBROBLAST GROWTH FACTOR ON DEVELOPING MOUSE HAIR, Diana L. du Cros, Department of Biological Structure, School of Medicine, University of Washington, Seattle, WA 98195

Acidic and basic fibroblast growth factors (aFGF and bFGF) appear to be involved in both proliferation and differentiation during skin development. In a study of the effects of these two growth factors on neonatal mouse skin, significant changes in hair follicle morphogenesis were noted. Newborn mice (B6C3-based strain) were given daily subcutaneous injections of either aFGF or bFGF and bovine serum albumin (BSA) at a dose rate of 1μg/g or 0.1μg/g body weight for 7 days. By day 7, areas of hairless unpigmented skin were observed surrounding the injection sites. This phenomenon persisted until about 14-16 days of age when the emergence of hair in the bald patches was first seen. Hair growth continued in the treated regions until they were covered about 4 days later. Hairs in these areas were significantly darker and appeared more dense than the surrounding body hair. Sites injected with BSA only were indistinguishable from the surrounding pigmented, hairy skin at all ages. Histological examination of skin from bFGF-treated mice at 23 days of age showed a dense dermis and a greatly thickened adipose layer with hair follicles extending deep into the adipose tissue; this is consistent with a stage of intense hair growth. Comparison with skin sections from control animals indicated that both follicle size and density had increased in the growth factor-treated mice. By 31 days, the skin in the FGF-affected regions appeared similar to that in surrounding areas, although the follicles remained larger and more numerous.

W 504 EPIDERMAL GROWTH FACTOR (EGF) STIMULATES PROLIFERATION BUT NOT DIFFERENTIATION OF EMBRYONIC PANCREATIC EPITHELIUM, George K. Gittes, MD., Philip E. Galante, William J. Rutter, PhD., and Haile T. Debas, MD., Department of Surgery and The Hormone Research Institute, University of California San Francisco, San Francisco, CA 94143-0660

The embryonic pancreas develops under the influence of the surrounding pancreatic mesenchyme and differentiates into both endocrine and exocrine pancreas. The pancreatic mesenchyme is thought to induce this differentiation through the release of a "mesenchymal factor". EGF is a potent cytokine in many developmental experimental systems. We hypothesized that EGF/TGFα may be the active agent in mesenchymal induction of embryonic pancreatic epithelia.

We isolated mouse embryonic pancreas epithelium from the surrounding mesenchyme using microdissection and trypsinization. Once isolated, we established the epithelium in organ culture suspended on a transparent Millipore filter at the air-media interface. Medium, with either 10% fetal calf serum or a serum-free defined additive, contained 0, 0.1, 1, 10, or 20nM EGF. As a positive control we added back the pancreatic mesenchyme in apposition to the isolated epithelium. After 10 days in culture, the area/volume index of tissue was measured and the tissues were fixed in paraformaldehyde, embedded in plastic, and assayed by immunohistochemistry for insulin and carboxypeptidase A.

We found that EGF stimulated a dose-dependent proliferation of epithelia, both in serum-containing media (7.6±5 for 20nM EGF, 1.5±2 for no EGF, mean area/volume index±SD) and in defined media (16.3±9 for 20nM EGF, 6.1±5 for no EGF). There was no evidence, however, in any of these conditions, of overt pancreatic differentiation as measured by staining for insulin and carboxypeptidase A.

W 505 STATES OF DETERMINATION OF MUSCLE PRECURSOR CELLS IN EARLY XENOPUS EMBRYOS STUDIED BY A SINGLE CELL TRANSPLANTATION TECHNIQUE

Kazuto Kato and J. B. Gurdon, Wellcome/CRC Institute of Cancer and Developmental Biology, University of Cambridge, Cambridge CB2 1QR, U.K.

We are studying the states of determination of muscle precursor cells in early Xenopus embryos using a single cell transplantation technique.

Single muscle precursor cells were isolated from various stages of embryos and transplanted into an ectopic position of host embryos. To distinguish transplanted cells from host cells, fluorescently-labeled dextran was used as a lineage marker. Host embryos were fixed when they became tadpoles and the fates of the transplanted cells were analysed using muscle specific antibodies.

Results obtained so far show that by the end of gastrulation many cells become determined as muscle while cells in the early gastrula are not yet determined. These results demonstrate that cell-cell interaction during gastrulation is important for muscle cell determination. We are now analysing further questions such as the nature of the interaction, the precise timing of determination and its correlation with the timing of MyoD activation and accumulation.

W 506 THE SIKVAV SITE OF LAMININ PROMOTES ANGIOGENESIS AND TUMOR GROWTH IN VIVO, Maura C. Kibbey, Derrick S. Grant, and Hynda K. Kleinman, Laboratory of Developmental Biology, National Institute of Dental Research, NIH, Bethesda MD 20892.

The formation of new blood vessels is critical in development, repair, and tumor growth. Endothelial cells normally contact basement membrane which is important for maintenance of vessel integrity and function. When synthetic peptides or polymers containing SIKVAV, a biologically active site from the laminin A chain, are premixed with Matrigel, a laminin-rich reconstituted basement membrane extract, and injected subcutaneously into mice, angiogenesis is stimulated in a dose-dependent manner. Two weeks post-injection was the peak period of angiogenesis. Control peptides containing a scrambled SIKVAV sequence were inactive. The Matrigel injected without peptide is relatively inert and does not contain significant numbers of endothelial cells until after two weeks in vivo. When SIKVAV-containing peptides are incubated with tumor cells, increased experimental metastases of melanoma cells to the lungs was observed, as well as increased collagenase IV activity and plasminogen activation (Sweeney et al., Cancer Metast. Rev. 10: 245, 1991). Since increased vascularization of tumors is correlated with tumor growth, we tested whether the SIKVAV peptide might also promote tumor growth through stimulation of tumor vascularization. When this peptide was premixed with B16F10 melanoma cells and Matrigel and injected subcutaneously, tumor growth was enhanced approximately 2.5-fold and vascularization was significantly increased over that observed with melanoma cells and Matrigel alone. These data suggest that the laminin-derived SIKVAV peptide is angiogenic in vivo and provide a new model for studying mechanisms of blood vessel formation and tumor vascularization.

W 507 CHARACTERIZATION OF A PRECHONDROGENIC AND A PATTERN-INDUCING CELL LINE FROM THE MOUSE LIMB BUD.

G. König, C. Shi, R. Anderson, B. Tonthat, M. Landry, and K. Muneoka. Department of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118

In order to obtain immortal cell lines which would express characteristics of mouse limb bud mesenchyme, Mouse Posterior Limb Bud cells were transfected with the pSV5-neo hybrid plasmid, which contains part of the polyoma virus genome. After G 418 selection, a number of immortal cell lines were obtained. Two of these cell lines were studied in more detail.

MPLB-1 is a fibroblastic cell line with a doubling rate of about 24 hr. When cultured at micromass density, MPLB-1 cells secrete an Alcian blue positive matrix and express alkaline phosphatase. In aggregates, these cells differentiate into histologically identifiably cartilage. Their chondrogenic potential, however, is not expressed at low cell density or in the internodular regions when co-cultured with dissociated limb bud cells in micromasses. These findings suggest that MPLB-1 is a prechodrogenic cell line whose differentiation into cartilage is regulated in a cell density-dependent manner.

MPLB-2 cells also secrete an Alcian blue positive matrix, but have a doubling time of roughly 2 days. When cells are aggregated and implanted into the anterior region of the chick wing bud, supernumerary digits are formed in about 50% of the cases. In this respect MPLB-2 implants mimic the effect of the zone of polarizing activity which is located in the posterior region of the limb bud and is hypothesized to produce a positional signal important for limb outgrowth. It thus appears that the MPLB-2 cell line has retained the positional signaling property of the cells they originated from, and therefore potentially represents a cell line which produces a morphogen relevant to limb development.

Supported by HD 23921 and a grant from the Monsanto Company.

W 508 FGF RECEPTOR REGULATION DURING DIFFERENTIATION OF MM14 MOUSE SKELETAL MUSCLE CELLS, Arthur J. Kudla,[1] Alan C. Rapraeger,[2] Alric López[1] and Bradley B. Olwin,[1] Departments of Biochemistry[1] and Pathology,[2] University of Wisconsin, Madison, WI 53706

Terminal differentiation of the MM14 mouse skeletal muscle cell line is repressed by members of the fibroblast growth factor (FGF) family. We have determined that acidic, basic or Kaposi FGF, but not keratinocyte growth factor or FGF-5, repress MM14 cell differentiation. Removal of FGF from proliferating cells results in an irreversible commitment to a post-mitotic phenotype, expression of muscle specific genes and fusion to form multinucleated myotubes. Because MM14 cells cease responding to FGF coincident with commitment to differentiation, we hypothesized that the causal event for MM14 commitment to differentiation is inactivation of FGF receptor(s). To address this hypothesis, we first determined which FGF receptors were expressed in MM14 cells. Polymerase chain reaction and Northern blot analyses indicated that syndecan, a heparan sulfate/chondroitin sulfate proteoglycan, and tyrosine kinase FGF receptor 1 (tkFR1), but not tkFR2, tkFR3 or tkFR4, were detectable in MM14 cells. We have previously demonstrated that FGF signaling is dependent on heparan sulfate and an additional cell suface FGF receptor, most likely tkFR1. To determine whether the loss of response to FGF correlates with a loss of FGF binding and a decline in FGF receptor levels, we examined the time course for loss of FGF response, FGF binding, tkFR1 and syndecan during differentiation. Loss of FGF binding correlated with loss of tkFR1 and syndecan mRNA, as well as tkFR1 protein. In contrast, syndecan protein was lost rapidly upon initiation of differentiation and did not parallel mRNA decline, suggesting post-transcriptional regulation of syndecan expression. Since the loss of MM14 response to FGF occurred prior to loss of FGF binding but after loss of syndecan protein, we are continuing experiments to determine whether the loss of syndecan protein plays a role in commitment of MM14 cells to terminal differentiation.

W 509 *IN VIVO* FOOTPRINTING OF A *XENOPUS* EPIDERMAL KERATIN GENE DURING DEVELOPMENT AND IN RESPONSE TO INDUCTIVE EVENTS, Peter H. Mathers and Thomas D. Sargent, Laboratory of Molecular Genetics, NICHD, National Institutes of Health, Bethesda, MD 20892.

The *Xenopus* keratin gene, XK81A1, is abundantly expressed in the epidermal cells of the developing embryo. During both mesodermal and neural inductions, cells fated to become epidermis are diverted towards new cell fates. This inductive influence results in repression of the XK81A1 promoter, a process mimicked by the treatment of animal cap ectoderm with growth factors such as activin. The XK81A1 gene is also repressed upon transition of the embryo to a swimming tadpole, some 2.5 days after fertilization. RNase protections were used to verify transcript levels in samples of various tissues and times during development. We have utilized PCR-mediated *in vivo* footprinting to analyze the protein:DNA interactions in these samples, identifying several elements that correlate with the active state of the promoter. Footprinting assays have looked at both dimethyl sulfate and copper phenanthroline protections in active and inactive epidermis, and non-expressing embryonic carcass, adult skin and adult blood. In addition, we are beginning to study the protein:DNA interactions in ectodermal animal cap cells treated with mesodermal and neural inducers, looking for induction-responsive elements within the promoter.

W 510 BOVINE BONE ACTIVIN ENHANCES BONE MORPHOGENETIC PROTEIN-INDUCED ECTOPIC BONE FORMATION, Yasushi Ogawa, David K. Schmidt, Ranga M. Nathan, Rosa M. Armstrong, Kathleen L. Miller, Steven J. Sawamura, Jill M. Ziman, Karen L. Erickson, Estelita R. de Leon, David M. Rosen, Saeid M. Seyedin, [1]Charles B. Glaser, [1]Ray-Jen Chang, [2]Anne Z. Corrigan, and [2]Wylie Vale, Celtrix Laboratories, Palo Alto, CA 94303, [1]Berlex Biosciences, South San Francisco, CA 94080, [2]The Salk Institute, La Jolla, CA 92037

A 25 kDa homodimeric protein was purified from demineralized bovine bone extract and identified as activin A by N-terminal sequencing and by cell culture assays. The yield of activin was 18-25 ug/kg bone powder. Activin enhanced formation of ectopic bone in rat subcutis when implanted in combination with partially purified bovine bone morphogenetic protein (BMP-2, BMP-3) in collagen/ceramic carrier. The implants, removed at 14 days, contained markedly elevated levels of alkaline phosphatase activity. Histological examination of the implants revealed an extensive formation of woven bone with very little cartilage. In contrast, combination of transforming growth factor-ß2 (TGF-ß2) and BMP promoted formation of bone with abundance of cartilage. The implants with BMP alone exhibited some osteoinductive activity, while the implants with activin alone showed no activity. These results demonstrate that bone is a rich source of activin and that activin plays an important role in modulating bone formation.

W 511 PlGF: A PLACENTA DERIVED ANGIOGENIC FACTOR , M. Graziella Persico, Valente Guerriero and Domenico Maglione, International Institute of Genetics and Biophysics (I.I.G.B.), CNR, 80125 Naples, Italy.

A human cDNA, coding for a protein related to the vascular permeability factor (VPF), was isolated from a term placenta cDNA library: we therefore named its product placenta growth factor (PlGF). PlGF is a 149-amino-acid-long protein and is highly homologous (53% identity) to the platelet-derived growth factor-like region of human VPF. By using N-glycosidase F, tunicamycin, and specific antibodies produced in both chicken and rabbit, we demonstrate that PlGF, derived from transfected COS-1 cells, is actually N-glycosylated and secreted into the medium. In addition, PlGF, like VPF, proves to be a dimeric protein. Finally, a conditioned medium from COS-1 cells containing PlGF is capable of stimulating specifically the growth of CPA, a line of endothelial cells, *in vitro* (Maglione et al 1991,PNAS 88: 9267-9271)

Furthermore,we have isolated, from a human choriocarcinoma cell line derived library, cDNA clones coding for a different form of PlGF. This new protein contains 20 additional amino acids, 50% Arg and Lys rich, inserted at the C-terminal. We are now characterizing the biochemical and functional properties of the new PlGF form.

This work was supported by a grant from the AIRC.

W 512 ANGIOGENESIS AND HEMANGIOMA, Werner Risau and Erwin Wagner*, Max-Planck-Institut für Psychiatrie, D-8033 Martinsried, FRG, and *Institute for Molecular Pathology, A-1030 Vienna, Austria

The cardiovascular system is laid down very early during embryonic development. Endothelial cells and blood cell precursor cells differentiate in mesodermal blood islands. The formation of blood vessels from in situ differentiating endothelial cells (vasculogenesis) and the sprouting from preexisting vessels (angiogenesis) contribute to the development of a functional vascular system. Proliferation of endothelial cells is a central phenomenon of both processes. We have analyzed the expression of genes encoding angiogenic growth factors (aFGF, bFGF, PD-ECGF, VEGF) using northern blot, PCR and in situ hybridization. The transient expression of VEGF in the brain neuroectoderm correlated with endothelial cell growth and angiogenesis during brain development.

The normal pattern of blood vessel development is dramatically altered by the expression of polyoma middle T oncogene in endothelial cells. Chimeric embryos derived from embryonic stem cells expressing polyoma middle T oncogen develop multiple lethal hemangiomas within the embryo and the yolk sac at mid-gestation. Middle T oncogen expressing endothelioma cells isolated from these embryos have a selective growth advantage in vitro and induced hemangiomas in syngeneic mice and in quail embryos in vivo. We recently found that hemangiomas can also be induced in c-src deficient mice suggesting that activation of c-src by the polyoma middle T oncogen is not responsible for the transformation of endothelial cells.

W 513 ISOLATION AND CHARACTERIZATION OF BMP-RESPONSIVE CARTILAGE AND BONE CELL PROGENITORS FROM MOUSE EMBRYO LIMB BUDS, Vicki Rosen, David McQuaid, R. Scott Thies, Leslie Kurtzberg, Joanna Capparella and John M. Wozney, Genetics Institute, Inc., 87 CambridgePark Drive, Cambridge, MA 02140-2387

Bone morphogenetic proteins (BMPs) induce bone formation in adult animals by affecting the differentiation of mesenchymal progenitors into cartilage and bone cells. The sequence of events that occurs after BMP implantation closely resembles that seen in endochondral bone formation during embryonic skeletal development. *In situ* hybridization studies using mouse embryo tissue indicate that BMPs are present in a spatial and temporal pattern consistent with their involvement in embryonic skeletal formation, suggesting that some of the target progenitor cells for BMPs may reside in the embryonic limb. To investigate this hypothesis, we immortalized limb bud cells from 9-13 dpc mouse embryos using v-myc, and tested the resulting clonal cell lines for their responsiveness to BMPs. Using this approach we have identified osteoprogenitor cell clones that, when treated with BMP-2, show increased alkaline phosphatase activity, develop a PTH-sensitive cAMP response and synthesize osteocalcin, all markers of the osteoblast phenotype. We have also identified separate populations of clonal cells that can be induced to a chondroblastic phenotype by BMP-2 as assessed by alcian blue staining of ECM, $^{35}SO_4$ incorporation into ECM, and production of mRNA for cartilage-specific proteoglycan core protein. In addition, a number of clonal cell lines appear capable of expressing both osteoblast and chondroblast phenotype markers in response to BMP-2 treatment. We believe that this approach will allow us to investigate the relationship between osteoprogenitor and chondroprogenitor cells during limb formation and elucidate the influence of BMPs on the differentiation of mesenchymal cells in the developing limb.

W 514 EFFECTS OF ELEVATED HERCULIN GENE EXPRESSION ON ACCUMULATION OF VASCULAR SMOOTH MUSCLE ACTIN, HERCULIN, AND MYOD mRNAS IN BC3H1 CELLS, Sandra B. Sharp, Barry Chess, Michael Mogassa, Edgar Enriquez, Maura Sylber, Maria Villalvazo, and Azieb Ghebremedhin, Department of Biology, California State University, LA, Los Angeles, CA 90032.

The ratio of vascular smooth (vsm) to skeletal (skm) muscle actin mRNA is higher in differentiated BC3H1 cells than in C2C12 or 3T3-MyoD1 cells. Of the four known myogenesis determination genes, BC3H1 cells express myf-5, myogenin, very low levels of herculin, and no MyoD. In contrast, in addition to myogenin, both C2C12 and 3T3-MyoD1 cells do express MyoD, and C2C12 cells also express herculin and myf-5. These observations have led us to test the hypothesis that the levels of vsm actin mRNA will be lowered and/or of skm actin mRNA will be elevated in BC3H1 cells when the level of herculin and/or MyoD expressed is elevated. To derive stable BC3H1 lines which express elevated levels of herculin mRNA, we have transferred a herculin expression construct containing a selectable marker for G418 resistance into BC3H1 cells . Of 23 G418 resistant clones analyzed, about half also expressed transcripts from the transferred herculin gene and some of these expressed elevated levels of mRNA from the endogenous herculin gene when compared to pools of control clones containing no herculin transgene. The elevated level of expression of herculin did not activate the endogenous MyoD gene. It had no effect on the level of vsm actin mRNA accumulation. These results show that if herculin expression elevated to the levels in these clones affects the vsm to skm actin mRNA ratio, it does not do so by decreasing vsm actin mRNA levels. We are currently assaying the level of skm actin mRNA to determine whether or not it is elevated. Supported by NIH-MBRS and NSF-RIMI.

This abstract has been submitted for simultaneous presentation at the FASEB Meetings, Anaheim, CA, April 5-9, 1992.

W 515 THROMBOSPONDIN AND SYNDECAN EXPRESSION IS ALTERED DURING TERMINAL DIFFERENTIATION OF HUMAN KERATINOCYTES, Nader Sheibani and B. Lynn Allen-Hoffmann, Department of Pathology, University of Wisconsin, Madison, WI 53706

Extracellular matrix proteins and their receptors most likely play important roles in control of proliferation and differentiation of stratified squamous epithelia. Alterations in the expression of these proteins may be important signals during terminal differentiation of human epidermal keratinocyes. We have examined expression of several matrix constituents during terminal differentiation of human epidermal keratinocytes in vitro. Kertinocytes were signaled to differentiate by removal from substrata and suspension in growth medium made semi-solid with methylcellulose. Keratinocytes rapidly lost the ability to form colonies in surface culture, after suspension in methylcellulose, and this was consistent with the increased percentage of cells producing cornified envelopes (ie. terminally differentiating). Adherent keratinocytes, cultured in the presence of a 3T3-feeder layer, expressed abundant thrombospondin mRNA. These cells also expressed low levels fibronectin, syndecan, α1 (I) and α1 (IV) collagen mRNAs. Suspension in methylcellulose resulted in rapid changes in the steady state mRNA levels of two basement membrane glycoproteins, thrombospondin and syndecan. The steady state mRNA level for thrombospondin decreased at least 10-fold after 6 h in suspension. In contrast, the steady state mRNA level for syndecan increased 3-fold. We did not detect any changes in the steady state mRNA levels for fibronectin and collagens after suspension-induced differentiation. These results indicate that changes in expression of these molecules is an early response of human keratinocytes to removal from substrata and suspension in methylcellulose. We are presently investigating the roles of these glycoproteins in differentiation of human keratinocytes.

W 516 Correlation between the expression of the transcription factor c-ets-1 and the expression of collagenase 1 and u-PA during angiogenesis and tumor invasion

Bernard VANDENBUNDER+, Nicolas WERNERT+*, Marie-Berthe RAES+, Bernard GOSSELIN*, Dominique STEHELIN+
+ CNRS URA 1160 - INSTITUT PASTEUR - 1 Rue Calmette - 59019 LILLE Cédex
* Service d'Anatomie et de Cytologie Pathologique C, CHU de Lille, 59037 LILLE Cédex, FRANCE

c-ets-1 is the cellular progenitor of the viral oncogene v-ets that is associated with v-myb in the genome of the avian acute leukemia virus E26. c-ets-1 proteins are localized in the nucleus and they activate transcription through specific ets-binding sequences (ETBS) exemplified by the Polyoma virus PEA3 motif.
During early organogenesis in the chick embryo, a transient expression of c-ets-1 is detected in migrating neural crest cells and a variety of cells of mesodermal origin. Throughout development, endothelial cells display a strong hybridization signal at the early stages of blood vessels formation. Epithelial cells are negative, irrespective of their germ layer origin. Interestingly, the expression pattern of c-ets-1 in human carcinomas displays the same general features. Epithelial neoplasic cells are negative whereas c-ets-1 transcripts are abundant in fibrocytes around invasive tumors as well as in endothelial cells of sprouting capillaries in the tumor stroma. In normal adult tissues fibrocytes and endothelial cells are negative. Thus c-ets-1 transcription occurs in situations where extracellular matrix degradation is essential.
It has been shown that the Ets proteins activate the promoters of stromelysin 1 and collagenase 1 genes in a transient cotransfection assay and the Ets-binding sequences are essential for the transcriptional activation of the urokinase-type plasminogen activator (u-PA) gene. Therefore, it is tempting to speculate that the c-Ets-1 proteins might regulate the transcription of genes coding proteases which are necessary for both angiogenesis and tumor invasion.

In human tumor, as well as in an experimental model of tumor in nude mice, we describe situations where the expression of c-ets-1 correlates with the expression of collagenase 1 or of u-PA genes in stromal fibrocytes. We discuss the significance of this result with respect to the existence of other transcription factors such as c-fos and c-jun that also take part in the regulation of collagenase 1 and u-PA genes transcription.

W 518 THE IMMEDIATE EARLY GENE *cyr61* ENCODES A HEPARIN BINDING PROTEIN WHOSE *in vivo* EXPRESSION CORRELATES WITH CHONDROGENESIS. G.P. Yang, T.P. O'Brien, A.S. Abler, and L.F. Lau. Dept. of Genetics, University of Illinois College of Medicine, Chicago, IL 60612.

The growth factor-inducible immediate early gene *cyr61* encodes a secreted protein that is associated with the cell surface and the extracellular matrix (ECM). The half-life of Cyr61 is at least 24 h in the ECM, but only 30 min on the cell surface. Cell surface-associated Cyr61 is rapidly degraded through a lysosomal pathway, consistent with a receptor-mediated event. Cyr61 in the ECM associates preferentially with heparan sulfate proteoglycans in a noncovalent manner. Another growth factor-inducible immediate early gene, *fisp12*, encodes a related protein which conserves all 38 cysteines found in Cyr61 (Ryseck *et al., Cell Growth and Diff.*, 2:225). The putative clone of a connective tissue growth factor activity from the conditioned medium of human umbilical vein endothelial cells encodes the human homolog of FISP12 (Bradham *et al., J. Cell Biol.*, 114:1285), suggesting that Cyr61 might have a related function.

Several immediate early genes appear to be involved in differentiation and development as well as proliferation, prompting us to examine the embryonic expression of *cyr61*. Analysis of staged mouse embryo RNA shows peak expression from 10.5- to 14.5-days of gestation. *In situ* hybridization of staged mouse embryos shows *cyr61* expression in cartilaginous portions of the prevetebrae in an anteroposterior sequence coincident with the timing and direction of somite maturation and differentiation. Expression of *cyr61* is also seen in other parts of the developing embryo including limbs, ribs, and craniofacial structures at the time they are differentiating into cartilaginous structures. These data suggest that Cyr61 might act as an autocrine or paracrine factor involved in chondrogenesis in the developing mouse embryo.

W 517 EMBRYONIC STEM CELL DERIVED CYSTIC EMBRYOID BODIES FORM VASCULAR CHANNELS: AN *IN VITRO* MODEL OF BLOOD VESSEL DEVELOPMENT, Rong Wang, Rebecca Clark, and Victoria L. Bautch, Dept. of Biology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Murine embryonic stem cells can differentiate *in vitro* to form cystic embryoid bodies (CEB) that resemble the mouse yolk sac. Yolk sac blood islands consist of immature hematopoietic cells surrounded by endothelial cells, the first identifiable vascular cells. Blood island development occurs in CEBs has not been well characterized. Our results show that CEBs differentiated *in vitro* developed blood islands initially, and subsequently these blood islands mature to form vascular channels containing hematopoietic cells. Phase microscopy demonstrated the presence of channels in mature CEBs grown in suspension culture, and high resolution light and electron microscopy showed that the cells lining these channels were endothelial cells. The channels appear less organized than the vasculature of the mature yolk sac. Hematopoietic cells were occasionally seen "flowing" through the CEB channels, although their numbers were reduced relative to the yolk sac. Analysis of primary CEB cultures showed the presence of cells with two characteristics of endothelial cells: approximately 30% of the cells labelled with flourescent acetylated low density lipoprotein and a small number of cells were positive for von Willebrand's factor by immunostaining. Thus we conclude that a primitive vasculature forms in CEBs differentiated *in vitro*, and that not only primary differentiation of endothelial cells but also some aspects of vascular maturation are intrinsic to this cell culture system. CEBs are therefore a useful model for the study of developmental blood vessel formation. We are currently investigating gene expression during CEB differentiation, with emphasis on those genes that may be involved in endothelial cell differentiation.

Neurogenesis; Hematopoiesis

W 600 *Abstract Withdrawn*

W 601 INTERLEUKIN-6 IS A COMPONENT OF HUMAN UMBILICAL CORD SERUM AND INDUCES HEMATOPOIETIC DEVELOPMENT IN EMBRYONIC STEM CELLS IN VITRO. Leslie G. Biesecker and Stephen G. Emerson, Divisions of Genetics and Hematology/Oncology, University of Michigan Medical School, Ann Arbor, MI 48109.

Embryonic stem cells (ESC) are derived from day 3-4 mouse blastocysts and are capable of totipotential differentiation. ESCs are maintained in the undifferentiated state by the addition of leukemia inhibitory factor and the removal of this factor leads to spontaneous differentiation. Culture of ESCs with human umbilical cord serum results in clusters of primitive erythropoiesis (primitive blood islands, PBI) in 5-30% of the embryoid bodies. We are utilizing the ESC system to study the role of hematopoietic growth factors (HGFs) in preimplantation hematopoietic development. Our first approach was to assay for HGF activity in the fluid from the cystic embryoid bodies (CEB). This fluid did not induce proliferation of the FDCP1 cell line which is responsive to many murine HGF's. Rather, CEB fluid caused a dose-dependent inhibition of FDCP1 proliferation. Based on these results, we assayed HUCS for cytokines known to have inhibitory effects under some conditions. ELISA for IL-6 was performed on pooled and individual HUCS samples. Pooled specimens ranged from 0-12.5 pg/ml and individual samples ranged from 0-820pg/ml. IL-6 was removed from the HUCS and this HUCS-(IL-6) was used in the ESC in vitro differentiation system and produced less hematopoietic development compared to control HUCS. Reverse transcription polymerase chain reaction analysis for IL-6 message was performed on differentiating ESCs. Our results show that IL-6 message is absent in undifferentiated ESCs and is present in differentiated embryoid bodies.

We conlude that this evidence supports the hypothesis that IL-6 is involved in early hematopoietic development. Further experiments are planned which will determine the effect of an increase in IL-6 receptor expression and interruption of IL-6 production in this system.

W 602 HOMEOGENES IN EMBRYONIC BRAIN, Edoardo Boncinelli, Antonio Simeone* and Massimo Gulisano, DIBIT, Istituto HS Raffaele, Via Olgettina 60, 20132, Milano, and * IIGB-CNR, Via Marconi 10, 80125 Naples, Italy

We cloned four vertebrate homeobox genes expressed in the embryonic brain. Two of them, Emx1 and Emx2, are related to *empty spiracles* (*ems*), and two, Otx1 and Otx2, are related to *orthodenticle* (*otd*). *ems* and *otd* are two genes controlling very anterior body regions during early *Drosophila* embryogenesis. Null mutations of them result in the deletion of specific anterior head structures and their products are expressed in a fairly anterior circumferential stripe. They have been recently cloned and shown to contain a homoeobox. All four vertebrate genes are expressed in the brain of mouse embryos. We characterised in detail the embryonic expression domains of Emx1 and Emx2. Both are expressed in the embryonic cerebral cortex in a developmental period, between day 10 and day 16 post coitum, corresponding to major events in cortical neurogenesis. In its full extension, days 12.5 to 13.5, their expression domain comprises cortical regions including promordia of neopallium, hippocampal and parahippocampal archipallium. In particular, Emx1 expression seems restricted to cortical regions, mainly but not exclusively hexalaminar in nature. At day 17 of development, cortical expression of the two genes is almost exclusively confined to hippocampal germinal layers. Since several homoeobox genes are believed to control cell identity with a regional pattern, it seems reasonable to speculate about a role of Emx1 and Emx2 in establishing the limits and identity of the embryonic cerebral cortex. Emx2, is also expressed in some embryonic neuroctodermal areas including olfactory placodes and olfactory epithelia.

W 603 FIBROBLAST GROWTH FACTORS (FGF) AND FGF RECEPTORS IN DEVELOPING RETINA, Yves Courtois, Kuyas Bugra, Nicole A. Fayein, Guy Fuhrmann, David Hicks, Edith Jacquemin, Maryvonne Laurent, Flore Renaud, Muriel Tcheng and Jean-Claude Jeanny, INSERM U.118, CNRS, Association Claude-Bernard, 29, rue Wilhem, 75016 Paris, France

We have studied the expression and role of FGF(s) in retinal development in vitro and in vivo. In vivo by in situ hybridization aFGF levels increase during differentiation of the respective neuronal layers. Its localization remains constant from late development to adult stages. mRNA levels quantified by PCR amplification coincide with protein accumulation (mostly nuclear localization) as observed by immunocytochemistry, with specific antibodies.

FGF-Rs have been localized by autoradiography of I 125-FGF binding sites, and by in situ hybridization or Northern blot analysis using FGF-R1 et FGF-R2 cDNA probes. Both receptors are expressed concomitantly in the retina and are especially abundant in ganglion cells.

In monolayers from dissociated newborn rat retinal cells bFGF increases the number of opsin expressing cells by six fold. EGF and NGF had no effect. Time-dose experiments indicate that during a short sensitive period bFGF is a specific photoreceptor differentiation factor.

W 604 ECTOPIC EXPRESSION OF *Wnt*-1 IN THE CENTRAL NERVOUS SYSTEM OF TRANSGENIC MICE. Mary E. Dickinson, Kevin L. Stark, Robb Krumlauf*, and Andrew P. McMahon, Department of Cell and Developmental Biology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110 and *Laboratory of Eukaryotic Molecular Genetics, Medical Research Council National Institute of Medical Research, London, NW7 1AA England. The *Wnt*-1 gene is expressed in the presumptive midbrain and spinal cord beginning at early somite stages (8.0 - 8.5 d.p.c.) and transcripts persist until much later in development (at least 14.5 d.p.c.). Previous studies have established the importance of the gene product during the development of the midbrain and the cerebellum (McMahon and Bradley; Cell 62:1073-1085), yet the potential role of *Wnt*-1 and related factors in other parts of the central nervous system (CNS) remains elusive. In order to examine the role of *Wnt*-1 in the developing CNS, we have chosen to misexpress this gene in a region specific manner using transgenic mice. Results of these experiments will be discussed.

W 605 PURIFICATION AND TISSUE LOCALIZATION OF THE HEPARIN BINDING, NEURITE-PROMOTING FACTOR P18 (PLEIOTROPHIN). Eva Engvall, Xiao-Rong Wu, Hong Xu, and Hannu Koho, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

P18 (HB-GAM, HBGF-8, Pleiotrophin) has been reported to have mitogenic, neurotrophic, and neurite outgrowth-promoting activity (Rauvala, EMBO J. 8:2933,1989, Li et al. Science 250:1690,1990). P18 is expressed at high levels in many tissues during embryonic development as evidenced by Northern blotting, but in the adult P18 mRNA can only be detected in the brain. We have prepared recombinant P18 in insect cells. Sf9 cells were cotransfected with wild type baculovirus and the pBlueBac vector containing a full length p18 cDNA made by PCR. Heparin affinity chromatography was used to purify P18 from the culture medium of insect cells infected with recombinant virus. The purified recombinant p18, when coated on a substrate but not when present in solution, promoted the outgrowth of neurites from embryonic day 8 chicken ciliary ganglia and cortical neurons. Antibodies were made against the purified recombinant P18 and against synthetic peptides derived from the N- and C-terminal sequences of P18. The antibodies were used in indirect immunofluorescence to stain sections of muscle, heart, tongue, skin, kidney, and brain from rats of different ages. In all tissues and at all ages, the predominant staining was found associated with neuronal processes. The neurite outgrowth-promoting activity and the tissue localization of P18 suggest that P18 is important for the formation and maintenance of axons.

W 606 CLONING AND ANALYSIS OF *MOTCH*, A MURINE HOMOLOG OF THE *DROSOPHILA* NEUROGENIC GENE *NOTCH*. F. Franco del Amo, D.E. Smith, P. Swiatek, M. Gendron-Maguire, R. Greenspan*, A.P. McMahon, and T. Gridley. Dept. of Cell and Developmental Biology, *Dept. of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

The choice of cell fate in the neurogenic region of the ectoderm of *Drosophila* embryos is under genetic control. In embryos homozygous for null mutations of the *Notch* gene essentially all of the cells in the neurogenic region become neuroblasts. Such embryos die with a vast hypertrophy of the nervous system and a corresponding absence of epidermal structures. The *Notch* locus in *Drosophila* encodes a large transmembrane protein that appears to mediate cell-cell interactions necessary for proper epidermal versus neural cell fate decisions in the neurogenic region of the ectoderm. To determine if a gene homologous to *Drosophila Notch* plays a role in early mouse development, we have screened an 8.5 day mouse embryo cDNA library with probes from the *Xenopus Notch* homolog, *Xotch* (Coffman, Harris, Kintner (1990) Science 249, 1438). We report here the isolation of cDNA clones encoding the mouse homolog of *Notch*, which we have termed *Motch*. We also characterize by ribonuclease protection analysis the expression pattern of *Motch* RNA in adult mice and postimplantation mouse embryos, and the induction of *Motch* RNA levels in tissue culture cells undergoing differentiation. An analysis by *in situ* hybridization of the spatial organization of *Motch* RNA expression suggests an important role for the *Motch* gene in patterning and differentiation in the early postimplantation mouse embryo.

W 607 THE MURINE RECEPTOR TYROSINE KINASE *nuk* IS CONCENTRATED IN A SUBSET OF CELL-CELL JUNCTIONS DURING EMBRYOGENESIS

Mark Henkemeyer, Jane McGlade, Peter Greer and Tony Pawson, Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, 600 University Avenue, Toronto, Ontario, Canada, M5G 1X5

We describe the cloning and embryonic expression pattern of a new murine receptor tyrosine kinase called *nuk* (neural kinase). *nuk* is very similar in structure to the the growing subclass of receptor tyrosine kinases which include *eph*, *eck*, and *elk*. Common features of these proteins include a cysteine-rich subdomain and fibronectin type III repeats in the extracellular domain and a fairly well conserved intracellular catalytic domain.

To begin to understand the developmental function of this receptor tyrosine kinase, the expression of *nuk* mRNA and protein during embryogenesis has been investigated using whole-mount RNA *in situs* and whole-mount immunohistochemical protocols. The results indicate that *nuk* is highly expressed in the neuroectoderm as early as 8.5 days post coitum. Later, during formation of the brain and neural tube, *nuk* is highly expressed in the telocoel (primative forebrain), midbrain flexure, optic vesicle and in the roof plate of the neural tube. In addition, *nuk* is expressed at elevated levels in the base of the developing heart.

Immunohistochemical staining with anti-*nuk* antibodies revealed that endogenous *nuk* protein is asymetrically located at the cell surface. The staining pattern is very filamentous and appears to be localized to the extracellular matrix separating different cell/tissue types. Electron microscopy studies indicate *nuk* protein is concentrated at some, but not all, cell-cell junctions. The presence of fibronectin type III repeats and the unusual localization of *nuk* protein suggest that this receptor tyrosine kinase may function to control cell-cell interactions during murine embryogenesis.

W 608 EXPRESSION OF NERVE GROWTH FACTOR IN SYMPATHETIC NEURONS LEADS TO DECREASED INNERVATION OF SYMPATHETIC TARGET TISSUES

Gary W. Hoyle[1], Eric H. Mercer[2], Richard D. Palmiter[2], and Ralph L. Brinster[1], [1]Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, [2]Department of Biochemistry and Howard Hughes Medical Institute, University of Washington, Seattle, Washington 98195.

Sympathetic neurons require the neurotrophin nerve growth factor (NGF) for survival. NGF is not synthesized by sympathetic neurons but rather the neurons acquire it from the tissues that they innervate. In vitro, sympathetic axons have the ability to grow along NGF concentration gradients, but the importance of NGF in determining the pattern of sympathetic innervation in vivo is not known. To investigate the requirement of NGF gradients in the development of sympathetic innervation, we disrupted the normal pattern of NGF production by expressing NGF in sympathetic neurons of transgenic mice. Mice were generated in which the human NGF gene was placed under control of the human dopamine β-hydroxylase (DBH) promoter, which brought about production of NGF in sympathetic neurons and their axons. Modified bacterial *lacZ* genes containing sequences for nuclear localization or axonal transport were placed under control of the DBH promoter and introduced along with the DBH-NGF gene to facilitate the detection of sympathetic soma or axons respectively. Sympathetic ganglia in mice carrying the DBH-NGF transgene were enlarged, as were the chain of fibers connecting the paravertebral sympathetic ganglia, the white and gray rami communicantes, and the nerves projecting to sympathetic target tissues. Aside from the size, the pattern of sympathetic axon growth from ganglia to target tissues was not affected, with the exception of sympathetic fiber growth into the spinal canal in some animals. The pattern of sympathetic axon growth within target tissues, however, was affected, since there was a marked decrease in sympathetic innervation in tissues that normally receive abundant sympathetic innervation. The observations are consistent with a model in which NGF gradients are not required to guide sympathetic axons to their targets, but are required for the establishment of the normal density and pattern of sympathetic innervation within target tissues.

W 609 MOLECULAR BASIS OF T CELL ACTIVATION VIA THE CD2 AND CD28 PATHWAYS : ROLE OF NF-kB ACTIVATION AND OTHER INDUCIBLE TRANSACTING FACTORS. Jean Imbert, Michèle Algarté, Régis Costello, Chantal Cerdan, Marie Bellosguardo, Carol Lipcey and Daniel Olive. INSERM U119 27 bd Leï Roure 13009 Marseille FRANCE.

Human T lymphocytes can be activated by mAbs directed against two adhesion molecules, CD2 and CD28. We have demonstrated previously that the simultaneous activation by CD2 and CD28 of purified resting T cells led to a long lasting (>3 weeks) proliferation. Furthermore, this proliferation is monocyte-independent and autocrine for IL-2. The monocyte-independence could be explained by a high and prolonged T cell synthesis of cytokines normally produced by accessory cells such as IL-1α, TNF-α and CSF-1. CD2+CD28 costimulation regulates cytokine and associated receptor genes both at the transcriptional and post-transcriptional levels. We are currently investigating which cisacting element(s) and transacting factor(s) are associated with the CD2+CD28 mediated transcription activation. The promoter-regulatory region of the major immediate-early gene of human cytomegalovirus is strongly responsive to CD2+CD28 as shown by a CAT assay after transfection of a chimeric plasmid CMV-CAT in Jurkat and purified T cells. As the CMV IE promoter contains several response elements identified in cytokine gene regulatory sequences (i.e.: AP1, CRE, NF-kB and SRE recognition sites), we are using this very sensitive assay in combination with a set of specific metabolic inhibitors in an attempt to determine the effects of CD2+CD28 triggering on inducible transactivators. To determine more precisely the role of NF-kB during the early and long lasting activation, experiments are in progress to analyze the effects of NF-kB inhibitors such as NACS and PDTC on IL-2, GM-CSF and IL-2Rα expression.

W 610 A NOVEL ENDOTHELIAL CELL ANTIGEN INVOLVED IN LYMPHOCYTE BINDING TO HIGH ENDOTHELIAL VENULES. Sirpa Jalkanen, Päivi Vuorela-Miilunpalo, Laura Airas, Jaakko Uksila, and Marko Salmi, Department of Medical Microbiology, Turku University, SF-20520 Turku, Finland

Extravasation of leukocytes from the blood is important in normal lymphocyte recirculation as well as in amounting adequate inflammatory responses in different tissues. This extravasation is critically dependent on proper interactions between leukocytes and vascular endothelial cells. In order to characterize novel endothelial cell molecules involved in the leukocyte migration, we have produced monoclonal antibodies (mAbs) against human endothelial cells by immunizing mice with synovial stroma. One of the mAbs obtained, 1B2, stains high endothelial venules (HEV) in frozen sections of synovium, peripheral lymph node and tonsil. Staining of appendix HEV is much weaker. Importantly, the 1B2-defined antigen is not expressed on normal or cytokine-activated human umbilical venule endothelial cells, nor on any of the leukocyte subsets. The molecular weight of the 1B2-defined antigen is approximately 90-100 kDa, when determined from iodinated tonsillar lysates under reducing conditions. The involvement of the 1B2-antigen in leukocyte-endothelial cell interaction has been tested by measuring leukocyte binding to tonsillar, synovial, peripheral lymph node and appendix HEV in the *in vitro* frozen section assay: the 1B2-antibody is capable of inhibiting lymphocyte binding to tonsillar, synovial and peripheral lymph node HEV 50-70%, and 30% to appendix HEV. It does not significantly affect the HEV-binding of neutrophils. Moreover, isolated 1B2 antigen supports lymphocyte binding, and this binding is inhibited by IB2 antibody. In conclusion, these data strongly suggest that the 1B2-defined antigen is a novel member of endothelial cell molecules involved in lymphocyte migration.

W 611 A MESSENGER RNA RELATED TO MAMMALIAN c-kit IN XENOPUS LAEVIS EMBRYOS, Kenneth R. Kao and Alan Bernstein, Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, M5G 1X5, Canada.

The dominant White spotting locus (*W*) in mice is known to encode the c-kit proto-oncogene. The protein encoded by c-kit (Kit) is a receptor tyrosine kinase whose ligand is known to be Steel factor (*Sl*). Mutations at the *W* or *Sl* locus all affect the hematopoetic, neural crest and germ cell lineages. Also, both c-kit and *Sl* transcripts are expressed in a complementary pattern in a wide variety of tissues. These results indicate that Kit and Steel form part of a cell-cell signalling pathway required for development of at least three cell lineages. We are interested in determining the cellular function of Kit in early development. To this end, we have isolated a Xenopus cDNA, that has significant sequence identity to mammalian c-kit in order to analyse early steps in frog development. Frog embryos are particularly suited to embryonic manipulation and therefore are useful models for studying the role of cell-cell interactions and the signal transduction mechanisms required for development. Using this cDNA, we describe the expression of a frog homolog to Kit, which we name Xkit.

W 612 *INDUCTION OF EMBRYONIC HEMATOPOIESIS IN XENOPUS* Clair Kelley[1] and Leonard Zon[2], [1]Department of Cell and Developmental Biology, Harvard University, and [2]Harvard Medical School, Division of Hematology, Children's Hospital, Boston, MA., Ventral mesoderm is induced by spatially distributed signals early in *Xenopus* embryogenesis. The most ventral mesoderm yields hematopoietic tissue. We have studied the expression of several transcription factors important early in hematopoietic development. We isolated and characterized *Xenopus* cDNA clones for the transcription factors GATA-1, 2 and 3. GATA-1 regulates many erythroid promoters and enhancers, and is absolutely required for erythroid development. GATA-2 is expressed in early hematopoietic cells and other tissues. GATA-3 is highly expressed in T-cells. mRNAs encoding these DNA-binding proteins are expressed about 4 hours after the onset of zygotic transcription. To determine if GATA-1, 2 and 3 organize the hematopoietic axis of the embryo before blood is histologically evident, we performed *in situ* analysis on staged embryos. GATA-1 is expressed in the region of the presumptive embryonic blood islands before embryonic globin. GATA-2 is initially highly expressed in the animal pole and rapidly localized to the ventral region which later expresses GATA-1 mRNA. GATA-3 is present in the pronephros region and in the second pharyngeal pouch; both subsequently contribute to thymopoiesis. GATA-2 and 3 are also expressed in the nervous system. PCR analysis shows that GATA-1 is initially expressed in the animal cap, and is later localized to the ventral marginal zone. Culture of multipotential cells of the animal cap has revealed that the hematopoietic program (GATA-1/globin expression) and the muscle program (MyoD/actin expression) are simultaneously expressed in a temporal pattern according to normal embryonic development. In the absence of mesodermal inducing factors, neither program is maintained. Although activin induces muscle formation, preliminary data suggest that the blood program is not maintained by either activin or bFGF. We believe that hematopoietic induction is therefore regulated by a distinct mesodermal inducing signal present in the ventral vegetal region of the embryo.

W 613 *Prd-like*: A NOVEL HOMEOBOX GENE EXPRESSED IN THE CEPHALIC NEURAL PLATE AND RATHKE'S POUCH OF THE EARLY MOUSE EMBRYO, Kathleen A. Mahon, Edit Hermesz, and Milan Jamrich, Laboratory of Mammalian Genes and Development, NICHD, NIH, and Division of Biochemistry and Biophysics, CBER, FDA, Bethesda, MD 20892

Embryonic patterning along the antero-posterior axis in *Drosophila* is controlled in part by a complex hierarchy of homeobox containing genes. The expression pattern of the known murine homeobox genes suggests that a similar gene network may control vertebrate pattern formation, particularly in defining regional identity in the developing central nervous system (CNS). We have isolated a new gene from the mouse that contains a homeobox similar to those of the *Drosophila paired* and *gooseberry* genes. "Prd-like" is unique in that its domain of expression is the most anterior of all known homeobox containing genes. *In situ* hybridization experiments have shown that expression of the "prd-like" gene in gastrulating embryos is quite dynamic. During early gastrulation (6.5 - 7.0 days), expression is seen in the endoderm at the prospective cephalic end of the embryo. A few hours later (7.5 days), transcripts are detected in the overlying anterior ectoderm, suggesting that expression in the ectoderm is induced by the underlying endoderm. By 7.75 days, transcription is confined to cephalic neural plate ectoderm only. As development proceeds, expression becomes progressively restricted to Rathke's pouch, the primordium of the anterior pituitary. Fate mapping experiments have previously shown that Rathke's pouch is derived from the most anterior ectoderm of the embryo. Transcripts accumulate in Rathke's pouch through day 13, but are undetectable by 15 days of embryogenesis, coincident with the differentiation of pituitary specific cell types. This striking pattern of expression suggests a role in the genesis of the pituitary. However, since expression is seen several days prior to the development of Rathke's pouch, and the domain of expression initially extends over a much broader area than that destined to become the anterior pituitary, it seems likely that "prd-like" is involved in a more general developmental function in the early stages of embryogenesis, such as defining the anterior region of the embryo.

W 614 A NOVEL, FORK HEAD DOMAIN CONTAINING GENE INVOLVED IN THE EARLY REGIONALIZATION OF XENOPUS BRAIN. Andra Miller, Marie Luise Dirksen and Milan Jamrich, Laboratory of Molecular Pharmacology, Division of Biochemistry and Biophysics, Center for Biologics Evaluation and Research, FDA, Bethesda, MD 20892

We have isolated a novel, fork head domain containing gene which appears to be involved in the regionalization of the Xenopus brain. This gene is activated in early gastrulae and its expression persists into adult brain. First signs of region-specific expression can be detected in mid-gastrula. At this, and consecutive stages, the major domain of expression appears to be the future midbrain area. In isolated tadpole brains this area can be identified as the optic tectum. Additional sites of expression are present in the forebrain, hindbrain and spinal cord. Interestingly, this gene is not expressed in the neural floor plate.

W 615 *nek*, a novel murine protein-serine/ tyrosine kinase primarily expressed in the nervous system. Benny Motro, Ken Letwin, Yaacov Ben-David, Lee Mizzen, Tony Pawson and Alan Bernstein. Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave. , Toronto, Ontario, Canada. M5G 1X5.

nek c-DNA was isolated from a mouse erythroleukemia cell expression library screened with antiphosphotyrosine antisera. The coding part of *nek* c-DNA is 2.2kb long and encodes a predicted 774 amino acid protein. The *nek* protein contains a N-terminal catalytic domain with motifs diagnostic of protein serine/threonine kinases and a large "tail" domain at the C-terminus. A search of the GENEMBL database revealed that *nek* is most similar to *nimA*, a serine/threonine kinase involved in the regulation of the G2-M phase transition in *Aspergilus nidulans*. The *nek* protein expressed in bacteria exhibited both serine/threonine and tyrosine kinase activity against a variety of bacterial proteins.

In order to get clues about the possible role of *nek* during development, we performed *in situ* RNA localization on mouse embryos and adult tissues. During embryogenesis *nek* is primarily expressed in the nervous system. In the central nervous system *nek* is expressed in spinal and cranial motor regions and at these sites the levels of expression peaked at 13.5-14.5 days postcoitum. In the peripheral nervous system, dorsal root, cranial, sympathetic and parasympathetic ganglia express *nek*. Placode-derived cranial ganglia neurons also express *nek*, indicating that the expression is not restricted to neural crest-derived cells. Outside the nervous system *nek* was found to be expressed only in the reproductive system.

W 616 ANALYSIS OF THE NEURAL-SPECIFIC GENE NRP-1 IN XENOPUS EMBRYOS

Marcia L. O'Connell, Martha Rebbert, Igor B. Dawid, Laboratory of Molecular Genetics, NICHD, NIH, Bethesda, MD 20892

A Xenopus laevis gastrula stage library was screened for genes expressed exclusively in the nervous system of adult frogs; the sequence of one such clone predicts an RNA-binding protein and has been designated nrp-1 (neural-specific RNA-binding protein 1, Richter et al. 1990, New Biol. vol. 2:556). The levels of nrp-1 mRNA rapidly rise in gastrula and neurula stage, and localization studies suggest that this gene may be a marker for neural induction. We have therefore pursued an analysis of the transcriptional regulation of this developmentally regulated gene. A series of overlapping genomic clones for nrp-1 were isolated that span the entire coding region, and several kilobases of the presumptive 5' flanking region. The intron/exon boundaries of these clones were determined, and indicate that nrp-1 has 15 exons. The translation termination codon is located in the penultimate exon, a feature of a subclass of hnRNP genes. Sequence analysis of the region 5' to the start site of transcription fails to show a TATA box, though there are several GC rich regions. Approximately 200 nucleotides of the upstream region has been fused to the chloramphenicol acetyltransferase (CAT) gene, and the construct injected into fertilized Xenopus embryos. This 200 nucleotide sequence is a strong transcriptional activator in neurula stage embryos, as demonstrated by a stimulation in CAT activity versus a promoterless control. The tissue and temporal specificity of this transcriptional regulator is under investigation.

W 617 Expression of Thrombospondin in the Developing Mouse Eye and Cell Adhesion of Isolated Retinal and Lens Cells. K.A. Rich, C. Spee, D.R. Hinton, and J.C. Blanks, Doheny Eye Institute and Departments of Ophthalmology and Pathology, USC School of Medicine, Los Angeles, CA 90033

Thrombospondin (TSP) is a large, adhesive extracellular matrix glycoprotein which appears during the genesis and remodelling of tissue. In the developing mouse embryo, TSP is observed throughout the central nervous system, while its distribution in adult tissues is much more restricted. The distribution of immunoreactive TSP was examined in developing mouse eyes and found to be uniformly present within the extracellular spaces of the early retinal neuroepithelium (day E10), confirming previous studies by others. In the present study, these observations were extended through to the post-natal period. From day E14 through to the neonatal period, TSP was observed to be prominent at the inner limiting membrane and in the ganglion cell layer, as well as in the developing lens. Staining intensity was dramatically reduced by adulthood. Attachment of dissociated cells from E12, neonatal and adult retina and lens to a peptide EWTPCSVTCGVGVRVRSR, derived from Region II of malarial CS protein and 80% homologous to sequences within TSP type-I repeats, was examined. This peptide was previously shown (Rich et al, 1990, Science 249:1574) to be highly adhesive for certain human hematopoietic tumor cell lines. In a cell adhesion assay, 70-85% of embryonic retinal and lens cells were observed to attach to wells coated with 1 ug peptide. Cells derived from neonatal retina and lens were somewhat less adhesive (60-75%), while those from adult mice were markedly less so (20-35% cell attachment). Similarly, the degree of cell attachment to substrate-bound TSP also declined with eye maturation. The human retinoblastoma cell lines Y79 and Weri-RB1 were only mildly adhesive to this peptide (20-40%), when compared to 5 different melanoma cell lines and certain hematopoietic cell lines (70-90% cell attachment). Thus the malaria-homologous domain of TSP appears to be a major attachment site within TSP for developing retinal and lens cells. Taken together with the immunolocalization of TSP, these data suggest an important role for TSP in eye development.

W 618 A NOVEL GENE TRANSFER TECHNIQUE TO TEST THE FUNCTION OF SPECIFIC GENE PRODUCTS DURING AVIAN NEURAL CREST DEVELOPMENT *IN VIVO*,

Kate M. Stocker, Gary Ciment and Anthony M.C. Brown, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR, 97201 and Cornell University Medical College, New York, NY 10021.

To determine the effect of expression of specific genes on nervous system and neural crest development, we have developed a novel technique for introducing genes into the developing avian nervous system *in vivo*. This technique involves (i) dissecting neural tubes from 2 day-old quail embryos, (ii) infecting the neural tubes in culture with a recombinant, helper-free retrovirus carrying the gene for ß-galactosidase (as a marker for infected cells) and the gene of interest, (iii) grafting the infected neural tube into the homologous region of the nervous system of an uninfected host chicken embryo *in ovo*, (iv) allowing the chimeric host embryo to continue development for several days, and (v) analyzing the chimeric embryo histologically to identify quail cells (i.e., graft-derived cells) and ß-galactosidase+ cells (i.e., retrovirus-infected graft cells).

The parental virus, "CXL", is derived from an avian spleen necrosis retrovirus and contains only the ß-galactosidase gene. CXL is packaged in an amphotrophic packaging cell line which allows for both high virus titers (approximately 10^6/ml of non-concentrated culture medium) and for its ability to infect avian cells. Titers of the virus can be increased further by Amicon filtration. Maximal levels of infection of quail neural tubes (including neuroepithelial and neural crest cells) are reached in approximately 2 hours and the percentage of blue cell clusters (representing clones probably derived from single "hits") increases linearly with increasing virus titer. Histological analysis of chimeric embryos shows that infected quail cells are found in the neural tube and in a variety of neural crest derivatives, including dorsal root and sympathetic ganglia, adrenal medulla, and melanoblasts. These results indicate that the expression of ß-galactosidase does not interfere with the normal development of these cells and that the CXL promoter functions in a relatively non-cell type-specific manner. This ability to drive expression in a variety of different cell types makes this virus an ideal vector for these types of studies.

W 619 REGULATION OF THE MAJOR NEURONAL GROWTH ASSOCIATED α-TUBULIN GENE, Tα1, BY NGF *IN VIVO* AND *IN VITRO*.

Jean G. Toma and Freda D. Miller, Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta T6G 2H7, CANADA.

Microtubules, which are assembled from α- and β-tubulins, play an essential role during neuronal process extension, and provide the substrate for axonal transport. We have previously demonstrated that two members of the α-tubulin multigene family that encode virtually identical proteins are differentially regulated in mammalian neurons; expression of Tα1 α-tubulin mRNA is specifically correlated with process outgrowth in developing and regenerating neurons, whereas expression of T26 mRNA is constitutive. Furthermore, expression of Tα1 mRNA is upregulated by NGF both *in vivo* and *in vitro*, whereas T26 mRNA levels are not altered. In order to address the regulatory elements responsible for inducing Tα1 α-tubulin mRNA in response to NGF, we have isolated the promoter region from a rat genomic library. The 5' promoter region thus isolated has been fused to β-galactosidase and chloramphenicol-acetyl transferase reporter genes. We have used PC12 cells to start to map the elements responsible for NGF-inducibility. The results obtained so far indicate that, in PC12 cells, endogenous Tα1 α-tubulin mRNA levels are increased within 2-6 hours following NGF addition. A similar increase in P75 NGF receptor mRNA was observed 6 hours following treatment with NGF, while the levels of T26 α-tubulin mRNA did not change. The observed increases in both Tα1 α-tubulin and P75 NGF receptor mRNAs are regulated at the transcriptional level, as determined using nuclear run-off. Transient transfection experiments indicate that 1.2 Kb of the Tα1 promoter is sufficient to confer NGF-inducibility in PC12 cells. To determine whether this promoter fragment confers NGF-inducibility *in vivo*, and to further characterize promoter elements responsible for coupling gene expression to neuronal growth, we have introduced a similar construct into transgenic mice. Analysis of these mice is currently underway.

W 620 TENASCIN AND ITS PUTATIVE FUNCTION IN PERIPHERAL NERVE DEVELOPMENT,

Bernhard Wehrle-Haller and Matthias Chiquet, Dept. of Biophysical Chem., Biocenter University of Basel, 4056 Basel, Switzerland

The extracellular matrix protein tenascin has a characteristic spatial and temporal expression pattern during development of the peripheral nervous system. During the major growth period of nerves, tenascin is strongly expressed by satellite cells and is deposited in the mesenchyme surrounding nerves. After the major nerve pattern is established, tenascin mRNA disappears from nerves in the upper limb, and is instead expressed in perichondrium and tendons (Wehrle and Chiquet, 1990, Development, 110, 401-415; Wehrle-Haller et al., 1991, Development, 112, 627-637). These findings imply a function of tenascin in nerve morphogenesis rather than in guiding. In order to examine the function of tenascin in nerve morphogenesis, we cultured explants of 6 day dorsal root ganglia, allowing neurites and satellite cells to migrate simultaneously over a given substrate (tenascin, laminin or fibronectin). Neurites extended immediately on fibronectin or laminin and were accompagnied by satellite cells migrating on the substrate. On tenascin, neurites grew after a lag phase of 5 to 10 hours, but with a similar velocity as on laminin and fibronectin. In contrast, satellite cells were not able to migrate on tenascin but instead moved on top of neurites. Furthermore neurites and satellite cells were confronted with borders of laminin/tenascin and fibronectin/tenascin. Neurites could cross borders from laminin to tenascin but were diverted away when they arrived at a low angle. Neurites at borders of fibronectin to tenascin were also able to cross, whereas satellite cells remained restricted to the fibronectin substrate. Neurite outgrowth on tenascin could be inhibited by either JG22 (a function blocking monoclonal antibody against ß1 integrins) or by heparin but not by chondroitin sulfate.

These in vitro studies showed that a tenascin substrate can specifically inhibit migration of satellite cells, whereas neural growth cones are able to adhere and to locomote on tenascin. ß1 integrins and heparansulfate cell surface proteoglycans are likely candidates to serve as receptors for tenascin on growth cones. We postulate that tenascin expressed by growing nerves restricts satellite cell locomotion to the surface of axons, thereby preventing them from migrating into the surrounding mesenchyme. However, tenascin does not inhibit axonal growth, and might still allow sorting of axons within the developing nerve.

Late Abstracts

ELEVATED EXPRESSION OF E-CADHERIN SUPPRESSES THE TRANSFORMED PHENOTYPE OF CHINESE HAMSTER OVARY CELLS AND PREVENTS FORMATION OF PRIMARY TUMORS IN NUDE MICE.

Deborah F.Aghib and Barry Gumbiner - Dept. of Pharmacology, University of California San Francisco, San Francisco CA 94143-0450-USA.

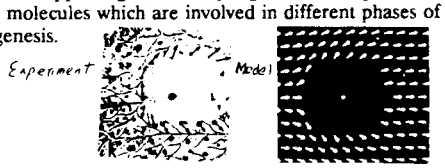
The role of E-cadherin expression in tumorigenesis was investigated using transformed Chinese Hamster Ovary (CHO) cells transfected with E-cadherin cDNA. Unlike parental CHO cells these transfectants express high levels of E-cadherin. A confocal microscope was used to obtain optical sections of E-cadherin distribution within the plasma membrane and of the actin cytoskeleton. E-cadherin appears properly localized at the cell surface. Moreover, the E-cadherin expressing cells exhibit cytoskeletal characteristics of normal cells when cultured in vitro. Furthermore, these cells are not tumorigenic when injected into nude mice. These results indicate that intercellular adhesion mediated by E-cadherin can influence the formation of primary tumors, possibly through regulation of cell growth. These data provide direct evidence that E-cadherin acts as a tumor suppressor molecule in CHO cells in vivo and also at the cytoskeletal level. Many changes occur in a cell after E-cadherin transfection, including the development of stress fibers, suggest the involvement of specific signalling pathways. Cellular transformation is associated to several measurable responses. The signals that induce these changes may result from the action of intercellular or matrix receptors. Our results suggest a role of E-cadherin down-modulation for the establishment of the transformed phenotype, raising the possibility that these two distinct receptor systems may eventually interact with the same downstream molecules or even be interdependent. The latest results involving E-cadherin in signal transduction will be discussed further.

Ectopic expression of BMP2 and BMP4 in transgenic mice, Manfred Blessing, C. Michael Jones and Brigid L.M. Hogan, Department of Cell Biology, School of Medicine, Vanderbilt University, Nashville, TN 37232

BMP2 and BMP4 are closely related members of the TGFβ family. In order to investigate possible functions of these molecules we ectopically expressed BMP2 and BMP4 cDNAs in transgenic mice. In one case we coupled these cDNAs to the bovine cytokeratin IV* promoter which directs transgene expression to the inner root sheath of the hair follicle. In the normal hair follicle only BMP2 is expressed. Its expression is restricted to the matrix cells which represent a transition between the epithelial cells of the inner and outer root sheaths and the trichocytes of the hair shaft. BMP2, therefore, might be a signal which triggers that switch. In that case, by expressing BMP2 or BMP4 in the inner root sheath we would expect the normal sequence of differentiation events being disturbed. In transgenic mice carrying the cytokeratin/BMP4 construct we find the hair follicle development abnormal and the whisker follicles fail to produce a proper hair shaft. In addition, due to the expression of the transgene in the epithelia of the oral cavity craniofacial abnormalities occur.

RETINOIDS, HOMEOPROTEINS AND ADHESION MOLECULES IN THE PATTERN FORMATION OF SKIN APPENDAGES C.M. Chuong, T.X.Jiang, S.A. Ting, R. B. Widelitz, and Y. S. Lee Department of Pathology, University of Southern California, Los Angeles, CA 90033

During the formation of feather placode, three epithelial domains are defined by tenascin and N-CAM. Integrin is in the dermal-epidermal junction. During feather bud formation, N-CAM, tenascin and XlHBox 1 antigen are enriched in the anterior but non-identical domains. Fibronectin is in the posterior buds and integrin is diffused distributed. Perturbation of embryonic chicken skin explant cultures with neutralizing antibodies showed that anti-integrin beta 1 and anti-fibronectin blocked epithelial - mesenchymal interaction, anti-N-CAM caused uneven segregation of mesenchymal condensation, and anti-tenascin inhibited feather bud growth. To further analyze the phenotyping determing factors, retinoic acid (RA) was added to cultures which made feather buds disoriented or transformed into scale like structures in a concentration dependent manner. Gradient-distributed RA created by a RA bead suggested that the new feather bud axis is determined by a combination of the original axis and a new axial force pointing centrifugally away from the RA source. The antero-posterior gradient of N-CAM and homeoprotein XlHbox 1 became diffusive. Endogenous retinoids in developing skins showed developmental stage - dependent changes. The results suggest that RA modulates endogenous morphogen(s) that determine the orientation and phenotype of skin appendages, and may regulate homeoproteins and adhesion molecules which are involved in different phases of skin morphogenesis.



BIOLOGICAL PROPERTIES OF BOVIN BRAIN PLEIOTROPHIN (bbPTN).

José Courty, Mylène Perderiset, Thi Thé Nguyen, Christiane Dorey, Marie Claude Dauchel and Denis Barritault.

Laboratoire de Biotechnologie des Cellules Eucaryotes. Université Paris Val de Marne. Avenue du Général de Gaulle. 94010 Créteil. France.

We have recently reported the purification from adult bovine brain of a novel growth factor that displayed a high affinity for heparin. Analysis of a partial amino acid sequence indicate that this protein is closely related to p18 or heparin binding growth associated molecule (HB-GAM) also referred to heparin binding neurotrophic factor (HBNF) or Pleiotrophin (PTN). Recently controversial results have been reported about the mitogenic activity of this molecule. Our freshly purified molecule exhibited *in vitro* growth factor activity for bovine brain capillary endothelial cells with maximal mitogenic effect at 100 pM concentration. Using a modified rabbit cornea model, we found that this molecule named bovine brain pleiotrophin (bbPTN), induced angiogenesis *in vivo*. A neovascular response was significantly detectable at dose as low as 2 nMol of bbPTN.

We have also found that bbPTN molecule added in culture medium with serum, displayed neurite outgrowth and increased tyrosine hydroxylase activity in dose dependant manner when tested on PC12 cells. Minimal effect was detected at 300 pM and reached a maximum at 3 nM of culture medium.

The precise relationship between neurite outgrowth activity and mitogenic activity at the cellular receptor level is in progress and warrants further investigation.

A MURINE 500 kDa ENZYME IS A PUTATIVE MEMBRANE PROTEIN OF THE MOUSE BRAIN AND DEFINES A NOVEL CLASS OF UBIQUITIN-CONJUGATING ENZYMES

HANS-PETER HAUSER and STEFAN JENTSCH (Max-Planck Institute, Tübingen)

The ubiquitin field has a growing impact on the understanding of cellular regulation. Recently, it has been shown that important cellular regulator proteins (cyclin, p53, MATα2 repressor, phytochrome) are rapidly degraded by the ubiquitin system. We have cloned a murine gene, *UbcM1*, encoding a novel ubiquitin-conjugating enzyme which is remarkable both for its structure and for its expression pattern.

UbcM1 is transcribed as an unusually long mRNA of about 16 kb. It contains one large open reading frame coding for a protein of roughly 500 kDa. The domain which is homologous to other known ubiquitin-conjugating enzymes is located within the C-terminal region leaving an N-terminal extension of 470 kDa.

The deduced amino acid sequence of *UbcM1* shows 9 putative transmembrane helices. The amphipatic character of these helices resembles structural features of membrane transporters or channels. It is tempting to speculate that UbcM1 might have a function in transport, possibly coupled to its ubiquitin-conjugating activity. A potentially outside the cell located RGD sequence might implicate a role of *UbcM1* in cell-cell interaction.

A developmental Northern shows strong expression of *UbcM1* in mouse embryos at least from day 8 on, whereas in the adult mouse expression seems to be restricted to the brain. *In situ* hybridization using murine embryos showed that expression is restricted mainly to brain ventricles and certain ganglia.

The presumed membrane localization and the brain-specific expression is particulary intriguing in the light of previous findings of ubiquitinated cell surface proteins including the PDGF and the hGH receptor and a very active ubiquitin system in neuronal tissues.

Being one of the largest proteins known this structurally novel enzym defines a complete new class of ubiquitin conjugating enzymes.

STIMULATORY EFFECTS OF MEMBERS OF rBMP-FAMILY ON PRIMARY BONE CELLS AND BONE IN VITRO, Hubert Mayer, Andrew Scutt, Christian Duvos and Gerhard Groß, Department of Genetics, Gesellschaft für Biotechnologische Forschung mbH, Braunschweig, Germany

Rec. proteins from three members of the BMP-family have been demonstrated to induce bone formation in an in vivo system. The underlying mechanisms of action of BMPs are not understood. We examined possible direct actions of rBMPs on primary pre-OB cells and bone culture in vitro. BMP2, 4, 5, 7 but not BMP3 increase DNA synthesis in a dose-dependent manner as TGFß1 with a half maximal concentration of 0.1 nM. BMP2 was also mitogenic on other cells as chondrocytes and skin fibroblasts. The stimulation of collagen synthesis is dependent on the cell type used. In bone culture, BMP2 has no effect on alkaline phosphatase activities and Ca^{2+} metabolism. It inhibits, however, the PTH 1-34 induced Ca^{2+} mobilisation. These results indicate that members of the BMP-family have differing effects on bone cells and bone. The influence of BMPs on osteoblastic phenotypes can be measured. These findings should permit the investigation of their distinct and unique regulatory activities in chondrogenesis and osteogenesis in vitro.

NEUROTROPHIC GROWTH FACTORS AND THEIR p^{75} RECEPTOR IN KIDNEY MORPHOGENESIS.

Karavanov, A., Palgi, J., Sainio, K., Arumäe U., Saarma, M., Sariola, H.
Department of Paediatric Pathology, Children's Hospital, University of Helsinki, SF-00290 Helsinki, Finland

The neurotrophin family consists of related growth factors (nerve growth factor,NGF; brain-derived neurotrophic factor, BDNF; neurotrophin-3,-4,-5; NT-3, NT-4 and NT-5) that possibly all bind to the p^{75} receptor (low-affinity nerve growth factor receptor, LNGFR).The members of *trk protooncogene family participate in high* affinity reception of neurotrophins. The neurotrophins are important in the development and maintenance of various neurons. However, the expression of these growth factors and LNGFR occurs in a variety of non-neuronal embryonic organs. We investigated the possible role of neurotrophins and their receptor in the differentiation of embryonic kidney tubules.

In situ hybridization showed a transient expression of LNFGR in the nephrons of the embryonic metanephric kidney. Antisense oligonucleotides to LNGFR mRNA completely blocked LNGFR expression and thereby kidney differentiation. Reverse transcription-PCR assay and RNA protection showed that three neurotrophins - NGF, BDNF, and NT-3- are expressed in the developing rat kidney . Their expression begins during the induction of the first nephrons and is developmentally regulated, but in a different manner. *In situ* hybridization reveals that these neurotrophins are transiently expressed by different cell types of the developing kidney. These data suggest the importance of the neurotrophic system for the differentiation of the embryonic kidney.

THE RECEPTOR FOR UROKINASE-TYPE PLASMINOGEN ACTIVATOR: REGULATION OF GENE EXPRESSION BY GROWTH FACTORS AND TUMOR PROMOTERS IN HUMAN CELL LINES.

Leif R. Lund, John Rømer, Ebbe Rønne, Vincent Ellis, and Keld Danø, Finsen Laboratory, Rigshospitalet, Strandboulevarden 49, DK-2100 Copenhagen Ø., Denmark.

The receptor for urokinase (u-PAR) is a key molecule in regulating plasminogen-mediated extracellular proteolysis and tissue destruction. In order to gain insight into the molecular mechanisms involved in in vitro regulation, we have compared the cell-specific expression and the effect of transforming growth factor-ß type 1 (TGF-ß1) on the expression of u-PAR mRNA in 10 different cell lines. The basal expression of u-PAR mRNA as well as its response to TGF-ß1 varied strongly between the different cell lines; However, 5 out of the 10 cell lines responded to TGF-ß1 by an increase in the u-PAR mRNA level. Among these, A549 cells were selected for a detailed elucidation of the molecular mechanisms involved in TGF-ß1 regulation of u-PAR mRNA expression. TGF-ß1 caused in A549 cells an early increase in u-PAR mRNA level, with a maximal 15-fold enhancement after 24 hour of treatment. The protein synthesis inhibitors cycloheximide and anisomycin also increased the level of u-PAR mRNA in a time- and dose-dependent fashion and, when both cycloheximide and TGF-ß1 were used, an additive effect was seen. Nuclear run-on experiments demonstrated a moderate (3-fold) increase in the u-PAR gene transcription rate after exposure of the cells to TGF-ß1 for 3 hours compared to a 12-fold increase in the mRNA level. mRNA stability experiments showed that TGF-ß1 treatment also increased the halflife of u-PAR mRNA 3-fold. Epidermal growth factor also strongly increased the u-PAR mRNA level in A549 cells in a dose- and time-dependent manner, like treatment with the tumor promoters phorbol myristate acetate and Okadaic Acid. In conclusion, these studies suggest that synthesis of u-PAR is regulated by a varity of growth factors and tumor promoters, like the other components of the plasminogen activation system. The pattern of regulation of u-PAR and of the other components of the plasminogen activation system appears to be different in different cell types. Further studies are required to elucidate the detailed molecular mechanisms behind cell-specific expression and regulation by hormones, growth factors and cytoqkines in vitro. In situ hybridization studies are necessary for a clarification of co-expression of growth factors and components of the plasminogen activation system in biological systems, involving tissue destruction; e.g. cancer invasion and wound healing.

RETINOIC ACID (RA), ACTIVIN, LIF, AND A NOVEL FGF-LIKE FACTOR INTERACT TO REGULATE THE DIFFERENTIATION OF EMBRYONIC CARCINOMA (EC) AND EMBRYONIC STEM (ES) CELLS.

Christine Mummery, Danny Huylebroeck*, Wouter van Inzen, Hans Slager, Janny van den Eijnden-van Raaij. Hubrecht Laboratory, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands and *Innogenetics SA, B-9052 Zwijnaarde, Belgium.

Among the first differentiation steps during mouse development are the formation of endoderm and mesoderm. Mesoderm begins to form at about 6.5d p.c. from the primitive ectoderm in a region adjacent to the visceral endoderm. EC and ES cells are frequently used as model systems for studying these early differentiation events. We have defined culture conditions for these cells in terms of exogenous retinoids, present at low concentrations in the fetal calf serum (FCS) of their media, by passing the FCS over activated charcoal (DCC-FCS). When DCC-FCS replaces FCS, spontaneous differentiation of one pluripotent EC cell line, P19, grown as an aggregate is completely eliminated; the cells become exquisitely sensitive to RA, forming mesoderm (beating muscle) at 10^{-9} M, neurons at 10^{-8} M and primarily endoderm at 10^{-7} M (Mummery et al., 1991, Diff.46:51). Further, under these conditions a new FGF-like factor secreted by visceral endoderm-like cells induces differentiation of P19 EC to endoderm and mesoderm; TGFβ promotes the mesoderm-inducing activity of this factor. By contrast activin A, a member of the TGFβ family that induces mesoderm formation in the amphibian embryo, inhibits P19 differentiation entirely (Van den Eijnden-van Raaij et al., 1991, Mech. of Dev.33:157). ES cells also become more sensitive to RA in DCC-FCS supplement ed media forming similar cell types as P19 at each RA concentration. Leukemia Inhibitory Factor (LIF) largely inhibits this differentiation but is permissive for the formation of endoderm. These effects as well as expression data for some of these factors during the differentiation of stem cells and in embryos will be presented . The results of these studies indicate that factors such as those described may interact during development and that it is probably the subtle balance between them that finally determines the state of differentiation of a particular cell in the embryo.

THE BINDING OF VASCULAR ENDOTHELIAL GROWTH FACTOR TO ITS RECEPTORS IS DEPENDENT ON CELL SURFACE ASSOCIATED HEPARIN-LIKE MOLECULES,

Gera Neufeld, Hela Gitay-Goren and Shay Soker, Department of Biology, Technion-Israel Institute of Technology, Haifa, 32000, Israel

Vascular endothelial growth factor (VEGF) induces the proliferation of endothelial cells, and is a potent angiogenic factor that binds to heparin. We have therefore studied the effect of heparin upon the interaction of VEGF with its receptors. Heparin, at concentrations ranging from 0.1 to 10 μg/ml, strongly potentiated the binding of ^{125}I-VEGF to its receptors on endothelial cells. Scatchard analysis of ^{125}I-VEGF binding indicates that 1 μg/ml heparin induces an 8-fold increase in the apparent density of high affinity binding sites for VEGF, but does not affect significantly their affinity towards VEGF. Cross-linking experiments showed that heparin potentiates strongly the formation of the 170, 195 and 225 kDa ^{125}I-VEGF/receptor complexes on endothelial cells. At high ^{125}I-VEGF concentrations (4 ng/ml), heparin enhanced preferentially the formation of the 170 and 195 kDa complexes. Preincubation of the cells with heparin, followed by extensive washes, produced a similar enhancement of subsequent ^{125}I-VEGF binding. The binding of ^{125}I-VEGF was completely inhibited following digestion of endothelial cells with heparinase, and could be restored upon the addition of exogenous heparin to the digested cells. The enhancing effect of heparin facilitated the detection of VEGF receptors on cell types which were not known previously to express such receptors. Our results suggest that cell surface associated heparin-like molecules are required for the interaction of VEGF with its cell surface receptors.

FGF RECEPTOR GENES ARE DIFFERENTIALLY EXPRESSED IN EMBRYONIC TISSUES DURING LIMB FORMATION AND ORGANOGENESIS IN THE MOUSE, Kevin G. Peters, Sabine Werner, Guofen Chen, David Ornitz* and Lewis T. Williams, Program of Excellence in Molecular Biology, Howard Hughes Medical Institute, Department of Medicine and the Cardiovascular Research Institute, University of California, San Francisco, CA 94147. * Department of Genetics, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115

The FGFs interact with a family of at least four closely related receptor tyrosine kinases that are products of individual genes. To investigate the role of FGFs in the growth and differentiation of embryonic tissues and to determine whether the individual FGF receptor genes might have specific functions, we compared the localization of mRNA for three FGF receptor genes, FGFR1 (the *flg* gene product) FGFR2, (the *bek* gene product) and FGFR3, during limb formation and organogenesis in mouse embryos (E9.5-E16.5). FGFR1 and FGFR2 were differentially expressed in mesenchyme and epithelium respectively. FGFR1 was expressed diffusely in mesenchyme of limb buds, somites and organ rudiments. In contrast, FGFR2 was expressed predominantly in the epithelial cells of embryonic skin and of developing organs. FGFR3 expression was limited to the developing CNS and to the cartilage rudiments of developing bone. The differential expression of the FGF receptor genes suggests that they are independently regulated and that they mediate different functions of FGFs during development.

DIFFERENTIAL HLH FAMILY GENES EXPRESSION IN L5 MYOBLASTS BY 3-DEAZAADENOSINE. Sigfrido Scarpa, Marco Lucarelli and Roberto Strom. Dipartimento di Biopatologia Umana e I Istituto di Clinica Chirurgica. Universita' di Roma "La Sapienza". Rome, Italy.

In previous work we showed a stimulatory effect of 3-deazaadenosine (3-DZA) on the differentiation of L5 myoblasts[1], exerted during the first 48 hours of culture. In this work we tested the expression of HLH family genes, regulating myoblast differentiation, after 3-DZA treatment. Northern hybridization with MyoD[2] and myf-4[3] probes was therefore performed under low stringency conditions in different L5 myoblast clones under fusing (1% FCS) and non-fusing (10% FCS) conditions, in the presence and absence of 3-DZA. Cultures treated with 3-DZA showed a conspiquous increase in the expression of these genes. This increase corresponded to additional myotubes formation and appearance of differentiation biochemical markers. The expression pattern of HLH family genes varied between clones, in relation to their differentiative ability. The effect exerted by 3-Deazaaristeromycin (3-DZari), another adenosine derivative, on the expression of HLH family genes was similar to the 3-DZA one, although 3-DZari did not correlate as closely as 3-DZA to differentiation. Some expression of HLH family genes appeared also in non-fusing medium additioned with 3-DZA or 3-DZari, although the expression was lower than in fusing medium and probably did not suffice to start the myogenic program. The most intriguing observation was the appearance of a second band in northern hybridizations, in some clones, and only in the presence of 3-DZA.

[1]-Proc.Natl.Acad.Sci.USA (1984) 81,3064. [2]-Cell (1987) 51,987. [3]-EMBO J. (1989) 8, 701.

SERUM RESPONSE FACTOR AND F-ACT-1/MAPF1 ARE CANDIDATE TRANS-ACTING FACTORS FOR BASAL AND GROWTH FACTOR-DEPENDENT TRANSCRIPTION OF THE SKELETAL α-ACTIN GENE IN CARDIAC MUSCLE CELLS. MD Schneider*†§, WR MacLellan*, T-C Lee†§, RJ Schwartz†§, TG Parker*. *Molecular Cardiology Unit, Depts. *Medicine, †Cell Biology, and §Molecular Physiology & Biophysics. Baylor College of Medicine, Houston, TX 77030 USA.

The skeletal α-actin gene (SkA) serves as a prototype of the genes active in embryonic but not adult myocardium, whose expression is reinduced by mechanical load and other trophic signals. In cardiac muscle, SkA transcription is subject to positive and negative regulation by basic and acidic fibroblast growth factors (bFGF, aFGF), respectively. To define DNA-protein interactions controlling SkA transcription in cardiac muscle cells, rat cardiac myocytes were transfected with mutations in the SkA promoter, including 3 motifs resembling the fos serum response element (SRE). The upstream, central, and proximal SRE each contributed to basal expression in cardiac myocytes. As isolated elements upstream from a neutral promoter, both the proximal SkA SRE (SRE1) and fos SRE were expressed at levels up to one-third that of the SkA promoter (nt -202/-11), in agreement with previous findings in skeletal muscle cells; neither was expressed in quiescent cardiac fibroblasts. Basic FGF augmented SRE1-CAT activity, yet aFGF produced no change; the fos SRE was induced by both. The transcriptional and mitogenic actions of aFGF were both contingent on the presence of a putative nuclear translocation motif. In cardiac myocyte extracts, two predominant DNA-binding proteins contact SRE1: serum response factor (SRF) and its competitive inhibitor, F-ACT-1/MAPF1. Binding of both was extinguished by a double transversion (GG→CC) of the 3' arm of the SRE core. A contiguous mutation of the F-ACT-1 site alone (retaining the SRE core) abolished F-ACT-1 binding but preserved recognition by SRF. Thus: (1) The SkA SRE1 and fos SRE each suffice for tissue-specificity in cardiac myocytes. (2) Sequences distinct from the c-fos SRE enable the SkA SRE1 to discriminate between signals generated by bFGF versus aFGF, in cardiac muscle cells. (3) Elements alternative to SRE1 alone are mandatory for aFGF to suppress the SkA promoter. (4) Differences in nuclear targetting are a potential basis for divergent effects of aFGF and bFGF on cardiac growth and SkA expression. (5) SRF and F-ACT-1/MAPF1 are candidate trans-acting factors for basal transcription of the SkA promoter, FGF signal transduction, or both, in cardiac muscle cells.

EXPRESSION OF RETINOBLASTOMA GENE IN DIFFERENTIATING EMBRYONAL CARCINOMA (P19) CELLS. R.S. Slack, P.A. Hamel, T.S. Bladon and M.W. McBurney. Univ. of Ottawa, Dept. Medicine, Cancer Research Program, Ottawa, Ont. Canada K1H 8M5 and The Hospital for Sick Children, Toronto, Ont. Canada M5G 1X8

The expression of the retinoblastoma (RB) gene was studied in P19 cells induced to terminally differentiate following treatment with retinoic acid (RA) and DMSO. Steady-state levels of RB mRNA were measured by RNase protection assays and RB protein was analysed by Western blotting. In rapidly dividing embryonal carcinoma (EC) cells very little RB mRNA and protein were detected. Following treatment with 1 uM RA P19 cells differentiate into neuroectoderm and a marked increase in RB mRNA was observed by 24 hours. These levels continued to rise, reaching a peak by 4-6 days. By 10 days the mRNA levels began to decrease. Western blotting showed a similar trend; very little RB protein was seen in untreated cells, but a marked increase was noted by 24 hrs, and reached a peak by 4-6 days. RAC65 cells are a clone of P19 cells selected for their inability to differentiate in response to RA. These cells carry a defective gene for the RA receptor - α and are unable to undergo neuronal differentiation in response to RA treatment. RA treated RAC65 cells did not contain elevated levels of RB mRNA indicating that differentiation is required to bring about the observed increase in RB expression following RA treatment. When treated with 1% DMSO, P19 cells differentiate to form mesenchymal cells including beating cardiac muscle. There was only slight increases in the levels of RB mRNA, and no peak at 4-6 days. Western blotting revealed similar results. In P19 cells therefore, the levels and pattern of RB expression is dependent on the differentiation state as well as the resultant cell type formed. Immunofluorescence is currently being carried out to more precisely define the cell type and stage of neural differentiation in which the elevated level of RB expression is found.

RECOMBINANT NUCLEAR FACTOR 1 AND OCTAMER TRANSCRIPTION FACTORS MEDIATE BASAL AND HORMONE RECEPTOR INDUCED TRANSCRIPTION FROM THE MMTV PROMOTER *IN VITRO*, Emily P. Slater, Thomas Preiss, Peter C. van der Vliet and Miguel Beato, Institut für Molekularbiologie und Tumorforschung, Marburg, Germany and University of Utrecht, Netherlands

The promoter of the Mouse Mammary Tumor Virus (MMTV) is well known in the context of hormonally regulated gene expression. In addition to the binding sites for steroid hormone receptors, those for Nuclear Factor One (NF-1) and Octamer Transcription Factor 1 (OTF-1) are also known to be important for transcription from the MMTV promoter. The influence of these sequence motifs was tested using highly purified NF-1 and OTF-1 or OTF-2 produced via a vaccinia virus expression system in *in vitro* transcription experiments. The templates comprised the MMTV-promoter region from position -270 to -20 with respect to the start of transcription, fused to an artificial region with the statistical composition C_2AT as the transcribed region. Depletion of HeLa nuclear extracts from NF-1 or OTF-1 was performed with specific DNA-affinity matrices. The recombinant NF-1, upon addition to the depleted extract was able to mediate transcription from the MMTV promoter to the extent of the normal extract. Experiments combining NF-1-depleted extracts with purified steroid hormone receptors *in vitro* confirm the finding that stimulated transcription can take place in the absence of NF-1. The ability of a recombinant NF-1 to stimulate transcription demonstrates that NF-1 is indeed a transcription factor for MTMV. Finally, these results show that an appropriate *in vitro* transcription system is helpful in studying the molecular mechanisms of gene regulation.

E-CADHERIN AS AN INVASION-SUPPRESSOR PROTEIN IN EPITHELIAL TUMOR CELLS, Kris Vleminckx[1], Luc Vakaet Jr.[2], Marc Mareel[2], Walter Fiers[1] and Frans Van Roy[1], [1]Laboratory of Molecular Biology and [2]Laboratory of Experimental Cancerology, University of Ghent, Ghent, Belgium

We previously described that the cell-cell adhesion molecule E-cadherin can act as a suppressor of invasion in epithelial tumor cells (Vleminckx et al., 1991, Cell 66, 107). A cDNA encoding E-cadherin was transfected into highly invasive epithelial tumor cell lines of either dog kidney or mouse mammary gland origin. Transfectants with a homogeneously high expression of E-cadherin showed a reproducible loss of activity in two types of in vitro invasion assays. Invasiveness of these transfectants could be reinduced specifically by treatment with anti-E-cadherin antibodies. Alternatively, a plasmid encoding E-cadherin-specific antisense RNA was introduced into noninvasive ras-transformed cells with endogenous E-cadherin expression. The resulting down-regulation, albeit partial, rendered the cells invasive.

In vivo, cells expressing E-cadherin, either from the endogenous or from the exogenous gene, formed partly differentiated tumors with E-cadherin expressed in differentiated areas only (Mareel et al., 1991, Int. J. Cancer 47, 922). Using in situ hybridization, we aim at clarifying at what level E-cadherin is down-regulated. Also, effort is being made in order to identify host factors which influence E-cadherin expression.

Control of Kidney Tubule Formation in Matrigel by Growth Factors, Mary L. Taub and Ho Jae Han, Department of Biochemistry, School of Medicine and Biomedical Sciences, State University of New York, Buffalo, NY 14214

The mechanisms controlling renal tubule formation in a reconstituted extracellular matrix derived from the EHS tumor (Matrigel) are being investigated. Previously, we have shown that primary rabbit kidney proximal tubule cells retain many differentiated functions when cultured in serum free medium supplemented with insulin, transferrin and hydrocortisone. When primary rabbit kidney proximal tubule cells are put into Matrigel in serum free medium, tubule formation occurs. Matrigel contains growth factors, as well as extracellular matrix proteins. When growth factors are removed from Matrigel by means of ammonium sulfate precipitation . Renal tubule formation no longer occurred, unless either EGF or TGF alpha was added to the culture medium. TGF beta further stimulated renal tubule formation in the presence of either EGF or TGF alpha. We have investigated the involvement of various signal transduction pathways in renal tubule formation, usign pharmacologic agents. While the phorbol ester TPA stimulated tubule formation in Matrigel, 8 bromocyclic AMP caused cysts, rather than tubules to formation. These results thus indicate the involvement of the protein kinase C, rather than the cyclic AMP pathway in the sequence of events leading to tubule formation. Stimulatory effects of pertussis toxin and okadaic acid on renal tubule formation were also observed, indicating the invovlment of G proteins and particular protein phosphatase(s) as well. These regulatory factors may stimulate renal tubule formation in vitro either by increasing the overall state of differentiation of the renal cells, and/or by increasing the ability of cells which are already differentiated to form tubules within this extracellular matrix.