GROWTH FACTORS AND THEIR RECEPTORS: GENETIC CONTROL AND RATIONAL APPLICATION

Organizers: Russell Ross, Tony Hunter and Antony Burgess
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Growth Factors and Their Receptors: Genetic Control and Rational Application

Structure and Function of Growth Factors and Their Receptors - I

C 001 TRANSFORMING GROWTH FACTOR-ALPHA: STRUCTURE EXPRESSION AND BIOLOGICAL ACTIVITIES, Rik Derynck, Patricia B. Lindquist, Timothy S. Bringman M.E. Winkler*, Department of Molecular Biology, Genentech, Inc., South San Francisco CA 90480. *Department of Protein Biochemistry.

cDNA analysis has indicated that human TGF-alpha is encoded as an internal part of a 160 amino acid precursor. This cDNA has been overexpressed in chinese hamster ovary cells and the resulting proteins were analyzed by immunoprecipitation. At least two types of glycosylated TGF-alpha are secreted in addition to an unglycosylated 50 amino acid form. These different forms are derived by alternative proteolytic cleavage from a transmembrane precursor. The cytoplasmic segment of this precursor is palmitoylated at one or more cysteine residues. The function of this palmitate linkage is unclear. Immunofluorescent examination of TGF-alpha producing cells shows that TGF-alpha or related molecules are specifically located at the cell surface and that interaction with specific anti TGF-alpha antibodies can result in internalization of the ligand-antibody complex.
The 50 amino acid form was expressed in E. coli. Purified and properly folded TGF-alpha was biologically active. Comparison of TGF-alpha with EGF shows that both molecules interact with the same receptor and exert similar effects. However, TGF-alpha appears to be a superagonist compared to EGF in several biological systems. It can be postulated that TGF-alpha and EGF may interact with the common receptor in a different way which could then be a basis for the observed differences in biological activities.

C 002 MULTIPLE CLASSES OF PDGF RECEPTOR RECOGNIZE DIFFERENT ISOFORMS OF PDGF Charles E. Hart*†, John W. Forstrom*, James D. Kelly*†, Robert A. Smith*, Russell Ross†, Mark J. Murray*, and Daniel F. Bowen-Pope†, (*)ZymoGenetics, Inc., 2121 N. 35th Street, Seattle, WA 98103 (†) Department of Pathology (SM-30), University of Washington, Seattle, WA 98195.

Platelet-derived growth factor (PDGF) is a potent mitogen for a variety of mesenchymal cells. In addition to its mitogenic activities, PDGF has been reported to stimulate both early and late events following its addition to cells in culture. Previous studies involving PDGF have been based upon the premise that there is only a single cell-surface receptor that binds all three isoforms of PDGF (AA, BB and AB). We have recently uncovered the existence of two populations of PDGF receptor that can be distinguished by their ligand binding specificity. We have designated these two classes of receptor as the B-receptor due to its ability to bind only BB homodimer, and the A/B-receptor, due to the ability of AA, BB and AB dimers to bind to this receptor. We have detected the two classes of receptor on several cell types including human dermal fibroblasts, mouse 3T3 cells and both human and rat smooth muscle cells. The B-receptor appears to be expressed at 10 fold greater levels than the A/B-receptor. We have also observed that it is the B-receptor which is responsible for the bulk of PDGF receptor phosphorylation.

It should be noted that most studies of 125I-PDGF binding have been done with PDGF iodinated by procedures that specifically label tyrosine residues. Since only the A-chain of PDGF contains tyrosine, only AA and AB dimers would be labeled in a mixture of PDGF purified from platelets. Since BB homodimers would not be labeled in this mixture the B-receptor would go undetected. Thus, when 125I-PDGF has been used as a probe for the PDGF receptor it has been the A/B-receptor being monitored while it is the B-receptor that has been observed when using phosphorylation as a method of detection.

It is not known whether these two receptor classes represent two distinct gene products, different post-translational modifications of a single gene product, or functional differences in a single molecule that result from different patterns of association with other cell-surface proteins. The identification of two classes of PDGF receptor may shed some light on why variable results have been obtained when specific properties of PDGF have been examined. Depending upon the composition of the PDGF used, either one or both of the receptor classes may be stimulated.

C 003 THE PROTEIN TYROSINE KINASE ACTIVITY OF EGF-RECEPTOR IS ESSENTIAL FOR SIGNAL TRANSDUCTION, Joseph Schlessinger*, Anne Marie Honegger*, Tom J. Dull, and Axel Ullrich, *Rorer Biotechnology, Inc., Rockville, MD 20850 and Genentech, Inc., South San Francisco, CA 94080

Cultured NIH-3T3 cells devoid of endogenous EGF-receptors were transfected with cDNA constructs encoding either the human EGF-receptor or an EGF-receptor mutant in which Lys721, a key residue in the ATP binding site, was replaced with an alanine residue. The mutant receptor was properly processed, and it displayed both high and low affinity surface binding sites. Similar to the wild type receptor, phorbol ester (TPA) abolished the high affinity binding sites. Furthermore, the initial rate of EGF internalization was similar for wild type and mutant EGF-receptors. However, unlike the wild type receptor, the mutant receptor did not display EGF-stimulatable protein tyrosine kinase activity, and was unable to undergo autophosphorylation or to phosphorylate exogenous substrates. Surprisingly, the mutant receptors were not down-regulated, but appeared to recycle in transfected cells. These data suggest that degradation of normal EGF-receptors after endocytosis is due to the kinase activity endogenous to this receptor (1). A single amino acid substitution rendered a "down-regulated" receptor into a receptor that can recycle from cytoplasmic compartment back to the cell surface (1). The addition of EGF to cells expressing wild type receptors caused the stimulation of various responses including, enhanced expression of proto-oncogenes c-fos and c-myc, "foci formation" and stimulation of DNA synthesis. However, in cells expressing mutant receptors EGF was unable to stimulate these responses, suggesting that the tyrosine kinase activity is essential for EGF-receptor signal transduction(2).

1. Honegger, A.M., Dull, T.J., Felder, S., Van-Obberghen, E., Bellot, F., Szapary, D., Schmidt, A., Ullrich, A., and Schlessinger, J. (1987). Point mutant at the ATP binding site of EGF-receptor abolishes protein tyrosine kinase activity and alters cellular routing. Cell, in press. Oct issue.
2. Honegger, A.M., Szapary, D., Schmidt, A., Lyall, R., Van-Obberghen, E., Dull, T.J., Ullrich, A., and Schlessinger, J. (1987). A mutant EGF-receptor with defective protein tyrosine kinase is unable to stimulate proto-oncogene expression and DNA synthesis. Mol. Cell Biol., in press. Dec. issue.

C 004 STRUCTURAL DETERMINANTS OF PDGF RECEPTOR FUNCTION, L.T. Williams, J.A. Escobedo, S.R. Coughlin, M.T. Keating, P.L. Orchansky, W.J. Fantl, and P.L. Lee, Univ. of Calif. Howard Hughes Med. Inst., San Francisco, CA., 94143.

The extracellular region of the mouse PDGF receptor consists of a series of five immunoglobulin-like domains that are involved in PDGF binding. The intracellular region consists of a tyrosine kinase domain (TK1) containing the ATP binding site, a kinase insert domain (KI) that separates tyrosine kinase domains, a second tyrosine kinase domain (TK2) and a carboxy terminal region (CT) of unknown function. A full-length human cDNA clone is highly homologous to the mouse sequence and has the same domain organization. Genomic clones show that the immunoglobulin-like domain boundaries coincide with intron and exon junctions.

The cDNA sequences for the human and mouse receptor have been expressed in their wild-type and mutated forms in cells that normally lack PDGF receptors. Expressed wild-type receptor binds all forms of PDGF, including AA and BB forms produced in a yeast expression system and AB heterodimer purified from human platelets. When activated by PDGF, the transfected receptor mediates a diverse group of early cellular responses as well as the "late" responses of DNA synthesis and cell division. The transfected receptor also undergoes a PDGF-induced conformational change which is detected by a conformation-specific antibody directed against cytoplasmic sequences. We have expressed the external domain of the receptor without the transmembrane or cytoplasmic sequences. This 70 kDa receptor fragment binds PDGF with high affinity, indicating that the immunoglobulin-like domains are sufficient for high affinity binding. A form of the receptor lacking the 90 amino acid KI region has also been expressed. This KI mutant mediates the early PDGF-induced cellular responses including binding, receptor internalization, receptor tyrosine phosphorylation, phosphatidylinositol turnover, pH change, and increase in calcium, but fails to mediate a proliferative response to PDGF. A form of the receptor that lacks kinase activity because of a point mutation at the ATP binding site, binds PDGF with high affinity but does not mediate any of the cellular responses to PDGF including the PDGF-induced conformational change in the receptor. This form of the receptor, however, is down regulated in response to PDGF. Other mutants that have deletions in the extracellular or carboxy terminal domains or alterations in the tyrosine phosphorylation sites have also been characterized. Taken together these studies show that the PDGF receptor binds multiple forms of PDGF through immunoglobulin-like receptor domains, and then undergoes a conformational change that is associated with an increase in tyrosine kinase activity that is essential for all of the cellular responses to PDGF except for receptor internalization.

Structure and Function of Growth Factors and Their Receptors - II

C 005 POSSIBLE INVOLVEMENT OF PDGF-LIKE GROWTH FACTORS IN AUTOCRINE STIMULATION OF CELL GROWTH, C.-H. Heldin[1], A. Hammacher[1], A. Ostman[1], C. Betsholtz[2] and B. Westermark[2], [1]Ludwig Institute for Cancer Research, Box 595, Biomedical Center, S-752 23 Uppsala, [2]Dept of Pathology, University Hospital, S-751 85 Uppsala, Sweden.

Platelet-derived growth factor (PDGF), is a dimer of two polypeptide chains, A and B. Examples have been found of A homodimers (e.g. a factor secreted by a human osteosarcoma cell line), B homodimers (e.g. the transforming product of simian sarcoma virus) and AB heterodimers (e.g. PDGF purified from human platelets). The frequent expressions of PDGF-like growth factors in normal, as well as transformed, cells suggest that such factors have roles in autocrine and paracrine stimulation of cell growth. For instance, out of 23 investigated human glioma cell lines, 23 and 16 expressed mRNA for the A chain and the B chain, respectively. PDGF-like factors secreted by one of these glioma cell lines were purified and characterized. Two different PDGF-like factors were resolved by HPLC reverse phase; one was identified as an A chain homodimer, whereas the other contained at least one B chain. All dimeric forms of PDGF binds to human fibroblasts and competes, at least partially, with ^{125}I-PDGF for binding. However, A homodimers have a lower mitogenic activity compared to B chain containing dimers. This finding, in combination with the observation that A chain homodimers remain preferentially cell associated suggests that different PDGF dimers have different functions in vivo.

C 006 FORCES INVOLVED IN EPIDERMAL GROWTH FACTOR RECEPTOR BINDING, Kevin H. Mayo, Department of Chemistry, Temple University, Philadelphia, PA 19122

Knowledge of the mechanism of epidermal growth factor (EGF) action requires an understanding of the forces involved in the interaction between the protein hormone and its cell receptor. After all, it is primarily the receptor binding process which triggers the cascade of cellular events that ultimately can lead to cell proliferation. In this paper, we present experimental data from several sources, i.e., one-dimensional and two-dimensional proton nuclear magnetic resonance (NMR) spectroscopy, photo-chemically induced dynamic nuclear polarization (CIDNP) proton NMR spectroscopy, and iodine-125 labelled EGF receptor binding kinetic studies, aimed at attaining some understanding of these forces. Proton NMR spectroscopic studies have yielded solution structure information on EGF from three species, i.e., mouse, human and rat. Since these three species can compete with eachother for cell receptor binding, a common receptor binding domain on EGF is likely. Comparison of these EGF solution conformations has indicated a number of conserved structural regions, one of which may be the receptor binding domain. The spatial arrangement of surface exposed amino acid residues at this binding domain partly defines those forces necessary for efficient receptor association and, probably, its specificity. The complement to these forces must arise from the receptor structure and its cell surface environment. The kinetics and thermodynamics of iodine-125 labelled EGF receptor binding to fibroblast cells, therefore, have also been investigated; these studies are used to probe the energetics of this protein-protein, i.e., EGF and receptor, interaction in a real cell situation. Photo-CIDNP NMR studies of EGFs have identified protein surface exposed aromatic residues which, in the presence of amphipathic micelles, become masked from the polar solvent by the hydrophobic micellar core; different species of EGF interact with these micelles in a similar way. Interestingly, iodine-125 labelled EGF receptor binding data and these micelle interaction data seem to render complementary results. Integration of these data may help define those forces involved in EGF receptor binding and aid in possibly designing more potent growth factors, prehaps by genetic engineering.

This research was supported in part by the National Institute of General Medical Sciences (GM-34662) and by the North Atlantic Treaty Organization (NATO) Scientific Affairs Division (RG.85/0424).

C 007 STRUCTURE AND FUNCTION OF ACIDIC FIBROBLAST GROWTH FACTOR, Kenneth Thomas, Guillermo Gimenez-Gallego, Sagrario Ortega, Jerry DiSalvo, Denis Soderman, David Linemeyer, Linda Kelly and John Menke, Dept. of Biochemistry and Molecular Biology, Merck Research Laboratories, Rahway, New Jersey 07065.

Acidic fibroblast growth factor (aFGF) is a protein mitogen for a wide variety of cells in culture, including vascular endothelial cells. In vivo the purified protein induces blood vessel growth and accelerates wound healing. Bovine and human aFGFs have been sequenced. As purified, both proteins are 140 amino acids long with only 11 substitutions between them. Although bovine and human aFGFs have three Cys residues, only Cys_{16} and Cys_{83} are common. The unique third Cys residues are at position 47 in bovine and 117 in human aFGFs.

This arrangement of one disulfide and one free cysteine residue in each aFGF could facilitate inactivation of the mitogen either by intramolecular disulfide reshuffling to produce one or both incorrectly disulfide bonded forms or by generation of intermolecular disulfides between aFGF molecules or with other proteins. Potentiation of the mitogenic activity of aFGF by heparin might, therefore result from preferential complexation of the correctly disulfide bonded protein molecules with the sulphated oligosaccaride, thereby pulling the conformation equilibrium in favor of the fully active form. Furthermore, the equilibrium between correctly and incorrectly disulfide bonded forms would be expected to be influenced by the location of the free Cys residue. In fact, heparin activates bovine aFGF about 4-fold and human aFGF over 100-fold in a Balb/c 3T3 mitogenesis assay.

To test the hypothesis that incorrectly formed disulfide bonds decrease the activity of aFGF in the absence of heparin, each of the three Cys residues of human recombinant aFGF were converted by site-directed mutagenesis to Ser residues. Changing either of the two conserved Cys residues (16 and 83) proposed to be involved in a disulfide bridge in the active conformation diminishes the activity of the protein in the absence of heparin even more than that observed for wild-type human r-aFGF. In contrast, modification of Cys_{117} abolished 90% of the heparin dependence of the wild-type form. Therefore, these data support the hypothesis that complex formation between heparin and aFGF minimizes formation of incorrectly formed intra- or intermolecular disulfide bonds.

Structure and Function of Growth Factors and Their Receptors - III

C 008 CHARACTERIZATION OF THE BIOLOGIC PROPERTIES OF HUMAN INTERLEUKIN-3, Yu-Chung Yang, Robert G. Donahue, Allan R. Mufson, Makio Ogawa*, and Steven C. Clark, Genetics Institute Inc., Cambridge MA 92140, and *Medical University of South Carolina and VA Medical Center, Charleston, SC 29403.

The molecular cloning of the human gene encoding interleukin-3 (IL-3) has enabled us to produce substantial quantities of this growth factor for analysis of its effects both in culture and in primate models. Because of its ability to support colony formation by many types of hematopoietic cells, IL-3 is also known as multi-lineage colony-stimulating factor. The IL-3-responsive target cells found in normal bone marrow overlap considerably with those responsive to another multi-lineage hematopoietic growth factor, granulocyte-macrophage colony-stimulating factor (GM-CSF). A direct comparison of the ability of these factors to support the proliferation of primitive blast cell colonies has revealed that IL-3 is more effective than is GM-CSF in generating human blast cell colonies. Furthermore, the resulting blast cell colonies from the IL-3-supported culture can be replated with higher efficiency than those from the GM-CSF cultures. These studies indicate that although the pool of target cells for the two factors overlap considerably, the IL-3-responsive cells include a population of more primitive stem cells with a higher proliferative potential than those found in the pool of GM-CSF-responsive progenitors. The effects of IL-3 on stimulating hematopoiesis in normal monkeys are consistent with the results of the clonogenic culture systems. Continuous intravenous infusion of recombinant IL-3 for 7 days resulted in a modest elevation in circulating white cell count, typically reaching a maximum of 2-3 fold 3 days after stopping the administration. The elevation was due to increased numbers of neutrophils, eosinophils, monocytes and lymphocytes. In comparison, GM-CSF has been found to elicit a rapid leukocytosis in the same model, typically reaching 5 fold within 2-3 days after the start of a 7 day infusion. To test the possibility that the IL-3 effects may be more restricted to earlier progenitors, we pretreated the animals for 6 days with IL-3 to increase the progenitor pool size then administered a suboptimal dose of GM-CSF to support the maturation of the IL-3-generated immmature cells. As expected, the elevation in circulating white cell count of the IL-3-pretreated animals to the minimal dose of GM-CSF was significantly greater to that in the those animals not receiving IL-3. These studies suggest that IL-3 may not be sufficient to support the complete developmental program of many of the hemopoietic progenitors and that this insufficiency is likely to be due to the acquisition of dependency of the developing cells to other growth factors such as GM-CSF.

C 009 INTERLEUKIN 1 SYNTHESIS, SECRETION, AND ACTION, Steven B. Mizel, Jill Suttles, Judith Giri, Fumihiko Shirakawa, Sho Ishikawa, Marcio Chedid, and Lucy Minnich, Department of Microbiology & Immunology, Wake Forest University Medical Center, Winston-Salem, NC 27106.

Previous studies have demonstrated that murine IL 1α is synthesized as a 33,000 m.w. precursor which is processed to the 17-19,000 m.w. forms found in the culture supernatant of activated macrophages. We have found that the murine IL 1α precursor lakcs a cleavable signal sequence and does not undergo co-translational translocation across microsomal membranes in vitro. Culture supernatants of the murine macrophage cell line, $P388D_1$, or from normal peritoneal macrophages collected within 0.5-3 hrs after stimulation contained the 33,000 m.w. precursor as the predominant form of IL 1α. Over an 18 hr period, the level of low m.w. IL 1 increased as the secreted precursor was processed by extracellular and/or cell surface-associated proteolytic enzymes. The rate of IL 1α precursor release was dramatically enhanced by calcium ionophores. The rapid release of IL 1 in response to calcium does not appear to be due to release of a membrane bound form of the protein, nor is there evidence that IL 1 is packaged and released from granules. The results of our studies indicate that the 33,000 m.w. precursor is secreted intact from activated macrophages via a novel, possible calcium-dependent pathway.

Following its release from cells, IL 1 specifically binds to a high-affinity receptor (Kd = 0.2nM) on responsive cells. The IL 1 receptor on mouse fibroblasts or human rheumatoid synovial cells exhibits an apparent m.w. of approximately 75-85,000. Using crude membrane preparations from Swiss 3T3 mouse fibroblasts we have found that the binding of IL 1 to its receptor is quite stable. When IL 1 was bound to membranes for 1 hr at 37°C and the membranes were washed and returned to 37°C, little if any dissociation of the IL 1 from its receptor was noted after 4 hrs. In whole cells, the IL 1-receptor complex is internalized at 37°C. The internalized receptor on T cells does not appear to re-cycle to the plasma membrane. IL 1 remains intact for at least 6 hrs after its internalization. Over this same time period, a significant portion of the internalized IL 1 accumulates in the nucleus.

Growth Factors: Intracellular Events

C 010 SPECIFIC ASSOCIATION OF AN 85 KD TYROSINE PHOSPHORYLATED PROTEIN AND A NOVEL PHOSPHATIDYLINOSITOL KINASE WITH CERTAIN GROWTH REGULATING TYROSINE KINASES, Lewis Cantley*, Malcolm Whitman*, David Kaplan+, Brian Schaffhausen*+, Leslie Serunian*, Tracy Keller+, Morris White# and Thomas M. Roberts+, *Dept. of Physiology and *+Dept. of Biochemistry, Tufts University School of Medicine, Boston, MA 02111, #Joslin Diabetes Clinic, Boston, MA 02115 and +Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

An 85 KD phosphoprotein was found to coimmunoprecipitate with the polyoma middle T/pp60$^{c\text{-}src}$ complex from cells expressing transformation competent middle T antigen but not from cells expressing transformation defective mutants of middle T. This protein could be separated from the middle T/pp60$^{c\text{-}src}$ immunoprecipitate by a detergent and high salt extraction and reimmunoprecipitated with an antibody directed against phosphotyrosine. An identical phosphoprotein was immunoprecipitated from PDGF stimulated Balb 3t3 fibroblasts with the anti-phosphotryosine antibody. Phosphoamino acid analysis of the 85 KD protein from $^{32}PO_4$ labeled cells revealed the presence of phosphotyrosine and phosphoserine. A novel phosphatidylinositol (PI) kinase activity coprecipitated with the 85 KD phosphoprotein from both middle T transformed cells and PDGF stimulated cells. The appearance of the PI kinase activity in the anti-phosphotyrosine immunoprecipates correlated with the appearance of the 85 KD phosphoprotein, both becoming detectable within 15 seconds of PDGF addition and reaching a maximum after 5 minutes. The 85 KD protein copurified with the PI kinase activity on an anion exchange column which separated the PI kinase activity from the PDGF receptor. The PI kinase which copurifies with the 85 KD protein is distinct from the major PI kinase activity observed in fibroblast membranes with respect to size, enzymatic properties and effects of inhibitors. In addition the phosphatidylinositol phosphates produced from these two enzymes are chemically distinct suggesting a novel pathway for PI turnover in growth factor stimulated and transformed cells.

C 011 TARGETS FOR SIGNAL-TANSDUCING PROTEIN KINASES, Tony Hunter, Kathy Gould, Clare Isacke, Peter Van der Geer, and Ellen Freed The Salk Institute, P.O. Box 85800, San Diego, California 92138

Protein phosphorylation is a major mechanism whereby signals are transduced from external stimuli into cellular responses. Many growth factor receptors are ligand-stimulatable protein-tyrosine kinases (PTK), while other PTKs, such as $pp60^{c\text{-}src}$, located on the inner face of the plasma membrane have also been implicated in signal transduction. Protein kinase C (PKC), a phospholipid/Ca^{2+}-dependent DAG-regulated protein-serine kinase also binds to the inner face of the plasma membrane where it forms a response pathway for external stimuli that induce the turnover of PI. Among the substrates we have identified for both the EGF receptor and $pp60^{v\text{-}src}$ is p81, an 81 kDa protein localized to the core of surface microvillar spikes. Together with Tony Bretscher (Cornell), we have obtained cDNA clones for p81, and the sequence of these clones is being used to elucidate a function for p81, and to assign the Tyr phosphorylation sites prior to mutagenesis. Among the PKC substrates we have identified is a 180 kDa surface glycoprotein against which we have raised several monoclonal antibodies. p180 is present in coated pits, a location suggesting that p180 may be a receptor. We have purified p180 by immunoaffinity techniques and shown that is a substrate for PKC, which phosphorylates p180 at a single major site that is also phosphorylated when cells are treated with TPA. We have obtained primary sequence information on several p180 tryptic peptides and are now trying to obtain molecular clones of p180. There is a family of cell adhesion receptors that recognize the sequence Arg.Asp.Gly in their ligand, which includes the vitronectin and fibronectin receptors. We have found that the vitronectin receptor β subunit is phosphorylated at a single Ser when cells are treated with TPA. Purified vitronectin receptor is phosphorylated in vitro by PKC at the same Ser, but we do not know if this phosphorylation is of functional consequence.

The signal transducing protein kinases are themselves substrates for other protein kinases. For instance the EGF receptor and $pp60^{c\text{-}src}$ are both phosphorylated by PKC. PDGF treatment of quiescent fibroblasts induces rapid phosphorylation of 5% of $pp60^{c\text{-}src}$ on an unknown Tyr in its N-terminal 18K, causing a slight gel retardation, an increase in phosphorylation of Ser 12 of all $pp60^{c\text{-}src}$ molecule, due to activation of PKC by PDGF-induced PI turnover, and phosphorylation of the altered form of $pp60^{c\text{-}src}$ at 2 novel, but unassigned, Ser phosphorylation sites. These changes correlate with a 3-fold increase in $pp60^{c\text{-}src}$ kinase activity. We are presently trying to determine whether the N-terminal Tyr in $pp60^{c\text{-}src}$ is phosphorylated by the PDGF receptor itself, and whether this phosphorylation plays a role in the mitogenic response to PDGF.

C 012 *EARLY EVENTS ELICITED BY BOMBESIN AND OTHER GROWTH FACTORS IN QUIESCENT SWISS 3T3 CELLS*, **Enrique Rozengurt, Ian Zachary, James Sinnett-Smith, Eewa Nånberg, Penella J. Woll and Jonathan Millar, Imperial Cancer Research Fund, P O Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.**

Regulatory peptides which act in an autocrine or paracrine fashion on adjacent cells are increasingly implicated in the control of cell proliferation. The amphibian tetradecapeptide bombesin and mammalian peptides structurally related to bombesin including gastrin-releasing peptide (GRP) are potent mitogens for Swiss 3T3 cells. These peptides bind to high-affinity cell-surface receptors which are distinct from those of other mitogens for these cells. A surface protein in Swiss 3T3 cells with apparent Mr 75000-85000 has been identified by chemical cross-linking as a putative component for the bombesin/GRP receptor. The affinity labelled protein binds to wheat germ lectin-sepharose columns from which it can be eluted by N-acetyl-D-glucosamine. Receptor bound ^{125}I-GRP is internalised and extensively degraded by these cells. However in contrast to other growth factors, peptides of the bombesin family do not cause down-regulation of their specific cell-surface receptors. Following binding the peptides elicit a complex array of early biological responses including a) phosphorylation of the 80K cellular protein, which reflects the activation of protein kinase C in intact 3T3 cells, b) phosphoinositide breakdown and mobilisation of Ca^{2+} from an intracellular store, which leads to a transient increase in the concentration of cytosolic Ca^{2+} and Ca^{2+} efflux, c) stimulation of activity of the Na^+/H^+ antiport, d) transmodulation of EGF-receptor affinity, e) enhancement of cAMP accumulation and f) increase in the expression of the cellular oncogenes c-fos and c-myc. The peptides of the bombesin family not only provide a novel and valuable model for the elucidation of the signal transduction pathways underlying cellular proliferation but also may play a role as autocrine growth factors for human small cell lung cancer cells.

Growth Factors and Their Receptors: Genetic Control and Rational Application

Growth Factors: Modulation of Gene Expression - I

C 013 FIBROBLAST GROWTH FACTOR REGULATION OF SKELETAL MUSCLE GROWTH AND DIFFERENTIATION. Steve Hauschka, Jean Buskin, Jeff Chamberlain, Chris Clegg, Cyndy Gartside, Jim Jaynes, Jane Johnson, Pat Noel, Brad Olwin, and Jennifer Seed. Dept of Biochemistry, Univ. of Washington, Seattle, WA 98195.

Clonal analysis of the myogenic cell lineage during chick and human limb development has disclosed a variety of muscle colony-forming (MCF) cells. All MCF cells are FGF responsive although some will grow in the absence of FGF; others exhibit absolute dependence on FGF for either their clonal survival or their subsequent differentiation. In all cases FGF initially represses the onset of terminal differentiation. However, even in the continuous presence of FGF, differentiation eventually begins. This suggests that cell density or extracellular matrix components may trigger differentiation independently of FGF removal. In a model cell culture system using a permanent line of mouse skeletal muscle myoblasts (MM14) purified bovine acidic and basic FGF both stimulate proliferation and repress terminal differentiation. bFGF is about 30 times more potent than aFGF--with half-maximal activity in clonal assays of 1 and 30pM respectively. Addition of low or high molecular weight heparin in the 100ng-1mg/ml range inhibits bFGF-mediated effects whereas heparin stimulates aFGF-mediated effects in the 1ng-1ug/ml range, and then becomes inhibitory in the 3ug-1mg/ml range. Such differential heparin-FGF effects on myoblasts could serve as an alternative in vivo mechanism for controlling muscle growth and differentiation. When FGF is removed from the medium of proliferating MM14 myoblasts, G1-phase cells withdraw from the cell cycle and undergo an irreversible commitment to a post-mitotic phenotype and initiate transcription of muscle-specific genes. Within one cell cycle (12.5h) the entire cell population becomes post-mitotic. One aspect of the post-mitotic mechanism appears to be the irreversible loss of specific growth factor receptors. FGFR and EGFR both disappear from the cell surface within 24h of FGF removal. While this would explain how the myocyte post-mitotic state is maintained, a current paradox is that cell surface FGFR and EGFR actually increase 2-3 fold during the first 12h of FGF deprivation. Thus at a time when the entire cell population is already committed to a post-mitotic phenotype, cell surface growth factor receptors are still present. Further studies of this problem will be described. At the level of gene regulation, FGF represses muscle-specific gene expression independently of cellular proliferation. Analysis of muscle-creatine kinase expression indicates that the gene is regulated via a tissue-specific enhancer element that activates transcription in the absence of FGF. The intracellular signal pathway between cell surface FGFR and muscle nuclear proteins that bind specifically to the enhancer element is under investigation.

C 014 THE GENOMIC RESPONSE TO GROWTH FACTORS, K. Ryder, S. Hartzell, and D. Nathans, Howard Hughes Medical Institute Laboratory and Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Lau and Nathans (Proc. Natl. Acad. Sci. 84:1182-1186, 1987, reported the identification by cDNA cloning of 10 genes that are activated rapidly and transiently following exposure of quiescent BALB/c 3T3 mouse cells to serum, platelet-derived growth factor, or fibroblast growth factor. Inhibitors of protein synthesis superinduce these "immediate early" genes by causing prolonged gene transcription and stabilization of the corresponding mRNAs. In general, this set of genes is regulated coordinately with c-fos or c-myc.

cDNAs derived from the mRNAs of three immediate early genes have been sequenced. One cDNA encodes a protein that is rich in serine and proline. It does not have detectable homology to any protein whose sequence is available for comparison. A second cDNA encodes a protein whose amino acid sequence suggests that it is a transmembrane protein, i.e, it has a hydrophobic N-terminal segment typical of a signal peptide, a segment with four potential N-glycosylation sites, a highly hydrophobic stretch, and a short C-terminal tail. Its sequence is similar to that recently described for human tissue factor. (Spicer et al Proc. Natl. Acad. Sci. 84:5148-5152, 1987).

The third cDNA encodes a protein homologous to that encoded by the avian sarcoma virus 17 oncogene v-jun. (We refer to this immediate early gene as jun-B.) Homology between the jun-B and v-jun protein is in two regions: one near the N-terminus, and the other at the C-terminus. The latter was shown by Vogt et al. (Proc. Natl. Acad. Sci. 84:3316-3319, 1987) to have regions of sequence similarity to the DNA-binding domain of the yeast transcriptional regulatory protein GCN4 and to the oncogenic protein fos. Southern blots of human, mouse and chicken DNA indicate that jun-B and c-jun are different genes, and that there may be other vertebrate genes related to jun-B and c-jun. These findings suggest that there is a jun family of genes encoding related transcriptional regulatory proteins. The jun-B protein, and perhaps other members of the jun family, may play a role in regulating the genomic response to growth factors.

C 015 REGULATION OF GENE EXPRESSION BY PLATELET-DERIVED GROWTH FACTOR,
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Much attention has focused upon induction of the c-myc and c-fos proto-oncogenes by platelet-derived growth factor (PDGF); however PDGF stimulates expression of between 10-30 other genes in appropriate target cells. The structure of these other PDGF-inducible genes (which we have termed "competence" genes) and their function in the cellular response to PDGF are largely unknown. We have examined the structure and regulation of the original PDGF-inducible competence genes isolated from Balb/c-3T3 cells. Our data may provide insights into function of these genes. The PDGF-inducible "JE" and "KC" genes encode secretory proteins with cytokine-like properties. In addition, PDGF induces other cytokine-like proteins including MCSF, EPA/TIMP and interleukin-6 (also known as type 2 beta interferon and BSF 2). The data highlight another parallel between the response of T-lymphocytes to antigens and the response of fibroblasts to PDGF. Just as a T cells secrete a panel of interleukins as a component of activation, it appears that fibroblasts secrete a panel of cytokines in response to PDGF.

Growth Factors: Modulation of Gene Expression - II

C 016 **REGULATION OF THE C-MYC ONCOGENE,** **Kenneth B. Marcu, Biochemistry Dept., SUNY at Stony Brook, Stony Brook, NY 11794-5215**

The murine c-myc gene is regulated at multiple levels in proliferating and differentiating cells. At the transcriptional level, c-myc is activated by serum and defined growth factors in fibroblastic cells. A block to transcriptional elongation within the gene's first exon severely down-regulates its expression in most cell types. The transcriptional block is located about 150 nucleotides upstream of the exon 1/intron 1 junction. Transcription initiation at the normal c-myc promoter appears to be essential for efficient transcriptional blockage. Serum stimulation of either confluent arrested or subconfluent serum deprived BAlB/c A31 fibroblasts activates the initiation of myc transcription but has no significant effect on the elongation block. Serum stimulation also has a strong positive though transient effect on myc expression at the post-transcriptional level. In contrast, EGF can activate myc expression largely by releasing the transcriptional block. The transcriptional block is greatly enhanced in NIH3T3 cells transformed by various strains of Abelson murine leukemia (A-MuLV) virus demonstrating that the contribution of this level of transcriptional control for regulated c-myc expression can vary greatly in normal and transformed cells.

Myc expression is down-regulated in most differentiating cells. In differentiating F9 teratocarcinoma cells, myc expression is negatively controlled solely at the post-transcriptional level while transcription initiation and elongation remain unaffected upon F9 differentiation. In mouse erythroleukemia (MEL) cells induced to differentiate with hexamethylene bisacetamide (HMBA), myc expression is initially down-regulated by a dramatic enhancement of the transcription block within 20 minutes of inducer addition while transcription initiation remains unaffected. However, this enhanced transcriptional blockage is only significant early in the MEL cell commitment period because post-transcriptional mechanisms eventually become solely responsible for myc down-regulation in these cells prior to the onset of βglobin gene transcription. This post-transcriptional control is probably mediated within the nucleus since little change in myc mRNA half-life is apparent. In summary, different levels of gene control differentially contribute to c-myc expression in various cell types.

C 017 C-FOS REGULATION AND FUNCTION. E. B. Ziff[1], M. E. Greenberg[1*], L. A. Greene[2], D. G. B. Leonard[1], R. Metz[1], and Z. Siegfried[1]. (1) Dept. of Biochemistry and Kaplan Cancer Center and (2) Dept. of Pharmacology, New York University Medical Center, New York, NY 10016-6402. We have studied the mechanism of transcriptional acativation of the c-fos gene by growth factors by purifying proteins which associate with regulatory sequences of the c-fos gene and by analyzing the structures of complexes of these proteins with DNA. Induction of c-fos gene transcription in quiescent 3T3 cells in response to serum requires a 20bp palindromic c-fos gene 5' non coding region DNA sequence, the dyad symmetry element (DSE) whose complex with a protein, the SRE binding factor (SRF), appears to be required for c-fos transcription activation (see 1) We have purified the SRF from HeLa cell extracts by a combination of conventional and DNA affinity column chromatography steps. This factor has an apparent M_r of 64kDa on denaturing SDS-PAGE and a similar (55-70kDa) as a native protein in solution when fractionated by FPLC superose-12 chromatography. In contrast, the complex of SRF with a synthetic 30bp DSE oligonucleotide fractionates on the superose column with apparent M_r of 250kDa ± 50kDa. These data together with the results of DSE mutation (1) suggest that the SRF can exist in solution as a monomer yet forms a multimeric structure in its associaion with the DSE.

When the model PC12 pheochromocytoma cell line is stimulated with nerve growth factor, c-fos transcription is induced within 10 min. After 24-48 hr the cells begin to assume a neuronal phenotype. To place the induction of c-fos within the context of PC12 differentiation we have identified additional genes regulated in PC12 by NGF. We discuss this program of gene regulation with respect to possible functions of c-fos and the functions of the induced genes in differentiated PC12 cells and in the adult rat nervous system.

1. Greenberg et al., (1987) Mol. Cell. Biol. 7:1217-25.
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Growth Factors and Growth Inhibitors in Neoplasia

C 018 THE HUMAN trk LOCUS: A NOVEL TRANSMEMBRANE RECEPTOR, M. Barbacid, D. Martin-Zanca, R. Oskam, F. Coulier, and G. Mitra, BRI-Basic Research Program, Frederick Cancer Research Facility, Frederick, MD 21701

trk is a new member of the tyrosine protein kinase family of hormone receptors. Nucleotide sequence analysis of trk cDNA clones revealed the three classical domains of growth factor receptors. The extracellular domain contains a putative signal peptide, 13 consensus N-glycosylation sites and 11 unclustered cysteines. The transmembrane region consists of a stretch of 26 hydrophobic amino acids. Finally, the 266-amino acid long kinase catalytic domain is followed by a short carboxy-terminal tail of 15 amino acids which includes a single tyrosine residue. The trk locus was first identified as part of an oncogene activated in a human colon carcinoma biopsy by a somatic rearrangement that fused a non-muscle tropomyosin gene with the transmembrane and catalytic domains of the trk proto-oncogene.

The primary translational product of the trk proto-oncogene is a glycoprotein of 110 kd with a polypeptide backbone of 79 kd. $gp110^{trk}$ is further glycosylated to yield the mature product, $gp140^{trk}$ which becomes anchored across the plasma membrane. Both $gp140^{trk}$ and $gp110^{trk}$ have in vitro autophosphorylating activity specific for tyrosine residues. The trk oncogene codes for a 70 kd molecule which also has an associated tyrosine kinase activity. $p70^{trk}$, unlike $gp140^{trk}$, does not appear to be associated with cellular membranes. Thus, suggesting that its transforming properties might be exerted by a mechanism involving phosphorylation of non-physiological substrates. To examine the role of tropomyosin sequences in the malignant activation of the trk oncogene. We have replaced them by other cytoskeletal (β-actin) or unrelated (β-globin) genes. The resulting chimeras completely failed to transform NIH3T3 cells in spite of directing the synthesis of proteins that retained tyrosine protein kinase activity. These results suggest that tropomyosin may contribute to the transforming properties of the trk oncogene by allowing $p70^{trk}$ to interact with a specific subset of cytoplasmic substrates.

Finally, we have observed that trk proto-oncogene sequences can efficiently recombine in vitro to yield novel transforming genes. We have identified thirteen new trk oncogenes by transfecting NIH3T3 cells with nontransforming plasmids carrying either the tyrosine kinase domain or the entire coding sequences of the trk proto-oncogene. Each of these oncogenes expressed a novel trk-related protein of aberrant size with tyrosine protein kinase activity. Biochemical characterization of these proteins indicates that whereas some are located in the cytoplasm others are transmembrane proteins. These results raise the possibility that the trk proto-oncogene may serve as a marker to identify multiple substrates whose unscheduled phosphorylation may contribute to malignant transformation.

C 019 GROWTH, DIFFERENTIATION, AND NEOPLASTIC TRANSFORMATION OF HUMAN BRONCHIAL EPITHELIAL CELLS. Curtis C. Harris, Brenda Gerwin, Ke Yang, George Mark, Tohru Masui, Andrea Pfeifer, Roger Reddel, and John F. Lechner, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892

We are investigating the molecular and cellular mechanisms controlling growth and differentiation of human bronchial epithelial cells and the dysregulation of these controls during the multistage process of carcinogenesis. Our results and those of others are consistent with the hypothesis that the preneoplastic and neoplastic bronchial epithelial cells have a selective clonal expansion advantage because of diminished responsiveness to negative growth factors and/or autocrine production of positive growth factors. Aberrations in regulation of the multistage pathway leading to terminal squamous differentiation of the bronchial epithelial cells are also observed. The role of activated proto-oncogenes in controlling proliferation and squamous differentiation is being studied by transfection of expression vectors containing ras, myc and raf in the sense or antisense orientation singly or in combination into normal, SV-40 immortalized or neoplastic bronchial epithelial cells. Activated ras and myc proto-oncogenes cause abnormalities in growth and squamous differentiation pathways of human bronchial epithelial cells.

C 020 REGULATION OF CELL PROLIFERATION BY TRANSFORMING GROWTH FACTORS, Harold L. Moses, Vanderbilt University School of Medicine, Nashville, TN 37232.

Two types of transforming growth factors (TGF) have been purified and well characterized, TGFα and TGFß. TGFα is a 5.6 kD single chain EGF-related molecule that binds to the EGF receptor and has biological effects very similar to those of EGF; it is mitogenic for most cell types including normal epithelial cells. TGFß is a 25 kD homodimer of 12 kD subunits that has its own specific cell surface receptors. While growth stimulatory for selected mesenchymal cells, TGFß inhibits proliferation of most cell types including normal epithelial cells. Using cultured skin keratinocytes as a model system for normal epithelial cells, the production of and response to TGFα and TGFß has been examined along with potential mechanisms of growth inhibition by TGFß. The keratinocytes are stimulated to proliferate by EGF and TGFα. TGFα is produced by adult and neonatal skin keratinocytes, and this production is autoregulated. TGFß, on the other hand, is a potent inhibitor of keratinocyte proliferation. The mechanism of growth inhibition by TGFß appears to involve selective inhibition of expression of growth factor inducible genes necessary for cell proliferation. The keratinocytes also synthesize and release TGFß, but in a latent form; the major regulatory step in TGFß action may be at the level of activation of the latent form. Normal autocrine stimulation by TGFα and autocrine inhibition by TGFß is implied and changes in this autocrine regulation may be important in neoplastic transformation of epithelial cells. Both increased autocrine stimulation by endogenous TGFα or decreased inhibition by TGFß could lead to an increased proliferative potential and thereby contribute to the neoplastic phenotype.

C 021 CSF-1 AND ITS RECEPTOR (THE c-fms PROTO-ONCOGENE PRODUCT) IN CELL TRANSFORMATION, Charles J. Sherr, Martine F. Roussel, Carl W. Rettenmier, Esther F. Wheeler, and Jean Michel Heard, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38105.

The macrophage colony stimulating factor, CSF-1 or M-CSF, is a homodimeric glycoprotein that stimulates the proliferation, differentiation, and survival of cells of the mononuclear phagocyte lineage. A human 1.6 kb CSF-1 cDNA specifies a polypeptide that includes an N-terminal signal peptide (residues -32 to -1), a biologically active moiety (residues 1 - ca. 165), a transmembrane spanning segment (residues 166-188) and a short C-terminal tail (residues 189-224). The precursor is dimerized through interchain disulfide bonds, and externalized on the plasma membrane as a 68 kd homodimer which is proteolyzed to yield a soluble 44 kd growth factor. Truncation of the precursor after residue 158 results in the production of a soluble, biologically active growth factor that is rapidly secreted. A 4 kb CSF-1 cDNA encodes a 554 amino acid polypeptide containing 298 additional amino acids interposed aminoterminal to the transmembrane segment. Intracellular proteolysis of the larger precursor within its unique domain results in the rapid secretion of a 86 kd homodimer. Cotransfection of the human c-fms gene together with these CSF-1 cDNAs into mouse NIH-3T3 cells induces morphologic transformation and tumorigenicity in nude mice. By contrast, introduction of the 1.6 kb CSF-1 cDNA into a CSF-1 dependent mouse macrophage cell line abrogates its factor dependence but does not induce tumorigenicity, suggesting that mature macrophages are relatively refractory to persistent autocrine signals which can transform fibroblasts.

The v-fms oncogene product differs from the normal CSF-1 receptor by scattered point mutations and by a truncation of its distal carboxylterminus that removes a presumed negative regulatory site of tyrosine phosphorylation. The altered receptor functions constitutively as a CSF-1 independent tyrosine kinase that is refractory to down modulation by CSF-1 and phorbol esters. Introduction of the v-fms gene into CSF-1 dependent macrophage or IL-3 dependent myeloid cell lines renders them factor independent and tumorigenic without affecting the synthesis, affinity, or turnover of normal CSF-1 or IL-3 receptors. To determine if the v-fms gene can directly initiate hematopoietic malignancies in animals, murine bone marrow cells infected with a v-fms-containing retroviral vector were used to reconstitute lethally irradiated mice. The majority of recipients contained dominant provirus-positive clones in their spleens which repopulated secondary lethally irradiated animals, giving rise to both erythroleukemias and B cell lymphomas. Thus, the v-fms gene can transform cells of several hematopoietic lineages both in vitro and in vivo, including those that do not normally express CSF-1 receptors.

Growth Factor Function In Vivo

C 022 **FIBROBLAST GROWTH FACTORS, THE EXTRACELLULAR MATRIX AND THE REGULATION OF THE NEOVASCULAR RESPONSE** *Andrew Baird, Pedro Cuevas, Ana Gonzalez, Naoya Emoto, Jean-Jacques Feige and Patricia Walicke*, Laboratories for Neuroendocrinology, Salk Institute, La Jolla CA and *Department of Neurosciences, University of California, San Diego, CA*

The acidic (a) and basic (b) fibroblast growth factors (FGF) are characterized by their wide distribution in tissues, their high affinity for heparin and their capacity to modulate the cell growth and function of a wide number of cell types. Of these biological activities, it is their capacity to stimulate neovascularization that has gained the most attention. In all experimental models of angiogenesis, bFGF is capable of eliciting a significant neovascular response suggesting that it may be an invaluable tool in the therapeutic application of growth factors. As an example, the administration of bFGF to the proximal stump of a severed sciatic nerve promotes the survival, growth and remyelination of the nerve possibly by increased vascularization to the tissue. When bFGF is administered in a similar paradigm to both the distal and proximal ends of the severed nerve, regenerating axons connect and the retrograde transport of horseradish peroxidase (HRP) to neurons in the lumbar spinal cord can be demonstrated suggesting successful regeneration of the tissue. Although similar effects can be seen in the absence of exogenous FGF, the rate of recovery is enhanced by the administration of the growth factor. Other examples of the *in vivo* applications of bFGF include regeneration of the optic nerve, vascularization of the vasa vasorum and angiogenesis in the CNS and under the kidney capsule. In each instance however, these procedures rely on the addition of exogenous growth factor to elicit a response. Little, if anything is known about procedures which might regulate the bioavailability of endogenous bFGF or aFGF even though they are present in the target tissues. To this end, the extracellular matrix (ECM) produced by endothelial cells has been used to examine the role that heparan sulfate and other related glycosaminoglycans (GAG) might play in regulating the secretion (crino-) and adhesion (-pexy) of FGF to the basement membrane. Results obtained with this model suggest that the binding of soluble factors in the ECM can be modulated by enzymatic degradation thus offering at least one mechanism to regulate the bioavailability of FGFs. Possible alternative mechanisms for accessing biologically active FGFs from the ECM include modifying the number of high affinity receptors on target cells and stimulating the formation of post translational modifications that might alter the affinity of FGFs for GAGs. Each of these mechanisms will be discussed.

Growth Factors and Their Receptors: Genetic Control and Rational Application

C 023 GROWTH FACTORS: CELL PRODUCTION, DIFFERENTIATION AND DEATH, Antony Burgess[1], Ralph Böhmer[1], Robert Maxwell[1], Gregory Johnson[2] and George Morstyn[1]. [1]Ludwig Institute for Cancer Research (Melbourne Tumour Biology Branch), PO Royal Melbourne Hospital, Vic. 3050, Australia; [2]Walter & Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Vic. 3050, Australia.

Hemopoietic growth factors (HGF's) induce the production of blood cells both in vitro and in vivo. Although it is easier to assess the relevant biological effects of the HGF in vitro, where so many of the variables can still be controlled and/or measured, the HGF's stimulate the target cells to both proliferate and differentiate so that the interpretation of even the simplest experiments can be complicated. For example, many laboratories have observed that cells of the murine myelomonocytic leukemia WEHI3B(D^+) produce colonies containing differentiated monocytes and neutrophils when these cells are incubated with granulocyte-colony stimulating factor (G-CSF). Furthermore, when WEHI3B(D^+) cells are treated with actinomycin D differentiated monocytes are produced. Analysis of the kinetics of WEHI3B(D^+) proliferation and differentiation at low cell densities ($<10^5$ cells/ml) indicated that G-CSF did not change the rate of proliferation nor were any differentiated cells produced. In contrast, actinomycin D (6.6 ng/ml) caused the WEHI3B(D^+) cells to: stop proliferating, initiate maturation along the monocyte pathway and subsequently die. The addition of G-CSF 20 h after the beginning of the actinomycin treatment delayed cell death for up to 3 days and resulted in an accumulation of mature monocytes. G-CSF does not appear to induce the differentiation of WEHI3B(D^+) cells directly, but rather permits the survival of mature monocytes produced as a result of autocrine stimulation when the cells are cultured at high density (e.g. in an agar colony).

The effects of growth factors in vivo will be even more difficult to interpret. Consequently, a numerical model of hemopoiesis based on growth factor stimulation of bone marrow has been developed. This model attempts to simulate cell production and growth factor synthesis, receptor binding and degradation in tissues at a wide range of cell concentrations (10^4 to 10^{10} cells/ml). This model is used to investigate the influence of GM-CSF and G-CSF on the cell trafficking between the bone marrow, blood and tissue pools as well as its influence on the life span of mature cells. The properties of the model are evaluated by comparing the simulations with the actual effects of the two HGF's on hemopoietic regeneration in mice and humans.

C 024 GROWTH FACTORS AND WOUND REPAIR, Katherine H. Sprugel, David G. Greenhalgh* and Russell Ross. Departments of Pathology and Surgery*, University of Washington, Seattle, WA 98195.

Repair of soft tissue injury is a complex sequential process involving cell migration, cell proliferation, cell differentiation, angiogenesis, and connective tissue deposition and remodeling. In considering the actions of growth factors in wound repair, two major questions arise. First, what role do endogenous growth factors play in the coordination of wound repair? Second, will growth factors, as pharmacologic agents, be useful in the clinical treatment of wounds? The reagents necessary to answer the first question are only just becoming available. However, recent successes in cloning and expressing a variety of growth factors on a large scale make it possible to consider therapeutic uses of these proteins and to address the second question.

By virtue of their effects in vitro on cell migration, cell proliferation, extracellular matrix synthesis and destruction, and immune cell function, growth factors can potentially affect tissue repair at many levels. A number of practical issues complicate the evaluation of the effectiveness of growth factors in promoting wound healing. The choice of wound type (e.g., incision, burn, or ulcer) and animal model can affect the ability to generalize results. Which growth factor(s) to apply, when to apply them, and in what vehicle are also significant variables. Complications arise when considering the effects of growth factor treatment in individuals with compromised wound repair. Finally, the question of adverse effects, local or systemic, immediate and long term, must be addressed. Experiences with PDGF will be used to illustrate strategies for assessing growth factor actions in wound repair.

Growth Factors and Diseases of Cell Proliferation - I

C 025 MECHANISMS IN INTERLEUKIN 3 MEDIATED GROWTH REGULATION OF EARLY MYELOID CELLS, James N. Ihle, Robert Isfort, John L. Cleveland and Yacob Weinstein, Basic Research Program, Frederick Cancer Research Facility, Frederick MD 21701.

Interleukin 3 (IL3) supports the proliferation and differentiation of early hematopoietic stem cells and cells committed to several myeloid lineages. IL3 also supports the in vitro growth of a variety of cell lines established from long term bone marrow cultures or from primary, retrovirus induced myeloid leukemias. All the IL3 dependent lines examined express a single class of high affinity receptors. By cross-linking and immunoprecipitation experiments, the primary IL3 binding protein has an apparent size of 65 kd which is smaller than most growth factor receptors. Although the IL3 binding protein differs in size from the typical tyrosine protein kinase containing growth factor receptors, a role for tyrosine protein phosphorylation in IL3 signal transduction has been hypothesized. This was initially based on the ability of a variety of oncogenes encoding tyrosine protein kinases to abrogate the requirements of the cells for IL3. In support of a role for tyrosine phosphorylation, stimulation of factor dependent cells with IL3 results in the rapid and specific tyrosine phosphorylation of a number of proteins detectable by isolation with monoclonal antibodies against phospho-tyrosine. The most rapidly phosphorylated protein is a membrane associated glycoprotein of 140 kd. Subsequently a series of cytoplasmic proteins become phosphorylated. In cells made IL3 independent by transformation with the tyrosine protein kinase containing oncogenes abl, src, fms and trk, similar cytoplasmic substrates were constitutively phosphorylated. In addition cells transformed with abl or src had higher levels of phosphorylation and additional substrates were phosphorylated. In factor dependent cells IL3 regulates the levels of transcripts of the c-myc gene and of the γ T cell receptor genes. Abrogation of IL3 dependence by abl, src, fms and trk oncogenes resulted in the constitutive expression of c-myc. In contrast only in abl and src transformed cells were the γ T cell receptor genes constitutively expressed. The results suggest that tyrosine phosphorylation is involved in IL3 signal transduction and that different pathways of phosphorylation may be involved in the regulation of γ T cell receptor and c-myc transcripts.

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C 026 REGULATION OF MYELOID AND LYMPHOID CELLULAR PROLIFERATION: DIFFERENTIAL EFFECTS OF TRANSFORMING GROWTH FACTOR β_1 ON NORMAL AND LEUKEMIC CELLS. Francis Ruscetti, Garwin Sing, Sandra Ruscetti, Larry Ellingworth, and Jonathan Keller, Lab. of Mol. Immunoregulation and Program Resources, Inc., BRMP., NCI-FCRF, Frederick, MD, Lab. of Genetics, Bethesda MD and Collagen Corp., Palo Alto, CA.

Transforming growth factors are polypeptides that can reversibly induce certain non-neoplastic cells to undergo anchorage-independent growth. One of these, TGF β_1, is a 25 Kd homodimeric protein with both growth-enhancing and growth-inhibitory properties. Moreover, it acts as an important immunomodulatory protein for cells of the immune system, inhibiting proliferation and immunoglobulin secretion of stimulated B cells, interleukin-2-dependent T cell proliferation as well as NK cytotoxicity. We recently examined the affects of TGF β_1 on the growth and differentiation of hematopoietic cells. Stimulation of fresh unfractionated murine bone marrow by IL-3 and human marrow by GM-CSF was inhibited by TGF β_1 but stimulation by G-CSF was not affected. Mouse and human hematopoietic colony formation was differentially affected by TGF β_1. CFU_{GEMM}, the most immature colonies were inhibited by TGF β_1 but CFU_G, CFU_M and CFU_E, the more differentiated unipotent granulocyte, macrophage and erythroid progenitors were not affected. In addition, the ability of CSF-1 to stimulate differentiated macrophage and erythropoietin to stimulate red cell maturation were not affected by TGF β_1, even though early events in hematopoiesis in which these molecules are co-factors were inhibited. TGF β_1 was found to inhibit IL-3 induced growth within 24 hours in murine leukemic cell lines. After 24 hours, the cells were still viable. Subsequent removal of the TGF β_1 results in the resumption of normal growth. In the case of NFS-60, a cell line that responds to IL-3, GM-CSF, G-CSF, CSF-1 and IL-4 (interleukin-4), TGF β_1 inhibited NFS-60 growth regardless of the growth factor used. Fractionation of mouse marrow by monoclonal antibodies into the most immature cells that still respond to IL-3 showed that the multipotent cell development but not unipotent cell development was inhibited by TGF β_1. Thus, the ability of TGF β_1 to block hematopoietic progenitor cell growth and differentiation depends on the differentiated state of the cell and suggests that TGF β_1 may be an important regulator of hematopoietic cell growth. Studies on the effect of TGF β_1 on the growth of fresh human leukemic cells and leukemic cell lines revealed that myeloid leukemias were inhibited but that erythroid leukemias were not sensitive to TGF β_1. In contrast, lymphoid leukemias, whose normal cellular counterparts were markedly inhibited by TGF β_1, were insensitive to TGF β_1. These results suggest that, at least in the case of lymphoid neoplasias, escape from the negative regulation of TGF β_1 may play a role in pathogenesis of these diseases.

C 027 TRANSFORMING GROWTH FACTOR-BETA: ROLES IN INFLAMMATION, REPAIR AND CANCER, Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892

We have previously suggested that peptide growth factors have a critical role in the pathogenesis of many proliferative and inflammatory diseases, including cancer, atherosclerosis, rheumatoid arthritis, psoriasis, idiopathic pulmonary fibrosis, scleroderma, and cirrhosis of the liver. Recent studies on the cellular and molecular mechanism of action of transforming growth factor-beta (TGF-beta) now indicate that this particular peptide may have an important role in many of these diseases. It is now known that TGF-beta can act as both a positive and negative autocrine or paracrine regulator for the growth of many types of cells. TGF-beta is synthesized by at least three types of inflammatory cells (platelets, macrophages, and lymphocytes), and may regulate the growth and differentiation of these very same cells, as in the case of lymphocytes. It is also the most potent known chemotactic agent for macrophages, being active at femtomolar concentrations. TGF-beta controls the growth and differentiation of both fibroblasts and endothelial cells, which often compose the bulk of many proliferative lesions. Particularly important is the ability of TGF-beta to regulate collagen gene expression, which is now known to be mediated by a functional binding site for the transcription protein, nuclear factor 1, on the collagen gene promoter. In collaboration with several clinical groups (Bert Glaser et al., Johns Hopkins; Ronald Wilder et al., NIH), we have begun to investigate the role of TGF-beta in proliferative and inflammatory diseases of the eye and joints, and preliminary results of these studies will be presented.

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Growth Factors and Diseases of Cell Proliferation - II

C 028 EXPRESSION OF A HEMOPOIETIC GROWTH FACTOR GENE IN TRANSGENIC MICE, Richard A. Lang[1], Donald Metcalf[2], R. Andrew Cuthbertson[3], Ian Lyons[3], Ed Stanley[1], Anne Kelso[2], George Kannourakis[2], D. James Williamson[2], Gordon K. Klintworth[4], Thomas J. Gonda[1] and **Ashley R. Dunn**[1]. [1]Ludwig Institute for Cancer Research (Melbourne Tumour Biology Branch), PO Royal Melbourne Hospital, Vic. 3050, Australia; [2]Walter & Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, Vic. 3050, Australia; [3]Howard Florey Institute for Experimental Physiology, Parkville, Vic. 3052, Australia; [4]Duke University Eye Center, Durham, NC 27710.

Transgenic mice have been established that carry the murine granulocyte-macrophage colony stimulating factor (GM-CSF) gene expressed from the Mo-MLV promoter. Transgenic mice exhibit elevated levels of GM-CSF in serum, urine, peritoneal cavity and eye. The eyes of transgenic mice are opaque at birth, contain accumulations of macrophages and develop retinal damage. Similar accumulations of macrophages are evident in the peritoneal and pleural cavities. Peripheral blood and bone marrow white cell counts of apparently healthy transgenic mice are similar to those of littermate control mice. Moreover, transgenic mice exhibit a normal frequency of granulocyte macrophage progenitors which display a normal responsiveness to stimulation by various hemopoietic growth factors.

After 6 weeks of age transgenic mice begin to die prematurely following a period of progressive weight loss and weakness in the hind limbs. At autopsy extensive cellular infiltration of striated muscle tissue is evident and is probably repsonsible for the premature death of these animals. Northern blot and hybridisation histochemical analysis of tissues from transgenic mice suggest that the macrophages themselves express the transgene. We are presently attempting to determine whether local tissue damage occurs by direct interaction with macrophages or, indirectly, by the production of certain biologically active molecules by constitutively activated macrophages.

C 029 PDGF: MACROPHAGES AND CELL PROLIFERATION, Russell Ross, Elaine W. Raines, David K. Madtes, Christopher D. Renz, University of Washington, Department of Pathology, Seattle, WA 98195

Both resident tissue macrophages and blood-borne monocytes can be activated to form several growth factors, including both chains of PDGF, TGF-alpha, FGF, IL-1, CSF-1, TGF-beta, and TNF. Induction of transcription and protein secretion occur to varying degrees and at different time intervals, depending upon the mode of activation. Monocyte infiltration is ubiquitous in all inflammatory responses and probably plays the key role in generation of these different growth factors dependent upon specific stimulation. The monocyte/macrophage is "activated" by lymphokines such as gamma-interferon. In addition, monocytes can become lipid-laden foam cells by incorporating lipid from diverse sources. It is not yet clear, however, whether foam cell formation represents monocyte activation. The growth factors released by activated macarophages can modulate proliferation and connective tissue formation by the local connective tissue cells and may be important in responses as diverse as wound repair, atherosclerosis, rheumatoid arthritis, and pulmonary fibrosis.

The messages for two of these growth factors, PDGF and TGF-alpha, are not expressed in circulating monocytes unless activated by agents such as endotoxin or Con A. Transcriptional activation and induction of secretion in cultured monocytes in response to activation by these agents appears to be transient. Modulation of PDGF and TGF-alpha gene expression and secretion of these mitogens by monocytes treated with gamma-interferon, CSF-1, thrombin, or after conversion to foam cells will be presented. These represent probable local mediators released by other activated cells during wound repair and various disease processes.

Late Additions

C 030 EFFECTS OF HEMATOPOIETIC GROWTH FACTORS IN VIVO, D.G. Nathan, The Children's Hospital, Boston, MA.

Primate recombinant IL 3 has multi-colony-stimulating activity in vitro and is a more potent stimulus of erythroid burst forming units than granulocyte macrophage colony stimulating factor (GM-CSF). In view of its potential importance as a hematopoietin in vivo, we wished to determine the mesenchymal cell sources of the human hormone. In blood mononuclear cells IL 3 messenger RNA (mRNA) was identified as early as six hours after activation with phytohemagglutinin (PHA) and phorbol myristate acetate (PMA) and continued to be expressed for up to 5 days. Production of IL 3 protein was confirmed by an IL 3 specific bioassay of the PHA/PMA mononuclear cell conditioned media on CML blast cells. After sequential depletion of monocytes and B cells, the remaining cell fraction consisting of T and NK lymphocytes was identified as the source of IL 3 mRNA. In addition, the mRNA was expressed by a proliferating helper T cell clone. Using conditions that are known to induce GM-CSF mRNA expression, we were unable to detect IL 3 in monocytes, fibroblasts and endothelial cells. The data demonstrate that activated T lymphocytes appear to be the sole source of IL 3 production among the mononuclear mesenchymal cells in blood and bone marrow. To study the role of different activation signals for IL 3 mRNA expression, we examined the stimuli necessary to induce the high IL 3 mRNA levels expressed in the gibbon T cell line MLA 144. This expression seems to be largely mediated through protein kinase C activation.

C 031 EPIDERMAL GROWTH FACTOR: STRUCTURE AND FUNCTION, Antony Burgess, Christopher Lloyd, Louis Fabri, Edouard Stanley, Sandra Smith and Edouard Nice. Ludwig Institute for Cancer Research (Melbourne Tumour Biology Branch), PO Royal Melbourne Hospital, Vic. 3050, Australia.

Derivatives of the murine epidermal growth factor (EGF), a 53-amino acid protein, have been produced by enzymic digestion, site-specific chemical reactions, and by bacteria. After trypsin digestion the EGF derivatives EGF_{1-48} (called EGF-T) and EGF_{1-45} (called EGF-T_2) were separated from the residual EGF and the C-terminal pentapeptide by reversed phase high performance liquid chromatography. EGF-T competes for binding to EGF receptors with the same efficiency as EGF. The EGF-T_2 derivative had no detectable receptor binding activity even at 100 nM. The in vitro mitogenic potencies of EGF and EGF-T for Balb/c 3T3 cells were indistinguishable. Treatment of EGF-T with carboxypeptidase Y yielded two derivatives EGF-T-(des-arg_{48}) and EGF-T-des(leu_{47}-arg_{48}). There was only a 3- to 4-fold diminution in the binding efficiency and mitogenic potency for EGF-T-des-arg_{48}. However, there was a considerable decrease in the binding efficiency and mitogenic activity of EGF-T-des(leu_{47}-arg_{48}). These results indicated that leu_{47} is an important determinant for the formation of the ligand receptor complex. Studies with a series of proteases indicated that the C-terminus of EGF was susceptible to enzymic digestion, however, the N-terminus appears to be folded into a conformation which prevents proteolytic digestion. Truncation of the N-terminus was achieved by preparing an EGF-T analogue by using a bacterial beta-galactosidase expression vector. ^{125}I-labeled EGF lys_{21}(4-48) bound to the EGF receptor with high affinity (Kd~4 pM) and had the same potency as EGF in the Balb/c 3T3 mitogenic assay. Although the lys for met substitution at position 21 did not affect the activity of EGF, disruption of the central anti-parallel-beta-sheet structure at met_{21} by treating EGF with cyanogen bromide reduced both the binding efficiency and mitogenic activity of EGF more than 1,000-fold.

Growth Factors and Their Receptors: Genetic Control and Rational Application

Molecular Biology of Growth Factors

C 100 INCREASED LEVELS OF A BOMBESIN-LIKE PEPTIDE IN THE BRONCHOALVEOLAR LAVAGE FLUID OF SMOKERS. S. Aguayo, M. Kane, T. King, M. Schwarz, L. Grauer and Y. Miller, Denver VA Medical Center, National Jewish Hospital and UCHSC, Denver, CO and Hybritech, San Diego, CA. Abnormal cell proliferation of the bronchial epithelium is a hallmark of tobacco-associated lung diseases. The mechanism by which this abnormal cell proliferation occurs is currently unclear but deregulation in production of growth factors could be implicated. We have examined bronchoalveolar lavage (BAL) fluid from smokers and non-smokers, using both high performance liquid chromatography (HPLC) and immunological techniques. BAL fluid from smokers frequently contains a variety of peptides not seen in non-smokers. We have identified one such peptide as being immunologically related to bombesin and its mammalian counterpart, gastrin releasing peptide (GRP). The concentration of this bombesin-like peptide (BLP) in the BAL fluid of smokers (mean 194.8 ng/ml) is significantly higher than the levels of BLP in the BAL fluid of non-smokers (mean 49.1 ng/ml), as determined by our ELISA ($p < 0.001$, Student's t-test). A peptide that elutes near the standard retention times for bombesin and GRP 14-27 in reverse phase HPLC is responsible for all the bombesin-like immunoreactivity contained in the BAL fluid. The levels of BLP in the BAL fluid are not related to the amount of protein or cellular components in the BAL fluid. Thus, tobacco smoking causes a specific increase of BLP in the lower respiratory tract of humans. Since bombesin is a growth factor for normal human bronchial epithelial cells, it is possible that the BLP detected by our immunoassay leads to increased proliferation of the bronchial epithelium in vivo.

C 101 **THE EGF SYSTEM AS PREDICTORS FOR PROGNOSIS OF GYNECOLOGIC CARCINOMAS**, Thomas Bauknecht, Universitäts-Frauenklinik, 78 Freiburg, Hugstetterstr. 55, FRG.

Studies on the expression of EGF receptors (EGF-R) and EGF like factors (EGF-F) in ovarian and breast carcinomas indicate an association with the aggressive behaviour of these carcinomas. In this study we analyzed the expression of EGF-R and EGF-F in ovarian, endometrial, breast, cervical and vulvar carcinomas and we correlated the expression with relevant clinical prognostic criterias. The EGF-R were measured by EGF two point binding assay, the EGF-F were analyzed by EGF radioreceptor assay (EGF RRA), EGF Ria and TGFα Ria.

Following correlations were found: increased binding sites of EGF-R in poorly differentiated squamous cell carcinomas; inverse relation of steroid hormone and EGF receptor status in breast, endometrial and ovarian carcinomas; a high number of lymph node negative, EGF-R^- steroid hormone $receptor^+$ breast carcinomas; high responserate on chemotherapy of EGF-R^+ ovarian carcinomas. The correlation of EGF-F levels with patients prognosis showed an association of EGF-F levels over 6 ng/mg with poor prognosis. The use of EGF RRA, EGF- and TGFα - Ria demonstrated that EGF-F consist of TGFα and components. From these results can be concluded that the parameters of the EGF system can be taken as indicators for prognosis. We assume that the growth patterns of some tumors are regulated in a para- or autocrine fashion of the EGF system.

C 102 SOLUTION STRUCTURE OF TRANSFORMING GROWTH FACTOR- BY NMR METHODS, Stephen C. Brown, Luciano Mueller and Peter Jeffes, Smith Kline & French Laboratories, Mail Code L-940, P.O. Box 1539 King of Prussia, PA 19406-0939. The proton NMR resonances of human Transforming Growth Factor alpha (TFG-) have been competely assigned and secondary structural elements were identified. Three dimensional structures generated using distance geometry algorithms will be compared to the reported structures of murine (1) and human (2) epidermal growth factors. These growth factors are reported to bind to the same receptor in-vivo, though they share only 30% sequence similarity (3). Potential interactions with this receptor will be discussed.

1) G.T. Montelione et al., Proc. Natl. Acd. Sci USA 84: 5226-5230 (1987).
2) R.M. Cooke et al., Nature 327: 339-341 (1987).
3) J. Massague, J. Biol. Chem 258: 13614-13620 (1983).

C 103 THE EFFECTS OF RECOMBINANT HUMAN G-CSF AND BETA-1 INTERFERON IN LEUKEMIA
C.L. Castiglia, M. Revel, M. Firpo, J.L. Gabrilove, L. Souza and M.A.S. Moore, Sloan-Kettering Institute, NY, NY 10021 and Amgen Corp., Thousand Oaks, CA 91320.
The single and combined effects of rhG-CSF and rh beta-1 interferon (B-1 IFN) upon human leukemic cell models has been studied at the cellular and molecular levels. Although G-CSF does not produce overt morphological change in monomyelocytic U937 cells (perhaps as a result of their committment to monocytic differentiation), 6 days exposure to 10,000 U/ml G-CSF does inhibit proliferation to 72-78% that of control cultures with viability maintained at greater than 95%. B-1 IFN, at a concentration of 500 U/ml, likewise inhibits U937 proliferation (to 42-46% that of control), but reduces viability to 80-90% by day 6. When U937 cells are treated with a combination of these two reagents for 6 days, proliferation drops to 26-28% of control, and viability declines to 66-78%. A two day pre-incubation with 500 U/ml B-1 IFN followed by exposure to 10,000 U/ml G-CSF in the continued presence of B-1 IFN further reduces proliferation, to 13% of control with 75% viability, whereas discontinuation of B-1 IFN allows to the cells to recover to 40% of control growth with 94% viability within 6 days. The effects of these manipulations on the expression of c-myc, c-fps, c-fms and c-Tac are currently under examination using Northern analysis and in situ hybridization.

B-1 IFN was ineffective in inhibiting proliferation of the promyelocytic HL-60 leukemic cell line. We believe this may in part be due to constitutive expression of the IFN-induced 2'5' oligo(A) synthetase in all HL-60 cells tested.

C 104 **EVIDENCE FOR EGF INDUCED RECEPTOR DIMERIZATION IN LIVING CELLS USING A CHEMICAL COVALENT CROSS-LINKING AGENT.**
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The receptor for epidermal growth factor is a transmembrane protein composed of an EGF-binding domain and a cytoplasmic kinase domain connected by a single hydrophobic stretch. We have used the soluble covalent cross-linking agent (EDAC) to show that EGF stimulates the dimerization of purified EGF-receptor, of EGF-receptor in membrane preparations and in intact A-431 cells. Three independent approaches including biosynthetic labeling, surface iodination and immunoblotting experiments were utilized to follow EGF-receptor dimerization in living A-431 cells. They provided consistent results indicating that EGF induced rapid dimerization of EGF receptor in living cells, suggesting that this process may play a role in transmembrane signalling mediated by EGF.

C 105 INTERCONVERSION OF cAMP AND TPA-RESPONSIVE DNA ELEMENTS, Paul J. Deutsch, James P. Hoeffler, Larry Jameson, Julia C. Lin, and Joel F. Habener, Massachusetts General Hospital, Boston, MA 02114.
Many genes which are transcriptionally regulated by the tumor promoter TPA via the protein kinase C pathway share a short 7 bp sequence in their 5'-flank similar to 5'TGA (C or G) TCA3'. A closely related 8 bp sequence, 5'TGACGTCA3', is found in many genes inducible by cAMP. Inasmuch as elevation of cAMP versus the activation of protein kinase C by growth factors can have similar or contrasting consequences, we sought to evaluate the degree of cross-talk between the two pathways at the level of gene activation. Our model system involved CAT constructs containing the alpha gonadotropin promoter transfected into JEG-3 choriocarcinoma cells. This promoter-cell pair is extremely receptive to cAMP-responsive enhancers. Constructs containing the above 8 bp sequence alone or in the context of the surrounding 10 bases present in the alpha, VIP or somatostatin (cAMP-responsive) genes, inserted 5' to the alpha promoter were stimulated 30-fold by cAMP analogues. The identical octamer, when surrounded by other short sequences, including those present in cAMP-unresponsive genes which contain the identical octamer, could not confer cAMP-responsiveness. Insertion of a single G into a minimally-sized TPA-responsive element containing the heptamer 5'TGACTCA3' was sufficient to entirely eradicate the 8-fold TPA-stimulatibility as well as confer 30-fold cAMP-responsiveness. The ability to actually interconvert TPA and cAMP-responsive sequences by the addition or subtraction of one base and comparisons of their preferences for certain contextual sequences over others has considerable physiologic and mechanistic implications.

C 106 GROWTH FACTOR LIKE DOMAINS IN LAMININ, P. End and J. Engel, in collaboration with G. Panayotou, MRC, London and R. Timpl, Max-Planck-Institut fur Biochemie.
Laminin a multifunctional glycoprotein present in all basement membranes acts as a mediator between cells and other components of the extracellular matrix and is discussed to be recognized by one or more receptors and to be of major importance in development and maintenance of cellular organisation. The cystein-rich motifs of the sequence of one of the three polypeptide chains of laminin show remote relation to epidermal growth factor (EGF). This prompted us to search for growth factor-like activities of laminin and fragments derived from it, as well as for possible interactions with the EGF-receptor. Fragment 1 comprises rod-like regions of the three short arms and is composed of about 15 cysteine-rich domains which show the resemblance to EGF. A larger fragement 1-4 comprises all three short arms, but lacks the long arm, which in turn includes fragments 3 and 8. Mitogenic activity comparable to that observed for EGF was indeed observed for laminin and those fragments of laminin which contain EGF-like domains (fragments 1-4 and 1) but not for fragments comprising other regions of the molecule (fragments 3 and 8). Thymidine incorporation was stimulated for Swiss 3T3 cells but not for a variant cell line NR6 which lacks the EGF-receptor. Direct interaction of laminin and its active fragments with EGF-receptor was demonstrated by equilibrium chromatography and other techniques. The expression of a localized growth factor activity on laminin is considered to be of importance in the formation of epithelial cell layers and in tissue repair.

C 107 STRUCTURE/FUNCTION STUDIES OF HUMAN EPIDERMAL GROWTH FACTOR BY SITE-DIRECTED MUTAGENESIS, David A. Engler, Rise K. Matsunami, Stephen R. Campion, Audrey Stevens and Salil K. Niyogi, The Univ. of Tenn. Oak Ridge Graduate School of Biomedical Sciences and The Protein Engineering Program, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.
A synthetic chimeric gene, containing the DNA coding for the human epidermal growth factor (EGF) and that for the signal peptide of E. coli alkaline phosphatase, was cloned into E. coli under the transcriptional control of the trp-lac (tac) promoter. Following induction with isopropylthiogalactoside, the secretion of EGF into the E. coli periplasmic space was confirmed by its specific binding to the EGF receptor and stimulation of the EGF receptor tyrosine kinase activity. Following purification to homogeneity, the primary sequence of the recombinant protein was determined and found to be identical to that of authentic human EGF as reported by Gregory and Preston (Int. J. Peptide Protein Res. 9: 107-118, 1977). Studies of structure-function relationships by specific alterations of targeted amino acid residues in the EGF molecule by oligonucleotide-directed mutagenesis will be reported. Results show that changes of several amino acid residues significantly alter receptor binding activity. (Operated by Martin Marietta Energy Systems, Inc., with the U.S. Dept. of Energy. DAE and RKM are supported by NCI and NIGMS predoctoral and SRC by NCI postdoctoral training grants to Univ. of Tenn. Oak Ridge Graduate School of Biomedical Sciences).

C 108 DISULFIDE DELETION STUDIES OF RECOMBINANT HUMAN TRANSFORMING GROWTH FACTOR ALPHA, Richard N. Harkins and John W. Brandis, Triton Biosciences Inc., Alameda, CA 94501.
Mutants of human Transforming Growth Factor-Alpha (hTGF-α) have been generated in which each of the three pairs of cysteine residues involved in disulfide bonds have been substituted to serine residues by site directed mutagenesis. Single base changes were made (TGC to TCC) in the DNA sequence for hTGF-α at amino acid coding positions Cys 8 and Cys 21, Cys 16 and Cys 32, and Cys 34 and Cys 43 by oligonucleotide synthesis of DNA fragments containing the desired cysteine to serine changes. The three mutant DNA coding sequences, designated A minus (Cys 8 Ser/Cys 21 Ser), B minus (Cys 16 Ser/Cys 32 Ser), and Ser), and C minus (Cys 34 Ser/Cys 43 Ser) were individually cloned and expressed in E. coli as fusion proteins containing 15 additional residues at the TGF-α NH_2 terminus. The mutant TGF-α fusion proteins were purified by reverse phase HPLC, treated with cyanogen bromide to remove the NH_2-terminal extension and to generate mature TGF-α, and refolded by air oxidation. The disulfide arrangement of each refolded mutant was determined by isolating and characterizing the disulfide peptides generated by thermolysin digestion and HPLC peptide mapping. Each of the mutants was tested for biological activity by competition for epidermal growth factor (EGF) receptors on A431 cells. The refolded B minus and C minus TGF-α mutants were completely unable to displace ^{125}I-EGF binding at concentrations of up to 18.0 and 26.3 μg per ml respectively. The refolded A minus protein was 0.4% as active as native refolded TGF-α in competing for binding to the EGF receptor.

C 109 AUTOCRINE STIMULATION OF INTRACELLULAR PLATELET-DERIVED GROWTH FACTOR RECEPTORS IN V-SIS-TRANSFORMED CELLS, Mark T. Keating and Lewis T. Williams, University of California, Department of Medicine, Cardiovascular Research Institute, Howard Hughes Medical Institute, San Francisco, CA., 94143.

Autocrine activation of platelet-derived growth factor (PDGF) receptors is the mechanism of transformation by the v-sis oncogene. Since the addition of PDGF does not transform normal cells, autocrine mechanisms may involve unique pathways of receptor activation. Using antireceptor and antiphosphotyrosine antibodies in metabolic labeling experiments, we have identified precursor, mature and activated forms of the receptor in v-sis-transformed cells. Activated receptor was detected 30 min after pulse labeling with ^{35}S-methionine in these cells and was resistent to trypsin. Thus, receptor activation occurred before the receptor could reach the cell surface. After activation the receptor was immediately diverted to a chloroquine and ammonium chloride sensitive degradation pathway. This pathway in v-sis transformed cells caused a dramatic shortening of receptor half life (10 min) compared to receptor half life in normal cells (3 hours). These findings show that intracellular activation of receptors by autocrine mechanisms can play a role in cell transformation.

C 110 INTERACTION OF THE EGF RECEPTOR AND p185, Yasuo Kokai, Kunio Dobashi, Jeffrey A. Cohen and Mark I. Greene, University of Pennsylvania, Philadelphia, PA 19104.

The neu oncogene encodes a cell surface glycoprotein, p185, with tyrosine kinase activity that appears to be a growth factor receptor. p185 is homologous to the epidermal growth factor receptor (EGFR) but is the product of a distinct gene. The normal and oncogenic neu proteins differ by a single Val to Glu substitution in the transmembrane domain. The tissue and stage-specific expression of p185 in rat development share some features with EGFR. Using cell lines expressing EGFR and either oncogenic p185(p185T) or proto-oncogenic p185(p185N), we confirmed that the EGFR and p185 are distinct. EGF did not bind to immunoprecipitated p185; p185 specific monoclonal antibodies did not bind to immuoprecipitated EGFR. EGF and the phorbol ester, TPA, stimulated the phosphorylation of p185N in vivo but reduced the phosphorylation of p185T in a dose dependent manner. Incubation of cells with EGF did not affect the level of expression of p185T or p185N. In contrast, TPA treatment increased surface expression of p185T. These findings suggest that the expression of function of p185 may be regulated via interaction with EGFR and the protein kinase C system, and that the transforming point mutation of p185 may alter these interactions.

C 111 COMPLIMENTARY DNA CLONING AND SEQUENCING OF TRANSFORMING GROWTH FACTOR BETA-1-RELATED MESSENGER RNAs FROM PORCINE LYMPHOCYTES, Paturu Kondaiah, Ellen Van Obberghen-Schilling, Robert L. Ludwig, Ravi Dhar*, Michael B. Sporn, and Anita B. Roberts. Laboratory of Chemoprevention and Laboratory of Molecular Virology*, National Cancer Institute, Bethesda, MD 20892.

Transforming growth factor beta-1(TGF beta-1) has previously been cloned, and the precursor sequences determined from human, mouse, bovine and porcine sources; a single species of 2.5 Kb mRNA related to TGF beta-1 has been reported. We now report, in porcine tissues, the existence of a prominent 3.5 Kb RNA species in addition to the 2.5 Kb RNA species and present data in support of alternate splicing and an alternate polyadenylation signal in porcine TGF beta-1 messenger RNA. We have isolated several cDNA clones which are related to TGF beta-1, among which there are cDNAs with a deletion in the coding region, which correspond exactly to the fourth and fifth exons reported for the human gene. In addition we have isolated a cDNA clone which shows a longer 3' untranslated region of 1.1 Kb, compared to the shorter sequence of 145 nucleotides in all other clones where the earlier polyadenylation signal is used. S1 nuclease mapping of RNA suggests the presence of an RNA species related to the alternately spliced RNA, while RNA blot analysis shows that the higher molecular weight messenger RNA species is related to the clone with the longer 3' untranslated extension. The sequences of all these cDNAs, together with the data on RNA expression, will be presented.

C 112 RELATIONSHIP BETWEEN CELL GROWTH, NUCLEAR ONCOGENE EXPRESSION AND DIFFERENTIATION IN MURINE ERYTHROLEUKAEMIA CELLS, Jean-Jacques LAWRENCE, Saadi KHOCHBIN, Etienne PRINCIPAUD, Bruno FRANZETTI, Agnès CHABANAS, Laboratoire de Biologie Moléculaire du Cycle Cellulaire, INSERM U 309, 85 X 38041 GRENOBLE CEDEX, FRANCE.

Murine Erythroleukaemia cells are derived from erythroid precursor after transformation by the Friend's viral complexe. They can resume a differentiation program under the action of HMBA. We have studied the variations in the cell cycle parameters, Histone H1° content and the expression of c-myc, c-myb, p53, in order to better understand the molecular events wich occur before commitment to differentiation. Cell growth underwent important modifications during the early times of induction of differentiation. A transient lenthening of both G1 and G2/M was observed, after which cells resumed a normal cell cycle, as compared to the uninduced population. Histone H1° was found to accumulate during this time, and decreased again as the cells reentered a normal proliferation cycle.

The three oncogenes tested showed a drastic fall in the accumulation of their mRNA's, as early as 1/2 hour after the input of the inducer. We studied the control level of such regulations : c-myc and c-myb mRNA's were both found regulated at a transcriptionnal level, without the prerequisite of de novo protein synthesis, (as seen in cycloheximide experiments). After 10 hours of induction c-myb mRNA remained at stationary low level, but c-myc mRNA started to accumulate again and reached a steady state level of about 50 % of the original value. p53 behaved differently in that the control level of the down regulation was post-transcriptionnal and involved the synthesis of a RNA component which is probably part of the degradation process specific for p53 mRNA. The study of the protein accumulation was possible for this oncogene, and was performed as a function of the time of induction and cell cycle proliferation. We found that, despite the fact that the cells reentered the S phase, the protein remainded at a low level, ruling out a direct role in the cell proliferation control. The results will be discussed in terms of a possible involvment of these nuclear proteins in the modulation of structural states of chromatin allowing an overall control on gene expression.

C 113 ORGANIZATION OF THE MOUSE PDGF RECEPTOR GENE, Pauline L. Lee, Jaime A. Escobedo and Lewis T. Williams, University of California, Department of Medicine, Howard Hughes Medical Institute, San Francisco, CA 94143.

The PDGF receptor (PDGFr) is comprised of discreet structural and functional domains. At the N-terminus, there is a signal sequence of 31 amino acids. The remainder of the extracellular region, the ligand binding region, has been shown by computer-assisted structural analyses to be arranged in five domains, each homologous to an immunoglobulin domain. The intracellular portion of the PDGFr contains a kinase domain split by an insertion of 107 amino acids. The C-terminus region of the PDGFr contains several possible autophosporylation sites.

To compare the organization of the receptor protein and the organization of the gene, we are currently examining the genomic sequence of the PDGFr. A mouse genomic library was screened with a 0.6 kb fragment of the PDGFr cDNA and 20 positive clones were isolated. Sequencing of a 13.5 kb clone that includes external domain coding sequences has shown that, for at least one of the immunoglobulin-like domains, the intron-exon junctions occur at the boundaries of the IgG-like domain. Further sequencing of the external domain and upstream regions is in progress.

C 114 INSULIN-ACTIVATED SERINE PHOSPHORYLATION OF AN AFFINITY-PURIFIED INSULIN RECEPTOR PREPARATION *IN VITRO*, Robert E. Lewis, G. Perry Wu, Richard G. MacDonald and Michael P. Czech, University of Massachusetts Medical School, Worcester, MA 01655.

Previous studies of insulin receptor phosphorylation *in vivo* demonstrated insulin-sensitive phosphate incorporation into tyrosine, threonine, and serine, whereas insulin receptor phosphorylated *in vitro* exhibits an insulin-dependent increase in phosphotyrosine alone. In the present studies the insulin receptor was sequentially purified on wheat germ agglutinin and insulin affinity resins followed by dialysis in 50% glycerol for 2-14h. Phosphorylation of the purified receptor in the prescence of 5mM [γ^{32}P]ATP, 1mM DTT, 3mM [Mn^{2+}], 10mM [Mg^{2+}], and 100nM insulin, followed by electrophoresis and autoradiography resulted in insulin-stimulated insulin receptor β subunit phosphorylation. Subsequent phosphoamino acid analysis by thin layer electrophoresis indicated insulin-stimulated incorporation of [^{32}P] into phosphotyrosine and phosphoserine. Densitometric scanning of the autoradiographs from phosphoamino acid analysis indicated that after 2h of phosphorylation in the presence of insulin, [^{32}P] can be incorporated into serine residues in the β subunit at a level equal to 25% of the amount of [^{32}P] incorporated into tyrosine. The appearance of phosphoserine may be dependent on prior tyrosine phosphorylation because addition of 5mM angiotensin II to the phosphorylation reaction prevented all measurable phosphorylation of the β subunit. Tryptic phosphopeptides of the labeled insulin receptor β subunit were separated on reverse-phase HPLC yielding 7-10 radioactive peaks. Labeled phosphoserine was found in the fourth peak, which appeared to contain additional peptides phosphorylated on tyrosine as well. *In vitro* serine phosphorylation of the purified insulin receptor is not affected by 1mM [Ca^{2+}] and/or 25 mg/ml phosphatidylserine. Under the conditions of our assay, a preparation of protein kinase C from bovine retinal rods did not phosphorylate the purified insulin receptor on serine above control levels. In conclusion, we have identified an insulin receptor-associated serine kinase activity *in vitro* which provides the opportunity to explore its identity and the mechanism by which it catalyzes phosphorylation of the insulin receptor tyrosine kinase in an insulin-dependent manner.

C 115 EXPRESSION OF HUMAN EPIDERMAL GROWTH FACTOR PRECURSOR CNDA IN TRANSFECTED MOUSE NIH3T3 CELLS, Barbara Mroczkowski, Martha Reich, Jonathan Whittaker, Graeme I. Bell and Stanley Cohen, Vanderbilt University School Medicine, Nashville, TN 37232 and Howard Hughes Medical Institute and Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL 60637.

Stable cell lines expressing the human EGF precursor have been prepared by transfection of mouse NIH3T3 cells with a bovine papilloma virus-based vector in which the human kidney EGF precursor cDNA has been placed under the control of the inducible mouse metallothionein I promoter. Synthesis of the EGF precursor can be induced by culturing the cells in 5 mM butyric acid or 100 uM $ZnCl_2$. The EGF precursor synthesized by these cells appears to be membrane associated; none is detectable in the cytoplasm. The size of the EGF precursor expressed by these cells is about 150-180,000 daltons which is larger than expected from its amino acid sequence suggesting that it is post-translationally modified, presumably by glycosylation. The EGF precursor was also detected in the conditioned media from these cells indicating that some fraction of the EGF precursor synthesized by these transfected cells is secreted. Preliminary data suggest that this soluble form of the EGF precursor may compete with ^{125}I-labeled EGF for binding to the EGF receptor. These cell lines should be useful for studying the processing of the EGF precursor to EGF as well as determining the properties and possible functions of the EGF precursor itself.

C 116 STUDIES OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS FOR EGF, EGFR, TGFA, GRL, FGFA, FGFB, IL2, TGFB, AND IL2R, JEFFREY C. MURRAY, H.A. ARDINGER AND R. SHIANG, UNIVERSITY OF IOWA, IOWA CITY, IOWA, 52242

Using a panel of 4-10 normal Caucasians and 60 different restriction enzymes, we have made a search for RFLPs for a variety of growth factors and their receptors. We have identified the following RFLPs:

EGF-HincII/SacI	FGFA-BclI
TGFA-BamHI/RsaI	FGFB-BglI
EGFR-HindIII/HaeIII/PstI	IL2-KpnI
XbaI/MboI	GRL-BclI

These polymorphic markers have been used in linkage studies on the long arm of chromosome 4 (EGF, IL2, and FGFB) and as candidate genes in linkage analyses of Reiger syndrome, neurofibromatosis and Van der Woude syndrome. Preliminary results suggest linkage of EGF and IL2 and FGFB in a family with Reiger syndrome. All polymorphic markers listed were excluded as candidate genes in neurofibromatosis. EGF, TGFA, GRL and FGFB were excluded as candidate genes in Van der Woude syndrome. EGFR shows linkage to Van der Woude syndrome with a lod score of 1.2 and a theta of 0. Additional studies are now underway to expand these linkage studies. RFLPs for growth factors provide *useful candidate gene markers in studies of genetic and developmental disorders.*

C 117 HTLV-II-INFECTED T-CELLS RELEASE GROWTH PROMOTING ACTIVITY FOR KAPOSI'S SARCOMA-DERIVED ENDOTHELIAL CELLS, Shuji Nakamura, Syed Z. Salahuddin, Barbara Ensoli, Peter Biberfeld, Phillip D. Markham, Flossie Wong-Staal, Lena Larsson and Robert C. Gallo, National Cancer Institute, Bethesda, MD. 20892, Karolinska Institute, Stockholm, Sweden.

Conditioned medium from T_4^+ cell lines (HTLV-II-CM) stimulated the growth of 6 of 6 KS-derived endothelial (KS-E) cells. These cultured cells are suggested to be lymphoendothelial in origin (Salahuddin et. al). This activity also stimulated the normal vascular endothelial (NE) cells. Some KS cell lines, although slow growing, have been cultured with help of this factor(s) for one year, and in large quantities. This has made it possible to compare to NE cells. Well known growth factors, including endothelial cell growth supplement (ECGS), acidic and basic fibroblast growth factors (FGF), epidermal growth factor (EGF), interleukin-1 (IL-1), tumor necrosis factor (TNF_α) and gamma interferon (IFN_γ) were also tested for their growth promoting effects. IL-1 and TNF_α stimulated the growth of KS-E cells, although their dose response was different from HTLV-II-factor(s) and they plateaued at lower cell dencity. ECGS, IFN_γ and other factors did not stimulate the growth of KS-cells. These well known factor(s) also had little or no effect on the growth of NE cells, with the exception of the strong effect of ECGS and EGF. HTLV-II-factor(s) had non or weak heparin binding activity and its molecular size was larger than IL-1 and TNF_α. This factor(s) is different from FGF, IL-1 and TNF_α, but whether it differes from other well known cytokines is under investigation. In summary, we developed an in vitro system for the study of KS. Our results show that KS-E cells have a different growth factor dependency than normal vascular endothelial cells. This system should provide further clues to our understanding of pathogenesis of KS.

C 118 EPIDERMAL GROWTH FACTOR INCREASES INOSITOL PHOSPHATE FORMATION IN DENSITY-ARRESTED BALB/c-3T3 CELLS PRETREATED WITH CHOLERA TOXIN AND ISOBUTYLMETHYLXANTHINE. N.E. Olashaw and W.J. Pledger, Vanderbilt University, Nashville, TN.

Epidermal growth factor (EGF) does not promote the proliferation of or stimulate phosphatidylinositol (PI) hydrolysis in density-arrested BALB/c-3T3 cells. Because increases in cellular cyclic AMP content render BALB/c-3T3 cells mitogenically responsive to EGF, we examined the effect of EGF and cyclic AMP elevating agents on PI turnover in these cells. We found that EGF stimulates the formation of inositol phosphates (IP) in cells pretreated with cholera toxin, an activator of adenyl cyclase, and isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor. Concomitant addition of EGF, cholera toxin and IBMX to cells did not increase IP levels, and pretreatment with both agents was required. Pre-exposure of cells to cholera toxin and IBMX also enhanced platelet-derived growth factor (PDGF)-stimulated IP formation; TGF ß, IGF I, TPA and the calcium ionophore, A23187, did not induce IP production in either pretreated or control cells. Pre-incubation of cells with cholera toxin and IBMX in the presence of cycloheximide or DRB (an inhibitor of mRNA synthesis) abrogated the permissive effect of these agents on EGF- and PDGF-stimulated IP production; these inhibitors did not affect EGF binding or the ability of PDGF to stimulate IP formation. Thus, EGF is capable of stimulating PI breakdown in BALB/c-3T3 cells if cells are pretreated with agents that increase cyclic AMP levels.

C 119 MECHANISM OF TUMOURIGENESIS BY POXVIRUSES: EXPRESSION OF AN EPIDERMAL GROWTH FACTOR-LIKE PEPTIDE BY MOLLUSCUM CONTAGIOSUM VIRUS. Colin D. Porter, Neil W. Blake and Len C. Archard, Biochemistry Department, Charing Cross and Westminster Medical School, London, W6 8RF, U.K.

Molluscum contagiosum virus (MCV) is a poxvirus of man which induces epidermal basal cell proliferation generating a benign skin tumour. We have purified virus from single lesions, characterised genomic DNA by restriction mapping and demonstrated two major subtypes of MCV.

In order to address the question of the possibility of cell transformation mediated by a viral gene product having functional homology to epidermal growth factor (EGF), we synthesized an oligonucleotide probe representing the conserved receptor-binding region of EGF-like peptides. Hybridisations with this probe demonstrated that both MCV subtypes have the potential to encode such a domain at equivalent genetic loci. However, there is no cross-hybridsation of the human EGF gene or the vaccinia growth factor gene with MCV DNA.

Restriction fragments spanning these loci were cloned and sequence analysis indicates that MCV encodes a peptide structurally homologous to EGF. We propose that expression of this gene is responsible for MCV-induced tumourigenesis and will report the effects of its expression in cultured cells.

C 120 DETERMINATION OF AMINO ACID SEQUENCE AND NUCLEOTIDE SEQUENCE OF cDNA CLONES FOR A NOVEL GROWTH REGULATORY FACTOR:MGSA, Ann Richmond[1], H.Greg Thomas[1], Joachim Spiess[2], Gail Flaggs[3], E. Balentein[1], and Rik Derynck[3], V.A. Medical Center and Emory University, Atlanta, GA[1]30033, Peptide Biology Laboratory, Salk Institute, La Jolla, CA[2] 92138, Department of Molecular Biology, Genentech, South San Francisco, CA[3] 94084.

Melanoma growth stimulatory activity (MGSA) is a novel growth regulatory peptide originally isolated from conditioned medium from the Hs294T human melanoma cell line. Though MGSA was originally described as an autocrine growth factor for human melanoma cells, immunoreactive MGSA has recently been demonstrated to be present in a number of non-melanocyte tissues exhibiting proliferative disorders. After purification to homogeneity by gel filtration, heparin sepharose chromatography and reverse phase chromatography, MGSA was subjected to gas phase sequencing and the amino acid sequence of 34 residues from the amino terminus was determined. Oligonucleotide probes based on this NH_2-terminal sequence data were used to probe cDNA libraries from melanoma and placenta and the nucleotide sequence for the mRNA was determined. From these data the sequence of the MGSA precursor and the MGSA protein have been deduced. The amino terminal amino acid sequence data and nucleotide sequence data demonstrate that MGSA represents a separate class of growth regulatory peptides with approximately 40% homology to a platelet factor involved in the inflammatory response.

C 121 STRUCTURAL CHARACERIZATION OF THE HUMAN PLATELET-DERIVED GROWTH FACTOR A-CHAIN cDNA AND GENE, Fredrik Rorsman[1], Timothy J. Knott[2], James Scott[2] and Christer Betsholtz[1], [1]Dept. of Pathology, University Hospital, Uppsala, Sweden, [2]Molecular Medicine Research Group, MRC Clinical Research Centre, Harrow, Middlesex HA1 3UJ, UK.

The human PDGF A-chain locus has been characterized by restriction endonuclease analysis and the nucleotide sequence of its exons determined. Seven exons have been identified spanning approximately 22 kb of genomic DNA. Alternative exon usage, identified by cDNA cloning, occurs in a human glioblastoma cell line and may give rise to two types of A-chain precursors with different C-terminals. The exon-intron arrangement is similar to that of the PDGF B-chain/SIS locus and seems to divide the precursor proteins into functional domains. Southern blot analysis of genomic DNA shows that a single PDGF A-chain gene is present in the human genome.

C 122 DIRECT IDENTIFICATION AND CHARACTERIZATION OF THE p75 SUBUNIT OF THE HIGH AFFINITY INTERLEUKIN-2 RECEPTOR.

Horacio Saragovi and Thomas R. Malek, University of Miami School of Medicine, Miami, FL. 33101.

The interleukin-2 (IL-2) receptor was initially defined in biochemical and cDNA cloning studies as a glycoprotein of M_r of 55,000 (p55). However, recent chemical cross-linking studies of radiolabeled IL-2 to IL-2 receptors have suggested that functional high affinity IL-2 receptors, which generally represent a small fraction (~5%) of total cell surface IL-2 receptors, may contain an IL-2 binding subunit of 75,000 daltons (p75). In the present investigation, we have studied the proteins closely associated with p55 in several cell populations that varied in their capacity to express high affinity IL-2 receptors. Cell surface proteins were radiolabeled by lactoperoxidase catalyzed iodination and treated with the reversible homobifunctional cross-linker dithiobis(succinimidyl propionate) (DSP). The proteins cross-linked to p55 were then precipitated with antibodies specific for p55 and analyzed on SDS-PAGE. A glycoprotein of M_r 75,000 (p75) was cross-linked to p55 from cells that bore high affinity IL-2 receptors. Three other proteins of apparent M_r of 100,000 (p100), 135,000 (p135), and 180,000 (p180) were also cross-linked to p55. In contrast, analysis of the p55 cross-linked proteins from the cell populations that expressed only low affinity IL-2 receptors revealed p100, p135, and p180, but not p75. Biochemical characterization of p75 showed that it is a cell surface glycoprotein modified by the addition of ~20,000 daltons of N-linked glycans, with a pI of 4.3 to 5.0. These results provide direct biochemical evidence for a close association of p55 and p75 in the formation of functional high affinity IL-2 receptors. The cross-linking of p100, p135 and p180 to p55 raise the possibility of a more complex IL-2 receptor subunit structure, although the detection of these three proteins on all cell populations indicates that in the absence of p75 they do not play a role in ligand binding affinity.

C 123 NUCLEAR PROTEINS INTERACTING WITH THE PROMOTER REGION OF THE HUMAN GRANULOCYTE/MACROPHAGE COLONY STIMULATING FACTOR GENE (DNA-BINDING PROTEINS/GENE EXPRESSION/TISSUE SPECIFICITY/GROWTH FACTORS/HEMOPOESIS, Mathew A. Vadas, Jennifer R. Gamble and M. Frances Shannon, Division of Human Immunology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia, 5000.

in a tissue specific as well as activation-dependent manner. The interaction of nuclear proteins with the promoter region of the GM-CSF gene that are likely to be responsible for this pattern of GM-CSF expression was investigated. We show that nuclear proteins interact with DNA fragments from the GM-CSF promoter in a cell specific manner. A region spanning two cytokine specific-sequences, cytokine-1 (5'GAGATTCCAC3') and cytokine-2 (5'TCAGGTA3') bound two nuclear proteins (nuclear factor (NF)-GMa and NF-GMb) from GM-CSF expressing cells in gel retardation assays. NF-GMb was inducible with phorbol myristate acetate and accompanied induction of GM-CSF message. NF-GMb was absent in cell lines not producing GM-CSF some of which had other distinct binding proteins. NF-GMa and NF-GMb eluted from a heparin sepharose column at 0.3 and 0.6M KCl respectively. We hypothesize that the sequences CK-1 and CK-2 bind specific proteins and regulate GM-CSF transcription.

C 124 EXPRESSION OF THE IL-2 RECEPTOR ON HUMAN BLOOD BASOPHILS,
Peter Valent, Hannes Stockinger*, Christoph Stain, Klaus Lechner, Walter Knapp* and Peter Bettelheim, 1st Medical Dept. and Institute of Immunology*, University of Vienna, Lazarettg.14, A-1090 Vienna, Austria.
Using a combined toluidine/immunofluorescence staining procedure we were able to establish the surface marker phenotype of human basophils. At least three activation linked structures were detected by moabs clustered as CD9, CD38 and CD25 (anti-IL-2 receptor) on normal and CML basophils. Immunoprezipitation using the anti-Tac antibody was performed on highly enriched CML basophils (complement lysis) revealing a 55 kd surface protein equivalent to the Tac peptide of the IL-2 receptor on human T cells. Quantitative binding assays using radiolabelled IL-2 indicated a majority of low-affinity IL-2 binding sites and about fifty high-affinity IL-2 binding sites. Furthermore using Northern blot analysis two mRNA bands of 3.5 and 1.5 kb were found to hybridize to a radiolabelled cDNA coding for the Tac peptide. Thus IL-2 receptors are actively synthesized by human basophils. Preliminary results of the functional significance suggest a possible role of IL-2 in the mediator release from human blood basophils.

C 125 STRUCTURAL AND FUNCTIONAL ANALYSIS OF p58, A PROTEIN PRODUCT RELATED TO THE TYROSINE KINASE pp56^{lck}. André Veillette[1], Ivan Horak[1], Anne Burkhardt[1], Neal Rosen[2], and Joseph B. Bolen[1]. Laboratory of Tumor Virus Biology[1] and Medical Breast Cancer Section[2], National Cancer Institute, Bethesda, MD 20892.

The tyrosine kinase gene lck is expressed in lymphoid cells and tissues as well as certain types of non-lymphoid human solid tumors such as colon carcinoma and small cell carcinoma of the lung. It appears to play a role in differentiated lymphoid functions and potentially in transformation as well as tumor progression. It primarily encodes a 56 kDa membrane associated product demonstrating tyrosine specific protein kinase activity. Using a specific anti-lck antiserum (recognizing a peptide sequence not conserved between the different members of the src-related tyrosine kinase family), we have found that most human cells expressing this tyrosine kinase also possess in addition to pp56 another related membrane associated protein migrating at 58 kDa on SDS-polyacrylamide gels. Its detection by immunoprecipitation is blocked by preincubation of the antiserum with the lck peptide. Peptide mapping revealed that p58 differs from pp56^{lck} in the amino-terminal half of the molecule and our data suggests that this alteration is related to p56 hyperphosphorylation and can be further increased in T-lymphocytes by treatment with phorbol esters. Interestingly p58 is phosphorylated in vivo on serine and tyrosine residues but lacks demonstrable in vitro phosphorylation in immune complex kinase assays. This suggests that p58 has reduced enzymatic activity compared to pp56^{lck}. The potential regulatory function of this alteration and its role in lymphoid signal transduction are currently being evaluated.

C 126 ISOLATION OF HIGH AFFINITY MONOCLONAL ANTIBODIES TO BIOACTIVE HUMAN ERYTHROPOIETIN AND A COMPARISON OF THEIR USE TO QUANTITATE EP LEVELS BY SENSITIVE RADIO-AND ENZYME IMMUNOASSAYS, Albertus Wognum, Peter M. Lansdorp, Connie J. Eaves and Gerry Krystal, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, Canada.
We have developed several monoclonal antibodies to human erythropoietin (Ep) using a bioimmunoabsorbentassay (BISA). In this assay, mouse antibodies are immobilized in microtitre wells previously coated with goat anti-mouse Ig antibodies, the wells are then incubated with human Ep, washed and Ep that has specifically bound to the antibodies is detected by measuring ^{3}H-thymidine incorporation of Ep-dependent spleen cells in these wells. The BISA is superior to conventional binding assays as RIA and ELISA, since 5 monoclonal antibodies isolated with these latter screening assays were not reactive to native Ep and 2 of 3 antibodies that were isolated with the BISA, and thus reactive to native Ep, could not be detected with ELISA. All 3 BISA-reactive antibodies were capable of neutralizing Ep bioactivity in vitro and were able to bind to urinary as well as recombinant Ep on immunoblots. Testing of the antibodies in radioimmunoprecipitation experiments yielded dissociation constants of 0.7 nM, 8 nM and 240 nM for the 3 antibodies, respectively. The two antibodies with highest affinity for Ep were used in a two-sided or sandwich radioimmunoassay (Ep-RIA) and in an enzyme immuno-assay (Ep-EIA) for Ep. The Ep-RIA has a sensitivity of 10 mU whereas the Ep-EIA can detect 1 mU, comparable to the detection limit of the in vitro bioassay. The Ep-EIA provides a sensitive, rapid and nonradioactive method for the measurement of Ep and should prove of considerable value in a variety of clinical and laboratory applications.

C 127 EXPRESSION OF INSULIN-LIKE GROWTH FACTOR (IGF) mRNAs IN HUMAN BREAST CANCER.
Douglas Yee[1], Kevin J. Cullen[1], Soonmyoung Paik[1], Arnold Schwartz[2], André Veillette[3], Francine Foss[1], Susan Bates[1], Roberto Favoni[1], Marc Lippman[1] and Neal Rosen[1]. Medical Breast Cancer Section[1] and Laboratory of Tumor Virus Biology[3], National Cancer Institute, Bethesda, MD. and Department of Pathology[2], George Washington University Medical Center, Washington, D.C. 20037
IGF-I and IGF-II are mediators of normal fetal and childhood growth and development. They have been reported to be expressed in some malignancies and we have shown that breast cancer cell lines produce immunoreactive IGF-I-like material. We have examined the mRNA expression of IGF-I and IGF-II in human breast cancer tissue and breast cancer cell lines. Northern analysis of polyA+ mRNA from breast cancer cell lines show multiple hybridizing species of mRNA. However using an IGF-IA probe in a sensitive RNAse protection assay, none of the cell lines express authentic IGF-I. This suggests the immunoreactive IGF-I-like material produced by these cell lines is a closely related IGF species. In contrast, RNA extracted from tumor tissues, fibroadenomas and surrounding normal breast have easily detectable IGF-I in an RNAse protection assay. In the breast cancer lines T47D and HBL100, IGF-II mRNA has been found to be expressed using Northern analysis and RNAse protection assay. In T47D the level of mRNA is increased by estrogens and decreased by antiestrogens. RNA extracted from tumor tissues shows that virtually all cancers tested express some IGF-II mRNA. In addition fibroadenoma and surrounding normal breast tissues also express IGF-II. These data suggest that IGF-I and IGF-II may act as paracrine or autocrine growth factors in proliferative breast disease.

C 128 MCF-7 BREAST CARCINOMA CELLS EXPRESSING TRANSFECTED TGF ALPHA SEQUENCES CONTINUE TO REQUIRE ESTROGEN FOR GROWTH AND TUMORIGENICITY.
R. Clarke, M. Lippman, P. Glanz, G. Wilding E Valverius S Bates R. Dickson and F Kern, Breast Cancer Section, Medicine Branch, NCI, NIH, Bethesda, MD 20892.
Estrogen (E2) treatment of MCF-7 cells results in an increase in the amount of TGF alpha mRNA and an increase in the secretion of a 30kd TGF alpha-like protein. Thus, E2 stimulation of both in vitro growth and tumorigenicity may be related to an autocrine loop involving the production of this growth factor. To explore this possibility, we have transfected MCF-7 cells with plasmids that contain the TGF alpha precursor peptide coding sequences linked to either the human metallothionein or the adenovirus major late promoter. Stable transformants expressing the TGF alpha sequences were isolated. These transformants constituitively express elevated levels of the appropriately sized mRNA as determined by Northern blotting and secrete elevated levels of a biologically active TGF alpha protein as determined by an EGF radioreceptor competition binding assay and NRK soft agar colony formation assay. However, transformants express estrogen receptor levels equivalent to nontransformed cells and respond to E2 by increased cell proliferation in vitro. Preliminary results indicate that transformed cells do not form progressively growing tumors in nude mice in the absence of E2. These results suggest that TGF alpha alone is not sufficient to fully induce the hormone-independent phenotype in hormone-dependent MCF-7 cells.

Intracellular Signals

C 129 THE IDENTIFICATION OF A REGION ON THE RAS MOLECULE SPECIFICALLY RECOGNIZED BY A GTPase ACTIVATING PROTEIN (GAP),
H. Adari*, D. Lowy†, B. Willumsen§, C. Der#, and F. McCormick*, *Cetus Corporation, Emeryville, CA; †NIH, Bethesda, Maryland; §University Microbiology Institute, Copenhagen, Denmark; and #La Jolla Cancer Research Foundation, La Jolla, CA.
Previous work in our lab has shown that extracts made from various mammalian cells contain a soluble protein (GAP) that interacts specifically with the N-ras proto-oncogene to greatly enhance its intrinsic GTPase activity. We have since observed that the GTPase activity of C-H-ras can also be stimulated by GAP, making it unlikely that GAP interacts with the variable C-terminal region of the ras protein (p21). We have examined various point- and deletion-mutants of H-ras for their sensitivity to GAP action in an attempt to identify the site of interaction between GAP and p21. The results indicate that the oncogenic mutations at positions 12, 59 and 61 cause a complete loss of sensitivity to GAP. These residues are thought to participate in the phosphoryl binding loop within the GTP binding site. Other mutations in the GTP binding site (in the purine ring binding region) do not seem to affect GAP recognition. Similarly, the non-transforming mutations at position 65, 74-77, 123-130 and 165-184, are still susceptible to GAP action. The partial loss of sensitivity to GAP observed with deletion mutants 96-104 and 101-109, is believed to be due to structural constraints and not to a partial loss of the GAP target site. In summary, our results so far suggest that GAP specifically recognizes the phosphoryl binding loop of p21. We are currently testing mutants in the putative effector binding region of p21 to determine whether GAP functions as the ras effector protein.

C 130 CHARACTERIZATION OF HIGH MOLECULAR WEIGHT PROTEIN RECOGNIZED BY PDGF-ANTISERA, Dana T. Graves and Harry N. Antoniades, Boston University Medical Center and Harvard School of Public Health, Boston, MA 02115.

We have demonstrated previously that a high molecular weight glycoprotein could be immunoprecipitated from metabolically labelled human osteosarcoma (U-2 OS) cells with anti-PDGF serum and that it appears to be derived from a different precursor than the 30 kd PDGF-like mitogen produced by these cells. The identification of high molecular weight proteins with anti-PDGF antibodies has been noted by other investigators. These findings were unexpected since the high molecular weight proteins are too large to be encoded by the PDGF structural genes. In experiments with metabolically-labelled U-2 OS human osteosarcoma and nontransformed NRK cells we now report that a 185 kd protein immunoprecipitated with anti-PDGF serum from these cells has the following characteristics: 1) It is a PDGF binding protein that is unrelated to alpha 2 macroglobulin. 2) It is a phosphoprotein. 3) It is phosphorylated in response to PDGF stimulation. 4) It is immunoprecipitated by both anti-phosphotyrosine and anti-PDGF antibodies. 5) It is not a general substrate of growth factor induced tyrosine kinase activity. 6) It is most likely a membrane or membrane associated protein. 7) Its presence in nontransformed cells is dependent on the additions of exogenous PDGF.

C 131 INTERFERON REGULATION OF GENE EXPRESSION AT POST-TRANSCRIPTIONAL SITES, R. Douglas Armstrong, La Jolla Cancer Research Foundation, La Jolla, CA 92037.

The ability of alpha-Interferon (alpha-Ifn) to selectively stimulate the release of nuclear mRNA of interferon-regulated genes to the cytoplasm was investigated. An in vivo assay designed to study the nucleocytoplasmic active mRNA transport under conditions which do not allow transcription was utilized for these studies. Nuclei were isolated from HL-60 human promyelocytic leukemia cells and suspended in a standard mRNA transport buffer at 37°, which contained either no alpha-Ifn or 100 IU alpha-Ifn, for 30 min. The supernatent was then isolated and divided into 3 aliquots which were subjected to analysis using standard Northern blot procedures. The efflux of 3 mRNA were determined: cMyc oncogene, dihydrofolate reductase (DHFR), and the alpha-Ifn regulated 56-K. Alpha-Ifn was found to selectively stimulate the efflux of the 56-K mRNA from the HL-60 nuclei, with efflux increased by 118% compared to control. The efflux of cMyc and DHFR from alpha-Ifn treated nuclei was only slightly altered, exhibiting increases of 35% and 18% respectively from control. These results demonstrate that alpha-Ifn can selectively alter the post-transcriptional processing and transport of mRNA for specific genes, and this may serve as a key receptor site for interferons.

C 132 EGF-STIMULATED DNA SYNTHESIS IS DEPENDENT UPON AN INFLUX OF EXTRACELLULAR CALCIUM, Alton L. Boynton, Timothy D. Hill, and Henrick Kindmark, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI.

Mitogenic stimulation of proliferatively quiescent cells is known to be inhibited by the removal of extracellular Ca^{2+}. This Ca^{2+} requirement was further investigated by determining the effect of a plasma membrane Ca^{2+} channel blocker on intracellular Ca^{2+} levels and DNA synthesis induced by EGF. T51B rat liver epithelial cell grown on glass coverslips and loaded with Fura-2 responded with elevated intracellular Ca^{2+} dose-dependently to EGF (5-50 ng/ml). Pretreatment with La^{3+} (50 μM) blocked completely the EGF-induced rise in intracellular Ca^{2+}. La^{3+} was unable to penetrate the plasma membrane as evidenced by a lack of change in the Fura-2 signal. Ca^{2+} mobilization from the ER pool stimulated by thrombin was unaffected by La^{3+} treatment. Confluent and proliferatively quiescent T51B cells treated for 24 hr with EGF (10 ng/ml) in 99% BME:1% BCS (1.8 mM Ca^{2+}) incorporated ^{3}H-thymidine into >75% of cell nuclei. In the presence of La^{3+} (50 μM) <5% of cell nuclei were found to be radiolabeled. These results indicate clearly that the EGF-induced influx of extracellular Ca^{2+} and subsequent DNA synthesis are abolished by plasma membrane channel blockage with La^{3+}. Therefore, a rise in intracellular Ca^{2+} appears to be a prerequisite for EGF mitogenic activity. Supported by NIH/NCI CA42942.

C 133 **THREE SIGNAL TRANSDUCTION PATHWAYS REGULATED BY EGF IN THE RAT HEPATOCYTE**, Patricia A. Connelly and James C. Garrison, Department of Pharmacology, University of Virginia Medical School, Charlottesville, VA 22908

Phosphorylation studies in ^{32}P-labeled rat hepatocytes have demonstrated that EGF treatment of these cells activates a tyrosine protein kinase, most likely to be the intrinsic EGF receptor tyrosine kinase, in addition to the activation of protein kinase C and Ca^{2+}/calmodulin-dependent protein kinases. Activation of all three kinases appears to involve only a single receptor type since phosphorylation of each class of substrate has a similar sensitivity to the concentration of EGF. Although the reversal of protein kinase C and Ca^{2+}-stimulated phosphorylation occurs rapidly, tyrosine phosphorylation of a cytosolic substrate, hpp 36, unique to EGF treatment, but apparently distinct from the lipocortins (calpactins), is stable for up to 30 min following stimulation and thus has the potential to be involved in regulation of gene expression by EGF.

C 134 HUMAN GM-CSF: RECEPTOR BINDING AND TRANSMEMBRANE SIGNALLING; J. DiPersio, P. Billing, S. Kaufman, P. Naccache*, P. Borgeat*, and J. Gasson; Division of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA; and *Unité de Recherche, Centre Hospitalier de l'Universite, Laval Quebec, Canada.

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) binds to high affinity receptors (Kd 30-50 pM) on all responsive hematopoietic cells, and is most highly expressed on mature neutrophils and eosinophils. The molecular weight of human GM-CSF receptor by cross-linking is 84 Kd. GM-CSF has multiple direct and indirect effects on neutrophils, but the mechanism of action remains elusive. Although GM-CSF does not directly induce changes in intracellular pH or Ca^{++}, pre-incubation of GM-CSF dramatically increases the intracellular acidification and levels of intracellular Ca^{++} after stimulation with FMLP, LTB_4, and PAF. In addition, pre-incubation of neutrophils with GM-CSF resulted in a profound depression of cellular alkalinization induced by chemotactic factors and phorbol esters, which was not due to a direct inhibitory effect on the Na/H^+ antiport. GM-CSF, a weak inducer of neutrophil arachidonic acid release, was found to prime neutrophils for increased arachidonic acid release in response to A23187, FMLP, LTB_4, PAF, and C5a. This priming effect was maximal after a 45-minute incubation at 23° with concentrations of GM-CSF $\geq$100 pM, and was not inhibited by pre-incubation of neutrophils with cycloheximide or indomethicin. The products of GM-CSF-primed neutrophil arachidonic acid release were found by radioimmunoassay and HPLC to be products of the 5-lipoxygenase system; specifically, LTB_4, its epimers, and the 20 omega metabolites of LTB_4.

C 135 $p21^{ras}$ INDUCED RESPONSIVENESS OF PHOSPHATIDYL INOSITOL TURNOVER TO BRADYKININ IS A RECEPTOR NUMBER EFFECT, Julian Downward, Jean de Gunzberg and Robert weinberg, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

Proteins encoded by ras genes have recently been reported to couple certain growth factor receptors to phospholipase C, the enzyme catalyzing phosphatidyl inositol breakdown. To investigate this hypothesis, the normal Harvey-ras gene was transfected into Rat-1 fibroblasts under the control of the SV40 early promoter. Several cell lines were selected that expressed very high levels of $p21^{ras}$. Phosphatidyl inositol (PI) turnover was measured in these cells in response to a wide variety of peptide factors; bradykinin was found to have a greatly enhanced effect on the $p21^{ras}$ overexpressors relative to the parental and control cells. Bradykinin receptor numbers were measured in these lines and found to be up to forty fold higher in the $p21^{ras}$ overexpressors than in the parental cells. Receptor numbers correlated well with the bradykinin dependent PI response. In a sub-cellular system, phospholipase C activities maximally stimulated by a non-hydrolyzable analogue of GTP were not significantly different between membranes from parental and transfected cells. These data indicate that the effects of $p21^{ras}$ on cellular responses to bradykinin are due to changes in receptor number rather than direct coupling by $p21^{ras}$ between the receptor and phospholipase C.

C 136 SIGNAL TRANSDUCTION IN PROLIFERATING ENDOTHELIAL CELLS: ROLE OF ARACHIDONIC ACID (AA) METABOLITES. V.Fafeur, F.Dray* and P.Böhlen, Universität Zürich, Switzerland. * Institut Pasteur, Paris, France

AA metabolites are potent biological mediators of the action of growth factors. The proliferation of endothelial cells is controlled by stimulatory and inhibitory factors, basic Fibroblast Growth Factor (bFGF) and Transforming Growth Factor-beta (TGFb) being the best known. Since the mode of action of bFGF or of TGFb is unclear, we asked whether AA metabolites are involved in mediating their effect. In a proliferative test assay, bovine aortic endothelial cells were treated with inhibitors of AA metabolism . Both nordihydroguaiaretic acid, a lipoxygenase inhibitor, or eicosatetraynoic acid, a blocker of AA metabolism, impaired the mitogenic effect of bFGF but indomethacin, a cyclooxygenase inhibitor, was ineffective. The inhibitory effect of TGFb, in the presence or absence of bFGF, was not modified by AA metabolism inhibitors. Within minutes, bFGF stimulated the release of AA from cells prelabeled with (3H)AA, but TGFb did not prevent this effect. These results indicates that AA metabolites, possibly lipoxygenase metabolites, are involved in bFGF-induced endothelial cell proliferation and that TGFB does not interfere in this early mitogenic event.(V.Fafeur is recipient of a grant from SANOFI).

C 137 PHOSPHORYLATION OF BASIC FGF: A NEW SUBSTRATE FOR PROTEIN KINASE C
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Basic fibroblast growth factor (bFGF) can be phosphorylated in vitro by purified protein kinase C (PKC). The reaction is phospholipid-dependent and the kinetic parameters of the reaction (km=1.4 μM, V_{max}=1.5 pmol/min) indicate that FGF is a good substrate. Human and bovine bFGF as well as bovine acidic FGF could be phosphorylated by PKC and, to a lesser extent, by cAMP-dependent protein kinase. Only serine was found to be the amino acid targetted by the phosphorylation of human bFGF by PKC. Although an analysis of the protein sequence indicated three potential phosphorylation sites for protein kinase C (-Ser/Thr-X-Arg/Lys), experiments using peptide fragments of bFGF identified Ser 108 as the major site of phosphorylation in vitro. It is interesting to note that Ser 108 is conserved in the sequences of int-2 and hst, two FGF-related oncogene products. The phosphorylated form of bFGF by PKC did not modify the binding of bFGF to heparin-Sepharose even though Ser 108 is located within the heparin binding site of the molecule. In vivo ^{32}P-labeling of bovine endothelial cells allowed us to isolate a 16kD protein whose phosphorylation is increased by TPA treatment and which may represent an intracellular phosphorylation of bFGF. A comparative analyses of the sites of in vitro and in vivo phosphorylation of bFGF will be presented. The possibility that some of the biological activities of bFGF may be regulated by phosphorylation is currently under investigation.

C 138 PDGF AND cAMP APPEAR TO INDUCE c-myc RNA BY DIFFERENT MECHANISMS, Kevin K. Frick and Charles D. Scher, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The human osteogenic sarcoma cell line MG-63 responds to TPA or PDGF treatment with an increase in c-myc RNA levels. In order to determine if other signal transduction pathways would also serve in c-myc induction in these cells, the ability of MG-63 cells to respond to an increase in intracellular cAMP was examined. Agents which stimulate adenylate cyclase (forskolin, cholera toxin), inhibit phosphodiesterase (IBMX), and cAMP analogues (dibutyryl cAMP) all caused an increase in c-myc RNA. The addition of manganous chloride ($MnCl_2$)(1 mM) also caused both an increase in cellular cAMP and an increase in c-myc RNA. Both c-myc RNA induction and cAMP levels in cells treated with forskolin increased in a dose-dependent fashion, indicating that these two factors are directly related. The maximum increase in cellular cAMP was only 2 to 3 fold over the resting level, suggesting that small changes in [cAMP] are sufficient for c-myc RNA induction. When the kinetics of cAMP levels after forskolin addition were studied, it was found that the increase in [cAMP] peaked at approximately 15 min after agonist addition, then returned to near the basal level within 60 min. In contrast, PDGF caused a slower increase in cAMP levels, with values either maximal or still increasing after 60 min. The kinetics of cAMP induction by PDGF imply that this second messenger is probably not the prime instrument in c-myc induction by PDGF.

C 139 SERUM-INDUCED ACTIVATION OF SODIUM/PROTON EXCHANGE DOES NOT INVOLVE KINASE C
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One of the earliest events to occur upon the addition of serum to quiescent cells is an activation of sodium/proton exchange. In the absence of bicarbonate, or at low external pH, this activation will lead to an increase in intracellular pH (pHin). In some systems, addition of kinase C activators, such as phorbol esters or di-octanoyl glycerol, will cause pHin to rise (1). This has prompted some researchers to hypothesize that sodium/proton exchange is physiologically activated through phosphorylation by serum induction of kinase C. We have examined this question by measuring the pHin of both primary human fibroblast (BoGi), BALB/c-3T3, Swiss-3T3 and NIH 3T3 cells using the fluorescence of pyranine (2). Phorbol esters or DOG induce intracellular alkalinization in BoGi cells, yet have no effect on the pHin of 3T3 cells. Sphingosine has been reported to inhibit kinase C both in vitro (3) and in vivo (4). In our system, sphingosine inhibits both cell growth (3T3 and BoGi cells) and the phorbol-induced alkalinization (BoGi cells). However, it has no effect on the serum-induced pHin change in either 3T3 or BoGi cells. We conclude that the serum-induced activation of sodium/proton exchange is independent of kinase C, and that phorbol-induced alkalinization is the result of promiscuous phosphorylation of the sodium/proton exchanger by kinase C.

(1) Reviewed in Moolenaar (1986) Ann. Rev. Physiol. 48, 363 (2) Giuliano and Gillies (1987) Analyt. Biochem. (in press) (3) Hannun et al. (1986) J. Biol. Chem. 261,12604 (4) Merrill et al (1986) and Wilson et al (1986) J. Biol. Chem. 261, 12610 and 1616

C 140 cDNA SEQUENCE OF HUMAN ENDONEXIN II: HOMOLOGY WITH LIPOCORTIN, CALPACTIN, AND PROTEIN II,
Harry T. Haigler*, David D. Schlaepfer*, Michael Jaye#, and Ruth Kaplan#, *University California, Irvine, CA 92717, and #Rorer Biotechnologies, Inc., Springfield, VA 22151.

Endonexin II is a member of the family of Ca^{2+}-dependent phospholipid-binding proteins known as annexins. We isolated and sequenced a cDNA clone for human endonexin II and showed that it contained a 960 nucleotide open reading frame. The deduced 320 amino acid sequence was in agreement with the previously determined partial amino acid sequence of the isolated protein. The partial sequence of bovine endonexin I could be aligned with the sequence of endonexin II to give 63% sequence identity. Endonexin II contained 58, 46, and 43% sequence identity to protein II, calpactin I (p36, protein I), and lipocortin I (p35), respectively. Like these other proteins of known sequence, endonexin II had a 4-fold internal repeat of approximately 70 residues preceded by an amino terminal domain that lacks homology with the repeated region. Comparing the amino terminal domains of these four proteins revealed that, in general, they have only limited homology; endonexin II had the greatest sequence identity (29%) with protein II in this region. Despite structural diversity in this domain, lipocortin I, calpactin I, and protein II are all phosphorylated at an analogous site by protein kinase C. Endonexin II was not a substrate for this enzyme even though it contained threonine at the analogous position. It is proposed that a lysine residue that is present in the three substrate proteins, but missing in endonexin II, is a part of the recognition site for protein kinase C.

C 141 CONSTITUTIVE TYROSINE-PHOSPHORYLATION IN CHRONIC MYELOGENOUS LEUKEMIA CELLS OF A 56kDa PROTEIN THAT IS ORDINARILY RELATED TO HEMOPOIETIC GROWTH FACTOR STIMULATION.
Richard D. Huhn, and A. Raymond Frackelton, Jr., Roger Williams General Hospital, Brown University, Providence, RI 02908.

Chronic myelogenous leukemia (CML) cells express an abnormal constitutively active protein-tyrosine kinase ($P210^{bcr\text{-}abl}$) as a result of the fusion of the *bcr* and *abl* genes in the Philadelphia chromosome (Ph^1). Ph^1-positive CML cell lines and peripheral blood mononuclear leukocytes have been shown to possess a characteristic spectrum of tyrosine-phosphorylated proteins by affinity chromatography on a monoclonal antibody to phosphotyrosine. These proteins may be substrates of the $P210^{bcr\text{-}abl}$ kinase and may be growth- or differentiation-related signal transducing proteins. In order to further characterize these proteins, we have examined proteins phosphorylated on tyrosine by stimulation of non-CML cell lines with specific hematopoietic growth factors. Colony-stimulating factor 1 (CSF-1, M-CSF) supports the growth and maturation of monocyte and macrophage cells in culture. Its receptor, the product of the c-*fms* proto-oncogene, is a ligand-stimulated protein-tyrosine kinase. Cells of the human choriocarcinoma cell line, BeWo, known to express the CSF-1 receptor, were metabolically labelled with $^{32}P_i$ and stimulated with recombinant human CSF-1, resulting in the specific phosphorylation of a 56kDa protein. Phosphopeptide mapping of this protein by staphylococcal V8 protease gave an identical pattern to that of the 56kDa phosphoprotein of CML cells, indicating that they are the same protein. Thus, the phosphorylation of a 56kDa protein ordinarily resulting from growth factor stimulation is seen to occur constitutively in CML cells. Further characterization of this protein and its possible relation to members of the protein-tyrosine kinase family will be presented.

C 142 NOVEL GENE INDUCTION IN RESPONSE TO DEFINED MITOGENIC SIGNALS, Steven G. Irving, Peter F. Zipfel, Kathy Kelly and Uli Siebenlist, NCI and NIAID, National Institutes of Health, Bethesda, MD 20892

A cDNA library, highly enriched for genes induced in the initial stages of mitogenic stimulation of human peripheral blood T cells, was constructed by subtractive cloning techniques. PHA and PMA were used to stimulate the cells which served as the RNA source for the cloning procedure; this combination of mitogens stimulates virtually all the cells in a given population of peripheral blood T cells. Therefore, the response of PHA/PMA induced genes to mitogenic monoclonal antibodies specific for defined cell-surface structures was examined in order to: 1) define regulatory groups of coordinately expressed genes, and 2) correlate or uncouple gene expression with mitogenesis.

The expression of ten novel induced genes, in response to various T cell mitogens, was examined by northern blot analysis. Monoclonal antibodies which are known to bind to different surface structures (OKT3, α-T cell receptor, α-T-11, 9.3), the mitogenic lectin, PHA, and chemicals which have mitogenic activity (PMA, Ionomycin) were used. Analysis of the expression of the genes assayed (either with or without the inclusion of PMA as a comitogen) enabled the subdivision of the genes into subgroups based on their responses to the various mitogens. Furthermore, groups of mitogenic stimuli could be discriminated, based on whether similar genes were induced by the agents used and on the presence or absence of a PMA (comitogenic) effect on the induction of the genes examined.

C 143 INTERLEUKIN 2 SIGNAL / RESPONSE COUPLING: SEARCH FOR SECOND MESSENGERS. S.J. LeGrue. The University of Texas M. D. Anderson Hospital, Houston, TX, 77030.

This study tested the hypothesis that the coupling of interleukin 2 (IL 2) binding to its specific membrane receptor with cellular proliferation is mediated by the generation of intracellular second messengers. The IL 2-dependant murine T cell line CTLL-2 was stimulated with recombinant human IL 2 (rhIL 2), and subsequent changes in intracellular Ca^{2+} ($[Ca^{2+}]i$), intracellular pH (pH_i), and membrane potential were assessed using fluorescent indicators. No increase in $[Ca^{2+}]_i$ was observed in IL 2-stimulated CTLL-2 cells using Fura-2. Similarly, no evidence was obtained to implicate either the Na^+/H^+ antiporter or the Na^+/K^+ ATPase in delivery of the proliferative stimulus, because no changes were detected in either pH_i or membrane potential subsequent to rhIL 2 stimulation. These fluorometric studies were confirmed by the failure of either amiloride (≤50 μM) or oubain (≤ 200μM) to significantly block growth. The role of protein kinase C (PKC) in IL 2 signaling was examined by pretreating the cells with high concentrations of phorbol ester (TPA) for 8 h prior to IL 2 stimulation to down-regulate intracellular PKC. Preincubation of CTLL-2 cells with 400 nM TPA had no effect on either baseline or stimulated thymidine incorporation, consistant with the static $[Ca^{2+}]_i$ following rhIL 2 binding. Finally, we evaluated the contribution of G-proteins to IL 2 signaling by pretreatment with pertussis toxin or cholera toxin (CT) to modulate G_i or G_s, respectively. Pertussis toxin (100 ng/ml) had no effect on IL 2-driven proliferation. Cholera toxin showed a time and dose dependent reduction in the absolute proliferative response (IC_{50} = 0.7 ng/ml at 4h), although the kinetics of the IL 2 response were unaffected. The effect of 1 to 10 ng/ml CT was maximal when added 6 h after rhIL 2, and was reversible if the CT as removed within the first 2 h after addition of rhIL 2. Together, these data suggest that the unique proliferative stimulus provided by IL 2 does not use the same types of intracellular second messengers generated subsequent to the binding of antigen or mitogen by T cells. (Supported by The University of Texas Cancer Foundation.)

C 144 MICROINJECTED, INTRACELLULAR INSULIN STIMULATES RNA SYNTHESIS IN AN INTACT CELL, David S. Miller, Lab of Pharmacol, NIH-NIEHS, Research Triangle Pk, NC 27709.

Circumstantial evidence suggests that internalized insulin may play some role in the overall mechanism of hormone action. Determining whether intracellular insulin does modulate metabolism requires experiments in which the hormone can be introduced into a cell with minimal disruption and in which metabolic effects are measured in the same cell; for most cells, small size makes such experiments technically difficult. I have found that Stage IV oocytes from Xenopus laevis (~800 um diameter) provide a test system in which to study the mechanism of insulin action. Like insulin-responsive somatic cells, these oocytes increased rates of RNA, protein and glycogen synthesis when exposed to nanomolar extracellular insulin. When oocytes were maintained under paraffin oil and insulin microinjected into the cytoplasm, ^{3}H-GTP incorporation into RNA increased. Doses as low as 15 fmoles/cell stimulated significantly and ~50 fmoles doubled incorporation (maximal effect). When oocytes were first exposed to external insulin (in Ringer's), then transferred to oil and microinjected with 19 fmoles insulin, the stimulatory effects of external and intracellular insulin were at least additive, suggesting separate sites and/or mechanisms of action. Finally, when oocyte nuclei were isolated under oil and dosed directly with insulin, GTP incorporation into nuclear RNA increased. The cell micro-injection data provide the first direct demonstration that intracellular insulin affects any metabolic process in an intact cell. The experiments with isolated nuclei suggest a nuclear site of action, which operates in the absence of plasma membrane or cytoplasm.

C 145 HEMATOPOIETIC GROWTH FACTOR-SPECIFIC ACTIVATION OF PROTEIN TYROSINE PHOSPHORYLATION IN INTERLEUKIN-3 DEPENDENT MURINE CELL LINES, Alex O. Morla, Jolanda Schreurs, Atsushi Miyajima and Jean Y.J. Wang, University of California, San Diego, La Jolla, CA 92093, and DNAX Research Institute, Palo Alto, CA 94304.
The multi-lineage hematopoietic colony stimulating factor, interleukin-3 (IL-3), is shown to stimulate the tyrosine phosphorylation of specific proteins in IL-3 dependent myeloid and mast cell lines. Purified murine IL-3 activates the tyrosine phosphorylation of proteins of molecular mass 95, 90, 70 and 55 Kda in all four cell lines and an additional 160 Kda protein is stimulated in the two mast cell lines. The half maximal concentration of IL-3 required for the stimulation of protein tyrosine phosphorylation correlates closely with the apparent K_d of IL-3 receptors in these cells. The IL-3 induced tyrosine phosphorylation is a rapid and transient event, reaching a maximum level within 5-10 minutes. The specificity of tyrosine phosphorylation of these proteins to IL-3 is shown by stimulation experiments with other growth factors. IL-2 had no effect on tyrosine phoshorylation in these cell lines, while granulocyte-macrophage colony stimulating factor stimulation resulted in only a small increase in tyrosine phosphorylation if any. On the other hand, IL-4 stimulated an increase in tyrosine phosphorylation of proteins of molecular mass 170 Kda, and 110 Kda in all cell lines tested. The stimulation of tyrosine phosphorylation by IL-3 and IL-4 was not mediated by the activation of protein kinase C. The finding that IL-3 stimulates tyrosine phosphorylation helps to explain the non-autocrine abrogation of IL-3 dependence caused by tyrosine kinase oncogenes.

C 146 ROLES OF CALMODULIN AND PROTEIN KINASE C IN REGULATION OF GENE EXPRESSION IN MITOGEN-ACTIVATED T LYMPHOCYTES, D.R. Morris, Department of Biochemistry, University of Washington, Seattle. The cellular levels of the mRNA coding for c-myc and ornithine decarboxylase (ODC) are rapidly elevated after activation of bovine T-cells with the mitogenic lectin concanavalin A (ConA). The regulation of the c-myc gene is largely at the transcriptional level, while that of ODC is post-transcriptional [Mol. Cell. Biol. 7, 3004 (1987)]. Activation of protein kinase C (PK-C) is both necessary and sufficient for the post-transcriptional elevation of ODC mRNA. Thus, ODC is induced by phorbol esters and its induction by ConA is blocked by H-7, but not W-7. The signaling pathways that results in stimulation of c-myc transcription are more complex and seem to involve a calmodulin-dependent reaction prior to a step dependent on activation of PK-C. Induction of c-myc by ConA is specifically inhibited by W-7 and also by Cd^{2+}. Phorbol esters by themselves do not activate transcription of c-myc, but induction of this gene by ConA is inhibited by H-7. We suggest that a calmodulin-induced change in chromatin structure must precede the activation of a transcription factor by a pathway involving PK-C. In conclusion, different genes induced early in the process of mitogenic activation are regulated at different levels and are coupled to different transmembrane signaling pathways involving calmodulin and PK-C.

C 147 SUPEROXIDE AS AN INDUCER OF INCREASE IN INTRACELLULAR pH, DNA SYNTHESIS AND EXPRESSION OF ONCOGENES, Kiyoshi Nose, Motoko Shibanuma and Toshio Kuroki, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan.
Polypeptide growth factors and tumor promoting phorbol esters stimulate quiescent cells to undergo progression through cell cycle, and the earliest responses after the stimulation include increase in intracellular pH (pHi) and production of superoxide. We examined the effect of superoxide generated by xanthine/xanthine oxidase (X/XOD) on pHi of U937 cells and on DNA synthesis in resting BALB/3T3 cells.
(1) 4ß-Phorbol dibutyrate increased pHi in U937 cells and this increase was inhibited by superoxide dismutase. X/XOD increased pHi in U937 cells comparable to that by the phorbol ester. (2) When 3T3 cells in quiescent state were treated with suitable concentrations of X/XOD, DNA synthesis was induced 12 to 24 hr after the treatment to a similar extent induced by serum. Labeling index was also increased, and these responses were inhibited by a specific inhibior for XOD (allopurinol). Accumulation of proto-oncogene c-myc and c-fos mRNA was induced 60 min after addition of X/XOD. These results suggest possible involvement of superoxide in the mitogenic stimuli.

C 148 TRANSFORMING GROWTH FACTOR-ß (TGFß) AND BASIC FIBROBLAST GROWTH FACTOR (FGF) REGULATION OF ENDOTHELIAL CELL ORNITHINE DECARBOXYLASE (ODC), Jack W. Olson, Bernhard Hennig and Mark N. Gillespie, University of Kentucky, College of Pharmacy, Lexington, KY 4053

Endothelial cells (ECs) have a major role in a number of responses such as angiogenesis, atherogenesis, inflammation and wound healing. A number of these EC functions are known to be regulated by a variety of growth factors including TGFß and FGF. Since cellular synthesis of proteins, RNA and DNA requires polyamines, we evaluated the significance of several intracellular signaling pathways in mediating FGF and TGFß activation of ODC, the rate limiting enzyme in polyamine biosynthesis. Four hours of exposure of cultured quiescent porcine pulmonary vascular ECs to either FGF or TGFß significantly increased ODC activity in a dose-response fashion, with maximum stimulation observed at 25 ng/ml (157±10 vs 2013±62 vs 475±25 pmoles CO2/60 min/mg protein; control vs FGF vs TGFß). Interestingly, 5 ng/ml of TGFß added 2 hours prior to a 4 hour incubation with 25 ng/ml FGF significantly incresed ODC activity in super additive fashion, but this response was not observed when TGFß was added at the same time as FGF. Since phorbol 12-myristate 13-acetate (PMA) or sn-1,2-dioctanoylglycerol significantly elevated ODC activity, the effect of the protein kinase C (pkC) inhibitor H-7 was examined. ODC stimulation by 25 ng/ml FGF was inhibited in a dose-related manor, with 10 µM H-7 producing a 72% inhibition. In contrast 10 µM H-7 had no influence on TGFß stimulated ODC activity. Down regulation of pkC, accomplished by a 16 hour PMA pretreatment, completely prevented the stimulation of ODC activity by either 3 µM PMA or 25 ng/ml FGF, but 5ng/ml TGFß significantly increased ODC activity. Thus further implicating FGF actions on ODC as pkC-dependent and TGFß as pkC-independent. Pretreatment with pertusis toxin did not alter control or FGF-stimulated ODC activity, but significantly inhibited TGFß-stimulated ODC activity by 25% suggesting partial involvement of a G protein in transducing the TGFß signal. The calmodulin antagonist W-7 signifcantly inhibited ODC activation by both FGF and TGFß. These data suggest that there are at least two different signal transduction mechanisms for ODC activation in ECs. Supported in part byPHS grant HL36404.

C 149 BIOCHEMICAL CHARACTERIZATION OF NOVEL trk ONCOGENE PRODUCTS AND THEIR PUTATIVE SUBSTRATES, Ralph Oskam*, Sylvia Giordano¶, Francois Coulier*, Paolo Comoglio¶, and Mariano Barbacid*. * BRI-Basic Research Program, Frederick Cancer Research Facility, Frederick, MD 21701, and ¶Dept. of Biomedical Sciences and Oncology, University of Turin Medical School, 10126 Turin, Italy.

We have recently identified thirteen new trk oncogenes generated during the course of gene transfer assays with normal trk proto-oncogene cDNA sequences. Each of these trk oncogenes code for proteins that retain and exhibited an intact carboxy terminus. Biochemical characterization of these novel trk oncogene proteins revealed two substantially different subsets of molecules. Whereas the products of trk V and trk IX were transmembrane glycoproteins, the remaining trk oncogene products appeared to be soluble cytoplasmic proteins. These findigns raised the possibility that the products of those in vitro generated trk oncogenes may interact with different substrates depending upon the nature of their NH_2-terminal activating sequences. To identify such substrates, we have studied the pattern of phosphotyrosine (P-Tyr)-containing proteins in normal and trk-transformed NIH3T3 cells using anti-P-Tyr antibodies. As control, we used cells expressing a non-transforming β-actin trk chimera which codes for a 70 kd protein that retains tyrosine kinase activity. At least five different substrates were found to be specifically phosphorylated in all trk-transformed cells. Three additional P-Tyr-containing proteins were identified in cells expressing transmembrane trk gene products independently of whether they were transformed (trk V oncogene) or not (trk proto-oncogene). Finally, only the transforming trk proteins were recognized by anti-P-Tyr antibodies suggesting that oncogenic activation of these molecules requires their phosphorylation in tyrosine residues.

C 150 CHARACTERIZATION AND PURIFICATION OF pI, A PDGF MODULATED NUCLEAR PROTEIN, Suhas Disa and Charles D. Scher, The Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA 19104.

The platelet-derived growth factor (PDGF) and other agents inducing competence including pituitary fibroblast growth factor (FGF) have previously been shown to stimulate density arrested BALB/c-3T3 cells to rapidly (within one hr) and selectively synthesize a 29 kD nuclear protein termed pI. Synthesis was inhibited by actinomycin D. The 29 kD protein appears to be a degradation product of a 31 kD PDGF modulated protein. The 31 kD pI is not solubilized from isolated nuclei by treatment with micrococcal nuclease or 2 M salt; solubilization requires the use of detergents or organic solvents suggesting that pI is an integral membrane protein. We have further characterized pI using reverse phase chromatography. Nuclei of PDGF-treated ^{35}S-methionine labeled BALB/c-3T3 cells were sonicated, extracted with 2 M salt and solubilized in formic acid/2-propanol. pI was purified by reverse phase HPLC on a Bondapack C18 column using a 5-40% propanol gradient in 60% formic acid. pI from PDGF (or FGF) treated cells eluted at a 20-21% propanol concentration demonstrating a high degree of hydrophobicity. Little pI was found in this fraction when cells were treated with platelet-poor plasma, demonstrating growth factor specificity. Treatment of cells with sodium arsenite induced the selective synthesis of a similar protein suggesting an overlapping pathway of growth factor and heavy metal related processes. C18 purified pI was iodinated and found to be about 20% pure using SDS-PAGE. Reverse phase chromatography on a C4 column yielded a product estimated to be 95% pure.

C 151 ACTIVATION OF S_6 KINASE BY SUBMITOGENIC LEVELS OF THROMBOSPONDIN, Tim Scott-Burden, Therese J. Resink, Ursula Baur and Fritz R. Buhler, Department of Research, University Hospital, Basel, Switzerland.

Multiple phosphorylation of S_6 *by a cytosolic kinase* (S_6 *kinase) appears to be a prerequisite for the activation of protein synthesis associated with the transition of cells from a quiescent to a proliferative state of growth.* S_6 *kinase itself has been shown to undergo activation, via phosphorylation, as a consequence of growth factor (EGF, IGF, PDGF inter alia) interactions with membrane receptors. Purified human platelet thrombospondin (TS) was shown to activate* S_6 *kinase in vascular smooth muscle cells in a dose- (1-9 µg/ml) and time-dependent manner. Down regulation of EGF and IGF receptors by prior treatment of cells with the growth factors did not reduce this effect. Kinase activation by TS was only marginally reduced in the presence of PDGF specific antibody at levels that totally inhibited PDGF (5 ng/ml) induced activation. Additionally, TS elicits a rapid dose-dependent phosphoinositide turnover response analogous to that of PDGF, EGF and IGF. Prior treatment of cells with TPA for 48 hrs in serum free culture medium resulted in a small enhancement of* S_6 *kinase activation by TS and the growth factors already mentioned but a complete loss in the ability of TPA to activate this enzyme.*

C 152 ACTIVATION OF CASEIN KINASE II IN RESPONSE TO INSULIN AND TO EPIDERMAL GROWTH FACTOR, James Sommercorn, Jenny A. Mulligan, Fred J. Lozeman, and Edwin G. Krebs, Howard Hughes Medical Institute and Department of Pharmacology, University of Washington, Seattle, Washington 98195. Insulin treatment enhances casein kinase II (CKII) activity in 3T3-L1 adipocytes and H4-IIE rat hepatoma cells, the magnitude of the activation varying from 30% to 150%. Activation of CKII was apparent after 5 min of exposure of 3T3-L1 cells to insulin, was maximal by 10 min, and persisted through 90 min. The insulin-stimulated activity was inhibited by low concentrations of heparin and was stimulated by spermine. Activation of CKII was effected by physiological concentrations of insulin (EC_{50} = 0.15 nM), suggesting that the effect is a true insulin response and not one mediated through insulin-like growth factor receptors. Epidermal growth factor (100 ng/ml for 10 min) also activated CKII in A431 human carcinoma cells, which is consistent with other observations that insulin and epidermal growth factor may have some common effects. Insulin stimulation of CKII activity was due to an increase in the maximum velocity of the kinase; the apparent K_m for peptide substrate was not altered. Enhanced activity did not appear to result from increased synthesis of CKII protein, because cycloheximide did not block the effect and because a Western blot, developed with antiserum to CKII, showed no effect of insulin on the cytosolic concentration of CKII. Because insulin-stimulated CKII activity was maintained after chromatography of cell extracts on Sephadex G25, it is unlikely that the effect is mediated by a low-molecular-weight activator of the kinase. Rather, the results are consistent with the possibility that insulin activates CKII by promoting a covalent modification of the kinase.

C 153 INHIBITORS OF PROTEIN KINASE C AND PHOSPHOLIPASE A2 BLOCK SIGNAL TRANSDUCTION BY V-SRC AND V-FPS, Rudolph Spangler and David A. Foster, The Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10021. The recently characterized 9E3 gene is transcriptionally activated in response to the kinase activity of v-src and v-fps. This activation is transformation-sensitive (1). To determine signal transduction pathways used by v-src and v-fps, we have tested the response of 9E3 to the kinase activities of v-src and v-fps in the presence of drugs that selectively block known signal transduction intermediates. We have found that H7, a specific inhibitor of protein kinase C, blocks the turn on of 9E3 induced by v-src and v-fps. This suggests that protein kinase C is required for the transduction of signals induced by v-src and v-fps. Consistent with this, TPA, which activates protein kinase C, leads to increased synthesis of the 9E3 message. We have also found that mepacrine, at concentrations reported to be specific for phospholipase A2, blocks the v-src and v-fps induced turn-on of 9E3. The TPA induction of 9E3 is also blocked by mepacrine, suggesting an involvement of phospholipase A2 downstream from protein kinase C in a transduction cascade. Thus far no differences between v-src and v-fps signal transduction have been detected for the induction of 9E3.
1) Sugano, S., Stoeckle, M.Y., and Hanafusa, H. (1987). Transformation by Rous sarcoma virus induces a novel gene with homology to a mitogenic platelet protein. Cell 49: 321-328.

C 154 CHANGES IN FREE INTRACELLULAR CALCIUM INDUCED BY PDGF AND FGF, Robert W. Tucker, David Chang and Kim Meade-Cobun, Johns Hopkins Oncology Center, Baltimore, Md 20205.
Increased free intracellular calcium (Ca_i) has been postulated to be one of the main regulators of cell growth and differentiation. In order to measure more precisely the changes of Ca_i in individual cells, we have used digital image analysis of intracellular Fura-2 fluorescence in quiescent BALB/c 3T3 cells stimulated with platelet-derived growth factor (PDGF)and fibroblast growth factor (FGF).Purified PDGF (1 - 8 U/ml) induced a rapid, sometimes biphasic increase in Ca_i that was dose-related to the percent of cells made competent by PDGF (subsequent growth in 1% platelet-poor-plasma). Purified and recombinant FGF (100 - 1600 pg/ml) also produced rapid, biphasic increases in Ca_i, but, in contrast to PDGF, the magnitude of Ca_i increases and the percent of cells responding (> 2 - fold Ca_i increase) was not related to the percent of cells made competent by FGF. For both growth factors, the initial rapid increase in Ca_i did not require extracellular calcium, whereas the second, more sustained increase did not occur when extracellular calcium was chelated by EGTA (1mM). Both nuclear and cytosolic [Ca^{2+}] increased with similar kinetics, implying that calcium changes in both the cell compartments could be important in the stimulation of mitogenesis. Intracellular calcium chelators (Quin 2, BAPTA) also inhibited both competence and Ca_i increases induced by PDGF, while the effect on FGF stimulation was less clear.Thus, two different polypeptide growth factors, both of which induce early mitogenic events (competence) must utilize different intracellular signals in the stimulation of gene expression and cell proliferation.

C 155 TRANSFORMING GROWTH FACTOR INDUCES CALCIUM MOBILIZATION IN A431 CELLS.
Larry A. Wheeler, Danon Goodrum & George Sachs, Dept. of Biological Sciences, Discovery Research, Allergan Inc./Herbert Labs, Irvine, CA 92715.
Transforming growth factor-α (TGF-α) is a mitogenic polypeptide that is structurally related to epidermal growth factor (EGF) and binds to the EGF receptor (J. Massaque, TIBS 10:237, 1985). To investigate receptor-effector coupling mechanisms for TGF-α, Ca^{2+} mobilization studies were undertaken with the Ca^{2+} sensitive fluorescent dye fura-2 in A431 cells. Addition of TGF-α induced a dose related (ED_{50}=7ng/ml), rapid biphasic rise in free cytosolic Ca^{2+} [$(Ca^{2+})_i$]. A maximal response (300-600nM) was obtained after 15-30 sec and slowly returned toward baseline over 20 min. When cells were suspended in Ca^{2+} free media and treated with TGF-α there was a rapid 5-fold increase in $[Ca^{2+}]_i$ that peaked within 20 sec but returned to baseline in 3 min. These studies show TGF-α was able to mobilize Ca^{2+} from both extracellular sources as well as release from intracellular stores. The putative TGF-α antagonist, sequence 34-43 did not block EGF or TGF-α-induced Ca^{2+} mobilization, nor did the EGF peptide fragment (20-31) induce Ca^{2+} mobilization. The phorbol ester TPA was used to explore further TGF-α induced Ca^{2+} mobilization. Studies with 0.3nM TPA for 2 min resulted in selective loss of the Ca^{2+} uptake through the plasma membrane but not intracellular Ca^{2+} signalling. TPA did not alter bradykinin induced Ca^{2+} mobilization that is due to Ca^{2+} release from intracellular stores. A phorbol ester that does not activate protein kinase C had no effect on the $[Ca^{2+}]_i$ response. These studies suggest that Ca^{2+} mobilization is an important action by which TGF-α induces its effects on cells and that compounds that alter Ca^{2+} mobilization can inhibit the actions of TGF-α on cell growth.

Receptors

C 200 CHARACTERIZATION OF HUMAN GM-CSF RECEPTORS ON NON- HEMATOPOIETIC CELLS. G.C. Baldwin, J. DiPersio, S.E. Kaufman, S.G. Quan, D.W. Golde, and J.C. Gasson.
Division of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA
Human GM-CSF (granulocyte-macrophage colony-stimulating factor) stimulates colony growth of myeloid progenitors, and enhances the differentiated function of mature effector cells. Small cell carcinoma cell lines (SCCL) have properties of APUD cells and high levels of the key APUD cell enzyme, L-aromatic amino acid decarboxylase. Although they have been hypothesized to be of neuroendocrine origin, SCCL were reported to be responsive to CSFs in vitro. We have characterized specific binding of the hematopoietic growth factor, GM-CSF, to SCCL. GM-CSF binds to SCCL via a single class of high affinity receptors (Kd ~35 pM, ~50 sites/cell). Biochemical cross-linking analysis shows a ^{125}I-cross-linked complex of 98,000 daltons, indicating a molecular weight for the GM-CSF receptor of 84,000 daltons. The estimates for receptor affinity and molecular weight are in agreement with our receptor studies of neutrophils, CML and HL-60 cells. In vitro, SCCL respond to GM-CSF in colony growth assays. It is important to assess the effect of CSFs on tumor cell lines, as the CSFs have recently been proposed for treatment of patients with solid tumors; among these, small cell carcinoma.

C 201 STRUCTURAL AND FUNCTIONAL MODEL OF THE PLATELET-DERIVED GROWTH FACTOR RECEPTOR EXTRACELLULAR DOMAIN, **J. Fernando Bazan[1,2], Robert J. Fletterick[2], Jaime A. Escobedo[2] and Lewis T. Williams[2], Univ. of California, Berkeley, CA 94720, and San Francisco, CA 94143**

Novel structure prediction and molecular modeling techniques are used to create a model for the tertiary structure of the extracellular region of the PDGF receptor (homologous to the CSF-1 receptor). This ligand-binding domain is found to be composed of five tandem β-rich immunoglobulin (Ig)-like repeats. These structural subunits are unconventional repeats of heterogeneous amino acid length, poor sequence similarity and, in the case of one Ig-repeat, lacking the distinctive Ig-intrachain disulfide-bridge. We examine the prospect that these receptors bind distinct growth factors through a typical antibody-antigen relationship utilizing a cleft between two Ig folds. In analogy to the generation of diverse antigen-recognition structures in the immune system, PDGF receptor and homologues are predicted to bind very different growth factors by minimal changes in key recognition loops. In addition, the structural composition of their N-terminal domains link them to a subset of the Ig superfamily that includes primitive cell-surface molecules such as NCAM, suggesting an alternative function for the PDGF receptor as a cell-adhesion molecule.

C 202 LIGAND INDUCED ASSOCIATION OF EPIDERMAL GROWTH FACTOR RECEPTOR WITH THE CYTOSKELETON OF A431 CELLS, Johannes Boonstra, Paul M.P. van Bergen en Henegouwen and Arie J. Verkleij, Dept of Molecular Cell Biology, University of Utrecht, Utrecht, The Netherlands.
Recently, evidence has been obtained in favor of a structural interaction between the epidermal growth factor (EGF) receptor and the Triton X-100 insoluble cytoskeleton of epidermoid carcinoma A431 cells (J. Cell Biol. 103: 87-94 (1986)). Here we present a further analysis of the properties of the EGF receptors associated to the cytoskeleton. Steady state EGF binding studies, analysed according the Scatchard method, indicate that EGF is bound to the cytoskeleton via high-affinity binding sites. Accordingly this class of receptors is further characterized by a slow dissociation of EGF from these receptors and by a rapid association of EGF to these receptors. After incubation of A431 cells for 2 hours at 4°C in the presence of EGF, scatchard analysis revealed a three-fold increase in the number of EGF-receptors associated to the cytoskeleton. These newly cytoskeleton associated receptors appeared to represent low-affinity receptors which demonstrated a fast dissociation of EGF from the receptors. The interaction of low-affinity receptors to the cytoskeleton, as induced by EGF, is apparently coincided with a clustering of receptors at the cell surface, as indicated by indirect immunofluorescence studies. Furthermore, these clusters apparently co-localize with patches of actin, indicating that actin plays a role in the interaction of EGF-receptors and the cytoskeleton. These results will be discussed in view of the recently proposed model of intermolecular activation of the EGF-receptor kinase by EGF.

C 203 Growth Hormone (GH) Promoted Tyrosyl Phosphorylation of GH Receptors.
C. Carter-Su, C. Foster and J. Shafer. Univ. of Michigan, Ann Arbor, MI. 48109
As a first step towards determining whether the GH receptor was a ligand-activated tyrosine kinase, we investigated whether GH promotes the phosphorylation of its receptor on tyrosyl residues. ^{125}I-GH was cross-linked to its receptor in intact 3T3-F442A fibroblasts. Up to 19% of the subsequently solubilized (0.1% Triton X-100) ^{125}I-GH-receptor complexes (Mr = 134,000) bound to and could be eluted from a highly specific phosphotyrosine binding antibody. When cells were labeled metabolically with ^{32}Pi, incubation of cells with GH (29 ng/ml) for 1 h at 24° resulted in a 7-fold increase in the amount of a 114K phosphoprotein (ppl14) which bound to the phosphotyrosine binding antibody. When higher concentrations of GH (2.2 ug/ml) were used, an increase in the amount of ppl14 was evident as early as 1 min after GH addition and peaked in less than 10 min. When solubilized cells that had been incubated with GH were treated with anti-GH antibody, ppl14 was immunoprecipitated, consistent with the notion that ppl14 is a GH receptor and remains complexed with GH when cells are solubilized. Phosphoamino acid analysis of ppl14 indicated tyrosyl phosphorylation. Studies of the interaction of ^{35}S-methionine labeled cell extracts with both phosphotyrosyl binding antibodies and anti-GH antibodies indicated that the GH receptor contains only 114,000 Mr chains and that the amount of phosphorylated GH receptor increased in response to GH.

C 204 GLYCOPROTEIN NATURE OF TRANSFORMING GROWTH FACTOR-β RECEPTORS. Sela Cheifetz and Joan Massagué, Univ. Mass. Med. Ctr., Worcester, MA 01655 USA

We have identified 3 cell surface glycoproteins with estimated sizes of 65 kDa (Type I), 85-110 kDa (Type II) and 280-330 kDa (Type III) that have the properties of receptors for TGFβ. From their sensitivity to endo-β-N-acetylglucosaminidase F (Endo F) and resistance to Endo H, Type I and Type II receptors both contain complex N-linked carbohydrate (CHO) chains which contribute approx. 5 and 15-25 kDa respectively to the size of the affinity labeled receptors as estimated by SDS-PAGE. Chemically removing all peripheral sugars with trifluoromethanesulfonic acid (TFMS) increased the mobility of the Type I and Type II receptors to the same extent as seen following Endo F treatment suggesting little if any O-linked CHO in these receptors. The situation is reversed for Type III where approx. half of the apparent molecular weight of the receptor is due to TFMS sensitive O-linked CHO and only 5-10 kDa of N-linked CHO. In addition, TFMS treatment routinely yielded two peptides with molecular sizes of 130 and 110 kDa independent of the cell line under investigation suggesting additional heterogeneity in the Type III receptor. Treatment of cells with tunicamycin, an inhibitor of N-linked glycosylation, revealed that the Type III receptor requires N-linked CHO for either expression on the cell surface and/or the ability to bind TGFβ whereas the aglyco Type I and Type II receptors are both expressed on the cell surface and are able to bind TGFβ. Thus despite some similarities in the ligand binding sites of the Type I and Type III receptors as reflected in peptide maps of these affinity labeled receptors, the Type I and Type III receptors are distinct proteins.

C 205 IDENTIFICATION OF SUBUNITS OF THE IL-1 RECEPTOR ON MOUSE AND HUMAN CELLS, R. Chizzonite, M. Griffin, K. Paganelli, P. Kilian, T. Truitt, and A.S. Stern, Roche Research Center, Hoffmann-LaRoche, Inc., Nutley, N.J. 07110

IL-1 receptors present on mouse and human cells have been characterized by chemical crosslinking of ^{125}I labelled human IL-1 alpha, IL-1 beta and mouse IL-1 alpha to the different cellular IL-1 receptors. Following the binding and crosslinking of ^{125}I-IL-1 to the respective cells, the ^{125}I-IL-1/IL-1 receptor complex was solubilized and either visualized directly by SDS-PAGE and autoradiography or immunoprecipitated with anti-IL-1 antibodies followed by SDS-PAGE and autoradiography. The results with mouse cells showed that human IL-1 alpha and beta yield crosslinked complexes of 120kD and 100kD, respectively, whereas mouse IL-1 yields both the 120kD and 100kD complexes. Both crosslinked complexes are blocked by adding excess IL-1 to the binding reaction. Similarily, both human IL-1 alpha and beta yield complexes of 100kD and 90kD when crosslinked to the receptor derived from human cells. These results indicate that the IL-1 receptor is composed of two subunits of 105kD and 85kD on mouse cells and 85kD and 75kD on human cells. However, further studies have demonstrated the presence of a single subunit receptor on specific types of mouse and human cells, suggesting the possibility that each subunit may independently bind IL-1 and be expressed in a tissue specific manner.

C 206 A PROTEIN TYROSINE KINASE ACTIVATED BY AUTOCRINE LOOP IN A HUMAN GASTRIC CARCINOMA CELL LINE, Silvia Giordano, Loredana Chiado'-Piat, Riccardo Ferracini, Maria Flavia Di Renzo, and Paolo M. Comoglio, Dept. of Biomedical Sciences & Oncology, University of Torino Medical School, 10126 Torino, Italy.

Phosphotyrosine (P-Tyr) antibodies have been successfully used to identify the phosphorylated forms of growth factor receptors and oncogene-coded tyrosine kinases. While screening human tumor cell lines using P-Tyr antibodies in "Western" blots, we detected in a gastric carcinoma cell line a protein of 150 Kd, which is heavily phosphorylated at tyrosine. This protein (p150) is labelled in vivo with ^{32}P-ortophosphate at tyrosine (at least three different sites) and serine residues. Furthermore, p150 is labelled by ^{125}I, under non-permeating conditions, and can be cleaved by mild trypsin treatment of intact cells. It is also labelled with tritiated glucosamine and retained by lectin-Sepharose. Thus, it appears to be a transmembrane glycoprotein with a domain exposed at the cell surface. Structural analysis performed under non-reducing conditions revealed that p150 is covalently linked to a polypeptide chain of 50 Kd, also exposed at the cell surface. Incubation with v32P-ATP of immunoprecipitates obtained with P-Tyr antibodies results in tyrosine phosphorylation of the 150 Kd subunit. P150 does not cross react with antibodies raised against known tyrosine kinases such as the EGF and PDGF receptors, or the proteins coded by the oncogenes src, abl, fes, fps and trk. P150 is rapidly dephosphorylated "in vivo" if the cells are treated with low pH or suramine, conditions known to dissociate ligands from their receptors. Moreover, in vitro tyrosine phosphorylation of p150 is significantly enhanced by the presence of serum-free medium conditioned by the cell line. These data indicate that this gastric tumor cell line expresses a 200 Kd two-chains complex with structural and physiological features similar to growth factor receptors and that the associated tyrosine kinase is activated through an autocrine loop.

C 207 PLATELET-DERIVED GROWTH FACTOR A-A, B-B, AND A-B FORMS ALL BIND TO AND ACTIVATE A PDGF RECEPTOR EXPRESSED BY A SINGLE cDNA CLONE. Shaun R. Coughlin, Jaime A. Escobedo, and Lewis T. Williams. Howard Hughes Medical Institute, University of California, San Francisco 94143.

PDGF purified from human platelets is a disulfide-linked heterodimer of two homologous polypeptide chains, designated A and B. Other cell types have recently been found to express distinct forms of PDGF, A-A and B-B homodimers. Whether these distinct forms of PDGF serve different functions is unknown. Using A-A and B-B homodimers produced in a yeast expression system and A-B heterodimer purified from platelets, we have shown that the A-A, B-B, and A-B forms of PDGF are all capable of binding to human and mouse fibroblast PDGF receptors, activating the receptor's tyrosine kinase, and eliciting mitogenesis. The degree of receptor occupancy required to elicit a given level of mitogenic response was similar for all three forms of PDGF, suggesting that all act as receptor agonists rather than as partial agonists or antagonists. Expression of mouse PDGF receptor cDNA in CHO cells, which do not normally express PDGF receptor or respond to PDGF, conferred responsiveness to all three forms of PDGF. These data suggest that the A-A, B-B, and A-B forms of PDGF are all capable of acting in a similar fashion via one PDGF receptor. Whether additional types of PDGF receptor exist that might distinguish among the three forms of PDGF is unknown.

C 208 RECEPTOR OLIGOMERIZATION IS NOT REQUIRED FOR THE ACTIVATION OF THE EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE PROTEIN KINASE*, Ingrid Northwood and Roger J. Davis, Department of Biochemistry, University of Massachusetts Medical School, 55 Lake Ave. N., Worcester, MA 01655.

The epidermal growth factor (EGF) receptor is a Mr=170Kd glycoprotein that consists of an extracellular domain that binds EGF, a single transmembrane spanning region, and an intracellular domain that exhibits tyrosine-specific protein kinase activity. The mechanism by which EGF activates the tyrosine protein kinase activity is of great interest because this process involves transmembrane signalling between two functional domains of the receptor. We have investigated the hypothesis that the mechanism of stimulation of the receptor tyrosine protein kinase activity is coupled to and requires the oligomerization of the receptor. Analysis of the effects of EGF on receptor oligomerization using two separate assays based on different physical principles indicated that EGF caused the association of Mr=170Kd monomeric receptors as aggregates (dimers and perhaps higher order oligomers). The methods used were sucrose density gradient centrifugation and chemical cross-linking. In each case the physical state of the receptors was analyzed by Western blotting. The results show that there is a strong correlation betwen the formation of oligomeric states of the EGF receptor and the activation of the tyrosine protein kinase. However, it was observed that the receptor oligomerization was not required for the activation of the kinase which could occur under conditions in which oligomers were not formed.

C 209 STRUCTURAL ANALYSES OF A cDNA CLONE CORRESPONDING TO THE HUMAN PDGF RECEPTOR. A. Eriksson, L. Severinsson, A. Morén, C.-H Heldin, L. Claesson-Welsh, Ludwig Institute for Cancer Research, Box 595, Biomedical Center, S-751 23 Uppsala.

The sequence of a cDNA clone, corresponding to the translated part of the human PDGF receptor was determined. At the protein level the highest homology between the murine (Yarden et al., Nature 232, 226-232) and human translated nucleotide sequence, 98%, is seen in the tyrosine kinase domain. The insert in the tyrosine kinase domain without homology to other kinases is 86% homologous to the corresponding mouse sequence; in the extracellular parts of the receptors, 79% of the amino acids are identical. A protein product of the expected size was immunoprecipitated using a PDGF receptor antiserum, after in vitro transcription and translation of the cDNA. The protein will be analyzed for functions associated with the PDGF receptor, e.g. induction of tyrosine kinase activity by addition of the ligand, PDGF. Transfection of various receptor constructs are currently performed in order to pinpoint receptor functions to the different structural domains.

C 210 PDGF RECEPTOR MUTAGENESIS: STUDY OF RECEPTOR FUNCTION IN CELL LINES EXPRESSING MUTATED FORMS OF THE PDGF RECEPTOR, Jaime A. Escobedo and Lewis T Williams, Univ. of Calif., Dept. of Med., Howard Hughes Med. Inst. San Francisco, CA., 94143
PDGF treatment of cells transfected with the mouse PDGF receptor cDNA elicits a diverse group of biochemical responses. The diversity of these cellular responses has raised important questions about the structure and function of the receptor. To correlate the structural domains of the receptor with its function we have generated several PDGF receptor mutants. The role of the kinase insert region (Ki) and the C-tail region (Ct) of the receptor in PDGF-mediated mitogenesis was studied in receptor mutants that lack the Ki region and the Ct region respectively. In addition, using site-directed mutagenesis we generated and ATP-binding site mutant (K602) and a receptor mutant in which the consensus tyrosine for autophosphorylation was changed. Stable CHO cells expressing mutated forms of the receptor were analyzed for: PDGF binding, autophosphorylation, phosphorylation of a cellular substrate, (p32), down regulation, phosphoinositide turnover and cell proliferation. All the mutants expressed comparable amounts of the receptor at the cell surface. Our results indicate that mutation at the ATP-binding site and C-tail deletion abolish the tyrosine kinase activity of the receptor and most of the responses to PDGF are absent, except for down regulation. This suggests that PDGF receptor can internalize by a mechanism independent of tyrosine phosphorylation. Mutation at Y825 produced a cell line in which some of the early responses to PDGF are present but these cells did not proliferate. In this mutant p32 failed to phosphorylate. Deletion of the Ki region resulted in a cell line in which early responses to PDGF are intact but the proliferative response is defective.

C 211 TRAPPING OF EPIDERMAL GROWTH FACTOR-INDUCED RECEPTOR DIMERS BY CHEMICAL CROSS-LINKING, Bradford O. Fanger, Judy E. Stephens and James V. Staros, Vanderbilt University, Nashville, TN 37232.
Receptors for epidermal growth factor (EGF), on exposure to EGF, form a dimeric species which can be trapped by cross-linking with the membrane-impermeant reagent bis[sulfo-succinimidyl] suberate. This Mr ~ 350,000 cross-linked receptor complex is detected in EGF-treated intact A431 and HeLa S3 cells and in freshly prepared Triton X-100 extracts of A431 cells; little or no dimeric species is cross-linked in the absence of EGF under otherwise identical conditions. The extent of the Mr ~ 350,000 species cross-linked in Triton extracts appears to correlate with stimulation of self-phosphorylation of the receptor under a variety of conditions, suggesting an important connection between receptor dimerization and kinase stimulation. EGF-induced dimerization is also observed in EGF receptors purified at a physiological pH by a novel procedure which uses 5% sodium deoxycholate to elute receptors from the EGF-affinity gel. This implies that EGF-induced dimerization is an intrinsic property of the receptor and does not require accessory proteins. However, when receptors are eluted from the affinity gel by exposure to 5 mM ethanolamine at pH ~ 10, they can be cross-linked into dimers in both the presence and absence of EGF. The difference in the aggregation state of receptors purified at physiological and elevated pH may explain the apparently opposite results reported by different laboratories concerning the role of EGF receptor aggregation in the activation of its tyrosyl residue-specific kinase activity. Supported by NIH grants DK25489, DK31880, and DK07061. B.O.F. current address: Merrell Dow Research Institute, Cincinnati, OH.

C 212 EXOGENOUS KINASE ACTIVITY IN THE WILD TYPE AND MUTATED FORMS OF THE RECEPTOR FOR PDGF. Wendy J. Fantl, Jaime A. Escobedo, and Lewis T. Williams, University of California, Dept. of Medicine, Howard Hughes Med. Inst., San Francisco, CA., 94143
One of the earliest events that occurs after PDGF binds to its receptor is activation of the receptor tyrosine kinase. The effects of autophosphorylation and of primary structural modifications of the receptor on kinase activity were examined quantitatively. Receptor was immunoprecipitated with an antibody raised to the external domain of the receptor. The kinase activity of the receptor was measured using $(Val)^5$ angiotensin as exogenous substrate. A 5 fold induction of kinase activity by PDGF was observed when cells were stimulated with PDGF prior to their solubilization, whereas only a 1.5 fold induction was observed when PDGF was added to solubilized preparations. In separate experiments kinase activity was measured in CHO cells transfected with wild-type and mutated forms of the receptor. A receptor mutant in which the ATP binding site was mutated had no tyrosine kinase activity stimulated by PDGF. Deletion of C-tail residues also caused a loss of tyrosine kinase activity. Mutation of a potential tyrosine phosphorylation site in the cytoplasmic domain reduced kinase activity. A mutated form of the receptor in which there is a deletion in the sequences which separate the kinase domains is currently under study.

C 213 **ALLOSTERIC PROPERTIES OF LECTIN AND EGF AFFINITY PURIFIED EGF RECEPTOR KINASE,** Shawn P. Fay and C. Fred Fox, Department of Microbiology and Molecular Biology Institute, University of California at Los Angeles, Los Angeles, CA 90024. EGF receptor purified by EGF affinity chromatography catalyzes two distinct processes in phosphorylation of both itself and substrates. Double reciprocal plots of ATP vs. initial velocity phosphorylation rate at 6.5 nM receptor reveal three distinct regions of kinetic behavior: 1) a high ATP affinity, low specific activity reaction process; 2) a low ATP affinity, high specific activity reaction process; and 3) a transition range at intermediate ATP concentration characterized by nonideal kinetic behavior. When receptor was incubated with ATP to achieve receptor phosphorylation prior to substrate addition, enhanced rates of subsequent substrate phosphorylation were not observed, providing no evidence for a requirement of EGF receptor phosphorylation for optimal receptor-catalyzed substrate phosphorylation. No substantial differences were seen in the two-dimensional maps of phosphopeptides derived from tryptic digests of EGF receptor phosphorylated to stoichiometries of 0.17 or 1.7, providing no evidence for an obligate ordered sequential phosphorylation of EGF receptor required for EGF receptor kinase activation.

Plots of log initial rate of receptor or substrate phosphorylation vs. log receptor concentration and Hill plots of these data to evaluate EGF receptor kinase activity as a function of receptor concentration indicate a unimolecular phosphorylation process with no cooperativity at low receptor concentration and high or low ATP concentration, and a multimolecular, cooperative process requiring both high receptor and high ATP concentrations. Similar findings were made for both the EGF-stimulated and unstimulated reactions, showing that EGF played no essential role in determining the order of reaction or in allosteric activation of receptor kinase. In phosphorylation of substrate, EGF stimulated kinase activity only 3-fold but specific activity was increased at least 30-fold as a function of receptor concentration. RRsrc substrate inhibited the phosphorylation of both receptor and substrate in the multimolecular reaction but not in the unimolecular reaction. Supported by NIH grants T32 CA09056 and DK25826.

C 214 **A UNIQUE CLASS OF VESICLES CHARACTERIZED EXCLUSIVELY BY A VERY HIGH AFFINITY CLASS OF EGF RECEPTORS INDUCED BY EGF.** **C. Fred Fox and Robert E. Williams, Department of Microbiology and Molecular Biology Institute, University of California , Los Angeles, Ca 900024.** Murine 3T3 cells, when incubated for 1 h at 37°C with [^{125}I]EGF and an inhibitor of lysosomal protease action, were characterized by two saturable classes of internalized EGF and these accounted for 94% of total [^{125}I]EGF binding. Very high affinity, K_d = 0.1 nM internalized [^{125}I]EGF and lower affinity, K_d > 1.5 nM internalized [^{125}I]EGF accounted for 34% and 60% of total specific [^{125}I]EGF binding, respectively. Concurrent incubation of cells with [^{125}I]EGF and a phorbol ester tumor promoter prevented detection of the internalized, very high affinity class of cellular EGF binding, but this was compensated by an increase in the quantity of internalized, lower affinity EGF binding. Membranes isolated from cells incubated with unlabeled EGF displayed K_d >1.5 nM lower affinity equilibrium [^{125}I]EGF binding only. When exposed to sonic irradiation prior to assay, these membranes displayed K_d = 0.1 nM very high affinity equilibrium EGF binding in addition to a K_d >1.5 nM lower affinity equilibrium EGF binding. The K_d= 0.1 nM very high affinity equilibrium EGF binding comprised a maximum of 5% of total specific equilibrium EGF binding to membranes. Sonicated membranes from cells not incubated with EGF or cells incubated concurrently with EGF and 12-O-tetradecanoyl-phorbol-13-acetate revealed the K_d >1.5 nM lower affinity EGF receptors only. Vesicles containing the very high affinity K_d = 0.1 nM EGF receptors were resolved physically from membranes containing K_d >1.5 nM lower affinity EGF receptors. The final membrane fractionation step employed adsorption to concanavalin A-derivatized beads and sedimentation through 6% Ficoll/D_2O to resolve vesicles containing K_d = 0.1 nM very high affinity equilibrium EGF binding from membranes displaying K_d >1.5 nM equilibrium EGF binding. A model proposing both the origin of and a physiological role for this very high affinity class of EGF receptors will be presented. Supported by NIH Grant USHHS 5 R01 DK25826.

C 215 CHARACTERIZATION OF THE HUMAN IL-4 RECEPTOR, Jean-Pierre Galizzi*, Hélène Cabrillat*, Odile Djossou*, Naoko Arai†, Takashi Yokota†, Ken-Ichi Arai† and Jacques Banchereau*
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Human recombinant IL-4 purified from mouse L cell transfection supernatants was radio iodinated to high specific radioactivity using a two-phase method without loss of biological activity. ^{125}I-IL-4 bound specifically to the Burkitt lymphoma Jijoye cells as well as to a panel of hemopoietic cell lines. Jijoye cells showed a high affinity for ^{125}I-IL-4 with an equilibrium dissociation constant : Kd ≈ 7 10^{-11} M, and displayed 1200-1400 specific receptors per cell at 4° C. Similar constants were found when equilibrium binding of ^{125}I-IL-4 was performed at 37° C in the presence of azide to prevent internalization. The equilibrium dissociation constant (Kd) corresponds to the IL-4 concentration which induces 50% maximal expression of CD23/FcεRL expression on Jijoye cells. At 4° C the association constant k1 is 2.9 x 10^6 M^{-1} s^{-1} and the dissociation constant k-1 is 1.3 x 10^{-4} s^{-1} (t 1/2 = 91 min.). No human recombinant lymphokine (IL-1, IL-2, IL-3, IFN-γ, IFN α2b, G-CSF, GMCSF) other than IL-4 were able to inhibit the binding of ^{125}I-IL-4 to its receptor. Murine IL-4 did not prevent binding of ^{125}I-IL-4, a finding consistant with the lack of biological cross reactivity between both species. Bound IL-4 was very rapidly internalized at 37° C and together with spontaneous dissociation of IL-4 into the medium, it resulted in the rapid disappearance of ^{125}I-IL-4 from the cell surface. Crosslinking studies demonstrated that IL-4 mainly bound to a 140 Kd polypeptide as well as 70 Kd polypeptides.

C 216 INSULIN-LIKE GROWTH FACTOR I IN CULTURED RAT ASTROCYTES: EXPRESSION OF THE GENE AND RECEPTOR TYROSINE KINASE.

S. Gammeltoft, R. Ballotti, F.C. Nielsen, W.D. Richardson and E. Van Obberghen. Dept. Clin. Chem., Bispebjerg Hospital, Copenhagen, Denmark; INSERM U145, Nice, France; Dept. Zoology, University College London, England.

Insulin-like growth factors (IGF's) I and II stimulate cell growth in several mammalian tissues. The cellular actions are mediated by interactions with two types of IGF receptor. Recently, gene expression and production of IGF's in mammalian brain have been reported, and two types of IGF receptor have been identified on plasma membranes of adult rat brain. The aim of the study was to investigate the expression of IGF I its receptor tyrosine kinase in primary cultures of fetal rat astrocytes, and to study the growth-promoting activity of IGF I. Northern blot analysis of poly (A^+) RNAs from astrocytes revealed an IGF I mRNA of 1.9 kb. Competitive binding and receptor labeling technigues revealed two types of IGF receptor in astroglial cells. Type I receptors consist of α-subunits (Mr 130,000) and β-subunits (Mr 94,000) with IGF I-sensitive tyrosine kinase activity. Type II receptors are monomeric proteins (Mr 250,000). DNA synthesis measured as thymidine incorporation was stimulated two-fold by IGF I and IGF II. Our findings suggest that IGF I is synthesized in fetal rat astrocytes and acts as a growth promoter for the same cells by activation of the type I IGF receptor tyrosine kinase. We propose that IGF I acts through autocrine or paracrine mechanisms to stimulate astroglial cell growth during normal brain development.

C 217 DIFFUSION OF CELL SURFACE RECEPTORS, Peter B. Garland, Amersham International, Little Chalfont, Bucks, England, HP7 9NA.

The lateral diffusion of cell surface receptors can be measured by the fluorescence photobleaching technique at the single cell level and a receptor occupancy of a few hundred copies per square micron of membrane. The technique measures translation over a distance of approx 1 um, and the diffusion coefficients so measured are relatively insensitive to molecular size or micro-aggregation. By contrast, rotational diffusion measurements are very sensitive to molecular size, but are difficult to perform on single cells or with equipment that is convenient for cell biologists. Solutions to the problems of sensitivity and convenience may come by adapting existing techniques (phosphorescence depolarization, depolarization of fluorescence depletion) from their current mode of time domain (pulsed) to frequency domain (phase modulation) measurement. Examples and comparisons will be presented.

C 218 AUTOCRINE ACTIVATION OF THE TYROSINE KINASE ASSOCIATED WITH THE BOMBESIN RECEPTOR IN SMALL CELL LUNG CARCINOMAS, G.Gaudino, M.Cilli, L.Gandino, D.Cirillo, L.Naldini and P.M.Comoglio, Dept. of Biomedical Sciences & Oncology, University of Torino Medical School, 10126 Torino, Italy.

The neuropeptide bombesin, and its mammalian homolog GRP, are known to be potent mitogens. Antibodies against phosphotyrosine (P-Tyr) identify in Swiss 3T3 fibroblasts a 115 Kd cell surface protein (p115) which becomes phosphorylated on tyrosine in response to bombesin stimulation of quiescent cells. p115 also becomes labelled at tyrosine residues when immunocomplexes obtained with P-Tyr antibodies are incubated with γ-32P-ATP. Bombesin enhances this in vitro phosphorylation. ^{125}I-Tyr_4-bombesin binds the immunocomplexes in a specific and saturable manner.

P115 has been partially purified through a procedure including solubilization with non-ionic detergents, lectin chromatography and affinity chromatography on bombesin-Sepharose, followed by specific elution. P115 co-purifies with a cell surface glycoprotein of 75-85 Kd to which ^{125}I-GRP covalently binds in cross-linking experiments performed with homobifunctional reagents. These data strongly suggest that p115 and the associated kinase activity participate in the structure and function of the bombesin receptor complex.

Phosphotyrosine antibodies also identify a 115 Kd phosphoprotein in four human SCLC lines producing bombesin, but not in a non-producer "variant" line. p115 from detergent treated SCLC binds to bombesin-Sepharose and becomes phosphorylated at tyrosine in the presence of γ32P-ATP and Mn^{++} ions. SCLC p115 is phosphorylated in an immunocomplex kinase assay, like the p115 kinase immunoprecipitated from mouse fibroblasts. However SCLC p115 does not require the presence of exogenous bombesin in the assay for activity. These observations are in agreement with the hypothesis of an autocrine activation of the bombesin receptor complex in human small cell lung carcinoma cells.

C 219 Expression of the EGF receptor cDNA in antigen-specific mouse cloned T cells: Down-regulation of the EGF receptor by T cell activation.
Daniel M Gorman and Atsushi Miyajima. Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology. 901 California Avenue, Palo Alto, CA94304.
To test the functional exchangeability of growth factor receptors between fibroblasts and T cells, we have introduced the human EGF receptor cDNA into antigen-specific mouse cloned T cells (MD13.5) which require IL-2 for growth, but do not express the EGF receptor. The resultant T cells expressed approximately 15,000 functional EGF receptors having an apparent affinity for EGF of 2.5 nM. Upon binding, EGF stimulated phosphorylation of the EGF receptor and was internalized. Similar to the EGF receptor on fibroblasts, where the binding of EGF is down-regulated by TPA or mitogens such as PDGF, the binding of EGF to those T cells was down-regulated by TPA or a T cell mitogen, ConA. Although these results suggest that the EGF receptor artificially expressed on the T cell is functional, EGF did not stimulate DNA synthesis. Thus, EGF could not replace the requirement of IL-2 for T cell growth, suggesting that signal transduction components required for EGF to triger proliferation might be missing in T cells.

C 220 GROWTH OF MAMMARY EPITHELIAL CELLS IN BREAST CANCER BIOPSIES CORRELATES WITH EGF-BINDING. R. Grosse and E. Spitzer, Central Institute of Molecular Biology, Department of Cellular Biochemistry, Academy of Sciences of the GDR, 1115 Berlin-Buch.
Microautoradiographic analysis of ^{125}J-EGF binding to tissue sections was applied to demonstrate that EGF was bound specifically and selectively to mammary epithelial cells. For 50 unselected primary breast carcinomas the quantitative parameters "EGF binding"and "number of mitoses" were estimated in serial 10 µm thick sections. The parameters were analysed by means of the rank correlation coefficient r_s and were found to be statistically significant correlated (p=0.05) A non-linear correlation analysis divides the 50 biopsies into two groups. One includes the biopsies from patients with positive lymph node status giving high degree of approximation to the non-linear model expressed by $y=P3+P1\ (1-e^{-P2\cdot x})$. The other group includes the biopsies from lymph node negative patients not fitting the non-linear model. The correlation between proliferative rate and EGF binding in breast cancer biopsies indicates that in lymph node positive patients growth of cancer is directly related to an EGF dependent acceleration of cell division. The EGF receptor status seems to be a new and valuable prognostic indicator for patients with bad prognosis Therefore, we have synthesized a biotinylated EGF probe (BioEGF). BioEGF behaves in binding experiments like EGF. Histochemical analysis of tissue sections proofs that BioEGF is as sensitive as ^{125}J-EGF or an anti-EGF receptor antibody.

C 221 CHARACTERIZATION OF MURINE IL-4 RECEPTOR, Nobuyuki Harada, Brian E. Castle and Maureen Howard, DNAX Research Institute, 901 California Ave., Palo Alto, CA, 94304.
Interleukin 4 (IL-4) is a T lymphocyte-derived glycoprotein with multiple biological activities. These include growth promoting activity on anti-IgM stimulated B cells, growth promoting activity on T cell lines or thymocytes, MAF (macrophage activating factor) activity, and regulation of isotype expression by activated B cells. Using a receptor-ligand binding assay, we have demonstrated the existence of high affinity IL-4 receptors on B cells, T cells, other hematopoietic cells, and non-hematopoietic cells. The Kd of this receptor is 20-60pM and the number of receptors range from 100 to 5000 on various cell types.

To attempt purification of the IL-4 receptor, we have established an assay for solubilized IL-4 binding proteins. E. coli expressed recombinant IL-4 was affinity purified using an anti-IL-4 blocking antibody and was then radioiodinated. This material was incubated with the solubilized plasma membrane fraction of HT-2 T cells (5000 R/cell). Free and bound ^{125}I were separated by immunoadsorption. The binding of ^{125}I-IL-4 was dependent on soluble protein concentration, and inhibited by IL-4 but not IL-2. Further characterization and purification of soluble IL-4 receptor is in progress.

C 222 ALLOSTERIC PROPERTIES OF LECTIN AFFINITY PURIFIED EGF RECEPTOR KINASE, Jennifer Haynes and C. Fred Fox, Department of Microbiology, University of California at Los Angeles, Los Angeles Ca. 90024.

Allosteric interactions between EGF receptor molecules participating in catalysis of EGF receptor "autophosphorylation" action have been reported to require binding of EGF to receptor (Yarden, Y., and J. Schlessinger, 1987, *Biochemistry* 26, 1434-1442). Previous experiments from our lab with a preparation of EGF receptor purified from A431 cells by a two step scheme involving both lectin and EGF affinity chromatography have demonstrated an allosteric activation of receptor kinase activity for both autophosphorylation and substrate phosphorylation in the presence or absence of EGF.

EGF affinity chromatography of receptor leads to capture of receptor in low yield, and the EGF affinity purification step does not preclude the selection for a receptor subspecies with properties which might differ from those of receptor purified by lectin affinity chromatography only. Therefore, we have characterized the properties of the EGF receptor purified by lectin affinity chromatography. Lectin affinity purified preparations of the receptor behave the same way in receptor catalyzed substrate phosphorylation as receptor adsorbed to and eluted from an EGF affinity column. Substrate phosphorylation specific activity increased as a function of EGF receptor concentration with EGF present or absent as described previously by Fay, *et al* (J. Cell. Biochem., supplement 11A, 1987) with EGF purified receptor. When added in excess (>20 nM EGF receptor) EGF receptor was inhibitory. The concentrations of receptor at the midpoint and at maximal allosteric activation (10 nM and 20 nM respectively) were the same with EGF present or absent with 1 mM RRsrc as substrate. Of equal interest was the finding that the specific activity of substrate phosphorylation was stimulated greater than 30-fold when EGF receptor concentration was varied, but only 3-fold by EGF addition. Supported by NIH grants T32 CA09056 and DK25826.

C 223 OVEREXPRESSION OF EGF RECEPTORS IN SQUAMOUS TUMORS IS ASSOCIATED WITH POOR SURVIVAL, F. Hendler, A. Shum-Siu, L. Nanu, B. Ozanne, University of Louisville, Louisville, KY 40292 & University of Texas Health Science Center, Dallas TX 75235.

We have studied EGF receptor in 76 biopsy specimens from lung and head and neck cancer using a quantitative radioimmunoassay on tissue sections. All squamous tumors studied have increased EGF receptors with a range of from 1.5 to 20 times the level detected in normal skin biopsy specimens. The overexpression of EGF receptors is associated with gene amplification in approximately 60% of tumors. All of the tumors that have 3 fold greater EGF receptor than skin have evidence of significant gene amplification. This subset of tumors are more undifferentiated tumors when compared to those with 1.5 to 3 fold increased receptor ($p < .04$). This overexpression is associated with a poor survival. If the EGF receptor is greater than 3 fold normal skin, the median survival is 9 months; if less than 3 fold, median survival is 25 months. When life tables are determined and the survival curves are compared, the survival is statistically different ($p < .001$). Those patients with EGF receptor values less than 3 fold normal skin can be subdivided into groups: those that do as poorly with a similar survival time to that of patients with very high EGF receptor levels and those who do well with a median survival of 34+ months. Studies are underway to determine what other biologic events may be affecting survival in patients with lower levels of EGF receptor. Thus, lung and head neck tumors can be biologically segregated on the basis of the EGF receptor into at least two groups. The EGF receptor level may be useful in designing experimental treatment programs as well as predicting patients prognosis.

C 224 ALTERED GROWTH CONTROL AND ENHANCED MORPHOLOGIC RESPONSE TO TUMOR PROMOTERS IN RAT FIBROBLASTS STABLY OVERPRODUCING PROTEIN KINASE C

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To study the role of protein kinase C (PKC) in growth control and tumor promotion, we have generated a series of rat fibroblast cell lines which stably overexpress a full-length cDNA encoding the $\beta 1$ form of this enzyme. Assays of these cell lines demonstrate that they contain a 20 to 53-fold increase in PKC activity, and also have an increase in high affinity phorbol ester receptors, when compared to control cells. Phosphorylation studies indicate that they display a marked increase in a 75 kilodalton ^{32}P-labelled protein, reflecting autophosphorylation of the overproduced PKC, as well as an increase in other phosphorylated proteins. Western blot analyses demonstrate that the cell lines primarily overproduce an intact form of $PKC_{\beta 1}$. These cell lines exhibit dramatic changes in morphology in response to treatment with phorbol esters such as TPA. Unlike control cells, which become refractory to the effects of TPA, the overproducing cell lines continue to respond to repeated TPA treatments, and display an elevated expression of certain TPA-inducible genes in response to this treatment. Furthermore, the cell lines overproducing PKC grow to a higher cell density in culture and display anchorage independent growth as well. Thus these cell lines will be useful for further defining specific physiologic functions of PKC and its role in growth control and tumor promotion.

C 225 FGF (BDGF, aFGF, bFGF, ECGF, HBGF's) RECEPTOR IS A 135-kDa PROTEIN TYROSINE KINASE, Jung San Huang, Ming-Der Kuo, and Shuan Shian Huang, Department of Biochemistry, St. Louis University School of Medicine, 1402 S. Grand Blvd. St. Louis, MO 63104.

Bovine brain-derived growth factor (BDGF) is a very potent mitogen with a broad spectrum of cell specificity. BDGF is also a potent chemotatic factor for fibroblasts, endothelial cells and astroglial cells. BDGF can promote the anchorage-independent cell growth of NRK cells in the presence of EGF. The neuron localization of BDGF indicates an important role of BDGF in the cellular physiology of neural tissues. The BDGF receptor in responsive cells appears to be an 135-kDa protein associated with a protein tyrosine kinase activity (Huang, S.S., and Huang, J.S. (1986) J. Biol. Chem. 261, 9568-9571). Since chemical and physical properties of BDGF resemble those reported for acidic FGF (aFGF), endothelial cell growth factor (ECGF)and heparin-binding growth factor-1 (HBGF-1), and BDGF and basic FGF (bFGF) compete for binding to the BDGF receptor, we have purified the solubilized BDGF receptor from bovine liver and examined the effects of these FGF growth factors on the autophosphorylation of the BDGF receptor. All FGF growth factors, including BDGF, aFGF, bFGF, ECGF, and HBGF's stimulate the tyrosine-specific autophosphorylation of the BDGF receptor. The stimulation of autophosphorylation of the BDGF receptor by these growth factors can be completely inhibited by their potent inhibitor, protamine. These results suggest that FGF receptor is a 135-kDa protein tyrosine kinase.

C 226 AMPLIFICATION AND POINT MUTATION OF THE PROTO-NEU ONCOGENE PLAYS AN IMPORTANT ROLE IN THE MULTIPLE-STEP TUMORIGENICITY, Mien-Chie Hung, Duen-Hwa Yan and Xiaoyan Zhao, Dept. Tumor Biology, Univ. of Texas System Cancer Center, Houston, TX 77030.

To study if the amplification of the proto-neu gene plays a role in tumorigenicity, we generated a NIH 3T3 transfectant (DHFR/G-8) that carried approximately 100 copies of the proto-neu gene (Hung et al., 1986 Proc. Natl. Acad. Sci. USA, 83:261-264). The DHFR/G-8 cells exhibit normal morphology. When analyzed by tumorigenicity using nude mice, the B104-1 cell, a NIH 3T3 transfectant that carries the activated neu oncogene, produced tumors within 10 days; the DHFR/G-8 cells did not produce tumors until 3 months after injections and the NIH 3T3 cells did not give any tumors after 3 months. The tumors produced by the injection of the DHFR/G-8 cells were excised and grew in culture. The cells (PNT-1 and PNT-2) derived from the tumors are of transformed morphology and highly tumorigenic. The results suggest that at least one more hit is required to render the DHFR/G-8 cells tumorigenic.

The DNA's from the PNT-1 and PNT-2 cells were transfected into NIH 3T3 cells. The transfection resulted in foci on the NIH 3T3 monolayer. Southern analysis indicated that the foci derived from the transfection contained the neu gene. The results suggest that the neu gene is the target of "the second hit." Using oligonucleotides as probes, "the second hit" was found to be a single point mutation changing a valine in the transmembrane domain of neu to a glutamic acid. Identical mutation at this position has previously been found in the rat neuroblastoma and glioblastoma induced by a chemical carcinogen, ethylnitrosourea. We conclude that the DNA region encoding transmembrane domain of neu is a "hot spot" for converting the proto-neu gene into an activated oncogene.

C 227 STUDIES OF THE BOMBESIN-LIKE PEPTIDE (BLP) RECEPTOR IN HUMAN SMALL CELL LUNG CARCINOMA NCI-H345 CELLS IN CULTURE. M. Kane, S. Aguayo, Y. Miller and L. Brown, Denver VA Medical Center and University of Colorado Health Sciences Center, Denver, CO.

H345 cells exhibit an autocrine growth system for BLP, since they contain and release immunoreactive BLP, and BLP stimulates (3H)-thymidine incorporation, proliferation, intracellular calcium release and phosphoinositide turnover. H345 cell growth in vitro and in vivo is inhibited by anti-bombesin monoclonal antibodies as well as a substance P analog which competes for the BLP receptor. Immunoreactive BLP content was measured by a very sensitive ELISA method in H345 cells and their conditioned medium at various times after plating. BLP content rose to a peak of 200 ng/10^6 cells at 48h after plating and fell to less than 10% of that value by 100 h after plating. For binding studies, HPLC analysis of radioligands revealed that 125 I - gastrin releasing peptide (GRP) (Amersham) eluted with authentic non-oxidized GRP, but all 125 I-(tyr4)-bombesin preparations analyzed were significantly oxidized. Specific uptake of 125 I-GRP in H345 cells at 22°C and 37°C increased with time and was approximately one fmol/10^6 cells/min. At 4°C, specific binding studies revealed a Kd of 0.5 nM. Purification of the BLP receptor has not been reported. Triton-solubilized H345 membranes possessed a 125 I-GRP binding peak by Sephadex G50 and Sephacryl S200 gel filtration analysis,and this binding was blocked by excess cold GRP. A unique 65 kDa band was visualized by silver staining of the reduced SDS-PAGE of dilute acid elutions of a GRP-agarose, but not blank, affinity gel. Further studies to characterize this protein, which may be the BLP receptor, are proceeding.

C 228 N-LINKED GLYCOSYLATION AND COMPLETE PROCESSING OF THE TYPE II INSULIN-LIKE GROWTH FACTOR (IGF) RECEPTOR ARE NOT REQUIRED FOR BINDING OF ^{125}I-IGF-II. Wieland Kiess, Lilly Lee, Matthew M. Rechler, and S. Peter Nissley, NIH, Bethesda, MD 20892

Rat C6 glial cells which have abundant type II IGF receptors were used to study the post-translational processing of this receptor and the effect of glycosylation on ligand binding to the receptor. Cells were pulse-labeled with ^{35}S-methionine, lysed and type II IGF receptor immunoprecipitated with a specific type II IGF receptor antiserum (3637). Analysis of the immunoprecipitate by SDS-PAGE with reduction of disulfide bonds showed a 235 kDa precursor which was processed into the mature 245 kDa receptor within 2 hours. Digestion of the 235 kDa precursor with endoglycosidase H (Endo H) produced a 220 kDa form, whereas the mature 245 kDa type II IGF receptor was resistant to cleavage by Endo H. Preincubation of C6 cells with swainsonine resulted in the synthesis of a receptor hybrid (240 kDa) which was cleaved by Endo H. Affinity crosslinking studies using ^{125}I-IGF-II and disuccinimidyl suberate as the crosslinker showed that the 240 kDa receptor species retained its ability to bind IGF-II. Pretreatment of C6 cells with 100 ng/ml tunicamycin resulted in the synthesis of a 220 kDa receptor species in addition to the mature 245 kDa receptor as shown by Western blotting with antiserum 3637. Affinity crosslinking studies showed that ^{125}I-IGF-II bound to both the mature 245 kDa and the 220 kDa receptor species. In conclusion, (1) the type II IGF receptor in C6 glial cells is formed as a 235 kDa precursor and processed into the mature 245 kDa form within 2 hours; in the absence of N-linked glycosylation the receptor is 220 kDa. (2) N-linked glycosylation and complete processing are not necessary for ligand binding to the type II IGF receptor.

C 229 EFFECTS OF MITOGENIC PROTEASES ON ACTIVATION OF THE INSULIN RECEPTOR TYROSINE KINASE. Judith Whipple Leef, Joseph Larner and Michael J. Weber, Departments of Pharmacology and Microbiology, University of Virginia School of Medicine, Charlottesville, VA 22908.

The protease trypsin has mitogenic and insulin-mimetic anabolic effects. It has also been shown to stimulate autophosphorylation and tyrosine kinase activity in partially and highly purified insulin receptor preparations. We have now examined the effects of trypsin on whole cell insulin receptor phosphorylation and tyrosine kinase activation. Adipocytes prelabeled with $^{32}P_i$ for 2 hrs were exposed to trypsin and receptors partially purified over WGA columns. Receptors were subjected to SDS-page, beta-subunits identified by autoradiography, bands hydrolyzed and phosphoamino acids separated by electrophoresis and quantified. Two- and 5-fold increases in P-Tyr were observed with 3 and 10 min. of trypsin treatment, respectively. Trypsin treatment of adipocytes also produced a time-dependent stimulation of tyrosine kinase activity as measured either in lectin extracts containing insulin receptors or by an immune-complex kinase assay. Comparison of insulin binding with tyrosine kinase activity in lectin extracts from trypsin-treated cells revealed the existence of a linear correlation between destruction of the alpha-subunit and activation of the beta-subunit. We therefore suggest that selective cleavage by trypsin of the insulin receptor alpha-subunit leads to stimulation of the tyrosine kinase activity of the beta-subunit, possibly by removal of inhibitory modulation. We further conclude that autophosphorylation of the insulin receptor and activation of its tyrosine kinase may be important to the insulin-mimetic anabolic effects of trypsin. We have extended this work on the insulin receptor to examine the effects of other proteases (such as thrombin, plasminogen activator and plasmin) which are mitogenic or capable of activating cell metabolism and are, therefore, potentially insulin-mimetic. Preliminary data suggest that neither plasmin nor thrombin activates the insulin receptor tyrosine kinase. Thus the insulin receptor is probably not involved in mitogenic or metabolic stimulation by these proteases. We are also screening for other factors secreted by transformed cells which could stimulate or interact with the insulin receptor.

C 230 A TYROSINE-KINASE DEFECTIVE MUTANT ALLOWS TO SEPARATE BETWEEN HIGH AFFINITIES, EFFICIENT ENDOCYTOSIS AND THE MITOGENIC RESPONSE. Etta Livneh, Nachum Reiss, Eva Berent, Axel Ullrich[+] and Joseph Schlessinger. Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel, and [+]Genentech Inc., 460 San Bruno Boulevard, South San Francisco, CA 94080, USA.

Cultured NIH-3T3 cells devoid of endogenous EGF-receptors were transfected with cDNA constructs encoding normal human EGF-receptor and with a construct encoding an insertional mutant of the EGF-receptor containing four additional amino acids in the kinase-domain after residue 708. Unlike the "wild type" receptor expressed in these cells which exhibits EGF-stimulatable protein tyrosine-kinase activity, the mutated receptor lacks protein tyrosine-kinase activity both in vitro and in vivo. Despite this deficiency the mutant receptor is properly processed, it binds EGF and it exhibits both high and low affinity binding sites. Moreover, it undergoes efficient EGF-mediated endocytosis. However, EGF fails to stimulate DNA synthesis and is unable to stimulate the phosphorylation of S6 ribosomal protein in cells expressing this receptor mutant. Hence, it is proposed that the protein tyrosine-kinase activity of EGF-receptor is essential for the initiation of S6 phosphorylation and for DNA synthesis induced by EGF. However, EGF-receptor processing the expression of high and low affinity surface receptors and receptor-endocytosis appears to require neither kinase-activity nor receptor autophosphorylation. Interestingly, phorbol ester (TPA) fails to abolish the high affinity state of this kinase-mutated receptor. Moreover, TPA is also unable to stimulate the phosphorylation on serine or treonine residues of this receptor mutant. We are currently examining the possible mechanisms that underlay the failure of this mutant receptor to be phosphorylated by protein kinase C.

C 231 DIRECT IDENTIFICATION AND CHARACTERIZATION OF THE p75 SUBUNIT OF THE HIGH AFFINITY INTERLEUKIN-2 RECEPTOR.

Horacio Saragovi and Thomas R. Malek, University of Miami School of Medicine, Miami, FL. 33101.

The interleukin-2 (IL-2) receptor was initially defined in biochemical and cDNA cloning studies as a glycoprotein of M_r of 55,000 (p55). However, recent chemical cross-linking studies of radiolabeled IL-2 to IL-2 receptors have suggested that functional high affinity IL-2 receptors, which generally represent a small fraction (~5%) of total cell surface IL-2 receptors, may contain an IL-2 binding subunit of 75,000 daltons (p75). In the present investigation, we have studied the proteins closely associated with p55 in several cell populations that varied in their capacity to express high affinity IL-2 receptors. Cell surface proteins were radiolabeled by lactoperoxidase catalyzed iodination and treated with the reversible homobifunctional cross-linker dithiobis(succinimidyl propionate) (DSP). The proteins cross-linked to p55 were then precipitated with antibodies specific for p55 and analyzed on SDS-PAGE. A glycoprotein of M_r 75,000 (p75) was cross-linked to p55 from cells that bore high affinity IL-2 receptors. Three other proteins of apparent M_r of 100,000 (p100), 135,000 (p135), and 180,000 (p180) were also cross-linked to p55. In contrast, analysis of the p55 cross-linked proteins from the cell populations that expressed only low affinity IL-2 receptors revealed p100, p135, and p180, but not p75. Biochemical characterization of p75 showed that it is a cell surface glycoprotein modified by the addition of ~20,000 daltons of N-linked glycans, with a pI of 4.3 to 5.0. These results provide direct biochemical evidence for a close association of p55 and p75 in the formation of functional high affinity IL-2 receptors. The cross-linking of p100, p135 and p180 to p55 raise the possibility of a more complex IL-2 receptor subunit structure, although the detection of these three proteins on all cell populations indicates that in the absence of p75 they do not play a role in ligand binding affinity.

C 232 COMPARISON OF A NUCLEAR EGF BINDING PROTEIN FROM NORMAL AND REGENERATING LIVER WITH THE EGF RECEPTOR.

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Previously we demonstrated an EGF binding protein located in the nucleus of hepatocytes. We compared this nuclear EGF binding protein from normal and regenerating liver with the EGF receptor located on the liver cell plasma membrane. A small percentage of both nuclear EGF binding protein and EGF receptor form covalent-like complex with the radiolabeled EGF molecule and both have the same molecular weight, about 180 KDa. With a monoclonal antibody against the EGF-receptor it was possible to identify on western blots of plasma membrane and nuclear protein the same 180 KDa protein. However the isolated and purified nuclei contain some additional proteins (between 60 KDa and 100 KDa) recognized by the antibody. 125-I EGF bound to the nuclear protein in both normal and regenerating liver was immunoprecipitated with antibody to EGF receptor. Binding assays with isolated plasma membranes or nuclei from normal or regenerating liver, did not resolve any significant difference in the KD's for EGF binding. However, the nuclei from normal rat liver show only one-tenth the number of binding sites per unit protein found in plasma membranes, and nuclei from regenerating liver have about 2/3 the binding sites found in normal nuclei. We speculate this reduction is due to preoccupation of the receptor by endogenous EGF.

C 233 GROWTH FACTOR EFFECTS ON MURINE PDGF RECEPTOR STEADY STATE MESSENGER RNA LEVELS.

Victoria R. Masakowski, Thomas Girard, Bruce E. Bejcek and Thomas F. Deuel, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110.

Synthetic oligonucleotides based on published PDGF receptor sequence (Nature 323:226) were used to screen a murine 3T3 fibroblast lambda gt11 cDNA library. A positive clone containing a 2 kilobase insert was isolated, characterized, and subcloned into the pT7/T3-19 vector. This plasmid was used to develop an S1 nuclease assay for facile detection of steady state PDGF receptor mRNA levels.

Balb/c 3T3 cells were grown to confluency and placed in serum free media for 48 hours. The media was then changed to media containing either 10% fetal calf serum or 100 ng/ml purified human PDGF. Total cellular RNA was isolated at 4 hour intervals over the next 24 hours. Characteristic elevations in c-myc mRNA levels were observed at 4 hours on Northern blots of the purified RNA, thus verifying the efficacy of the mitogenic stimulation. Neither serum nor PDGF produced alterations in receptor message levels as detected by S1 nuclease assay. These data, together with previous studies showing down regulation of cell surface PDGF receptors in response to ligand, suggest a post-transcriptional mechanism of regulation of PDGF receptor synthesis. This finding is in marked contrast to the effects of another growth factor, EGF, on its receptor messenger RNA levels, which are elevated in response to EGF treatment (J. Biol. Chem. 262:7932), and suggests very different mechanisms by which growth factors may regulate the expression of their receptors.

C 234 ACTIVATION OF THE EGF RECEPTOR BY A MONOCLONAL ANTIBODY DIRECTED AGAINST THE CARBOXY-TERMINUS OF THE RECEPTOR.

Deborah J. McCarley, Alan Wells*, J.M. Bishop*, and Randall C. Schatzman, Cancer and Developmental Biology, Syntex Research, Palo Alto, CA 94304 and *G.W. Hooper Foundation, University of California, San Francisco, CA 94143.

Monoclonal antibodies were generated against the epidermal growth factor receptor (EGF-R) by immunizing mice with a bacterially expressed fragment of the EGF-R from the transmembrane domain to the carboxy-terminus. Two monoclonal antibody secreting hybridomas (291-3A and 291-4A) were isolated and subcloned. Both antibodies recognized native and denatured forms of the EGF-R and the erb-B gene product as demonstrated by immunoprecipitation and Western blot analysis. In an immune complex kinase assay of either EGF-R or erb-B protein, antibody 291-4A allows both autophosphorylation and phosphorylation of exogenously added substrates, whereas antibody 291-3A does not. In a soluble kinase assay using detergent extracts of A-431 shed vesicles antibody 291-4A activated in a concentration dependent manner the autophosphorylation of EGF-R and phosphorylation of exogenously added substrates to the same extent that 62 nM EGF did. At similar antibody concentrations 291-3A had no effect on kinase activity. Delivery of the antibodies into A-431 cells by fluid phase pinocytosis yielded results similar to that seen in vitro: 291-4A activated receptor kinase activity, whereas 291-3A did not. In order to further examine the mechanism by which antibody 291-4A activated the EGF-R, Fab fragments were prepared. While the Fab fragments still recognized the EGF-R as determined by immunoprecipitation, they were unable to activate the EGF-R kinase activity in the soluble kinase assay. This result suggests that either the dimerization of the EGF-R is necessary for autophosphoyrlation to occur or that a conformational change required for autophosphorylation does not occur with the Fab fragment.

C 235 CHARACTERIZATION OF IL-3 RECEPTORS ON HUMAN ACUTE MYELOGENOUS LEUKEMIA CELL LINE KG-1, R.A. Mufson, T.G. Gesner, Katherine Turner, Christine Norton, Yu-Chung Yang and Steven Clark, Genetics Institute Inc., 87 Cambridge Park Drive, Cambridge, 02140. Human interleukin-3 (IL-3) was biosynthetically labelled with [^{35}S] methionine in Chinese hamster ovary (CHO) cells containing amplified copies of the human IL-3 cDNA. We have studied the interaction of this labelled recombinant IL-3 with the human acute myelogenous leukemia cell line, KG-1. [^{35}S] IL-3 bound to KG-1 cells in a time dependent, saturable and specific manner at 4°C. Scatchard transformation of binding isotherms performed at 4° C demonstrated the existence of a small number (175-250) of binding sites with an apparent dissociation constant of 70-105 pM. After a temperature shift from 4°C to 37°C, surface bound [^{35}S] IL-3 was rapidly internalized and processed into a trichloroacetic acid soluble form. Experiments to address the specificity of the IL-3 binding site indicated neither human IL-2, M-CSF, erythropoietin, transferrin, bovine insulin, nor murine nerve growth factor compete for [^{35}S] IL-3 binding. Only human and gibbon recombinant IL-3, and surprisingly human recombinant GM-CSFcould effectively compete.Competition by GM-CSF was found to be concentration dependent, but followed kinetics different from the sigmoidal competition curve seen with IL-3.

C 236 EXPRESSION OF GROWTH FACTOR RECEPTORS IN HUMAN COLON ADENOCARCINOMA CELL LINES, U. Murthy, M.A. Anzano and R. Greig, Department of Cell Biology, Smith Kline & French Laboratories, King of Prussia, PA 19406

The expression of receptors for EGF/TGF-α, TGF-ß, IGF-I and PDGF was examined in human colon carcinoma cell lines. Radioligand binding and immunoprecipitation analysis revealed EGF/TGF-α receptors in all cell lines tested. These receptors were biochemically functional since challenge with EGF/TGF-α increased receptor autophosphorylation. TGF-ß (from both human and porcine sources) blocked DNA synthesis in non-neoplastic rat epithelial cells, but was without detectable effect on 90% of the colon carcinoma cell lines. Chemical crosslinking of ^{125}I-TGF-ß to its receptors demonstrated an aberrant receptor subtype in 4/5 colon carcinoma cell lines. Radioreceptor binding and crosslinking studies revealed IGF-I receptors in all colon carcinoma lines examined. Although PDGF was produced and secreted by certain colon cell lines, receptors for this ligand were not detected. These results suggest that uncontrolled proliferation of colon adenocarcinoma cells may reflect loss of negative regulation as well as production of progression/competence factors such as TGF-α, PDGF and IGF-I.

C 237 CELL TYPE DISTRIBUTION AND INTERNALIZATION OF THE FIBROBLAST GROWTH FACTOR RECEPTOR. Bradley B. Olwin, Patricia Noel, Dorit Bader and Stephen D. Hauschka, Department of Biochemistry, SJ-70, University of Washington, Seattle, WA, 98195.
The fibroblast growth factor (FGF) family of polypeptides are known mitogens for a wide variety of cells and are positive and negative regulators of cellular differentiation. To understand more concerning the overall role of FGF in development, we have surveyed a number of cell and tissue types for FGF receptor (FGFR), and have studied FGFR internalization in fibroblasts and skeletal muscle cells. Scatchard analysis of human, rat, mouse, and hamster cells revealed high affinity binding sites for FGF ranging from 700-30,000 sites per cell with K_ds of 8-60 pM. Anionic (aFGF) and cationic (bFGF) forms of bovine FGF compete for ^{125}I-labeled bovine aFGF binding to all cell types examined except hemopoetic cells which do not possess FGFR. Chemical crosslinking of ^{125}I-aFGF to intact cells revealed a strikingly uniform 165 kDa polypeptide receptor in human, rat, mouse, and hamster cells. In contrast, FGFR were not detectable in adult bovine, human placenta, or chicken tissues, while embryonic mouse and chick tissues exhibited high affinity ^{125}I-aFGF binding sites. Analogous to several other growth factor receptors, FGF addition to intact cells at 37° C causes rapid receptor-mediated internalization and degradation of ^{125}I-aFGF. Swiss 3T3 cells and MM14 skeletal muscle myoblasts internalized 50% of the bound ligand in 10 and <1 min, respectively. When MM14 cells become committed (irreversibly lose their FGF-responsiveness) to terminal differentiation by removal of FGF from the growth medium, the cell surface FGFR disappear totally by 24 h after FGF withdrawal. During this period the rate of FGFR internalization remains identical to that in proliferating myoblasts.

C 238 EXPRESSION AND CHARACTERIZATION OF THE EXTRACYTOPLASMIC PORTION OF THE MOUSE PLATELET-DERIVED GROWTH FACTOR RECEPTOR. Patricia L. Orchansky, Jaime A. Escobedo and Lewis T. Williams, University of California, Cardiovascular Research Institute and Howard Hughes Medical Institute, San Francisco, CA., 94143.

The coding sequence of the external domain of the PDGF receptor, truncated just outside the transmembrane region, was cloned into an SV40 early promotor expression vector. Plasmids containing truncated or wild type receptor coding sequences were transfected into chinese hamster ovary (CHO) cells devoid of endogenous PDGF receptor and stable transfectants were selected. The truncated receptor transfectants expressed 10-50 fold more receptor protein than the wild type transfectants. Both proteins were recognized by extracellular domain antireceptor antibody. The expressed truncated receptor had a molecular weight of 70-80kDa on SDS-PAGE, whereas the entire receptor had the expected molecular weight of 180kDa. The extracellular domain transfectants bound ^{125}I-PDGF with approximately the same affinity as the wild type, indicating that the PDGF binding site is located in the extracytoplasmic portion of the receptor and that cytoplasmic and transmembrane sequences are not necessary for high affinity PDGF binding.

C 239 Biochemical Characterization of the Murine IL-3 Receptor with a Polyclonal Antibody, E.W. Palaszynski[1], J. Keller[2,3] and F. Ruscetti[3], George [1]Washington Univ., [2]Program Resources Inc. and [3]LMI BRMP NCI-FCRF Frederick, MD. Rabbit polyclonal sera were generated against plasma membrane preparations extracted from the IL-3 dependent cell line FW 311. The resulting anti-FW311 sera is capable of competing with ^{125}I-IL-3 for its receptor assessed by radioreceptor assays for IL-3. The anti-FW311 sera is also capable of precipitating detergent extracted preparations of ^{125}I-IL-3 cross-linked to its receptor on 32D-Cl23 SRC. When electrophoresed and exposed to film, resulting autoradiograms yield three major bands precipitated by the anti-FW311 sera. These are 152, 132 and 72 kilodaltons after subtraction of ^{125}I-IL-3 (28,000). Normal rabbit serum precipitates nothing and these same three bands occur when cross-linked preparations are electrophoresed with no immune precipitation. Detergent extracted membranes from cells containing the IL-3 receptor can compete with the anti-FW311 sera in a concentration dependent manner for cross linked ^{125}I-IL-3 to its receptor while membrane preparations known not to contain the IL-3 receptor, do not compete. Furthermore, when surface labelled poteins from 32D-Cl23 SRC are precipitated with anti-FW311 sera, three major bands result upon autoradiographic analysis. These are 150, 130 and 70 kilodaltons which correspond to the molecular weights precipitated from ^{125}I-IL-3 cross-linked to its receptor. Thus the anti-FW311 antibody appears to recognize the murine IL-3 receptor and could represent a valuable research tool in further caracterization of the receptor.

C 240 INTERLEUKIN-4 BINDS TO MURINE FIBROBLASTS: RECEPTOR CHARACTERIZATION AND INDUCTION OF COLONY STIMULATING ACTIVITY PRODUCTION. Linda S. Park, Robert J. Tushinski, Diane Y. Mochizuki and David L. Urdal, Immunex Corporation, Seattle, WA 98101.

We have previously demonstrated the presence of receptors for IL-4 on a broad spectrum of cell lineages, and now report the presence of receptors for IL-4 on murine fibroblast and epithelial cells as well. In all cases IL-4 binding exhibited a single class of high affinity receptor (<2000 receptors per cell) with a K_a of 10^9 - 10^{10}. Affinity crosslinking experiments on L929 cells resulted in the identification of a single receptor species with an average M_r of 139,000 ± 12,000, similar to that found on human adherent and nonadherent cells but larger than that of M_r 60,000 to 75,000 previously reported on murine cells. Further experiments revealed that with sufficient protease inhibitors, receptor subunits of M_r 138,000 to 145,000 could also be detected on murine nonadherent cell lines. To examine the possibility that BSF-1 might stimulate production of colony stimulating factors by fibroblasts, monolayers of BALB-c/3T3 cells were maintained in the presence or absence of IL-4 and the culture media tested for capacity to support proliferation and colony growth of hematopoietic precursor cells from mouse bone marrow. The production of colony stimulating activity was stimulated significantly, as measured by both assays, within one day following addition of IL-4, and the levels detected increased with increasing time of incubation with IL-4. This observation expands the repertoire of activities that have been attributed to IL-4 and may help explain the diverse effects that IL-4 displays.

C 241 INDUCTION OF NERVE GROWTH FACTOR RECEPTORS ON CULTURED HUMAN MELANOCYTES, M Peacocke, M Yaar, CP Mansur MV Chao* and BA Gilchrest. USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA. 02111 and Dept. of Anatomy and Cell Biology, Cornell University Medical College, New York, NY 10021

The nerve growth factor receptor (NGFR) is a 70-80,000 dalton protein found on the surface of many neural crest derived cells. It has been well characterized in pheochromocytoma cells, sympathetic nerve cells and melanoma cells. To date, efforts to identify this receptor on the surface of the human melanocyte have not been fruitful. In our tissue culture system, when melanocytes are stimulated with phorbol 12-tetradecanoate 13-acetate (TPA), the initially tripolar/stellate cells convert within hours to cells with prominent nuclei, scarse perinuclear cytoplasm and long, dendritic processes projecting toward adjacent cell bodies. We observed that this morphological phenomenon corrleted with the appearance on the cell surface of a linear pattern of immunofluorescence staining with the monoclonal antibody ME20.4, known to recognize the human NGFR. Subsequently immunoblotting studies identified a protein of molecular weight 70,000-80,000 daltons in TPA-treated cells. This band was not seen in unstimulated cells. The gene for the NGFR receptor has recently been cloned, and northern blot analysis of melanocyte total cellular RNA using the NGFR cDNA probe demonstrated increasing levels of the 3.8 kilobase NGFR mRNA in melanocytes stimulated with TPA up to 12 hours post stimulation but an absence of this mRNA species in unstimulated melanocytes. Thus, these data establish the ability of cultured human melanocytes to express the NGFR, given the appropriate stimulation, and suggest a role for NGF in the human pigmentary system.

C 242 INSULIN-DEPENDENT TRANSMEMBRANE SIGNALLING OF THE INSULIN RECEPTOR KINASE, J.E. Pessin, L.J. Sweet, B.D. Morrison, M.L. Swanson and P.A. Wilden, The University of Iowa, Iowa City, IA 52242.

We have begun to investigate the molecular mechanism by which extracellular insulin binding to the α subunit of the insulin receptor results in the propagation of a transmembrane signal that activates the intracellular β subunit kinase domain. In these studies, the native $\alpha_2\beta_2$ heterotetrameric disulfide-linked insulin receptor complex was dissociated into a functional αβ heterodimeric state by treatment with a combination of alkaline pH and DTT. Examination of the kinetics of autophosphorylation demonstrated that both basal and insulin stimulated autophosphorylation occurred in a dilution-independent manner for the $\alpha_2\beta_2$ complex (intramolecular) but occurred in a dilution-dependent manner for the αβ complex (intermolecular). Insulin treatment of the isolated αβ complex was also observed to induce the complete reassociation to an $\alpha_2\beta_2$ state when analyzed by nondenaturing Bio-Gel A-1.5m gel filtration chromatography. However, nonreducing SDS-PAGE of ^{125}I-insulin affinity crosslinked and ^{32}P-autophosphorylated αβ heterodimers demonstrated that the insulin-dependent reassociation to the $\alpha_2\beta_2$ state occurred both covalently and non-covalently under these conditions. A comparison between the time course of insulin-dependent reassociation with that of substrate kinase activity indicated that protein kinase activation directly correlated with the formation of the covalent $\alpha_2\beta_2$ complex from the isolated αβ heterodimers. The non-covalent reassociation of the αβ heterodimers was found to be reversible and to be dependent upon the continuous presence of both a divalent metal ion (Mn or Mg) and an adenine nucleotide (ATP, ADP or AMPPCP). In contrast, the divalent metal ions or NaATP separately were incapable of inducing the non-covalent association between the αβ heterodimers. These results document that Mn/MgATP and insulin modulate the interactions between the heterodimeric insulin receptor subunits in a manner necessary for the insulin-dependent regulation of insulin receptor protein kinase activity.

C 243 THE HUMAN IFN-GAMMA RECEPTOR IS LOCATED IN PROXIMITY TO THE C-ROS ONCOGENE ON CHROMOSOME REGION 6Q16 TO 6Q22, Klaus Pfizenmaier, Katja Wiegmann, Peter Scheurich, Martin Krönke, Michel Aguet°, and Ugur Ücer, Clinical Research Group BRWTI, Max-Planck-Society, 3400 Göttingen, FR Germany; °Institute of Immunology, Zürich, Switzerland.
Specific high affinity IFNγ-receptors are constitutively expressed on some normal and most malignant cells of various tissues. Both hyperexpression of IFNγ-receptors and receptor negative cells have been found in lymphoid malignancies, characterized by frequent deletions or translocations of the long arm of human chromosome 6, which harbour several oncogenes. As earlier studies mapped IFNγ binding-capacity to chromosome 6, we were interested to know which part of chr 6 is involved. Employing human-rodent cell hybrids, we have analyzed human IFNγ-receptor expression by ligand binding-studies as well as by binding of a receptor-specific mAb (A6). The data obtained show i) co-ordinate binding-capacity for IFNγ and antibody A6 as well as competition in binding to hybrid cells, indicating that the A6-defined protein is capable of high affinity IFNγ binding and thus represents at least part of the IFNγ-receptor; ii) concordant segregation of IFNγ-receptor expression with presence of chromosome region 6q16 to 6q22, on which the ros oncogene, a member of the src-gene family, is located. Thus the IFNγ-receptor gene is apparently located in proximity to an oncogene and in a region of frequent aberrations, which might be seen in a causal relationship to the deregulated expression of IFNγ-receptors in some lymphoid tumors. However, unresponsiveness of chr. 6 hybrid cells to human, but not to mouse IFNγ, indicates lack of signal transduction of the human IFNγ-receptor protein coded for by chr. 6 and suggests the possibility of a complex structure of IFNγ receptors comprised of functional subunits coded for by distinct chromosomes.

C 244 PRESENCE OF SOMATOMEDIN RECEPTORS ON PRIMARY HUMAN BREAST AND COLON CANCERS. Michael N. Pollak, James F. Perdue, Kathy Baer, and Martine Richard, McGill University Cancer Centre and Department of Oncology, Jewish General Hospital, Montreal, Quebec, H3T 1E2.
Competitive binding techniques were used to study the interaction of insulin-like growth factor I (IGF-I) with a plasma membrane-enriched subcellular fraction purified from primary breast and colon carcinoma specimens obtained at surgery. The presence of specific binding sites for IGF-I was detected in all tumour specimens studied. Scatchard analysis and competition studies with insulin and insulin-like growth factor-II (IGF-II) revealed the presence of specific IGF-I receptors, showing a K_d of approximately 2 nM. These results are consistent with the hypothesis that somatomedins play a role in determining the proliferative behaviour of human breast and colon tumours, and suggest that recent laboratory studies showing dependance of neoplastic cells on somatomedins for optimum proliferation may have clinical relevance.

C 245 DENSITY-INDUCED DOWN REGULATION OF GROWTH FACTOR RECEPTORS A. Rizzino, P. Kazakoff, E. Ruff, C. Kuszynski. Eppley Institute, Univ. Neb. Med. Ctr. Omaha, NE
Regulation of growth factor receptors is not fully understood. Growth factors can selectively reduce the number of their own receptors by a process known as down regulation and some growth factors influence receptors for other growth factors by a process known as receptor transmodulation. In the current study, we have observed a general effect on growth factor receptors that has not been previously reported: the binding of four different growth factors is reduced as cell density is increased. This effect was initially observed for the binding of TGF-β to five different cell lines. Scatchard analysis of the binding data indicates that the reduction in TGF-β binding results from a decrease in the number of receptors and not from a change in receptor affinity. Examination of several cell lines, under conditions where TGF-β binding is reduced, revealed that EGF binding, PDGF binding and FGF binding are also reduced. In addition, we have determined that the reduction of binding is not due to a reduction in cell spreading. Detailed analysis of our data strongly suggest that the effect of cell density on growth factor receptors differs from receptor down regulation and receptor transmodulation. Therefore, we propose that this process be referred to as density-induced down regulation of growth factor receptors. At present, the role of density-induced down regulation of growth factor receptors is unclear, but it likely to influence cell proliferation.
(Supported by Nebraska Dept. of Health 87-38, HD 19837 and CA 36727)

C 246 CHARACTERIZATION OF THE ENDOTHELIAL CELL GROWTH FACTOR (ACIDIC FIBROBLAST GROWTH FACTOR) RECEPTOR OF CORONARY VENULAR ENDOTHELIAL CELLS. Margaret E. Schelling, James R. Hawker Jr., and Harris J. Granger, Texas A&M University College of Medicine, College Station, TX 77843.
Coronary angiogenesis involves the proliferation of coronary venular endothelial cells. The kinetics of binding of iodinated Endothelial Cell Growth Factor (acidic Fibroblast Growth Factor) to coronary venular endothelial cells was determined. Binding was saturable and specific, as evidenced by competition studies. Scatchard analyses of the binding data were used to determine the number of receptor sites per cell. For the binding of ECGF to coronary venular endothelial cells, the K_d was 5.10 x 10^{-10} M and there were 34,800 binding sites (ECGF receptors) per cell. ECGF binds to aortic endothelial cells with a K_d of 9.3 x 10^{-10}M, eachcell has 25,000 receptor sites. In competitive binding studies, unrelated mitogens such as Epidermal Growth Factor did not compete in the binding of ECGF to its receptor on coronary venular endothelial cells, but basic Fibroblast Growth Factor did compete. Heparin at concentrations from .1 ug/ml to 8 ug/ml was found to potentiate the binding of ECGF to its receptor. Heparin at any concentration was found to inhibit the binding of b-FGF to its receptor. Covalent crosslinking studies indicated the molecular weight of the receptor to be 110 Kda, which differs from the molecular weight of 150 Kda reported for the ECGF receptor isolated from lung endothelial cells (J. Biol. Chem. 261:7581). B-FGF was found to compete in the covalent crosslinking of ECGF to its receptor on coronary venular endothelial cells.

C 247 REGULATION OF TNF-RECEPTOR EXPRESSION CONTROLS TNF-SENSITIVITY, Peter Scheurich, Bettina Thoma, Rainer Unglaub, and Klaus Pfizenmaier, Clinical Research Group "BRWTI" of the Max-Planck-Society, 3400 Göttingen, FR Germany.
It is evident from numerous studies that tumor cells show a differential sensitivity to TNF-mediated cytostasis/cytotoxicity. Mechanisms controlling this differential responsiveness may be effective both at the level of TNF receptors and at post-receptor levels. We here show that regulation of TNF-receptor expression and function contributes to control of TNF-sensitivity. While IFN-gamma enhances TNF-receptor expression on a broad spectrum of tumor cells, IL-2 in conjunction with antigen receptor crosslinking is required for de novo induction and maintenance of TNF-receptor expression in normal resting T-lymphocytes. In contrast, in both normal and malignant cells of various tissue origins, selective activation of protein kinase-C by stimuli such as oleyl acetyl glycerol provokes an immediate and complete loss of TNF binding capacity. As down-regulation of TNF-binding is neither due to internalization of nor due to shedding of TNF-receptors, our data suggest that PKC directly phosphorylates TNF-membrane receptor proteins, thereby causing a greater 100-fold decrease in binding-affinity and irreversible inactivation of phosphorylated receptors. This is parallelled by a conversion of TNF-sensitive cells to a resistant phenotype. Transient stimulation of PK-C allows recovery of binding capacity by de novo synthesis receptor proteins. Upon reexpression of receptors in these cells, TNF-sensitivity is fully recovered.

C 248 EPIDERMAL GROWTH FACTOR RECEPTOR GENE ABNORMALITIES IN HUMAN NON-SMALL CELL LUNG CANCER, P.M. Schneider, M.-C. Hung, M.A. Tainsky, A. Gazdar, R.S. Ames, and J.A. Roth, Departments of Thoracic Surgery and Tumor Biology, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030
Quantitative and qualitative changes of the Epidermal growth factor receptor (EGFR) gene have been linked to tumor initiation and progression in various primary human neoplasms and tumor cell lines. We analyzed 47 patients with non-small cell lung cancer (NSCLC) and 6 NSCLS-derived cell lines for the presence of abnormalities in the EGFR gene. The genomic organization of the EGFR gene in the primary tumors and lymph node metastases was compared with paired uninvolved lung tissue from each patient. DNA was extracted from fresh tissue specimens and cell lines, digested with Eco RI, and analyzed by Southern blot hybridization to a P32 labelled human EGFR cDNA probe that encodes almost the entire extracellular and tyrosine kinase domain of the receptor. In addition, the tissue samples were screened for alterations in the c-myc and N-myc genes. EGFR gene abnormalities were detected in tumor samples from 25 (53%) of the patients. Five patients (10%) showed amplifications, 2 (4%) exhibited rearrangements, and 20 (42%) demonstrated loss of a 4.2 Kb Eco RI fragment in their tumor and/or lymph node metastasis compared to DNA isolated from uninvolved lung tissue. Lymph node metastases from 5 of 6 patients (83%) showed loss of this fragment although it was present in 2 of the primary tumors. The 4.2 Kb fragment was absent in the 6 NSCLC cell lines. This fragment hybridized with the specific probe for the tyrosine kinase domain of the receptor under high stringency conditions and did not cross-hybridize with the human neu gene. No abnormalities in the c-myc and N-myc genes could be detected in the patients. We conclude that EGFR gene abnormalities are frequent and may play an important role in initiation or progression of human NSCLC.

C 249 A PUTATIVE ANTI-IL3 RECEPTOR ANTIBODY, Jolanda Schreurs, Atsushi Miyajima, and Ken-ichi Arai, DNAX Research Institute, Palo Alto, CA 94304. Minoru Sugawara, Chigusa Hattori, Emiko Tezuka, Sumie Tamura, and Yumiko Ohta, Nippon Roche Research Center, Kamakura, Kanagawa 247, Japan. Interleukin-3 (IL-3) is a growth factor for multi-potential hematopoietic cells. Ohta et al. (in press) have shown that hybridomas from autoimmune MRL/lpr mice secrete antibodies with IL-3-like activities. Our analysis of these antibodies strongly indicates that one, M7B15.1-F9 or (F9), interacts with the IL-3 receptor or with part of an IL-3 receptor complex. Our evidence is as follows: 1) F9 (γ_1 isotype) is a full agonist (80-100% IL-3 efficacy) with a potency of 0.5-2.0 nM in proliferation assays using 8 different IL-3 dependent cell lines of mast or myeloid origin; 2) A variant cell line, NFS60.8, which requires 30-fold more IL-3 for maximum proliferation also has a 100-200 fold increased concentration requirement for F9; 3) The antibody is not additive with IL-3 in bioassays, suggesting that the same or similar transduction mechanism is used; 4) The specificity of the agonist effects of this antibody were investigated in radioligand binding assays. Pre-incubation of 3 different cell lines with F9 leads to greater than 90% competition of the [125I]IL-3 specifically bound, with an IC50 of about 200 nM; it has no effect on [125I]IL-4 binding to these same cells; 5) The converse is also true. Purified IL-3 blocks the binding of non-Fc$_\gamma$ receptor associated [125I]-F9 antibody; IL-2 has no effect. Determination of the molecular mass of this antigen is in progress.

C 250 THE MAJOR TGF-β RECEPTOR IS A LIPOPHILIC PROTEOGLYCAN.
Patricia R. Segarini,† Ralph Sanderson,‡ and Saeid M. Seyedin.† †Collagen Corporation, 2500 Faber Place, Palo Alto, CA 94303 ‡Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305

Both forms of transforming growth factor-beta (TGF-β), TGF-β1 and TGF-β2 bind to several components at the cell surface. On fibroblast-like cells there are 3 classes of binding species, namely, low M_r, 50-80 kDa, intermediate M_r, 115-140 kDa, and the major binding species, which are heterogeneous in size with an average M_r of 250 kDa and are glycosylated with heparan sulfate and chondroitin sulfate chains. Furthermore, these proteoglycan receptors are highly hydrophobic, supporting the assumption that they are integral membrane components. Removal of the glycosaminoglycan (GAG) chains yields products that migrate on 4-10% gradient PAGE at the same size as the intermediate sized binding components suggesting that the intermediate species represent nonglycosylated core proteins of the intact molecule. In situ digestion of Swiss 3T3 cells with GAG-specific degradative enzymes eliminates the 250 kDa binding species and enriches the intermediate sized binding species, suggesting that TGF-β1 and TGF-β2 do not bind to the glycosaminoglycan chains and that these sugars are not necessary for interaction of the factors with the binding components. The basis for the multiple binding species is unclear, but the data suggest that the high and intermediate M_r species range in the extent of their glycosylation. The presence of glycosaminoglycan chains on the major species indicates that it has the potential for interaction with the extracellular matrix.

C 252 CLONING OF THE MOUSE INTERLEUKIN 1 RECEPTOR, J.E. Sims, D. Urdal, C.J. March, S.M. Call, J. Jackson, A. Akelis, and S.K. Dower, Immunex Corporation, 51 University Street, Seattle, WA 98101.
Interleukin-1α and interleukin-1β play a central role in the regulation of immune and inflammatory responses. They regulate the metabolism of cells through a common plasma membrane receptor protein. In this study, it is demonstrated that the IL-1 receptor from detergent solutions of EL-4 cells can be stably adsorbed to nitrocellulose with full retention of IL-1 binding activity. This assay system was used to monitor the purification of the IL-1 receptor. IL-1 receptors extracted from EL-4 6.1 C10 cells can be bound to and specifically eluted from IL-1α coupled to Sepharose. The affinity chromatography method resulted in the identification, by silver staining of polyacrylamide gels, of a protein of M_r 82,000 that was present in fractions exhibiting IL-1 binding activity. Further purification by lectin affinity chromatography and by reversed phase HPLC has yielded material of sufficient purity to consider protein sequence analysis.

C 253 ESTROGEN REGULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR MESSENGER RNA, George M. Stancel, Russell B. Lingham and David S. Loose-Mitchell, Department of Pharmacology, The University of Texas Medical School at Houston, Houston, TX. 77025.
We have been investigating the possible role of growth factors in steroid mediated growth in vivo, and have demonstrated previously that estradiol (E2) causes a three fold increase in epidermal growth factor (EGF) receptors in uterine membranes (J. Biol. Chem. 260:9820, 1985). We now report that the increase in uterine EGF receptor levels following E2 is due to an increase in the steady-state levels of EGF receptor mRNA. A rat EGF receptor cDNA (kindly provided by Dr. Axel Ullrich, Genentech, Inc.) was used to prepare an antisense RNA probe. Following a single E2 injection to immature rats, uterine EGF receptor mRNA levels, as determined by RNA blots, increase between 1 and 3 hours, remain elevated at 6 hours, and decline between 12 and 18 hours. This change in mRNA levels precedes the increase in functional EGF receptors which is elevated 6 to 12 hours after hormone treatment and declines between 18 and 24 hours after steroid administration. The increase in EGF receptor mRNA is specific for estrogenic steroids since progesterone, dexamethasone, dihydrotestosterone, and an inactive isomer of estradiol (17-alpha estradiol) do not produce this effect. The E2 mediated increases in EGF receptor mRNA levels are blocked by actinomycin D but not by puromycin. Taken together these results indicate that E2 regulates the level of epidermal growth factor receptor by increasing the steady-state concentration of the receptor mRNA in vivo. (Supported by NIH grants HD-08615, DK-38965 and RR-01685 for computer use.)

C 254 REGULATION OF P185^{neu} BY ONCOGENIC ACTIVATION AND BY EGF, David F.Stern, Dept. of Pathology, Yale Medical School, New Haven, CT 06511.
p185^{neu} is a receptor-like protein encoded by the neu/erbB-2 proto-oncogene. p185 is closely related to the epidermal growth factor (EGF) receptor but does not bind EGF. We have found that incubation of Rat-1 cells with EGF stimulates tyrosine phosphorylation of p185. The EGF-stimulated tyrosine phosphorylation of p185 and of the EGF receptor occurred with similar kinetics and EGF dose-responses, and both phosphorylations were prevented by down-regulation of the EGF receptor with EGF. Since p185 does not bind EGF, these results suggest that p185 is a substrate for the EGF receptor kinase. Incubation of cells with EGF before lysis stimulated the tyrosine phosphorylation of p185 in immune complexes. This suggests that EGF, acting through the EGF receptor, can regulate the intrinsic kinase activity of p185.

The rat transforming variant of p185 differs from its normal cellular counterpart by a single amino acid replacement in the transmembrane domain. We have found that this transforming protein has higher tyrosine kinase activity in vitro than normal p185. Furthermore, cell lines expressing high levels of the transforming protein, but not cells expressing high levels of the normal protein, contained novel phosphotyrosine-containing proteins. These findings support the simple model that the oncogenic activation of p185 results in increased p185 effector activity.

C 255 DISSOCIATION OF THE LIGAND AND DEPHOSPHORYLATION OF THE ACTIVATED PLATELET DERIVED GROWTH FACTOR RECEPTOR. E.Sturani, R.Zippel, L.Morello, L.Alberghina. Department of General Physiology and Biochemistry, University of Milano, Via Celoria 26, 20133 Milano, Italy.

The receptor for PDGF is endowed with tyrosine-specific protein kinase activity and becomes phosphorylated in tyrosine upon ligand binding. Antibodies against phosphotyrosine (P-tyr) recognize the phosphorylated form of the receptor in Western blots as a sharp band of 170 Kd. In Swiss 3T3 fibroblasts the ligand-induced phosphorylation of PDGF receptors at 37°C is followed by dephosphorylation, while a 4° C the phosphorylated form is more stable. A mild acid treatment of stimulated intact cells, which removes the surface-bound ligand, causes the rapid loss of phosphate from the P-tyr groups of the receptor. Suramin which induces the dissociation of the PDGF-receptor complex also lowers the P-tyr content of the PDGF receptor. These data suggest that, when the ligand-receptor complex is dissociated, the kinase activity of the receptor is switched off, and the tyrosine-bound phosphate decreases due to the action of phosphatases. On the other hand the cleavage of the extracellular domain of the receptor by treating PDGF-stimulated cells with trypsin gives rise to two proteolytic fragments, which remain phosphorylated at tyrosine.

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C 256 IDENTIFICATION OF THE BOMBESIN RECEPTOR BY MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES. Anders Sundan, Apothekernes Lab. A.S. Harbitzalleen 3, 0275 Oslo 2, Norway.

Cross-linking experiments with iodinated gastrin releasing peptide (GRP) have recently identified cell surface binding molecules for bombesin/GRP migrating in SDS-gel electrophoresis with apparent MW 69 kD in intact and Triton X-100 extracts from quiecent Swiss 3T3 cells. By immunization of mice with a monoclonal antibody directed against the receptor-binding domain in bombesin/GRP, and subsequent hybridoma clone selection for binding activity against Swiss 3T3 extracts, we eventually was able to identify antibodies which inhibited GRP binding to intact 3T3 cells. One of these antibodies, termed BRICF6 and of IgG1 subclass, reacted strongly with a 69 kD polypeptide in Western blots of Swiss 3T3 extracts. Furthermore, after cell surface iodination of quiecent 3T3 cells this antibody precipitated a molecule with apparent MW 69 kD. The immuno-precipitation could be inhibited by an excess of GRP, and the 69 kD polypeptide could be eluted from Sepharose-protein A-antibody BRICF6 complexes with GRP. The results indicate that in Swiss 3T3 cells GRP/bombesin bind a putative receptor polypeptide with apparent MW 69 kD. Furthermore, if the receptor is a complex containing other subunits these are not accessible to cross-linking agens in intact cells, or cell surface iodination.

C 257 RECEPTORS FOR B CELL STIMULATORY FACTOR 2 (BSF-2/IL-6): REGULATION OF THE EXPRESSION AND BIOCHEMICAL CHARACTERISTICS

Tetsuya Taga, Yoshikazu Kawanishi, Yuuich Hirata, Toshio Hirano and Tadamitsu Kishimoto, Institute for Molecular and Cellular Biology, Osaka University, Suita, Osaka 565, Japan.

Receptors for B cell stimulatory factor 2 (BSF-2-R or IL-6-R) were studied using radioiodinated recombinant BSF-2. There was a single class of receptors with a Kd of 3.4×10^{-10}M on EBV-transformed human B-LCL CESS and with a number of 2,700 per cell. Binding of ^{125}I-BSF-2 to CESS was competitively inhibited by unlabeled BSF-2 but not by IL-1, IL-2, IFN-β, IFN-γ and G-CSF, indicating the presence of the receptors specific for BSF-2. Four T cell lines examined did not express BSF-2-R but normal resting T cells did. BSF-2-R were not present on normal resting B cells but expressed on activated B cells fitting the function of BSF-2 which acts on B cells at the final maturation stage to induce immunoglobulin production. Two plasmacytoma lines examined expressed BSF-2-R, fitting the function of BSF-2 as plasmacytoma growth factor. The plasmacytoma line U266 expressed two types of BSF-2-R with different Kd, one was 1×10^{-11}M and the other was 8×10^{-10}M. Results from a saturable binding and following cross-linking study with dissuccinimidyl-suberate and a photo-affinity labeling study using photoactivatable, heterobifunctional, cleavable ^{125}I-labeled Denny-Jaffe reagent indicated that BSF-2 receptors on U226 were composed of two polypeptide chains of 120 kD and 80 kD.

C 258 INCREASE OF PDGF RECEPTOR EXPRESSION WHEN CELLS ARE CULTURED, Anders Tingstom, Louis Terracio, Lars Rönnstrand, Carl-Henrik Heldin, Lena Claesson-Welsh, Keiko Funa and Kristofer Rubin, Ludwig Inst for Cancer Research and Dept. of Medical and Physiological Chemistry, Uppsala, Sweden and Univ. of South Carolina, Columbia, SC.
The receptor for platelet derived growth factor (PDGF) is expressed on many mesenchymally-derived cells in vitro. Recently we have raised monoclonal antibodies recognizing the external domain of the PDGF receptor and we are currently using them to analyze the regulation of PDGF receptor expression. Histochemical staining of pig uterus revealed the presence of PDGF receptors on cells surrounding the glands and the coiled arteries of the endometrium, but very little or no staining on the myometrium. The same pattern was seen on isolated cells; myometrial cells were all negative while many endometrial cells showed staining specific for the PDGF receptor. When maintained in culture, the previously negative myometrial cells became positive for the PDGF-receptor after a couple of days, and after a week a completely dense monolayer of strongly positive cells was seen. The histochemical findings were supported by immunoblotting and autophosphorylation studies done on isolated membranes from myometrial and endometrial cells. In situ hybridization for PDGF receptor mRNA showed a higher synthesis in the endometrium than in the myometrium. These results indicate that the response to PDGF stimulation is not only dependent on the availability of the ligand but also on the expression of the receptor.

C 259 CHARACTERIZATION OF A HIGH TITER RETROVIRUS CARRYING THE HUMAN erbB PROTO-ONCOGENE ENCODING THE NORMAL EGF RECEPTOR, Thierry J. Velu, Laura Beguinot, William C. Vass, Ira Pastan, and Douglas R. Lowy. National Cancer Institute, Bethesda MD 20892.
By inserting a normal human EGF receptor (hEGFR) cDNA into a retroviral vector, we have generated a high titer retrovirus (up to 3×10^7/ml). Chronically infected NIH3T3 cells expressed up to 4×10^5 hEGFR/cell (normal NIH3T3 cells have 10^4 hEGFR/cell). In the absence of EGF, they grew similarly to control NIH3T3 cells, but they grew twice as fast as control cells in the presence of 20 ng/ml EGF and reached a higher saturation density. The dose dependent growth response to EGF of cells grown in serum free medium can be used as a highly sensitive bioassay for the quantitative assessment of EGF and TGFα. Its sensitivity for EGF is 100 pg/ml. Uninfected NIH3T3 cells can be used as controls for the specificity of growth stimulation. In acute infection of NIH3T3 cells, the EGFR virus induced EGF dependent foci of morphologically transformed cells and EGF independent foci in cells expressing TGFα. Infected cells formed colonies in soft agar, but only when EGF was added to the medium. Nude mice (which contain endogenous EGF) inoculated with cells expressing high levels of hEGFR developed tumors with a 55 day latency; the latency was shorter (35 days) when mice were given exogenous EGF (5 μg daily and subcutaneously). Control cells were non-tumorigenic. We conclude that high levels of hEGFR can induce ligand dependent transformation and contribute to tumorigenicity. These results suggest that the high number of EGFR found in a variety of human tumors may play a direct role in the pathogenesis of these diseases.

C 260 SMOKING-RELATED ALTERATIONS IN EPIDERMAL GROWTH FACTOR AND INSULIN RECEPTORS IN HUMAN PLACENTA. S-L. Wang, G. W. Lucier*, R. B. Everson* and K. T. Shiverick, University of Florida, Gainesville, FL 32610 and NIEHS*, Research Triangle Park, NC 27709
Studies characterized insulin and EGF receptors in human placental tissue from smokers and non-smokers. Specific binding of [^{125}I]-labeled insulin and EGF to placental membranes was not different between smokers compared to non-smokers. In wheat germ agglutinin-purified preparations of solubilized membranes from non-smokers, EGF stimulated the active phosphorylation of M_r 170,000 (170K) and 140K protein bands which was half-maximal (EC_{50}) at 5×10^{-8} M. In extracts from smokers, however, phosphorylation of these two protein bands was barely detectable over a range of 0 to 10^{-6} M EGF. Thus, both basal and EGF-stimulated phosphorylation of the 170 and 140K bands was markedly decreased in placental membranes from smokers. In contrast, insulin stimulated the phosphorylation of a 95K protein which was immunoprecipitated with anti-insulin receptor antibody in membrane preparations from both non-smokers and smokers. Dose-response curves for autophosphorylation indicate that EC_{50} values were 2.6 and 7.0 nM insulin for non-smokers and smokers, respectively. Laser densitometry scan of the 95K band on autoradiograms further showed that maximal [^{32}P]-incorporation was 30% greater in smokers compared to non-smokers. Analysis of the insulin-dependent phosphorylation of an exogenous substrate, poly(Glu,Tyr) (4:1), showed a similar pattern of values for non-smokers *vs* smokers. These results indicate that insulin receptor autophosphorylation and tyrosine kinase activity were normal or increased, while EGF-stimulated kinase activity was markedly decreased in placental membrane proteins from smokers. Western blot analysis using an antibody to the EGF receptor showed the presence of immunoreactive bands of 126K and 150-170K in receptor preparations from non-smokers, while only 126K protein was detected in preparations from smokers. Thus the smoking-related deficiency in EGF receptor autophosphorylation appeared to be due to the absence of 150-170K receptor protein. In conclusion, maternal cigarette smoking is associated with selective alterations in two major pathways involved in cell growth and differentiation in human placenta.

C 261 A CASCADE OF TYROSINE AUTOPHOSPHORYALTION IN THE β-SUBUNIT ACTIVATES THE PHOSPHOTRANSFERASE OF INSULIN RECEPTOR, Morris F. White, Steven E. Shoelson, Henry Keutmann and C. Ronald Kahn, Joslin Diabetes Center and Harvard Medical School, Boston, MA 02215.
The insulin receptor is a tyrosine-specific protein kinase that undergoes autophosphorylation on at least 5 tyrosyl residues in its β-subunit during insulin binding to the α-subunit. This reaction activates a phosphotransferase in the β-subunit. To study this process, we purified the insulin receptor from Fao hepatoma cells and carried out phosphorylation in solution. Two phosphorylation sites are in the C-terminus: Tyr-1316 and Tyr-1322. Removal of this domain by mild trypsinolysis had no effect on the activation of the tyrosine kinase suggesting that these residues do not play a regulatory role. To understand the events which activate the kinase, we arrested the autophosphorylation cascade of the intact receptor with an antiphosphotyrosine antibody (αPY). The αPY inhibited autophosphorylation of the C-terminal domain, but had no effect on the first two autophosphorylation events which occurred in the *Tyr-1150 domain* at Tyr-1146 and either Tyr-1150 or 1151 (2Tyr(P)-form); however, this did not activate the kinase. Removal of the antibody allowed all three of these tyrosyl residues to be phosphorylated (3Tyr(P)-form) which activated the kinase in the presence or absence of the C-terminal domain. In the intact cell, the 2Tyr(P)-form of the Tyr-1150 domain predominated. We conclude that (1) autophosphorylation of the insulin receptor begins by phosphorylation of Tyr-1146 and either Tyr-1150 or Tyr-1151, and (2) progression of the cascade to phosphorylation of the third tyrosyl residue activates the phosphotransferase during *in vitro* assay; (3) *in vivo*, the 2Tyr(P)-form predominates suggesting that progression of the autophosphorylation cascade to the 3Tyr(P)-form is regulated during insulin stimulation. Thus, multisite phosphorylation in the Tyr-1150 domain may be an important control site for transmission of the insulin signal in the intact cell.

C 262 TGF-β Receptors of Normal Hepatocytes and Hepatomas, Robert H. Whitson, Brian I. Carr, Thomas J. Fielder, Wee Ling Wong and Keiichi Itakura, Beckman Res.Inst., City of Hope, Duarte, CA 91101. Using a combination of affinity crosslinking and competitive binding assays, we have compared the TGF-β receptors of normal rat hepatocytes to those of several rat and human hepatoma lines. Four molecular weight species of TGF-β receptors have been identified on the surface of freshly isolated rat hepatocytes. In addition to the 280 kDa, 85kDa and 65kDa receptors commonly seen in many other cell types, hepatocytes have the somewhat rarer 135 kDa form as well. Hepatocytes are also unusual in that the binding affinity of the 280 kDa receptor is extremely low (Kd=2500 pM). Each of the hepatoma lines has a distinctive array of TGF-β receptors, with different combinations of the hepatocyte forms and variable binding affinities. Several of the cell lines (SK Hep 1, an adenocarcinoma, Hep 3B, a human hepatoblastoma, and H4IIE and MH_1C_1, rat hepatoma lines derived from Morris and Reubers hepatomas, resp.) had the same low affinity 280 kDa receptor as the hepatocytes. The other hepatomas, (the human hepatocellular carcinomas, HUH-7 and PLC/PRF, the human hepatoblastoma, Hep G2, and the rat hepatoma, MH_1C_1) had 280 kDa receptors with a higher affinity for TGF-β (Kd=100 pM). The Hep G2 line had 15-20 fold as much TGF-β binding as the hepatocytes, due to the presence of more than 70,000 high affinity 280 kDa receptors per cell. Hep G2 also has a 110 kDa TGF-β receptor. The biological consequences of different arrays of TGF-β receptors in different cells are not clear.

C 263 EXPRESSION OF THE <u>DROSOPHILA</u> EGF RECEPTOR HOMOLOG, Ronald Wides, Naomi Zak, Eyal Schejter, Erez Raz, and Ben-Zion Shilo, Dept. of Virology, The Weizmann Institute of Science, Rehovot 76100,Israel.
The <u>Drosophila</u> EGF receptor (DER) gene is homologous to both the human EGF receptor and the <u>neu</u> gene. The primary amino acid sequence shows that it is 170 amino acids larger than these two proteins, due to the presence of a third cysteine rich domain. Antibodies were raised against the C-terminal portion of the DER protein. These antibodies recognized a protein of the expected size (190 KD) produced by Cos cells and <u>Drosophila</u> tissue culture cells transfected with DER cDNA constructs.

The structure of the cDNA clones indicates the use of two sets of 5' exons. Each of the two splicing alternatives is driven by its own promoter. Gene fusion constructs consisting of each of the two DER promoters and the CAT structural gene were used to assay promoter activity. Both promoters were active when injected into <u>Drosophila</u> embryos and when transfected into <u>Drosophila</u> tissue culture cells. The promoter activities were comparable to each other in both assay systems. In order to quantitate the utilization of each exon <u>in vivo</u>, RNAse protection experiments were performed. Comparable levels of expression of the two alternatives were found at all stages of fly development.

Since the two DER alternatives are used throughout development, it is not clear whether each has a specific role. It is possible, however, that the N-terminal differences specify differences in ligand recognition by the two alternative proteins. We are using the structural information on DER in conjunction with genetic techniques to study the role of DER during development.

C 264 EXPRESSION OF HUMAN NERVE GROWTH FACTOR RECEPTOR (hNGFR) IN MDCK CELLS FROM TRANSFECTED cDNA. A.PATZAK, M.CHAO and E.RODRIGUEZ-BOULAN. Dept.of Cell Biology and Anatomy, Cornell University Medical College, New York, NY, 10021

The nucleotide sequence of the cDNA encoding the hNGFR has been determined and transfection of a full-length cDNA in mouse fibroblasts resulted in stable expression of NGFR, recognized by monoclonal antibodies and binding of 125I-NGF (Johnson et al, 1986, Cell 47, 545). We transfected MDCK cells with hNGFR cDNA and isolated different clones expressing the protein by a red blood cell rosetting assay. Northern blots revealed the presence of hNGFR mRNA in the transfected cells. Immunoprecipitation of (35S)-cystein labelled extracts detected a 65 kD protein in transfected but not in normal MDCK cells. Immunofluorescence on monolayers of the transfected cells grown on filters detected hNGFR in the apical as well as in the basal membrane. The same kind of experiments are currently under progress on MDCK cells transfected with truncated hNGFR cDNA. Immunolabelling on ultrathin and semithin frozen sections of monolayers transfected with hNGFR cDNA with a monoclonal antibody detected only a slightly polarized expression, up to twice higher in the apical than in the basal membrane. The receptor is totally extractable with Triton X-100. Experiments are currently under progress to investigate the pathways followed by hNGFR to the cell surface as well as its possible role in transcytosis of NGF.

Supported by NIH grants GM-34107 and NS-21072.

Modulation of Growth Factor Gene Expression

C 400 CONSTITUTIVE OVER-EXPRESSION OF A NOVEL GROWTH-REGULATED GENE IN TRANSFORMED CHINESE HAMSTER AND HUMAN CELLS, Anthony Anisowicz, Lee Bardwell, and Ruth Sager, Dana-Farber Cancer Institute, Boston, MA 02115.

Subtractive hybridization was used to isolate a gene expressed at moderately high levels in a tumorigenic Chinese hamster embryo fibroblast cell line (CHEF/16) and at very low levels in a closely related non-tumorigenic cell line (CHEF/18). The cloned cDNA was sequenced and showed close homology to human connective tissue activating peptide III (CTAP III). The human tumor cell cDNA hybridizing to the CHEF gene was isolated, sequenced, and found to have closer similarity to the CHEF gene than to CTAP III. Thus the cloned genes seem not to be the homologs of CTAP III, but a closely related, novel gene, which was named gro. Steady state levels of the gro message were found to be higher in rapidly growing CHEF/18 and undetectable in confluent cells indicating a tight regulation by growth status in normal cells. Cycloheximide pretreatment of CHEF and human cells showed a superinduction of gro message. Nuclear run-on assays indicate that the steady state levels in CHEF/16 and CHEF/18 can be accounted for by differences in transcriptional rate. Incubation of CHEF/16 cells in the presence of actinomycin D indicate that the message is quite stable ($t_{\frac{1}{2}}$ ≈8 hours). The expression of gro mRNA was determined during reinitiation of growth by serum of serum starved CHEF/18 cells. The message appeared 30 minutes after growth reinitiation, peaked at 1 hour, and was down to basal levels at 2 hours. Thus the gro gene is growth regulated and might play an important role in cellular growth control. Studies of the regulation of gro expression in human cells will be presented.

C 401 REEXPRESSION OF IGF-II FETAL TRANSCRIPTS IN HUMAN PRIMARY LIVER TUMORS, Elisabetta CARIANI*, Chantal LASSERRE, Danielle SEURIN, Bernard HAMELIN, François KHEMENY*, Dominique FRANCO*, Christian BRECHOT, INSERM U 75. CHU Necker, PARIS, *Hôpital Louise Michel, Evry, FRANCE.

Insulin-like growth factor II (IGF-II) is a polypeptide mitogen which is thought to be involved in fetal growth. An enhanced IGF-II expression, as well as deletions of the short arm of chromosome 11, where IGF-II gene is located, were evidenced in embryonic tumors such as Wilms' tumor. Since rearrangements at the same level were also observed in hepatoblastoma and a few hepatocellular carcinomas, a similar tumorigenic mechanism can be hypothesized in primary liver cancer. Thus, we analyzed IGF-II transcription in 35 primary liver cancers (PLC) and 12 benign liver tumors (BLT). For 32/35 PLC and 11/12 BLT one or more adjacent non-tumorous areas were examined. A quantitative analysis made by slot-blot on 20ug of total RNA allowed to detect a marked increase of IGF -II mRNA in 8 PLC (including adjacent cirrhotic tissue from 3 samples) and in no BLT as compared to normal adult liver. Further study by Northern blot on total or poly (A) + RNA of 15 PLC and 10 BLT showed two kinds of transcripts: the first, an unique band of 5.3kb, in normal liver, 3/15 PLC and 8/10 BLT; the second, three bands of 6,5 and 2kb, was detected in fetal liver, in 12/15 PLC and 2/10 BLT. This study indicates that: 1) an elevation of IGF-II mRNA can be shown in approximately 25% of primary liver cancers; 2) IGF-II overexpression is associated to a fetal pattern of transcripts, which is consistent with a reactivation of fetal IGF-II promoter, possibly due to tumorous cells' dedifferentiation; 3) the reexpression and/or increase of IGF-II fetal transcripts is mainly observed in malignant hepatocytes and in some potentially "pretumorous" states (such as cirrhosis and some adenomas).

C 402 IDENTIFICATION AND PARTIAL CHARACTERIZATION OF AN EGF-INDUCED GENE EXPRESSED LATE IN THE CELL CYCLE. Maureen T. Cronin and Carol L. MacLeod, Univ. of Calif., San Diego, Cancer Center, Department of Medicine, La Jolla, CA 92093.
When cells expressing the EGF receptor are exposed to EGF, biochemical and physiological responses are observed within minutes after ligand-receptor binding. These changes are rapidly reversed with removal of EGF. Mitosis will not occur in EGF-responsive cells without continuous exposure for about 8 hours. Studies to identify genes expressed during these early cellular events have identified abundant transcripts regulated early in the EGF response, but prior to commitment to mitosis. Using subtractive hybridization enriched cDNA probes obtained from cloned A431 epidermoid carcinoma cells a late responding, EGF-regulated gene has been identified. The gene is maximally expressed after about 12 hours of continuous EGF exposure at a time when the cells are irreversibly committed to mitosis. Five low abundance transcripts ranging in size from 4.7 to 1.1 kB in length are detected by Northern blot analysis of cytoplasmic or total RNA prepared from tumor cells and from normal fibroblasts. Southern blot analysis indicates that the gene is present as a single copy and is rearranged in the A431 clonal cell lines and solid tumors grown from these cell lines in athymic mice. Partial sequencing of the cDNA clone has shown no similarity with any known gene.

C 403 KAPOSI'S SARCOMA (KS)-DERIVED ENDOTHELIAL CELLS EXPRESS GROWTH FACTOR GENES WITH ROLE IN NEOANGIOGENESIS. B. Ensoli*, L. Larsson*, S.Z. Salahuddin*, S. Nakamura*, P. Biberfeld+, B. Beaver*, F. Wong-Staal*, and R.C. Gallo* *Laboratory of Tumor Cell Biology, NCI NIH , Bethesda, MD, US. +Department of Pathology, Karolinska Institute, Stockholm, Sweden

KS-derived cell lines were developed from AIDS-KS patients. The long term culture was made possible by using conditioned media (CM) from HTLV-IICD4$^+$ transformed cell lines, which contain novel growth promoting factor able to sustain KS cell growth. CM and/or cellular extracts from KS cell lines possess a variety of growth promoting endothelial and fibroblast cells, KS cell lines themselves, hematopoietic cells, IL-1-like activity, and neoangiogenic activity demonstrated by in vitro and in vivo assays. To further characterize these activities we studies the gene expression of these cell lines for a number of growth and regulatory molecules. By Northern blot hybridization with antisense oligonucleotide probes or full-length probes we showed KS cells to express abundant levels of mRNA for basic fibroblast growth factor (bFGF), interleukin-1B (IL-1B), and intermediate levels of mRNA for acidic fibroblast growth factor (aFGF), IL-1 , Transforming Growth Factor-B (TGFB), Granulocyte-Monocyte Colony Stimulation Factor (GM-CSF) and Platelet Derived Growth Factor -B (PDGF-B). No detectable levels of mRNA were present for TGFa, Tumor Necrosis Factor a and B (TNFa and B), Interleukin-2 (IL-2), Monocyte-Colony Stimulation Factor (M-CSF-1), or Interferon-gamma (INF-gamma). A cDNA library from a KS endothelial cell lines enabled us to clone and sequence the transcripts for bFGF and confirmed it to be bFGF. We suggest that following initial, as yet unknown, event(s) that leads to the establishment of a first focus of "KS cells", the over-production of one or a combination of these growth factors might cause or maintain the KS lesion via different pathways: by autocrin growth stimulations of KS cells, by paracrine growth stimulation of normal endothelial and fibroblast cells, and by continued local activation of inflammatory cells.

C 404 MODULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR GENE EXPRESSION BY TRIIODOTHYRONINE AND TRANSFORMING GROWTH FACTOR-ß IN A HUMAN BREAST CARCINOMA CELL LINE. J. A. Fernandez-Pol, P.D. Hamilton, D. L. Theodoro, V. M. Schuette and D. J. Klos, Laboratory of Molecular Oncology, VA Medical Center and Dept. of Medicine, St. Louis University, St. Louis, Mo 63106.

We have investigated the actions of triiodothyronine (T3) and/or transforming growth factor ß (TGFß) on epidermal growth factor (EGF) receptor(R) gene expression in MDA-468 human breast carcinoma cells. Using the cDNA clone pE7 (Merlino et al,Science, 224:417, 1984) as a hybridization probe, we have found that exposure of MDA-468 cells to T3 and/or TGFß results in enhancement of the accumulation of EGF-R mRNA induced by EGF. Two possible mechanisms could account for the observed increases in steady state levels of the EGF-R mRNA induced by T3 and/or TGFß: 1) increased transcription rate or 2) altered mRNA stability-i.e. decreased half-life. We have determined the in vitro transcription rate of the EGF-R gene and the half-life of the EGF-R mRNA. Our data suggest that the levels of EGF-R mRNA are regulated by T3 and/or TGFß by both transcriptional and post-transcriptional mechanisms. The results are consistent with the hypothesis that the actions of T3 and/or TGFß on MDA-468 cell growth may be mediated , at least in part, by modulation of EGF-R gene expresion.

C 405 EXPRESSION OF HUMAN IL-2 GENE TRANSFECTED INTO NIH-3T3 AND HELA CELLS. Alberto Gulino*, Antonietta Farina*, Angela Gismondi*, Angela Santoni*, Luigi Frati* and Nikki J. Holbrook§, *Dept. of Experimental Medicine, University La Sapienza, Rome, Italy and §Laboratory of Molecular Genetics, National Institute of Aging, Baltimore, MD, 21224.
IL-2 gene expression is tightly controlled and limited to antigenic stimulation of mature T cells. In order to study the cell specific regulation of IL-2 gene we have co-transfected the human IL-2 gene, including 2.0 kb of 5' flanking sequences, together with pSV2neo into mouse NIH-3T3 fibroblast and human Hela cells. Selected stable transformants had integrated an intact and not rearranged human IL-2 gene into their genome. Cytoplasmic RNA hybridizing with human IL-2 cDNA was observed in these transformants but not in untransfected or pSVneo-transfected cells. IL-2 mRNA was significantly increased by a 4 h treatment with 2 ug/ml cycloheximide. This IL-2 mRNA was larger (1.4 kb) than IL-2 mRNA of human T cells, although smaller than RNA containing unspliced intact introns. No alternative promoters or polyadenylation signals appeared to be used by these cells, but some intronic sequences appeared to be present in the mRNA. These transformants secreted low levels of bioactive IL-2, whose production was insensitive to TPA, Ca^{++} ionophores and mitogens, while untransfected or pSVneo-transfected cells did not. We conclude that the human IL-2 gene transfected into NIH-3T3 and Hela cells is expressed constitutively and might splice and/or process differently than endogenous gene in T cells.

C 406 Expression of two c-fms-related gene products in rat myoblasts.
J. HAREL, S.A. LEIBOVITCH, M.P. LEIBOVITCH and M. GUILLIER. Laboratoire d'Oncogénèse Moléculaire (UA 1158 CNRS) Institut Gustave-Roussy,94802 Villejuif, France.
Two c-fms-related transcripts, 2.0 and 3.7 Kbs long were found to accumulate during the growth of tissue-cultured rat myogenic cells (L6α1 subline) as in L6α1 cell-derived transformants (Leibovitch et al. Cancer Res. 1986,46,4097). In the present study both transcripts wer found to be down-regulated agter L6α1 cells stop dividing to form myotubes and to be consistently expressed in the various lines of profilerating myoblats examined and sligtly expressed in fetal or perinatal muscles. The 3.7 Kbs transcript appears to span the three domains of the putative gene product whereas the 2.0 Kbs transcript only corresponds to the intracytoplasmic domain. In cell-free translation assays the 2.0 Kbs and 3.7 Kbs RNA species were shown to code fo two v-fms-related antigens of 69 Kd and 116 Kd respectively. Preliminary data indicated that both proteins are synthesized in myoblasts and that the 116 Kd protein is the short-lived precursor to a 170 Kd kinase. Altogether these data plead for a role of c-fms-related gene(s) expression in normal or neoplastic growth of muscular stem cells.

C 407 STUDIES ON THE NGF GENE EXPRESSION BY NORTHERN BLOT AND IN SITU HYBRIDIZATION IN MOUSE AND CHICKEN, Angelica Keller, Charles Auffray, Institut d'Embryologie du CNRS, Nogent sur Marne 94130, France, in collaboration with Bertrand Bloch, Faculté de Médecine, Besançon, France.

The aim of this study is to determine which are the organs and cell types which are responsible for nerve growth factor (NGF) synthesis in the early chick embryo, where this neurotrophic activity has been first demonstrated. The 3' exon of the chicken NGF gene has been isolated, using a murine cDNA probe (Wion et al. 1986). In the murine and chicken sequences coding for the mature β-NGF, 80% of the nucleotides are identical. Both mouse and chicken coding sequences have been subcloned in a pSP64 plasmid, permetting their transcription into cRNA. Various types of probes and labelling methods have been used both for Northern blot analysis and in situ hybridization, in order to achieve the highest sen-sitivity and specificity of these methods. Various brain regions and organs have been analysed both in mouse and chick and the presence of βNGF mRNA demonstrated in both brain and heart. Probes includes 45-mer oligonucleotides, double stranded cDNA and cRNA. Use of the cRNA probes represent the most sensitive method - in our hands -for in situ mRNA detection.
Ref: Molecular cloning of the avian β-nerve growth factor gene: transcription in brain. Wion, D, Perret, D, Frechin, N, Keller, A, Behar, G, Brachet, P and Auffray, C. (1986) FEBS Lett. 203, 82-86

C 408 IDENTIFICATION OF 40S RIBOSOMAL PROTEIN S6 PHOSPHORYLATION SITES, Joachim Krieg,Jan Hofsteenge and George Thomas,Friedrich Miescher-Institut, CH-4002 Basel, Switzerland.

Stimulation of quiescent cells to proliferate by growth factors and oncogenes leads to the multiple phosphorylation of 40S ribosomal protein S6. The phosphates are all incorporated into serine residues and appear to be added in a specific order. In vivo the addition of five phosphates seems to either trigger or facilitate an increase in the rate of initiation of protein synthesis and to be tightly coupled to the altered expression of specific mRNAs. Under conditions in vitro where two phosphates are incorporated by the cAMP-dependent protein kinase, the sites of phosphorylation have been shown to be adjacent to one another within the COOH-terminal region of the molecule. It has been speculated, that all the sites of phosphorylation may be clustered in this locus of the protein. We show here, that up to five moles of phosphate are incorporated into rat liver ribosomes after treatment of animals with cycloheximide and that all the phosphates reside in a 32AA cyanogenbromide fragment at the C-terminal end of the molecule. To identify which serines are phosphorylated, we have isolated this phosphopeptide by reverse phase HPLC, converted the phosphoserines to S-ethylcysteines and identified directly the five sites of phosphorylation by amino acid sequence analysis.

C 409 EXPRESSION OF GROWTHFACTOR GENES AND TRANSCRIPTIONAL REGULATION OF THE C-FOS GENE IN MOUSE EMBRYONIC STEM CELLS. Wiebe Kruijer, Siebe van Genesen, Alie Feyen and Christine Mummery. Hubrecht Laboratory, Netherlands Institute for Developmental Biology, 3584 CT Utrecht, The Netherlands.

We have investigated the expression of growth factor genes and examined the transcriptional regulation of the fos gene in mouse embryonic stem cells. Undifferentiated P19 embryonal carcinoma (EC) cells express the TGFβ gene as a unique mRNA, while the genes encoding the A and B chains of PDGF, the insulin-like growth factor types I and II and basic fibroblast growth factor are not expressed. Differentiation of P19 EC cells with retinoic acid, results within 24 hours in high levels of expression of the genes encoding the A and B chains of PDGF and IGF II. Similar expression patterns were found in other EC cell lines, including F9, C1003, PC13 and in embryonic stem (ES) cells. In synchronized P19 EC cells, a short pulsed treatment with retinoic acid, early in G1 of the cell cycle, results in the simultaneous loss of the transformed phenotype of EC cells and commitment to express growth factor genes. The gene regulatory aspects of this interplay will be discussed.

Since EC cells can proliferate autonomously in the absence of serum growth factors, we examined the functionality of signal transduction leading to induction of the c-fos gene. P19 EC cells as well as several other EC cell lines are resistent to induction of the c-fos gene following treatment with PMA. This resistence is not trivial, as P19 EC cells express phorbol ester receptors and induce the c-myc gene in response to PMA. The unresponsiveness of the fos gene in EC cells is transcriptionally regulated and correlates with the presence of a distinct set of nuclear fos enhancer binding proteins, not found in differentiated cells.

C 410 Interleukin-1 induces c-fos protooncogene expression in cultured human endothelial cells,

F.Colotta,M.G.Lampugnani,N.Polentarutti,E.Dejana,A.Mantovani,Istituto "Mario Negri",Milano,Italy. In the present study we have evaluated the expression of c-fos protooncogene in normal human endothelial cells (HEC) by Northern blot analysis. HEC do not show neither constitutive nor cycloheximide-induced expression of c-fos protooncogene. When HEC were treated with cytokines known to modulate a number of specialized functions of these cells,we observed that,unlike interferon-γ,interleukin-1 (IL-1) and tumor necrosis factor (TNF) were able to induce appreciable levels of c-fos in HEC. Both IL-1 α and IL-1 β induced c-fos transcripts in HEC. Maximal levels of c-fos mRNA induced by IL-1 were found after 1 hour of treatment,with undetectable levels at 4 and 7 hours. c-fos induction in HEC by IL-1 and TNF may play a role in the acquisition of functional properties induced in HEC by these cytokines.

C 411 ABERRANT ACTIVATION OF LYMPHOKINE GENES IN MYELOID LEUKEMIA, Kevin B. Leslie, Sabariah Schrader and John W. Schrader. The Biomedical Research Centre, University of British Columbia, Vancouver, BC, Canada and The Walter and Eliza Hall Institute, Melbourne, Victoria, Australia.

We have documented 4 instances where aberrant activation of a T-cell lymphokine gene has occurred in a myeloid leukemia. In vitro experiments with variants of non-leukemogenic, pan-specific hemopoietin or interleukin-3 (IL-3)-dependent cell-lines demonstrated that the onset of leukemic behavior coincided with the activation of the IL-3 gene. The aberrant production of IL-3 was probably involved in the pathogenesis of a second myeloid leukemia, WEHI-3B, in which the IL-3 gene is rearranged. Subclones of a third myeloid leukemia, WEHI-274, could be divided into three distinct classes with respect to lymphokine production. Class I demonstrated autocrine growth behavior mediated by IL-3 with a 5' re-arrangement of the IL-3 gene introducing an abnormal promotor to produce an 8 kb mRNA species instead of the normal 1.3 kb and the gene was rearranged at its 5' end. Class III did not produce any autostimulatory factor and lacked IL-3 or GM-CSF gene transcripts or rearrangements. All clones of WEHI-274, however, were monoclonal with respect to a common c-myb rearrangement suggesting that all clones were derived from a common ancestral cell. Both IL-3 and GM-CSF analogues have been synthesized chemically - the therapeutic potential of antibodies and antagonists should soon be evident.

C 412 EXPRESSION OF GROWTH FACTORS IN HUMAN LEUKEMIC CELLS, M.D. Minden, C. Yee, J. De Souza, A. Biondi. Ontario Cancer Institute, Toronto, Ontario, Canada.

Recently we and others have reported that human acute myeloblastic leukemia cells can express mRNA for a variety of growth factors including granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage (GM-CSF), IL 1, macrophage (M-CSF) and tumor necrosis factor (TNF). In essence the results of the studies have been similar however the frequency of samples expressing the various genes has varied. Such variation does not appear to be due to differences in the age of the patients or the FAB classification of their disease. One possible difference between the groups is the way in which the cells are handled, for example in some studies the cells have been cultured for a short period of time before RNA was extracted. To determine whether this might affect the results we have begun to study the expression of TNF in AML blast cells. RNA was extracted from the T-cell and macrophage depleted leukemic blast cells of patients before and after incubation in conditioned medium obtained from a bladder carcinoma cell line (5637-CM) known to express G-CSF, GM-CSF and IL-1. We found that in some cases that TNF mRNA was never expressed, in other cases TNF was expressed only after incubation with 5637-CM and in other cases TNF was expressed without incubation with 5637-CM.

From these studies we conclude that leukemic blast cells can express mRNA for TNF but that this expression can be modulated. The ability to modulate the expression of growth factor genes in leukemic cells may be of therapeutic use however it is also necessary to recognize the ability of cell handling to modulate the expression of these genes when studying their "constitutive expression" in leukemic cells.

C 413 IDENTIFICATION OF HUMAN GM-CSF REGULATORY SEQUENCES. S.D. Nimer, E. Morita, M.J. Martis, W. Waschman, and J.C. Gasson, Division of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA.

Expression of the human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene is tightly controlled. To determine the molecular events involved in the induction of the GM-CSF gene following T-cell activation, we prepared and analyzed deletion mutant constructs containing 5' flanking sequences of the GM-CSF gene linked to the chloramphenical acetyltransferase gene. Our results demonstrate a complex pattern of regulation, involving both positive and negative regulatory regions. We have localized T-cell-specific promoter activity to a 90 bp region flanking the GM-CSF initiation site (from bp -53 to bp +37), and using crude nuclear extracts and DNase I footprinting techniques, have demonstrated protection of sequences contained within this region. Mutagenesis studies are in progress to further identify the regulatory nucleotides involved.

C 415 TRANSCRIPTION OF THE C-FOS GENE, Ron Prywes, Tobe M. Fisch and Robert G. Roeder. Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, New York 10021

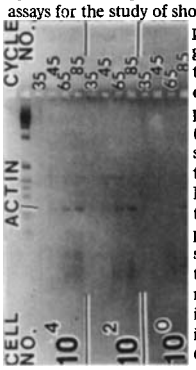
Transcription of the c-fos proto-oncogene is induced rapidly by a number of mitogenic agents including serum, growth factors and cyclic AMP. An enhancer like element at -300 in the promoter is responsible for serum and epidermal growth factor regulation of the gene. Gel mobility shift and footprint assays have identified a factor (SRF) which binds to the c-fos enhancer element.

We have purified SRF from HeLa cell nuclear extracts by DNA affinity chromatography. The purified protein had a molecular weight of approximately 62,000 daltons and contained the binding activity, as demonstrated by the renaturation of the activity from an SDS-polyacrylamide gel slice. When added to an in vitro transcription system with Hela cell nuclear extract the purified factor was capable of specifically stimulating transcription from an enhancer containing plasmid template. This demonstrates that SRF is a positively acting transcription factor.

We have also found a strong effect both in vitro and in vivo of a sequence element around -60 in the c-fos promoter. This element is similar to the binding site for ATF (in several adenovirus early genes) and an element in the somatostatin gene thought to be involved in cAMP regulation of that gene. We have found, however, that deletion of this element in the c-fos gene does not impair the ability of a transfected c-fos plasmid to be transcriptionally induced by cAMP.

C 416 A METHOD FOR STUDYING SINGLE CELL GROWTH FACTOR PHENOTYPES. Daniel A. Rappolee*, +David Mark and *Zena Werb. *Laboratory of Radiobiology and Environmental Health, UCSF, San Francisco, CA 94143, and +Department of Molecular Biology, Cetus Corporation, Emeryville, CA 94608.

A great challenge in biology is the study of obligate cellular cooperation defined in metazoans by the circuitry of growth factor ligands and receptors. We now report a method superior to in situ hybridization, Northern blot analysis or S1 protection assays for the study of short-lived, low copy number growth factor transcripts, which allows characterization of growth factor phenotypes in single cells. We first purify RNA by an adaptation of the CsCl/GuSCN density gradient centrifugation technique. Purified RNA is copied into cDNA during two cycles of reverse transcription (RT) primed by oligo-dT. Products of first strand synthesis are amplified in 60-90 cycles of polymerase chain reaction (PCR) using specific 5′- and 3′-oligonucleotides as primers. Reaction products are fractionated by electrophoresis in agarose and visualized by ethidium bromide staining (Fig. 1). Product authenticity is verified by a) size, b) restriction analysis, c) Southern blot, and d) sequence. Using this technique, we are elucidating pathologic and physiologic growth factor circuits that control macrophage growth factor phenotypes in wound healing and nerve regeneration. Lipopolysaccharide or acetylated low density lipoproteins stimulate macrophage synthesis of TFG-α transcript. A strong signal is seen between 3 and 9 h after stimulation. This induction is seen in a population of fluorescence-activated cell sorter-purified, F4/80 (macrophage-specific marker) positive stimulated macrophages. We also found TFG-β, IL-1-α, PDGF-A chain and G-CSF transcripts in these macrophages, but not NGF or EGF transcripts. NGF transcripts were seen in 99% pure macrophages by Northern blot analysis or by RT-PCR analysis. When stimulated cultured or freshly isolated macrophages were first cell sorted by FACS, no NGF mRNA was detected. Our results indicate that fibroblasts synthesize NGF transcripts under macrophage control. Supported by DOE-OHER contract no. DE-AC03-76-SF01012.



C 417 THE RAT OSTEOGENIC SARCOMA CELL LINE UMR 106 PRODUCES IGF-II, AN IGF BINDING PROTEIN, BUT NOT IGF-I, Raoul P. Rooman, T. Kenney Gray, Pauline K. Lund and Judson J. Van Wyk, University of North Carolina, Chapel Hill NC 27514.

We investigated the production of IGF binding proteins (IGF-BP), IGF-I, and IGF-II in the rat osteogenic sarcoma cell line UMR 106. Serum-free DMEM/F12, supplemented with insulin, transferrin and selenium was conditioned by the cells for 72 hrs. After overnight incubation of medium with ^{125}I-IGF-I, peak radioactivity eluted from a neutral Sephadex G-50 column at an apparent molecular weight of 38k. In a charcoal assay, maximal ^{125}I-IGF binding activity was detected in fractions with an apparent molecular weight of 30k. Treatment of the cells with lnM estradiol resulted in no detectable change in the molecular weight or amount of the secreted IGF-BP. After acid chromatography of media to separate free IGF's from IGF-BP, no small molecular weight IGF-I immunoreactivity was detectable. Likewise, poly(A+) RNA extracted from estrogen treated UMR 106 cells and assayed by Northern blot hybridization contained no detectable IGF-I mRNA. Taken together, these findings suggest that UMR 106 cells do not secrete or synthesize significant quantities of IGF-I. In contrast, UMR 106 cells synthesize 4.7, 3.9, 2.1 and 1.2kb mRNA species that hybridize with a human IGF-II cDNA probe. We conclude that UMR 106 synthesizes IGF-II and an IGF-BP but not IGF-I. The role of IGF-II in these cells is unknown but it might function as an autocrine growth factor as proposed in some cases of Wilm's tumor and colon carcinomas.

C 418 DIFFERENTIAL EXPRESSION OF INSULIN-LIKE GROWTH FACTOR GENES IN THE RAT CENTRAL NERVOUS SYSTEM, Peter Rotwein, *Susan K. Burgess, Jeffrey D. Milbrandt, and James E. Krause, Washington University School of Medicine, St. Louis, Mo. 63110 and *Glaxo Inc., Research Triangle Park, N.C. 27709.

Insulin-like growth factors I and II (IGF-I and II) are polypeptide mitogens which play fundamental roles in mammalian growth and developmental processes. Recent studies suggest that both IGFs may enhance survival and promote differentiation of components of the vertebrate nervous system. To investigate whether IGFs are produced in the central nervous system (CNS) and thus may act as autocrine or paracrine growth modulators, a sensitive solution-hybridization assay was used to assess the expression of each gene in rat brain and spinal cord. Messenger RNAs for both IGFs are synthesized throughout the CNS of adult rats, but exhibit distinct regional differences for each growth factor. IGF-I mRNA is 8-10 times more abundant in the cervical-thoracic spinal cord and in the olfactory bulb than in whole brain, and is enriched 3-fold in the midbrain and cerebellum. IGF-II mRNA is minimally enriched in the medulla-pons and cerebellum, but is 3-5 times less abundant in the midbrain and corpus striatum than in total brain. During CNS development the content of IGF-I and IGF-II mRNA is highest at embryonic day 14 and declines by 3-4 fold at birth to levels found in adult brain. Embryonic neurons and glia synthesize IGF-I mRNA during short-term cell culture; only glia produce IGF-II mRNA. These observations show that IGF-I and IGF-II are differentially expressed in the developing and adult CNS, and suggest that each IGF may play a unique role as a potential autocrine or paracrine growth factor or neuromodulator in the mammalian nervous system.

C 419 TRANSFORMATION OF NIH 3T3 CELLS BY OVEREXPRESSION OF THE HER2 GENE INDUCES CELLULAR RESISTANCE TO MACROPHAGES AND TUMOR NECROSIS FACTOR-α, Robert M. Hudziak, Gail D. Lewis, Thomas E. Eessalu, Bharat B. Aggarwal, Axel Ullrich and H. Michael Shepard, Genentech, Inc., So. San Francisco, CA 94080.

Functional characterization of oncogene products which induce cellular transformation has progressed rapidly in recent years. However, the mechanisms by which the transformed cell may escape destruction by host immune defenses are poorly understood. A key component of early host defense against tumorigenesis is thought to be the activated macrophage, which kills sensitive target cells primarily through the production of the monokine, tumor necrosis factor-α (TNF-α). In this paper we demonstrate that amplified expression of HER2, which encodes a receptor-like protein ($p185^{HER2}$) with homology to the human epidermal growth factor receptor (HER1), and which has recently been shown to be amplified in about 30% of human breast carcinomas, can induce resistance of NIH 3T3 cells to the cytotoxic effects of macrophages or TNF-α. Induction of resistance to TNF-α by amplified expression of $p185^{HER2}$ is accompanied by alterations in the binding of TNF-α to its receptor.

C 420 DIFFERENTIAL EXPRESSION OF TYPE-2 HEPARIN-BINDING GROWTH FACTOR mRNA IN NORMAL AND TRANSFORMED HUMAN CELLS. Gary D. Shipley*, Mark D. Sternfeld*, Robert J. Coffey, Jr.** and Mark R. Pittelkow#. *The Oregon Health Sciences University, Portland, OR 97201; **Vanderbilt University, Nashville, TN 37232; #Mayo Clinic/Fnd., Rochester, MN 55905.

Type-2 heparin-binding growth factor (also known as basic fibroblast growth factor and eye-derived growth factor-1), HBGF-2/bFGF, is a potent polypeptide mitogen for normal human cells, including dermal fibroblasts, proliferative keratinocytes and melanocytes. We have examined poly-A+ RNA isolated from these cells as well as RNA from normal human lung fibroblasts and from mammary epithelial cells for expression of HBGF-2/bFGF transcripts, utilizing a bovine HBGF-2/bFGF cDNA clone (Abraham et al., Science 223:545, 1986). The results of these studies indicate that the fibroblasts, but not the epithelial cells, express mRNA coding for HBGF-2/bFGF. At least four separate transcripts of 7.0, 3.5, 2.2 and 1.5 kb are present in both dermal and lung fibroblasts. The abundance of all four of these transcripts is increased 10-15 fold within 4 hours of treatment of quiescent fibroblasts with fresh fetal bovine serum. Our results suggest that in skin and perhaps in mammary gland and lung, expression of HBGF-2/bFGF mRNA is cell-type specific and confined to stromal cells. The gene may be regulated in fibroblasts during response to injury and contribute to the proliferation of several cell types. HBGF-2/bFGF mRNA is expressed in some, but not all tumor cells. An astrocytoma (HTB-14), a hepatoma (SK-HEP-1) and a squamous cell carcinoma (SCC-25) express all four HBGF-2/bFGF transcripts. However, transcripts were not detected in two colon carcinoma cell lines (WIDR, SW620) or in a melanoma cell line (M14) under similar conditions. Thus, aberrant expression of HBGF-2/bFGF could contribute to the malignant phenotype in some, but not all, of these tumor cells. Supported by USPHS grant CA42409 (GDS) and the Mayo Clinic/Foundation (MRP).

C 421 POSITIVE AUTO-REGULATION OF TRANSFORMING GROWTH FACTOR-BETA 1, Ellen Van Obberghen-Schilling, Nanette S. Roche, Kathleen C. Flanders, Michael B. Sporn, and Anita B. Roberts. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

Transforming Growth Factor-beta 1 (TGF-beta) regulates either the growth, differentiation, or function of nearly all cell types. We now report that TGF-beta increases steady state levels of its own message in many different normal and transformed cells in culture. Accumulation of TGF-beta mRNA can be detected within 3 hrs of addition of the peptide to cells and enhanced message levels persist as long as TGF-beta is present in the culture medium. This auto-induction is half-maximal at 10pM TGF-beta, and maximal stimulation corresponds to a 2-3-fold increase in transcript levels. In normal rat kidney (NRK) cells, the rise in TGF-beta mRNA is accompanied by a parallel (approximately 3-fold) increase in secretion of TGF-beta protein into the medium of treated cells, as detected by immunoprecipitation of biosynthetically-labeled ^{35}S-TGF-beta using specific anti-TGF-beta antibodies. Treatment of NRK cells with either EGF or PDGF also results in an increase in TGF-beta mRNA; although its functional significance it not yet known, this may serve as a negative feed-back regulator of growth of the cells. Alternatively, in inflammation and tissue repair, where TGF-beta functions to activate fibroblasts to secrete extracellular matrix proteins, auto-induction of TGF-beta in its target cells at the site of an injury may be an important mechanism to amplify and sustain the response to the peptide following its initial release by platelets.

C 422 IMMUNOREGULATORY EFFECTS OF DIFFERENT SIGNALS ON REGULATION OF GENES IMPLICATED IN T CELL PROLIFERATION, C.Vaquero,F.Paillard,G.Sterkers,G.Bismuth*,E.Gomard and J.P. Lévy,U152 27 rue du Fbg St Jacques 75014 PARIS,*Lab.d'Immunol.91 Bd de l'Hôpital 75013PARIS
The regulation of genes implicated in T cell proliferation was studied in different human T cell populations:preactivated PBL,CD4$^+$T cell clones and CD8$^+$T cell lines all maintained in IL-2.The different stimuli were,soluble anti-CD3,PMA,anti-CD3+PMA,anti-CD2 or specific antigen.We investigated the modulation of messengers coding for IL-2 receptor (IL-2 R) and immunoregulatory lymphokines (IL-2 and IFNγ) as well as those coding for T cell receptorα and γ chains expressed in the three cell types.Depending on the stimulus and the cell type, transient accumulation of IL-2R mRNA alone or IL-2R as well as lymphokine messengers was triggered.Thus,these stimuli varied in their ability to induce different steps of T cell activation:minimal stimulation allowed expression of IL-2R,additional signal(s) were necessary to induce immunoregulatory lymphokines.Proliferation was observed,when the three messengers were induced,and appeared to be independant of IL1β mRNA expression.Additionnally, proliferation was associated with coordinate down regulation of T cell α and γ chain mRNA. Finally we examine the role of IL-2 on gene regulation during CD2 mediated T cell activation.When anti-CD2 activation took place,72h after the last addition of rIL-2,IL-2R mRNA expression occurred accompanied by low IFN γ and undetectable IL-2 mRNA expression.In contrast when the cells were primed 24h with IL-2 prior activation,high level of IL-2 and IFN mRNA was observed.Thus exogenous IL-2,in addition to IL-2R mRNA, upregulates IL-2 and IFNγ mRNA.Interestingly accumulation of IFNγ mRNA is biphasic, rising the possibility to be upregulated as well, by endogeneously secreted IL-2.

C 423 EXPRESSION OF GROWTH FACTORS AND ONCOGENES IN NORMAL AND TUMOR DERIVED HUMAN MAMMARY EPITHELIAL CELLS, Deborah Zajchowski, Nelly Pauzié, Vimla Band, Andrew Wasserman, and Ruth Sager, Dana-Farber Cancer Institute, Boston, MA 02115
In an effort to understand the etiology of human breast cancer, we are characterizing the growth of normal and tumorigenic human mammary epithelial cells in tissue culture. The establishment, in our laboratory, of a culture medium which supports the growth of normal cell strains (derived from patient reduction mammoplasty specimens) as well as established tumor cell lines has facilitated studies which compare the responses of these cells to different growth promoting and inhibitory agents in the same medium. As an initial step in this investigation, we have assessed the expression of genes, whose products have been implicated in the control of cell proliferation, by quantitating the steady-state levels of their mRNA by northern blot analysis. We have found that the expression of the proto-oncogenes Ha-ras, Ki-ras, c-myc, and c-erbB2 and of transforming growth factor β (TGF-β), is not significantly different in actively growing normal vs. tumor cells. On the contrary, the amounts of mRNA corresponding to the EGF receptor homolog, erbB and to transforming growth factor α (TGFα), as well as to a growth regulatory gene called gro (Anisowicz et al., PNAS, 1987) are reduced in the transformed cells. The significance of these results will be considered in view of published evidence of active EGF binding molecules and TGFα activity in these cells.

C 424 GM-CSF GENE REGULATION IN MURINE T CELLS. Matthias Bickel, Stephan E. Mergenhagen, Sharon M. Wahl and Dov H. Pluznik, NIH, NIDR, LMI, Bethesda, MD 20892.
The coordinated expression of granulocyte-macrophage-CSF (GM-CSF) and interleukin 3 (IL3) by activated T lymphocytes suggests common regulatory mechanisms of synthesis. However, cyclosporin A (CsA), an immunosuppressive drug, inhibits the production of interleukin 2 (IL2) and IL3 but not GM-CSF. We used this drug to study the regulatory mechanisms of CSF production by activated murine T lymphocytes. Two T cell lines, the thymoma EL-4 and the T cell hybrid 2B4, were treated with CsA and mitogen. When the culture supernatants were assayed on IL3 (DA-1) and GM-CSF or IL3 (PT-18) dependent cell lines, IL3 activity could not be detected following CsA treatment, but substantial growth activity was observed on the PT-18 cell line. Antibodies to recombinant GM-CSF were shown to neutralize the biological activity retained after CsA treatment. To dissociate between differential synthesis and/or secretion of these two CSFs by CsA, GM-CSF gene expression was evaluated. Northern analysis of RNA from 2B4 cells demonstrated GM-CSF mRNA in stimulated cells regardless of CsA treatment. Nuclear transcription analysis showed transcriptional activity even in the presence of CsA as well as in unstimulated cells. These data suggest that differential control mechanisms exist for the regulation of IL3 and GM-CSF synthesis in spite of recently reported common regulatory sequences in the 5' and 3' end of both genes. Furthermore the regulation of the GM-CSF gene expression clearly has a post-transcriptional component, which might be the key to understanding the lack of the inhibitory effect of CsA on GM-CSF production.

Growth Factors-Growth Inhibitors-Cellular Interactions

C 500 GROWTH FACTOR PRODUCTION BY HUMAN COLON ADENOCARCINOMA CELL LINES, M.A. Anzano, D. Rieman, W. Prichett, D. Bowen-Pope and R. Greig, Dept. of Cell Biology, Smith Kline & French Laboratories, 709 Swedeland Road, King of Prussia, PA 19406 and Dept. of Pathology, University of Washington, Seattle, WA 98105.

Conditioned media from 18 human colon adenocarcinoma cell lines were analyzed for transforming growth factor types alpha and beta (TGF-α and ß) and platelet-derived growth factor (PDGF) using anchorage independent growth and radioreceptor assays. TGF-α (> 15 pM), TGF-ß (> 20 pM) and PDGF (> 15 pM) was observed in 13, 13 and 5 cell lines, respectively. Three liters of conditioned medium from highly tumorigenic (HT-29, DLD-1 and SW-620) and non-tumorigenic (SKCO1) colon cell lines and from non-neoplastic rat kidney (NRK-52E) and intestinal (IEC-6) epithelial cells were HPLC-purified and assayed for TGF-α-like activity. The highly tumorigenic colon cell lines produced 3 to 18-fold (soft agar assay) and 9 to 12-fold (radioreceptor assay) more TGF-α-like material compared to the non-tumorigenic colon cells and non-neoplastic rat epithelial cells. NRK-52E did not produce detectable TGF-α activity. RIA analysis of peak fractions (as identified in the EGF-radioreceptor binding assay) revealed only TGF-α immunoreactivity; EGF was not detected. These results suggest that TGF-α plays an important role in controlling the proliferation of human colon adenocarcinoma cells and that TGF-α antagonists may serve as useful tools to examine the pharmacology of tumor cell growth.

C 501 CHARACTERIZATION OF GROWTH FACTORS PRESENT IN HUMAN BONE MATRIX, David J. Baylink, Subburaman Mohan, Thomas A. Linkhart, & John C. Jennings. Depts of Med, Biochem, Physiol, & Peds, Loma Linda Univ & Pettis VA Hosp, Loma Linda, CA 92357.
Recent studies suggest that bone derived growth factors are important in the local regulation of bone volume. We have recently purified and characterized human skeletal growth factor (SGF), a low molecular weight polypeptide growth factor from human bone matrix. The rationale for the present study was the finding of mitogen activity in human bone extracts not ascribable to SGF. Matrix proteins were extracted from human bone, either by demineralization with 10% EDTA (EDTA extract), or with 10% EDTA + 4M Guan. HCl inhibitors (G-EDTA extract). EDTA and G-EDTA extracts were desalted and assayed as follows: SGF was determined by an RRA using ^{125}I-SGF binding to embryonic chick calvarial cells in monolayer culture and purified SGF as standard. IGF-I was determined by an RIA using rabbit polyclonal antiserum from NIH and human synthetic IGF-I. EGF was determined by an RRA using ^{125}I-EGF binding to NRK cells. TGF-β was determined by EGF dependent NRK colony formation in agar culture using purified human TGF-β (from Anita Roberts, NIH) as standard. PDGF was estimated by determining the mitogenic activity of the G-EDTA extract with and without PDGF inhibitory antibodies. FGF was assayed by RIA. The results are given below:

Growth Factors are:	SGF	b-TGF	IGF-I	PDGF	FGF	EGF
ng/µg G-EDTA protein	1.05±0.15	0.38±0.12	0.07±0.009	0.055±0.01	detectable	undetectable
µg/g bone powder	1.26	0.46	0.085	0.067		

Our results indicate that human bone matrix contains (a) in addition to SGF, TGF-β, IGF-I, PDGF and FGF but not EGF, and (b) much more SGF and TGF-β than IGF-I and PDGF. Based on these findings and our previous work showing that these growth factors stimulate bone cell proliferation, we speculate that local bone formation is regulated through the coordinated actions of several growth factors.

C 502 THE GLIOBLASTOMA CELL DERIVED T CELL SUPPRESSOR FACTOR (G-TsF) IS A MEMBER OF THE TRANSFORMING GROWTH FACTOR β FAMILY. S. Bodmer, C. Siepl, K. Frei, R. deMartin, B. Haendler, E. Hofer and A. Fontana. Section of Clinical Immunology, University Hospital, Haeldeliweg 4, CH-8044 Zurich, and Preclinical Research, Sandoz AG, CH-4002, Basel, Switzerland. We previously reported the release of a T cell suppressor factor (G-TsF) by human glioblastoma cells in vitro (J. Immun. 132, 1837-1844, 1984 and 134, 1003-1009, 1985). We now have purified this protein to homogeneity by chromatography on hydroxylapatite and Pro-RPC, cation exchange chromatography on Mono-S and a final reversed phase FPLC on Pro-RPC. The factor was identified as a protein with a molecular weight of 12.5 kd. Amino-terminal sequence analysis by gas phase sequencing demonstrated a 55% homology to human transforming growth factor β within the first 20 amino acids (EMBO J. 6, 1633-1636, 1987). As observed with the semipurified G-TsF in 1984, the purified factor as well as transforming growth factor β was found to inhibit the growth promoting effect of interleukin-2 (Il-2) when testing on an Il-2 dependent ovalbumin specific mouse T-helper cell line, as well as the concanavalin A induced thymocyte proliferation. We also report the cloning of the complementary DNA for human G-TsF, which reveals that G-TsF shares 71% amino acid homology to transforming growth factor β . In analogy to transforming growth factor β, G-TsF apparently is synthesized as the carboxy-terminal end of a precursor polypeptide which undergoes proteolytic cleavage to yield the 112 amino-acid-long mature form of G-TsF. If released by glioblastoma cells in vivo, the factor may contribute to impaired immunosurveillance and to the cellular immunodeficiency state observed in patients. Supported by the Swiss National Foundation (3.930-0.87)

C 503 GROWTH FACTOR EXPRESSION IN NORMAL, BENIGN AND MALIGNANT BREAST TISSUE, R.Charles Coombes (1,2), Trevor J.Powles (2), Maureen T.Travers (1), Yunus A.Luqmani (1), Peter J.Barrett-Lee (1), and Uta Berger (1). (1) Ludwig Institute for Cancer Research, St George's Hospital Medical School, Cranmer Terrace, London, SW17 ORE (2) Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, U.K.

The levels of messenger RNA (mRNA) for several growth factors, known to be synthesised by breast cancer cells in vitro, were examined in 51 malignant and 19 non-malignant human breast biopsies. Transforming growth factor-β (TGF-β) mRNA was more abundant in breast cancers compared to non-malignant breast tissue ($p<0.05$). Transcripts for both transforming growth factor-α (TGF-α) and its receptor, epidermal growth factor receptor (EGFR) were found more often in oestrogen receptor (ER) negative than ER positive carcinomas ($p<0.05$ and $p<0.001$ respectively). Insulin-like growth factor-II (IGF-II) mRNA was present in all non-malignant tissue but was found at much lower levels in only 52% of carcinomas ($p<0.001$). EGFR was also found in all non-malignant breast tissues, compared to 42% of carcinomas. Platelet-derived growth factor (PDGF) A and B chain transcripts co-existed in all normal and benign tissue and the majority of carcinomas. This differing pattern of growth factor expression in malignant compared to benign and normal breast tissue suggests that some growth factors, particularly TGF-α and TGF-β may have an important role in the control of growth of human breast cancers, particularly those that are hormone independent.

C 504 INTERLEUKIN-1 INHIBITS AND GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR STIMULATES PROLIFERATION OF HUMAN OSTEOGENIC SARCOMA CELLS, Shoukat Dedhar and Paula Galloway, Cancer Control Agency of B.C., 600 West 10th Avenue, Vancouver, B.C., V5Z 4E6.

Utilizing an assay containing serum-free defined medium we have found that cell proliferation of MG-63 human osteosarcoma cells was stimulated by recombinant granulocyte-macrophage colony stimulating factor (GM-CSF)and platelet derived growth factor (PDGF) but was inhibited by recombinant interleukin 1β (IL-1β) in a dose dependent manner. In contrast, the cell proliferation of differentiated osteoblast-like variants (MG-63.3A) (Dedhar et al, J. Cell. Biol., 1987, in press) was not altered by GM-CSF or IL-1β, but was stimulated by PDGF and estradiol. In addition, the MG-63 osteosarcoma cells secrete a PDGF-like factor which is not produced by the more differentiated MG-63.3A osteoblasts. The conditioned medium of these latter cells however inhibits the cell proliferation of the MG-63 osteosarcoma cells. The inhibition of cell proliferation of MG-63 osteosarcoma cells by IL-1β was associated with morphological changes and increased expression of the cell surface receptor for fibronectin. Furthermore, MG-63 cells pre-exposed to IL-1β and TGF-β, for 5 hours became insensitive to growth stimulation by GM-CSF and PDGF. The negative effect of IL-1β on PDGF growth stimulation was associated with the down-regulation of PDGF receptors. These data indicate that normal and neoplastic cells of the osteoblast cell lineage synthesize and respond to different growth factors, which include hematopoietic growth factors and steroid hormones.

C 505 MULTIPLE TRANSCRIPTS FROM THE POTENTIAL ONCOGENE int-2 WHICH ENCODES A PROTEIN WITH HOMOLOGY TO FIBROBLAST GROWTH FACTOR, Clive Dickson, Rosalind Smith, David Wilkinson, Andrew McMahon and Gordon Peters, Imperial Cancer Research Fund Laboratories, London, England.

Int-2 is a common proviral integration locus in tumors induced by the mouse mammary tumor virus. Proviral insertions are found adjacent to the int-2 gene and correlate with its transcriptional activation. Int-2 RNA has not been detected in normal adult mouse tissues, but expression has been localised to the mesoderm and parietal endoderm of mouse embryos at 7.5 days of gestation. Transcription of int-2 can be induced in embryonal carcinoma cells following treatment with retinoic acid and dibutyryl cAMP. The pattern of transcription is complex but reflects the situation seen in mouse mammary tumors. Predominantly four RNA species of 2.9, 2.7, 1.8 and 1.6kb are observed. Ribonuclease protection experiments indicate that the RNAs are initiated at multiple sites spanning a 200bp region from the domains of two distinct promoters. Two alternative polyadenylation sites contribute to, and can explain, the remaining complexity of transcription. However, despite these variations in structure, all the species of RNA encode the same protein product with a calculated molecular mass of 27,000 daltons. The predicted primary structure of int-2 shows significant homology to the family of growth factors/oncogenes typified by basic fibroblast growth factor.

C 506 UNTRANSFORMED CELLS INHIBIT THE FOCUS FORMATION OF RADIATION AND ONCOGENE TRANSFORMED C3H/10T 1/2 CELLS. V. Drozdoff, L. Zeitz and E. Fleissner, Sloan-Kettering Institute, New York, NY 10021.

We have previously shown that the constitutive expression of an exogenous c-myc gene introduced into C3H/10T 1/2 cells both increases the sensitivity of the cells to growth stimulation by peptide growth factors and increases the susceptibility of the cells to malignant transformation by ionizing radiation. Radiation directly induces transformation in C3H-myc cells, in contrast to the parent 10T 1/2 cells in which an additional event is required during proliferation of irradiated cultures. The growth of transformed clones arising in irradiated dishes of C3H-myc cells appeared, however, to be inhibited by the presence of untransformed cells. Reconstruction experiments utilizing mixed cultures of untransformed 10T 1/2 cells and radiation-transformed C3H-myc cells were performed to analyze the nature of this inhibition. The G418 resistance of the C3H-myc cells allowed the growth of transformed C3H-myc clones in these cultures to be determined by drug selection upon replating. These studies indicated that the growth rate of transformed cells during the proliferation of the mixed cultures was unaffected but that the normal cells strongly suppressed the ability of the transformed cells to form foci in the dishes. The extent of this suppression was sharply reduced by prior irradiation of the normal cells seeded into the dish. These results indicate that growth inhibition of transformed cells may be induced by intercellular contact with normal cells, possibly by factors produced in those cells, and that the modulation of the transformed phenotype may critically affect the interpretation of in vitro transformation assays.

C 507 POSITIVE AND NEGATIVE REGULATORS OF PRIMITIVE HUMAN HEMOPOIETIC PROGENITORS, Allen C. Eaves, Johanne D. Cashman, Dagmar K. Kalousek and Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C., Canada, V5Z 1L3.

Neoplastic progenitors obtained from most patients with CML and AML decline over time in long term culture (LTC), an approach being used to purge leukemic cells for autologous marrow transplantation. Since these cells are actively cycling when taken directly from patients, whereas their primitive normal counterparts are in G_0, we have begun studies of the role of the LTC adherent layer in regulating progenitor cycling to determine whether alterations in cell-cell interactions explain these various differences in neoplastic cell behaviour. LTC are fed by regular or mock half media changes every 7 days and cycling of progenitors examined 2 and 7 days after feeding. Following a regular medium change only primitive normal progenitors return to a quiescent state by day 7, and then only if they are located in an adherent layer containing mesenchymal cells of marrow origin. In contrast, the proliferative activity of neoplastic progenitors is not negatively regulated by adherent marrow cells, even when these are derived from normal donors. To explore the mechanism underlying this control of normal progenitor proliferation, we have tested a variety of mesenchymal cell activators for their ability to influence hemopoietic progenitor cycling in the LTC system. Many such molecules, including IL-1, which rapidly elicits growth factor release from LTC adherent layer cells, also stimulate primitive progenitor cycling. However, this effect cannot be obtained by simple addition of crude or pure GM-CSF preparations to LTC. Another factor, TGF-β, acts as a negative, but non-toxic, regulator. These findings suggest complex mechanisms of normal stem cell regulation and hence a variety of potential genetic targets for neoplastic change.

C 508 SYNTHETIC PEPTIDES INHIBIT CELLULAR RESPONSE TO EPIDERMAL GROWTH FACTOR.

Deborah A. Eppstein[1], Y. Vivienne Marsh[1], Brian B. Schryver[1], Sherry R. Newman[1], John J. Nestor Jr.[1], Giulia Taraboletti[2], and Lance A. Liotta[2]. [1]Institute of Bio-organic Chemistry, Syntex Research, Palo Alto, CA 94304, and [2]NCI, Laboratory of Pathology, Bethesda, MD 20205.

We have demonstrated that neutralizing antibodies to the epidermal growth factor (EGF) receptor, or a small synthetic decapeptide analog based on the third disulfide loop of transforming growth factor-α (TGF-α), inhibit estrogen or EGF/TGF-α stimulated growth of human mammary adenocarcinoma MCF-7 cells. The growth inhibitory effect of this TGF-α peptide was conformation specific, as reduction of the disulfide bond completely abolished activity. In probing the mechanism whereby the TGF-α peptide inhibited the EGF/TGF-α stimulated cell growth, we found no competition by this peptide for binding of iodinated EGF or TGF-α to cell surface receptors. However, the radio-iodinated TGF-α peptide bound to a cell surface component that in turn was not competed by EGF. The internalization of this peptide and its effect on EGF post-receptor binding events will be discussed.

Homology of this peptide with a 10 amino acid sequence in the laminin B1 chain led us to investigate the possible role of laminin/laminin receptor in EGF mediated cell growth. Analogous laminin synthetic decapeptide analogs likewise inhibited EGF-stimulated growth. Furthermore, antibodies to the laminin receptor inhibited EGF-stimulated vs. basal cell growth. These results suggest a specific cell receptor-extracellular matrix interaction that influences the cellular response to EGF.

C 509 STEROIDAL REGULATION OF EGF RECEPTOR LEVELS IN HUMAN BREAST CANCER CELL LINES

Tania M. Ewing, Masafumi Koga and Robert L. Sutherland, Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, N.S.W. 2010, Australia.

Human breast cancer cells in culture secrete a number of autocrine growth factors. Steroid hormones are known to modulate breast cancer cell proliferation and to regulate the production of these factors. Regulation of autocrine pathways can occur at the receptor level. In order to understand the effects of steroid hormones on autocrine pathways we have studied the effects of some steroids on EGF receptor levels in breast cancer cell lines.
In cell lines expressing high levels of progesterone receptor (PR) e.g. T 47D, progestins increase the concentration of EGF receptor greater than 2-fold, as measured by Scatchard analysis. Specificity studies with a series of progestins indicated this was a PR-mediated response. Interestingly, in cell lines lacking PR, similar increases in EGF receptor levels were observed following incubation with glucocorticoids. The sensitivity and magnitude of this response in five cell lines was highly correlated with the level of glucocorticoid receptor (GR). Furthermore, in these GR+ but PR- cell lines the increase in EGF receptor was due to ligands acting via the GR while specific progestins like ORG 2058 were ineffective. In all cases the increase in EGF receptor binding was due to an increase in the number of high affinity binding sites without a change in affinity. These differences were apparent when EGF binding was assayed at both 4 and 37C indicating that the effect was not due to steroid-induced changes in receptor internalization and/or degradation.
This up-regulation of EGF-R could be due to direct effects of the steroid on EGF receptor synthesis and degradation, and/or indirect effects on the production of autocrine growth factors like α- and β-TGF which are known to have effects on EGF receptor synthesis. It appears that the gene(s) controlling EGF receptor levels in these breast cancer cells contain the hormone response element that binds both PR and GR since the major factor determining the steroid specificity of the increase in EGF receptor levels appears to be the steroid receptor environment.

C 510 ^{125}I EGF BINDING ON HUMAN BREAST ADENOCARCINOMA CELL LINES. EFFECT OF 1.25(OH)2VIT.D3, N. Falette, L. Frappart, M.F. Lefebvre, D. Poujol and S. Saez,

Centre Leon Berard, 69373 Lyon cedex 08, FRANCE.

Epidermal Growth Factor (EGF) binding sites have been investigated in 2 human breast cancer cell lines : MCF.7 and BT.20 which are sex steroid responsive and non responsive respectively. Both contain specific receptors for 1.25(OH)2Vit.D3 and are growth inhibited by this steroid at low concentration (10^{-10}M to 10^{-8}M).
MCF.7 cells contain EGF receptors. Scatchard analysis of the binding data obtained on whole cells reveals one single class of sites (1.5 to 2×10^4/cell) binding ^{125}I EGF with a high affinity (2.2×10^{-10}M ± 0.77). Treatment of the cells in culture with 10^{-9}M 1.25(OH)2Vit.D3 for up to 8 days does not modify either the number of sites or the Kd.
BT.20 cells contain a very high number of EGF binding sites : 5.5 to 8×10^5 sites/cell. Scatchard analysis of the data obtained on whole cells shows two classes of sites binding ^{125}I EGF with a low (Kd = 1.5 nM) and a high affinity (Kd = 0.3 nM). The high affinity receptors represent about 1/3 of the total number.
Treatment of BT.20 cells with 1.25(OH)2Vit.D3 at 10^{-8}M produced a 2 to 3 fold increase of both classes of ^{125}I EGF binding sites. This increase is progressive after 24 up to 72 hr of treatment.When the experiments are performed on membrane subcellular fractions, ^{125}I EGF binds to one class of sites (Kd = 4×10^{-9}M) and the 1.25(OH)2Vit.D3 induced increase of sites is similar to that observed on whole cells.
These data suggest that the EGF receptors are modulated by 1.25(OH)2Vit.D3 in BT.20 cells but not in the sex steroid responsive MCF.7 line.

Growth Factors and Their Receptors: Genetic Control and Rational Application

C 511 INHIBITION OF A431 CELL GROWTH BY β TGF AND EGF. A. Goldenberg, J. Castagnola, M. Cronin, C. MacLeod, and J. Mendelsohn. Memorial Sloan-Kettering Cancer Center, NY, NY and University of California, San Diego Cancer Center, San Diego, CA.
β TGF is known to inhibit the proliferation of a number of epithelial cell lines. We have examined the effect of β TGF on the proliferation of the A431 squamous cell line, which has 1-2 x 10^6 EGF receptors (EGFR) per cell, and is strongly inhibited by nanomolar concentrations of EGF. After four days of exposure to 20 pM β TGF, A431 cell growth was inhibited by 25-45%. Exposure of cells to 20 pM β TGF in the presence of 0.5 or 5.0 nM EGF produced greater inhibition of proliferation than exposure to either growth factor alone, measured by cell count and thymidine incorporation (TI). Growth was arrested in G0/G1 phase. We also studied the effect of β TGF and EGF on TI in three A431 cloned variant cell lines designated A1S, A1N, A5I, which have growth that is stimulated, not affected, and inhibited by EGF, respectively. Simultaneous exposure of the A1N or A5I variants to 100 pM β TGF and 10 nM EGF inhibited TI to a greater extent than exposure to either factor alone. Although β TGF inhibited the A1S cells by no more than 18%, it completely reversed the stimulatory effect of EGF on this variant. Scatchard analysis showed that A431 cells grown in the presence of 20 pM β TGF for 2, 4 or 7 days expressed the same number of EGFR per cell as control cells, with no change in the K_D for EGF. β TGF did not alter the capacity of EGF to activate autophosphorylation of the EGFR on tyrosine residues. These results suggest that exposure to β TGF produced an inhibitory effect on A431 proliferation which was independent of the effect of EGF, could reverse EGF-induced stimulation, and could augment EGF-induced inhibition of growth.

C 512 PRODUCTION OF TRANSFORMING GROWTH FACTORS ALPHA AND BETA BY ORAL SQUAMOUS CELL CARCINOMAS AND HUMAN KERATINOCYTES. Martin R. Green[1] and Maxine Partridge[2]. [1]Unilever Research, Colworth Laboratory, Sharnbrook, Bedford, U.K. MK44 1LQ, [2]Charing Cross Sunley Research Centre, London W6 8LW. We have examined oral squamous cell carcinomas (SCC), human skin and cultured keratinocytes, for the expression of EGF, TGF-alpha and TGF-beta. Using northern blot analysis the 5.0Kb preproEGF was absent from all samples though a variety of other bands were seen, the significance of which is unclear. However 4.5Kb TGF-alpha transcripts were found in varying levels in all 10 tumours examined and, using RIA we also detected TGF-alpha protein in 9 out of 10 tumour extracts (0.55 to 9.0ng/g wet weight). TGF-alpha transcripts were also detected in keratinocytes but not in skin samples while TGF-alpha protein was detected high passage (P9) (but not low passage) cultured keratinocytes (0.32 ng/10^7cells), conditioned media from high (P9) and low passage (P3) keratinocytes and skin (0.35 to 1.5 ng/gm wet weight). As we have been made unable so far to detect transcripts for TGF-alpha in skin the source of this molecule in this tissue remains equivocal. The secretion of TGF-alpha by keratinocytes (P3) was greatly enhanced by the addition of 10 ng/ml EGF to the culture medium (1.0ng/10^7 cells/24h to 3.8 ng/10^7 cells/24h). In preliminary experiments we have also detected a high level of TGF-beta transcripts in some SCC, fetal human fibroblasts and a lower level of transcripts in keratinocytes, the latter again enhanced by 10 ng/ml EGF in the culture medium. Autocrine and/or paracrine TGF-alpha production may play a role in SCC development. Manufacture by keratinocytes of TGFs-alpha and -beta in response to external stimuli such as EGF, could have a significant role in regulating keratinocyte proliferation and maturation.

C 513 SYNERGISTIC INTERACTION OF ERYTHROPOIETIN, GM-CSF, AND IL-3 WITH AN ERYTHROPOIETIN-RESPONSIVE CELL LINE, L.J. Guilbert, L. Francescutti, D.R. Branch, University of Alberta, Edmonton, Alberta, Canada T6G 2H7
We have shown that erythropoietin (Ep) stimulates proliferation, but not differentiation, of the IL-3-, GM-CSF-responsive cell line DA-1 (Blood 69:1782, 1987). Growth stimulated by Ep or GM-CSF is suboptimal and transient (peaks at 48h of culture), whereas growth stimulated by IL-3 is sustained and strong. To determine whether subpopulations were being stimulated, DA-1 cells were cloned in the factors alone and in combinations. Clones were found only in cultures containing IL-3 or the combination of GM-CSF and Ep. Ep and GM-CSF stimulated transient and suboptimal growth in all clones. Using the clone, E5, isolated from Ep/GM-CSF cultures that most strongly responded to Ep, GM-CSF, but not IL-3, synergized with Ep to sustain growth beyond 48h of culture. Synergy was absolute at 96h of culture; cells died with Ep or GM-CSF alone but grew at log phase in the combination. IL-3 added at suboptimal levels (stimulating growth at 24h equal to maximal Ep or GM-CSF) synergized with GM-CSF but not with Ep. The concentration dependency of factors acting in synergy at 96h was the same as alone at 24h. Thus the three hematopoietic growth factors fall cleanly into two complementation groups (Ep/IL-3 and GM-CSF) according to ability to synergize, members of these groups do not synergize by altering their apparent interaction affinities, and the difference between sustained and transient growth is not a function of the initial level of stimulation, but presumably of the number of occupied receptors. These results suggest that Ep and its receptor, and GM-CSF and its receptor, send qualitatively different mitogenic signals to DA-1 cells.

C 514 RESPONSES OF CULTURED MELANOCYTES TO DEFINED GROWTH FACTORS, Ruth Halaban, Yale University, New Haven, CT 06510.

Normal melanocytes, unlike normal fibroblasts and other cell types, require specific growth factors in order to proliferate in vitro, in addition to those present in serum. The substances that promote melanocyte proliferation also promote their pigmentation, therefore, cell division and the expression of at least some differentiated functions are not mutually exclusive for melanocytes. As of now, the only known natural growth factor for normal human melanocytes is basic fibroblast growth factor (bFGF). However, bFGF is an effective mitogen only in the presence of substances that increase intracellular levels of cAMP, such as cholera toxin or isobutylmethyl xanthine. There is evidence that a second mitogen for melanocytes, yet unidentified, whose function is probably to increase intracellular levels of cAMP, is present in bovine pituitary extract. bFGF is produced by keratinocytes, suggesting a paracrine mechanism by which melanocytes are stimulated in the skin. In addition, cells from metastatic melanomas that grow in culture without specific additives produce bFGF, and inhibiting bFGF antibodies injected into melanoma cells inhibit their growth. Thus, deregulation of bFGF expression in melanomas may contribute to their malignant state. Abnormal melanocytes, such as those derived from dysplastic nevi and primary melanomas, are dependent on lower concentrations of both factors or on only one of them for proliferation, indicating that the initial event of malignant transformation is not the acquisition of full independence from factors for growth in vitro.

C 515 NEONATAL RAT HEART NONMUSCLE CELLS PRODUCE FACTOR(S) CAUSING MUSCLE CELL HYPERTROPHY IN CULTURE, Curtis J. Henrich and Paul C. Simpson, University of California, and VAMC (111C), San Francisco, CA 94121.

Heart muscle cell (MC) hypertrophic response to a variety of factors has been studied in cultures of neonatal rat heart MCs which contain 5-15% nonmuscle cell (NMC) contamination. It is therefore of interest to investigate the effects of NMCs on cultured MCs. Co-culture of neonatal rat heart MCs with increasing numbers of heart NMCs reveals two effects of NMCs: 1) increased MC size (after 2 days average cell area up to 30% greater than control cultures with no added NMC) and 2) at higher NMC: MC ratios (> 0.5:1), MCs become organized into beating masses of cells. To elucidate the cause(s) of these effects, MCs were observed in the presence of NMC-derived extracellular matrix (ECM) or conditioned medium from NMC maintained in serum-free culture. MC hypertrophy was determined by measuring surface area and/or incorporation of radioactive amino acids into protein, both of which have been shown to reflect cell volume. Culture of MCs on ECM from NMC caused slight increase in size (approximately 10% over control), whereas addition of NMC conditioned medium (50%) caused substantially greater hypertrophy, a 30-90% increase in MC size in 1-3 days, without causing beating. Passage of conditioned medium over heparin-Sepharose removed this growth-promoting activity, which subsequently eluted between 0.5-1.0M NaCl. Thus, NMC produce a heparin-binding growth factor(s) which stimulates MC hypertrophy. NMC may influence heart MC behavior in the following ways: 1) direct cell-cell interaction, 2) production of ECM component(s), 3) production of soluble heparin-binding growth substance(s).

C 516 REGULATION OF ANTI-TUMOR CYTOLYTIC T-LYMPHOCYTE GENERATION BY INTERLEUKIN 4, Branka Horvat, Elizabeth Skinner and Patrick M. Flood, Yale University School of Medicine, Howard Hughes Medical Institute, New Haven, CT 06510.

The majority of epithelial cell tumors induced by ultraviolet light are strongly immunogenic when transplanted into normal immunocompetent syngeneic mice. These tumors can induce strong tumor specific cytolytic T lymphocytes (CTL) responses both *in vivo* and *in vitro*. We have used one such tumor 1591-RE, to investigate the role of recombinant interleukin 4 (IL-4) on the generation of anti-tumor CTLs. IL-4, known as a B cell stimulatory factor, has been shown to express multiple biological functions, including B cell, mast cell and T cell stimulation. Our results have shown that both recombinant and native purified IL-4 can enhance the production of 1591 specific CTLs in the primary mixed lymphocyte - tumor cell cultures (MLTC). The enhancing effect mediated by IL-4 in the generation of the anti-tumor CTLs is very similar to the stimulatory activity of the recombinant interleukin 2 (IL-2). Conversely, the monoclonal antibody to the IL-4, 11B11, significantly effects the level of anti-1591 CTL activity. This effect appeared to be different than the effect seen when anti-IL-2 monoclonal antibody S4B6 was used to block CTL generation. In addition, differential effects of IL-4 and anti IL-4 antibody on CTL activity were seen in the presence or absence of $CD4^+$ cells. These data suggests that IL-4 can enhance the generation of anti-tumor cytolytic T lymphocytes and may play an important physiologic role, either directly or indirectly, in their activation.

C 517 MURINE SPLEEN CELLS PRODUCE A MAST CELL GROWTH ENHANCING ACTIVITY (MEA) DIFFERENT FROM BOTH IL-3 AND IL-4/BSF-1, L.Hültner, J.Moeller and P.Dörmer, GSF-Institut für Experimentelle Hämatologie, D-8000 München 2, FRG

Permanent interleukin 3 (IL-3)-dependent "mucosal type" mast cell lines have been established from mouse bone marrow using pokeweed mitogen (PWM)-stimulated spleen cell conditioned medium (SCM) as a source of IL-3. In the presence of SCM proliferation of these cloned cell lines significantly exceeded not only the level obtained with pure IL-3 but also the higher plateau observed with a combination of IL-3 and IL-4 suggesting the presence of an additional mast cell growth enhancing activity (MEA) in SCM. 32 D cl. 23 cells (IL-3 responsive but unresponsive to MEA and IL-4) and monoclonal anti-IL-4 antibodies (derived from 11B11 hybridoma cells) specifically neutralizing the effects of IL-4 were the tools utilized to screen for MEA-activity using cultures of MEA-responsive mast cells during biochemical separation of serum-free SCM. PWM, GM-CSF, G-CSF, CSF-1, hu IL-1α, IL-2, hu TGF-β, hu TNF-α could not substitute for MEA in a standard proliferation assay. In our purification procedure (chromatography by a strongly acidic cation exchanger and Procion Red ligands coupled to agarose followed by HPLC gel filtration) MEA (apparent MW of 26 kD) peaks separately from both IL-3 and IL-4. In medium conditioned by TPA-induced EL-4 cells we readily detected IL-4 but not MEA activity.

C 518 ALTERATION IN GROWTH REGULATION IN CELLS OVEREXPRESSING A MITOGEN-RESPONSIVE GENE. Johnson, Mark D., Housey, Gerard M., Kirschmeier, Paul T., Hsiao, W.L. Wendy, and Weinstein, I.B. Cancer Center, Columbia Univ., N.Y.C. 10032.

We have recently isolated a cDNA sequence corresponding to a gene (designated TPA-S1 or phorbin) whose expression is induced in C3H 10T1/2 cells treated with 12-O-tetradecanoylphorbol-13-acetate (TPA)(Johnson et al., Mol. Cell. Biol. 7:2821, 1987.). We have described the induction of phorbin by TPA, PDGF, EGF and serum, and the role of protein kinase C activation in the regulation of phorbin expression. To determine the effect of over-expression of phorbin on cellular phenotype, a full-length phorbin cDNA was cloned into a retroviral expression vector (pMV7) that contains the neo gene. This construct was then transfected onto psi-2 cells and viral supernatants were used to infect NIH/3T3 cells. Northern blot analysis of neo^+ clones demonstrated a significant elevation in the levels of phorbin RNA, especially in clones 7 and 8. Clones 7 and 8 displayed an altered pattern of growth when compared to neo^+ control cells infected with pMV7 virus lacking the phorbin cDNA sequence. These clones did not grow to a uniform confluent monolayer, but instead formed large clumps of cells that were highly resistant to trypsinization. Clone 7 and 8 cells also displayed anchorage-independent growth since they formed large colonies (0.5 mm) in 0.3% soft agar with an efficiency of 80-90%. Addition of conditioned medium from log phase cultures of these cells to normal fibroblast cultures demonstrated a significant but transient alteration in cell morphology. The morphologic changes were similar to those of the phorbin overexpressing clones. These results indicate that overexpression of phorbin may play an important role in cell transformation.

C 519 **THE EFFECT OF TRANSFORMING GROWTH FACTOR β1 ON EARLY HEMATOPOIETIC PROGENITOR CELL GROWTH. J. R. Keller [1], G.K. Sing [2],L. Ellingsworth[3], I. K. McNiece [4], P.J. Quesenberry [5] and F. W. Ruscetti [6]. [1] P. R. I. Inc., [2,6] L.M.I., FCRF Frederick, MD. [3] Collagen Corp., Palo Alto, CA., [4,5]U.of Va., Charlottesville, VA.Studies in our laboratory using transforming growth factor β1 (TGF-β1) have demonstrated that TGF-β1 is a potent inhibitor of interleukin-3 (IL-3)-induced murine bone marrow growth and differentiation. Specifically, TGF-β1 inhibits IL-3-induced multipotential colony formation (CFU-GEMM), but it does not effect the formation of more differentiated colonies such as erythropoietin (Epo)-induced colonies, CFU-E, suggesting that TGF-β1 selectively inhibits early hematopoietic progenitors, while the later progenitors are insensitive. To test this hypothesis, other early progenitors were studied. First, Thy-1 negative (Thy-1-) hematopoietic progenitors were isolated from bone marrow and stimulated with IL-3 to generate Thy-1 positive (Thy-1+) progenitor cells. The results demonstrate that the Thy-1+ cells give rise to CFU-GEMM and CFU-GM when stimulated by IL-3 and Epo, and that TGF-β1 inhibits both colony types, while monolineage colonies (CFU-M or CFU-G) in the same culture are unaffected. Also, TGF-β1 inhibits the transition of Thy-1- to Thy-1+ cells. Second, high proliferative potential (HPP) progentitor cells that are among the earliest hematopoietic progenitors measurable in vitro are also inhibited by TGF-β1. Thus, TGF-β1 is a selective regulator of the early events of hematopoieisis.**

C 520 TUMOR ANGIOGENESIS INHIBITORS, Gary Koppel, Russell Barton, Steve Briggs, Joseph Parton, Jess Bewley, Lilly Research Laboratories, Indianapolis, Indiana, 46285

The phenomenon of angiogenesis or neovascularization has been the cornerstone of embryonic development. Without question, the process of blood vessel formation has been a prerequisite for subsequent organ development. Indeed, normal processes of adult organisms also have neovascular components, e.g., 1) regeneration of tissue, 2) the healing of wounds, 3) collateral blood vessel formation in the heart, and 4) vascular changes accompanying growth and repair of peridontal tissue. The most carefully documented neovascular reaction has been tumor-induced angiogenesis, the process by which a solid tumor elicits the growth of blood vessels thus assuring successful tumor maintenance. A substance derived from (L) ascorbic acid has been prepared which has been identified as an effective inhibitor of tumor-induced angiogenesis. A mouse in vivo model was developed to screen and identify drugs for inhibition of neovascularization. Subsequent studies in tumor-bearing animals determined the antitumor characteristics of this class of substances. The ascorbic acid derivatives were determined to be non-cytotoxic. Additional studies were carried out to evaluate the effect of the inhibitors on a normal wound healing process. Finally, the activity of these angiogenesis inhibitors in other disease models mediated by the inflammatory process were evaluated.

C 521 PROTEIN IMMUNOBLOT STUDIES OF THE INSULIN-LIKE GROWTH FACTORS AND THEIR BINDING PROTEINS, Phillip D.K. Lee, David R. Powell, Hans Bohn, Choh-Hao Li, Francis Liu, & Raymond L. Hintz. The Children's Hosp Kempe Research Center, Denver, CO 80218 (PDKL); Baylor College of Med, Houston, TX 77054 (DRP); Behringwerke, Marburg, FRG (HB); Univ California, San Francisco 94143 (CHL); and Stanford University, Stanford, CA 94305 (FL, RLH).

Insulin-like growth factors (IGF) I and II are circulating peptides which mediate the mitogenic actions of growth hormone. Both IGFs have MWs of ±7500, and are comprised of A,B,C & D regions. The IGFs are synthesized as prohormones with N-term extensions and C-term E-peptide regions. Two forms of human IGF-I prohormone, differing in E-peptide regions, are known as IGF-IA and IB. In order to determine whether IGF prohormones circulate and, if so, whether they are bound to IGF binding proteins (BP), we conducted immunoblotting studies using antisera to the following human antigens: IGF-I (aI), IGF-IA E-peptide (aIAE), IGF-II C-peptide (aIIC), IGF-II (aII), IGF-II E-peptide (aIIE), and BP from HepG2 cells (aHBP) and placenta (aPBP). Serum samples were separated by SDS-PAGE (5-20% gradient) and transferred to nitrocellulose. Membranes were blocked with BSA, labelled with antisera, and developed using the ABC system (Vector). Labelled bands were compared with MW standards and pure IGF in parallel lanes. All of the BP and IGF antisera labelled bands at ±30K and ±200K, indicating presence of IGF/BP and IGF-prohormone/BP complexes. The IGF-I antisera labelled additional bands at ±7K (aI), ±20K (aI and aIAE), and 40-50K. The IGF-II antisera (aII, aIIC, aIIE) recognized a distinct band at ±26K in the reduced samples, probably representing IGF-II prohormone. No 7K band was seen for IGF-II in these studies. Our data indicate that IGF and IGF-prohormones circulate in association with BP and that the major MW forms of the IGFs and complexes may differ from those previously recognized. Our data also indicate that the BP portion of the IGF/BP complexes are antigenically similar for the large (200K) and small (30-40K) complexes.

C 522 ISOLATION OF EGF-LIKE PEPTIDES IN RAT PROSTATES, Dean Li, Hiroki Nanri, Melissa Chu and Thomas F. Deuel, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110.

Growth factor activity was purified from homogenates of rat prostate by acid extraction, ion exchange chromatography, and gel filtration. These peptides have molecular weights of 35 kDa and 25 kDa when applied to non-reducing SDS-PAGE gels. The growth promoting activity of these peptides was stable to heat and acid but was not stable to reduction. Preliminary sequence data for the 25 kDa protein suggested that it was an EGF-like peptide. Incubation of both peptides with either anti-mouse EGF antibodies or anti-EGF receptor antibodies blocked their mitogenic activity on NRK fibroblasts. Northern analysis indicated the presence of EGF messenger RNA in the prostate gland of rats. Whether these polypeptides from rat prostate are EGF bound to specific binding proteins or result from alternative splicing or differential processing is not clear.

C 523 TRANSFORMING GROWTH FACTOR-β REGULATION OF PLASMINOGEN ACTIVATOR INHIBITOR TYPE-1.

Leif R. Lund (1), Peter A. Andreasen (1,2), Andrea Riccio (3), Francesco Blasi (3), and Keld Danø (1). (1) Finsen Laboratory, Copenhagen, Denmark, (2) Institute of Biochemistry C, University of Copenhagen, Denmark, (3) Institute of Microbiology, University of Copenhagen, Denmark.

The mechanisms behind transforming growth factor-β (TGF-β) -induced stimulation of type-1 plasminogen activator inhibitor (PAI-1) production in WI-38 human lung fibroblasts have been studied, using a full length cDNA probe for PAI-1. An early increase in the PAI-1 mRNA level is seen reaching a maximal 50-fold enhancement after 8 hours of TGF-β treatment. Quantitative studies of the effect of TGF-β on PAI-1 levels in cell extracts and culture media, using monoclonal antibodies against PAI-1, are consistent with the effect on PAI-1 mRNA. Urokinase-type and tissue-type plasminogen activator (u-PA and t-PA) levels are decreased in the culture media of TGF-β treated cells concomitantly with the increase in PAI-1 accumulation. These findings suggest that a primary effect of TGF-β is an overall decreased extracellular proteolytic activity. TGF-β has previously been reported to stimulate production of fibronectin and procollagen. These proteins are potential targets, either direct or indirect, for degradation by plasminogen activators. The concerted action of TGF-β on these proteins and on PAI-1 and plasminogen activator levels is directed towards the same goal, and might constitute an important aspect in an overall protective effect of TGF-β on the extracellular matrix.

C 524 TRANSFORMING GROWTH FACTOR BETA ACTIVATES PHOSPHATIDYLINOSITOL METABOLISM AND PROTEIN KINASE C IN CULTURED BRAIN ASTROCYTES.

Jasna Markovac, Patricia L. Robertson, and Gary W. Goldstein, University of Michigan Medical Center, Ann Arbor, MI 48109.

Astrocytes appear to play a critical role in the maintenance of ion homeostasis in the brain. Little is known about the regulation of astrocyte function. Transforming growth factor type beta (TGFβ) appears to have many regulatory functions in a variety of cell types. We investigated the possibility of a role for TGFβ in mediating astroctye function by measuring its influence on the metabolism of phosphatidylinositol lipids(PI), an important pathway for cellular signal transduction. Using astrocyte cultures obtained from neonatal rats, we found that picomolar concentrations of TFGβ stimulate the production of inositol,phosphates from ^{3}H inositol labeled PI lipids 103% above basal levels (30.7 ± 0.4 cpm/μg protein vs 15.1± 1.7 cpm/μg protein). Because one of the products of PI turnover is the generation of diacylglycerol, which, in turn, can activate protein kinase C, we investigated TGFβ effects on this regulatory enzyme. Astrocytic cultures exposed to TGFβ showed a redistribution of protein kinase C activity from the cytosolic to the membrane fraction. Protein kinase C in the control cultures was mainly found in the cytosol (5.5 ± 0.3 pmol/mg/min, vs 2.0 ± 0.7 pmol/mg/min in the particulate). Following 45 min incubation with picomolar concentrations of TGFβ, the enzyme activity was translocated to the membrane fraction (1.0 ± 0.3 pmol/mg/min in the soluble vs 8.4 ± 1.3 pmol/mg/min in the particulate). These results suggest that TGFβ, working through the PI and protein kinase C second messenger system, may regulate the function of astrocytes in the brain.

C 525 MOLECULAR CHARACTERIZATION AND PARTIAL PURIFICATION OF CSF-1 FROM HUMAN PLACENTAL TISSULAR EXTRACTS.

Martin V., Lanotte M., Tayot J.L. and Uhlrich S. Hopital Saint-Louis, Paris; Institut Mérieux, Lyon, France.

CSF-1 specifically regulates the proliferation, differentiation, survival and biological functions of cells of the mononuclear-phagocytic lineage. We demonstrate that human placental extracts represent a potent natural source of this hemopoietic regulator. Tissue-bound regulators were separated from tissular pulp using an industrial-scale extraction that yielded large amounts of biological activities. We describe the biochemical characterization and the partial purification of CSF-1 from this source. This includes: DEAE-Spherodex chromatography; con-A affinity chromatography; DEAE-5PW and Phenyl-5PW HPLC; electrophoretic separation and immunoblotting. The average M.W. estimated by gel filtration is 60 kd, and the pI is 4.0 on chromatofocusing. This factor stimulates the proliferation of predominantly monocytic macrophage colonies from both human and murine bone marrow cells and is detected by a specific human CSF-1 RIA. This activity is resistant to heat treatment at 60°C for 30 mn but destroyed at 100°C or by a two hour incubation with 0.1M B-ME. It is note worthy that a high molecular weight form of CSF-1 (70-90 kd) has been isolated and is supposed to be inserted into cell membranes. This form could also be present in placental extracts.

C 526 SEQUENCE-SPECIFIC PROTON MAGNETIC RESONANCE ASSIGNMENTS FOR HUMAN RECOMBINANT TYPE-ALPHA TRANSFORMING GROWTH FACTOR (TGF-α), Gaetano T. Montelione,[†], Michael B. Sporn,[§] and Gerhard Wagner[†], [†]Institute of Science and Technology, Biophysics, The University of Michigan, Ann Arbor, MI 48109, [§]Laboratory of Chemoprevention, National Cancer Institute, NIH, Bethesda, MD 20205.

Two-dimensional (2D) ^{1}H-NMR spectra have been obtained for human recombinant TGF-α in H_2O solution at pH 3.5, 30°C. The data collected include 2D two-quantum filtered correlated (2QF-COSY), relayed coherence-transfer (RELAYED-COSY), two-quantum (2Q-SPECTRA), nuclear Overhauser effect (NOESY), and total correlation (TOCSY) spectra. Many sequence-specific proton resonance assignments are determined from these data. The NOESY spectra provide evidence for some compact three-dimensional structure. These NMR data will provide the basis for determining the polypeptide chain-fold of TGF-α. Temperature dependent measurements of 1D-NMR spectra indicate that there are no major conformational changes over the range 28° to 68°C at pH 3.8 in ^2H_2O.

C 527 PARTIALLY PURIFIED EXTRACT FROM APLASTIC ANEMIA PATIENTS CONTAINS STIMULATORY ACTIVITY FOR MOUSE HEMATOPOIETIC PROGENITOR CELLS IN VITRO AND IN VIVO, T. Abe, J. P. Fuhrer, M. D. Bregman, A. Kuramoto (*) and M. J. Murphy, Jr., Hipple Cancer Research Center, Dayton, OH 45439-2092 and (*) Hiroshima University, Hiroshima, Japan.
A partially purified urinary extract from patients with aplastic anemia induced significant stimulation of BDF_1 mouse hematopoietic progenitor cells in vitro and in vivo. The urine was partially purified by Sephadex G50, DEAE-cellulose, 90% ethanol precipitation and ConA-Sepharose column chromatography. When the urinary extract was assayed in vitro for CFU-gm and BFU-e (in methyl cellulose), CFU-e and CFU-gm (in plasma clots), and for CFU-meg (in fibrin clots), significant increases in colony formation of CFU-mix, CFU-gm, CFU-meg and BFU-e in both bone marrow and spleen cells were observed. The clonal results from in vivo assays also documented a significant increase in the numbers of CFU-mix, CFU-gm, CFU-meg and BFU-e from mouse bone marrow and spleen cells. Moreover, three consecutive daily i.p. injections of partially purified extract produced a significant increase in white blood cells, platelets and hematocrit values in rat peripheral blood. Initial results from reverse-phase HPLC purification indicate a molecular species of 41,000 daltons.

C 528 DIFFERENTIAL PROLIFERATIVE EFFECTS OF TRANSFORMING GROWTH FACTOR-BETA (TGFb) ON HUMAN HEMATOPOIETIC PROGENITOR CELLS, Louis M. Pelus and Oliver G. Ottmann. Sloan Kettering Institute, New York, N.Y. 10021
TGFb functions as a negative regulator of cell growth and differentiation in numerous cell sytems including hematopoiesis. We employed in vitro cultures of highly enriched hematopoietic progenitor cells to investigate the direct effects of TGFb1 and TGFb2 on erythroid (CFU-E, BFU-E), granulocyte-macrophage (CFU-GM) and multilineage (CFU-GEMM) colony forming cells. In the absence of exogenous colony stimulating factors (CSF), neither TGFb1 nor TGFb2 supported progenitor cell growth. In the presence of recombinant (r) or natural CSFs, picomolar concentrations of TGFb1 inhibited growth of CFU-E, BFU-E and CFU-GEMM, but enhanced gowth of day 7 CFU-GM. Inhibition of CFU-E and BFU-E by human and porcine TGFb1 was similar, and ranged from 17±8% at 50 pg to 73±3% at 1 ng (4 expts). The sensitivities of CFU-E and BFU-E to inhibition by TGFb1 were equivalent and independent of the type of burst promoting activity employed (rIL3 vs 5637 CM). Inhibition of CFU-GEMM ranged from 79±22% at 0.25 ng to 98±3% at 1 ng. The inhibitory effects of TGFb1 were progressively lost when addition was delayed for 40-120 hrs, suggesting an action during early cell divisions. In contrast, human TGFb1 enhanced the number of CFU-GM by 20±12% at 0.1 ng to 154±12% at 1 ng, when plateau levels of rGM-CSF, rG-CSF and rIL3 were used as a co-stimulators. Using rG-CSF alone, TGFb1 enhanced CFU-G growth by 23±8% at 0.25 ng to 44±26% at 5 ng (4 expts). Porcine platelet TGFb2 was without effect in all systems examined. These results support the hypothesis that TGFb plays a role in the regulation of hematopoiesis by differentially affecting progenitor cell lineages, by direct action on the colony forming cells.

C 529 MONOCLONAL ANTI EGF RECEPTOR ANTIBODIES INHIBTI THE GROWTH OF TUMOR CELLS IN NUDE MICE, E. Aboud-Pirak*, E. Hurwitz*, F. Bellot+, J. Schlessinger*+ and M. Sela*, *Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel, +Rorer Biotechnology Research Center, Meloy Laboratories, Rockville, MD 20850, USA.
Overexpression of EGF receptor was implicated in various types of human cancers, notably epidermoid and squamous carcinoma cell lines. Therefore, we have explored the possibility to utilize monoclonal antibodies against the external region of the EGF receptor (108.4 of JqG 2a class) for their possible use for affinity therapy of tumor cells rich in receptors on their surface.

The binding of 108.4 Mab to EGF receptor on KB cells (human oral epidermoid carcinoma) was analysed by cell sorter analysis and showed homogeneity of receptor expression in at least 90% of the cells. Radioiodinated 108.4 Mab, when injected intravenously to nude mice bearing xenografts of KB cells, localized specifically at the tumor mass.

When mice were treated with the 108.4 Mab one day after tumor inoculation, retardation of tumor development was observed. The $F(ab)'_2$ fragment of the antibody was slightly less efficient but still active in retardation of tumor growth, indicating that no immune mechanisms demanding participation of the Fc portion of the antibody are involved. Preliminary experiments show that the growth factor itself increases the in vitro growth of the KB cells, suggesting that the antitumoral activity of antibodies in vivo may due to some perturbation of the factor-receptor interaction.

C 530 **PURIFICATION OF A PDGF-LIKE GROWTH FACTOR RELEASED FROM M4 METASTATIC FIBROSARCOMA** Vittoria Cioce, Giorgio Ferrari, Elisa Vicenzi, Ida Biunno* and Andreina Poggi. Istituto di Ricerche Farmacologiche Mario Negri and *Istituto Nazionale dei Tumori, Milano, Italy.
In vitro proliferation of tumor cells seems to be supported by secretion of autocrine growth factors. We report here that cells from a chemically-induced murine fibrosarcoma with high metastatic ability (M4), lacking the ability to bind ^{125}I-PDGF, released in the conditioned medium a growth factor similar to PDGF. Conditioned medium from M4 cells (10^9 cells) was applied to a Sulphadex G50 column and eluted with a gradient of increasing ionic strength. The fractions were tested for their ability to stimulate ^{3}H-Thymidine incorporation in NIH 3T3 cells. The peak of mitogenic activity was eluted with 0.65 M NaCl. The active fraction appeared to migrate as a 30 kd protein; its activity was 54% inhibited by anti-PDGF IgG's (kindly provided by Prof. A. Wasteson, Linkoping, Sweden). A 3.9 kb transcript in poly (A) + RNA hybridizing with vSIS probe was found in M4 cells. In conclusion, further suggesting that they produce a growth factor with chemico-physical characteristics similar to PDGF.(Supported by Italian CNR, Progetto Oncologia, Contract n. 86.00680.44).

C 531 **PDGF PLASMA LEVELS IN PATIENTS WITH PROGRESSIVE SYSTEMIC SCLEROSIS.** A. Pandolfi, M. Florita*, G. Altomare* and A. Poggi. Mario Negri Institute for Pharmacological Research, Milan and * Department of Dermatology, Milan University Medical School, Milan, Italy.
Progressive systemic sclerosis (PSS) is a connective tissue disorder characterized by vascular lesions, fibrosis, inflammation and diffuse sclerosis in various areas. Its etiology is not clear. Vascular lesions, due to deposition of immunocomplexes or release of cytotoxic factors, might be the origin of this disease. As a consequence of vascular damage, platelets of sclerodermic patients might be activated and release Platelet Derived Growth Factor (PDGF) which could act as a mediator of the fibrotic process characteristic of PSS. In this study we evaluated the mitogenic activity of platelet poor-derived serum (PDS) in a group of sclerodermic patients in comparison with a group of normal subjects matched for age and sex. Measurement of 3H-thymidine incorporation into NIH 3T3 cells showed that PDS of patients, (5% vol/vol) had a greater mitogenic activity than PDS of control subjects (4912±666 cpm, 2776±641 cpm, respectively mean±SD of 8-10 subjects, $p < 0.05$ Duncan's test). When anti-PDGF IgG (kindly provided by Professor Russel Ross, Seattle, USA) was used, an inibitory effect (about 42%) on the mitogenic activity of PDS of sclerodermic patients was observed. These results indicate that the increased mitogenic activity observed in sclerodermic patients might indeed be due to PDGF, suggesting a possible pathogenetic role of this peptide in PSS. This work was partially supported by Italian CNR, Progetto Finalizzato Oncologia, Contract. no. 86.00680.44

C 532 REGULATORY FACTORS OF CFU-S PROLIFERATION. Françoise Sainteny, Evelyne Lauret, Dominique Dumenil, Joanna Wdzieczak-Bakala, Maryse Lenfant*, Emilia Frindel - INSERM U.250, Institut Gustave-Roussy, 94805 Villejuif - *ICSN/CNRS, 91190 Gif s/Yvette.
In the last few years, we have presented data demonstrating that, contrary to previous hypotheses, hemopoietic pluripotent stem cell (CFU-S) proliferation is controlled by humoral factors that are in equilibrium in normal situations. The entry of quiescent CFU-S into cycle after various treatments is under the control of excess stimulating factors which are different from IL3, EPO, CSF, and which are secreted by the surviving cells. The inhibition of stimulated CFU-S to enter DNA synthesis is obtained by a factor extracted from fetal calf bone marrow. Details concerning this factor will be presented in the poster of M. Guigon et al. This inhibitor (a tetrapeptide) is now completely characterized. The synthetic material has identical biological properties to the natural molecule concerning inhibition of CFU-S entry into DNA synthesis. The other parameters have not as yet been tested. Preliminary data suggest that this inhibitor may be active at the GO-G1 phase.

C 533 KAPOSI'S SARCOMA (KS)-DERIVED ENDOTHELIAL CELLS: ROLE OF LYMPHOENDOTHELIAL CELLS IN PATHOGENESIS OF KS, Syed Z. Salahuddin, Shuji Nakamura, Peter Biberfeld Phillip D. Markham and Robert C. Gallo, National Cancer Institute, Bethesda, MD. 20892, Karolinska Institute, Stockholm, Sweden. Although no direct relationship between HIV-infection and the development of Kaposi's sarcoma (KS) has been established, AIDS patients have high incidence of KS. Previous to its recognition as AIDS-associated disease, KS was primarily found in elderly men of Eastern Europe, and Mediterranean people. Geographical distribution in Africa and transplantation or immunosuppressive therapy-associated disease has also been reported. While there are clinical differencies among these KS, such as indolent or aggressive course, and nodular or infiltrative lesion, important histological characteristics are commonly observed among them. This histologically complex lesion consists of dividing spindle cells and endothelial cells, fibroblasts, smooth muscle cells and infiltrating inflammatory cells. Another feature of the lesion is a new vascular formation. So far, the origin of KS cells and its pathophysiology remains unknown. In order to develop a better understanding of KS and its pathogenesis, cloned cell lines from KS lesion were established in cell culture. Morphological, biological, immunological and cyto-chemical analysis demonstrated that these cells are endothelial cells possibly of lymphatic orgin. These KS-derived endothelial (KS-E) cells induced neo-angiogenic activities on chicken chorioallantoic membrance and also other biological activities able to stimulate and support the growth of KS-E cells and normal vascular endothelial cells in vitro. When inoculated into nude mice, KS-E cells induced the formation of lesions composed entirely of murine cells exhibiting morphological and histological properties similar to cells characteristically found in human AIDS-KS lesion. Based on these observation, a model for KS pathogenesis was developed proposing that following an initial, unknown event(s), effector or regulator cells including lymphatic endothelial cells (spindle cells) are able by the release of multiple biological activities to support and/or regulated the development of KS lesion in multifocal fashion.

C 534 MITOGEN INDUCIBLE GENES IN HUMAN T CELLS, Ulrich Siebenlist[1], Peter Zipfel[1], Steven Irving[2] and Kathleen Kelly[2], National Institutes of Health, LIR, NIAID[1] and IB, NCI[2], Bethesda, Md. 20892
In order to understand the cellular response to a growth promoting agent, we have identified in excess of 60 unique genes which are induced by mitogens in resting, primary human peripheral blood T cells. These genes were cloned by making a subtractive library, to enrich for induced genes and then by screening with subtractive probes. We have studied various aspects of regulation, tissue specificity and function of the activatable genes. Groups of genes have been defined that are reponsive to distinct stimuli, such as mitogenic antibodies to specified cell surface structures on T cells. An additional regulatory category of genes has been identified based upon the inhibition of their mitogen-caused activation in the presence of the immunosuppressive drug cyclosporin A. Although many of the genes isolated from the T cell library are inducible in human fibroblasts as well, a number of others are more restricted in their tissue specificity. We have identified several genes whose expression is constitutively turned on upon infection with HTLV I, a human retrovirus that stimulates polyclonal proliferation of normal T cells. We are investigating the effects of the tat transactivator protein on the regulation of expression of these mitogen-inducible genes.

C 535 PDGF STIMULATES TYROSINE KINASE ACTIVITY OF PDGF RECEPTOR PRECURSORS WHICH ACCUMULATE WITH MONENSIN AND SWAINSONINE TREATMENT OF NRK FIBROBLASTS.
Neil J. Silverman, Bruce E. Bejcek, Rodney S. Kawahara, and Thomas F. Deuel, Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110.
NRK fibroblasts were treated with monensin, a carboxylic acid ionophore that interferes with intracellular transport and terminal glycosylation, or with swainsonine, an inhibitor of mannosidase II in Golgi, in order to accumulate incompletely processed forms of the PDGF receptor and to test their ability to exhibit PDGF-stimulated tyrosine kinase activity. Membranes were prepared from NRK fibroblasts which were down regulated for six hours with 500ng/ml PDGF in DMEM and then treated for 18 hours with 1.5uM monensin or 5uM swainsonine. The membranes were phosphorylated with γ^{32}P- ATP in the presence or absence of PDGF. Immunoprecipitation of solubilized ^{32}P-labelled membranes with anti-PDGF receptor or antiphosphotyrosine antibodies resulted in the identification of 160kD and 168kD receptor precursors from monensin and swainsonine treated cells, respectively, but the 180kD mature receptor was not identified. The phosphorylation of both pre-receptors were markedly enhanced in the presence of PDGF. Therefore, PDGF is recognized by pre-PDGF receptors, and stimulates their intrinsic tyrosine kinase activity, raising the possibility that the sis gene product can stimulate the pre-PDGF receptor internally during processing in cells which express both the PDGF receptor and the sis gene.

C 536 INSULIN-LIKE GROWTH FACTOR-I AND SOMATOSTATIN MODULATE BOVINE GROWTH HORMONE RELEASING HORMONE (bGRF)-STIMULATED GROWTH HORMONE SECRETION IN VITRO, J.W. Tanner, N.H. McArthur and T.H. Welsh, Jr., Texas A&M University, College Station, TX 77843.
Development of technologies to selectively modulate expression of the bovine growth hormone (GH) gene is important in regulating animal growth processes. Serum-free primary cultures of bovine adenohypophyseal cells were used to study GH secretion in response to hypothalamic neurohormones, steroidal anabolic agents and growth factors. The hypothalamic neurohormone bGRF (10^{-12} M-10^{-7} M) stimulated ($P<.05$) GH secretion. Basal and bGRF-stimulated GH secretion were increased 14- and 41-fold, respectively, by IGF-I treatment (100 ng/ml). The estrogenic steroid-like growth promotant zeranol (10^{-6} M) increased bGRF-stimulated (10^{-7} M) GH secretion by 260%. In contrast, the hypothalamic neurohormone somatostatin (SS, 10^{-7} M) inhibited ($P<.05$) both basal and bGRF-stimulated GH secretion by 37 and 34%, repectively. Forskolin (10^{-5} M, an adenyl cyclase activator) increased ($P<.05$) GH secretion 150% but co-treatment with SS inhibited this effect by 68%. Similarly, $(Bu)_2$cAMP-stimulated GH secretion was reduced 61% by concomitant SS treatment. At 10^{-6} M, phorbol-12-myristate-13-acetate (PMA, a diacylglycerol mimetic/protein kinase C activator) increased ($P<.05$) GH secretion 4-fold. Co-administration of 10^{-7} M SS did not inhibit ($P>.10$) PMA-stimulated GH secretion. Somatostatin inhibition of the transduction of bGRF's biological effects on GH synthesis/secretion appears to be via the adenyl cyclase-cAMP-protein kinase A rather than the protein kinase C route. These data provide insight regarding mechanisms whereby growth factors, anabolic agents and hypothalamic neurohormones modulate bovine GH secretion.

C 537 AUTOCRINE INHIBITION OF DNA SYNTHESIS IN MOUSE EMBRYO FIBROBLASTS BY A 45000 DALTON SECRETED PROTEIN, T.Nagashunmugam and G.Shanmugam, Cancer Biology Division, School of Biological Sciences, Madurai Kamaraj University, Madurai, INDIA-625 021.

Cells synthesize and secrete a variety of growth factors and inhibitors into their growth medium. To learn about the nature of these factors, Swiss mouse embryo fibroblasts were labelled with 35S-methionine and the secreted proteins were analysed. The monolayers were labelled at different time intervels after synchronizing them with medium containing 0.5% serum for 48 hrs followed by 16 hrs of hydroxyurea (HOU) treatment. Among the several secreted proteins, a polypeptide of 45000 dalton was barely visible in the conditioned medium of cells that synthesized DNA at peak levels. The quantity of 45 K protein was higher in the medium of HOU arrested cells and the level of this protein gradually declined as the cells entered into DNA synthesis. The amount of 45 K protein in the medium was found to be more as the cells left the S phase and entered into G2 and M phases. Releasing the HOU arrested cells with the 45 K protein containing medium inhibited DNA synthesis. The 45 K protein was purified using ion-exchange high pressure liquid chromatography. Purified 45 K protein was found to inhibit DNA synthesis in mouse embryo fibroblasts released from HOU arrest.

C 538 Study on Sensitivity of H^3-TdR Transormed C_3H 10T 1/2 CL8 Cell and Mouse Ascites Carcinoma Cells to Epidermal Growth Factor and DBcAMP. Licheng Xu, Tan-jun Tong and Chang-ying Chang, Dept. Biochem.,Beijing Medical University,Beijing,China

Since the tyrosine-specific phosphorylation of some proteins in cell is closely associated with control of cell growth and proliferation, and the sensitivity of cells to growth factors may be altered when the cells are transformed, the effects of epidermal growth factor (EGF) and dibutyryl cAMP (DBcAMP) on incorporation of H^3-TdR into DNA of various mouse ascites carcinoma cells were observed. It was found that there are some differences of sensitivity to growth factors among different mouse ascites carcinoma cells. Considering this might be connected with malignity of carcinomas, we compared sensitivity of C_3H10 T1/ 2_3CL8 (a normal fibroblast from embryo of C_3H mouse, designated as NC_3H10) with that of H^3-TdR transformed C_3H10 T1/2 CL8 (designated as TC_3H10)to growth factors, and the tyrosine specific phosphorylation of cellular proteins of these cells was also assayed. We found that the sensitivity of TC_3H10 to EGF decreased obviously in comparison with NC_3H10 and the stimulation rate of H^3-TdR incorporation of TC_3H10 by EGF was less than 1/4 that of NC_3H10 while tyrosine-specific phosphorylation of protein in TC_3H10 increased obviously, but that of NC_3H10 could rarely be measured. Meanwhile it was demonstrated that DBcAMP was able to inhibit incorporation of H^3-TdR into NC_3H10 and TC_3H10 as well as EGF-induced incorporation of H^3-TdR . Our results showed that radioactive carcinogen, like biological and chemical carcinogens, could also change sensitivity of cells to growth factors and induce imbalance of tyrosine-specific phosphorylation of proteins of transformed cells. Perhaps this is a general way of different carcinogen-induced transformation. Thereby our results maybe provide some theoretical basis of molecular biology for radioactive tumorigenesis theory.

C 539 ISOLATION AND PARTIAL CHARACTERIZATION OF A HIGH AFFINITY HEPARIN-BINDING PROTEIN FROM BOVINE PITUITARY AND TESTIS , Naoto Ueno and Andrew Baird, The Salk Institute for Biological Studies, La Jolla, California 92037.

The major contaminants that are present in partially purified preparations of fibroblast growth factor (FGF) have been identified. A 26,000 dalton heparin-binding protein has been isolated from bovine pituitaries, and 26,000 and 20,000 dalton species from bovine testes by means of heparin-Sepharose affinity chromatography. The proteins from both tissues extracts coeluted with endogenous basic FGFs from the affinity columns at a NaCl concentration of 1.0-2.0 M. They were separable from basic FGF by either ion-exchange or reversed-phase HPLC. Gas phase amino-terminal amino acid sequence analysis of ther pituitary-derived heparin-binding protein (HBP) has been determined as: Ala-Pro-Asp-Ser-Val-Asp.-Tyr-Arg-Lys-Lys-Gly-Tyr-Val-Thr-Pro- Val-Lys-Asn-Gln-Gly-Gln-Xaa-Gly-Ser-Xaa-Trp-Ala-Phe-Ser-. Amino acid composition of testis-derived HBP was very similar to that of pituitary counterpart. Amino-terminal sequence analysis of the first 24 residues of the testis-derived HBP revealed an identical amino-terminal structure to pituitary-derived HBP. Because this sequence is very homologous to those of papain, cathepsin B, cathepsin H, and especially that of recently characterized cathepsin L the results emphasize the importance of achieving homogeneity in preparing basic FGF for biological studies.

C 540 FUNCTION OF MARROW STROMA CELL SUBPOPULATIONS. Hong Wang and Arthur K. Sullivan. McGill Cancer Centre, McGill University and Division of Hematology, Royal Victoria Hospital, Montreal, CANADA H3G 1Y6.

Maturation of blood cells in the bone marrow is controlled by a complex interaction of precursors with supporting cells and with soluble growth factors. However, the important sources of physiologic factor production have not been clearly identified, nor is it well understood what regulates the regulators. One mechanism has been described (JR Zucali, et al. Blood 69:33, 1987) by which macrophages can be stimulated to release IL-1, leading to further stimulation of fibroblastoid cells to produce colony stimulating factors (CSF). Previous work from this laboratory has shown that fibroblastoid cells cultured from the bone marrow of BN rats bear surface antigens different from fibroblasts grown from most other peripheral sites (e.g., lung), as determined by antibodies ST3 and ST4. The distribution of the ST3 antigen is similar, but not identical, to that of OX-7 (Thy 1.1). To assess the relative contribution of CSF production by the different populations, homogeneous layers of ST3+/ST4- (marrow-derived) and ST3-/ST4+ (lung-derived) cells were expanded in culture. Preliminary results indicate that the ST3+, but not the ST4+ fibroblast was stimulated by macrophage conditioned medium to release stimulatory factors for granulocyte/macrophage colonies. This suggests that there may be a distinct, antibody-defined subpopulation of supporting cells which can function in a regulated manner to produce certain hemopoietic growth factors.

C 541 MYOCARDIAL ACIDOSIS RELEASES BASIC FIBROBLAST GROWTH FACTOR: A POSSIBLE MECHANISM OF ISCHEMIA INDUCED ANGIOGENESIS. E.Y. Yang, W. Casscells, P.E. Karasik, F. Bazoberry, S.E. Epstein, NHLBI, Bethesda, MD 20892.
The angiogenic growth factors, acidic and basic fibroblast growth factor (aFGF and bFGF), are present in normal myocardium. Using ex vivo incubation of rat myocardium in normal saline as a model of myocardial ischemia, we showed that basic fibroblast growth factor is released from myocardium following 60 minutes of incubation. Radioimmunoassay, using a polyclonal antisera against amino acids 1 to 24 and sensitive to 40 pg, revealed bFGF levels rose from 8.85 $\pm$ 0.38 pg/mg in controls to 17.40 $\pm$ 2.53 pg/mg in ex vivo injured hearts ($p<0.001$). Although bFGF could be synthesized and released from ischemic myocardial cells, we hypothesized that pre-existing stores of bFGF in the extracellular matrix are made more bioavailable by myocardial acidosis. We therefore extracted control and ex vivo injured hearts in pH extraction buffers ranging from 7.5 to 4.5 to determine whether decreasing pH releases bFGF and, if so, what pH simulates the ischemia mediated bFGF release. With decreasing pH, myocardial bFGF levels in the control hearts rose significantly ($p<0.01$) towards the levels of the ischemia injured hearts. Below pH 6.5 bFGF levels in the two groups were essentially equivalent. We conclude that myocardial acidosis can simulate ischemia/infarction stimulated release of bFGF. Given that pH 6.5 is the lower limit of tissue pH in vivo during myocardial ischemia, it is probable that ischemia induced acidosis causes release of bFGF from extracellular matrix, which may then play a role in stimulating coronary collateral growth.

C 542 DECREASED KINASE C DEPENDENT PHOSPHORYLATION OF AN 80KD PROTEIN IN TRANSFORMED BALB/c 3T3 CELLS, Henry C. Yang, Harbor-UCLA Medical Center, Torrance, CA. 90509
Three BALB/c 3T3 fibroblast cell lines which were transformed by benzo(a)pyrene (BPA31), 7,12 dimethylbenz(a)anthracene (DA31) and Kirsten sarcoma virus (KA31) were studied for the appearance of a kinase C dependent 80kD phosphoprotein (pp80) after low serum arrest and serum restimulation. In the untransformed (A31) cells, pp80 increased 7-10x at 3 hours after serum or phorbol 12-myristate 13-acetate (PMA) stimulation. In all 3 transfomred cell lines, pp80 was markedly reduced at the end of low serum arrest and increased only slightly after serum or PMA stimulation.Pp80 was immunoprecipitated by an antibody to the kinase C dependent 87kD phosphoprotein found in bovine brain synaptosomes (1), suggesting homology between these two phosphoproteins. The total amount of pp80 and unphosphorylated 80kD protein (p80) in BPA31 cells was similar to that in A31 cells. In vitro kinase C enzyme assay revealed a small transient rise in activity in A31 cells 1 hour after serum stimulation. The 3 transformed low serum arrested cell lines had kinase C activity equal or greater than that of low serum arrested A31 cells but did not demonstrate a rise in kinase C activity 1 hour after serum stimulation. PMA treatment of A31 cells did not cause a rise in cellular kinase C activity in vitro. The results suggest that reduced p80 phosphorylation during G_0 arrest and exit is a common finding in transformed 3T3 cells and that reduced pp80 is due to altered in vivo activity of kinase C for the p80 substrate or possibly accelerated dephosphorylation of pp80.
1. Albert, K.A., S.I. Walaas, J.K.-T. Wang, and P. Greengard. (1986) Widespread occurrence of "87 kDa", a major specific substrate for protein kinase C. Proc. Natl. Acad. Sci. U.S.A. 83:2822-2826.

C 543 INHIBIN AND FSH RELEASING PROTEIN MODULATE ERYTHRODIFFERENTIATION, John Yu*, Li-en Shao*, Victor Lemas*, Alice L. Yu*, Joan Vaughan++, Jean Rivier++ and Wylie Vale++, Research Institute of Scripps Clinic*, Uni. of Cal. at San Diego+, and The Salk Institute for Biological Studies++, La Jolla, CA 92037. Inhibin, a hypophysiotropic hormone which selectively suppresses the secretion of pituitary follicle-stimulating hormone (FSH), has recently been isolated from gonadal fluids and characterized as heterodimeric proteins consisting of an α subunit and one of two β subunits (β_A or β_B). FSH releasing protein (FRP) or activins were also purified and shown to be a dimer consisting of two inhibin β-chains. In the present studies, FRP and inhibin were found to modulate induction of hemoglobin accumulation of a human erythroleukemic cell line, K562, and proliferation of erythroid progenitor cells in human bone marrow culture. FRP was highly potent (50% effective concentration $\simeq$ 30pM) in inducing K562 to undergo differentiation and to produce twice as much hemoglobin as chemical inducers on a single cell basis. In the presence of erythropoietin, FRP potentiated the colony formation of erythroid progenitors in a dose responsive manner. Furthermore, inhibin was functionally antagonistic to the FRP-induced hemoglobin accumulation in K562 cells and proliferation of progenitor cells in bone marrow cultures. Thus, these proteins may constitute a novel humoral regulatory control for erythropoiesis involving two types of related protein dimers with functionally opposite effects.

C 544 PROLACTIN: ROLE IN T CELL PROLIFERATION, Li-yuan Yu-Lee, Departments of Medicine and Cell Biology, Baylor College of Medicine, Houston, TX 77030.
As a model system for analyzing the mitogenic effect of prolactin on T cell proliferation, we have been studying the rat Nb2 T lymphoma cell line which requires very low quantities of prolactin (pg/ml) for growth. One approach has been to characterize the expression of oncogenes (*c-myc, c-fos*) and growth-related (ODC) genes after prolactin stimulation of quiescent NB2 T cells. Both *c-myc* and ODC mRNAs are induced rapidly by 2 hr, with maximum stimulation occurring by 4 hr. A recently-identified, prolactin-inducible heat shock 70 homologue, Nb29, mRNA is also induced maximally between 4-6 hr. Treatment with protein synthesis inhibitors, cycloheximide or anisomycin, led to a super-induction of *c-myc* mRNA but inhibited ODC and Nb29 heat shock mRNAs. These studies show that *c-myc* and ODC gene expression, although responsive to the same hormonal stimulation, is regulated by different mechanisms, involving labile protein factors which may act as either activator (ODC) or inhibitor (*c-myc*) on gene transcription, post-transcriptional processes and/or mRNA stability. Nuclear run-on transcription assays indicated that ODC gene expression appears to be regulated primarily at the transcriptional level while *c-myc* and Nb29 are regulated at both the transcriptional and post-transcriptional levels by prolactin. Experiments on modulating the expression of these genes are underway to obtain insights into the intracell signalling pathways involved in the early events following prolactin induction. A second approach has been to construct a cDNA library using the lambda ZAP system, to isolate novel lymphocyte-specific, prolactin-inducible gene sequences which may be important for T cell proliferation. Supported by ACS BC425

C 545 DETECTION OF MULTIPLE GLYCOSYLATED FORMS OF INTERLEUKIN-3 USING POLYCLONAL AND MONOCLONAL ANTIPEPTIDE ANTIBODIES. Hermann J. Ziltener, Barbara Fazekas de St. Groth, Kevin B. Leslie, John W. Schrader, The Biomedical Research Centre, UBC, Vancouver, BC, V6T 1W5 and The Walter and Eliza Hall Institute, Melbourne, Australia.
T cell derived interleukin-3 with was biosynthetically labelled with [^{35}S] methionine and affinity-purified using monoclonal or polyclonal anti-IL-3 antibodies. SDS-Page analysis of the purified IL-3 preparations revealed the presence of three bands with apparent Mr of 32000-36000, 27000-31000 and 21500-22500 respectively. Elution of gel slices as well as high performance gel permeation chromatography demonstrated that each of the three [^{35}S] labelled species was active in the standard IL-3 bioassay. Biosynthetic labelling in the presence of tunicamycin revealed only one band, comigrating on SDS-PAGE with synthetic IL-3 at an apparent Mr of 16000, thus indicating that the heterogeneity of Mr forms is due to different patterns of N-linked glycosylation. IL-3 derived from the myeloid leukemia WEHI-3B was also affinity-purified and part of it labelled with ^{125}I. Autoradiography after SDS-PAGE showed a significantly different Mr pattern with a broad band between Mr 29000 and 45000 and a major peak around Mr 35000. Elution of bioactivity from gel slices of a parallel track containing unlabelled IL-3 confirmed this Mr distribution. IL-3 produced by transient expression in Cos 7 cells was also analysed using the above techniques. The Mr pattern of this IL-3 was found to be similar to that of the T cell derived material.

Biological Action of Growth Factors on Cells, Tissues and Organs

C 600 **EXPRESSION OF HIGH AFFINITY INTERLEUKIN 2 RECEPTORS ON HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAS OF IMMATURE PHENOTYPE.** Michèle Allouche, Vassilis Georgoulias, Cristina Varela-Millot and Claude Jasmin. I.N.S.E.R.M. U268, Hôpital Paul Brousse, B.P. 200, 94800 Villejuif, France.

We have studied fresh leukemic cells from two patients with T-cell acute lymphoblastic leukemia (T-ALL) of an immature phenotype ($CD7^+$, $CD1^-$, $CD2^-$, $CD3^-$, $CD4^-$, $CD8^-$). Leukemic cells could be induced to express 40-97 % interleukin 2 receptors (IL 2-R), as shown by indirect immunofluorescence with anti-Tac monoclonal antibody, after 24-48h culture in the absence (one case) and in the presence (both cases) of 50 ng/ml Phorbol Myristate Acetate (PMA). Note that PMA did not induce phenotypic differentiation of leukemic cells. Binding studies with radioiodinated IL 2 demonstrate that such Tac^+ leukemic T cells bear an average of 50 IL 2-R per cell with a Kd of 5-20 pM, i.e. of high affinity. It is also possible, however, that only a proportion of leukemic T cells bear a higher number of high affinity receptors whereas the remainder only bear low affinity IL 2-R. In one case we also demonstrated specific internalization of IL 2 by the cells. The function of these IL 2-R on immature leukemic T cells will be discussed.

C 601 αFGF DISTRIBUTION IN THE BRAIN AND IN THE EYE, Barritault D. (1), Groux B. (1), Caruelle D. (1), Caruelle J.P. (1), Gaudric A. (2), (1) University Paris XII, Dept Biotechnology, Av. du General de Gaulle, and (2) Dept Ophtalmology, Hopital Intercommunal, 94000 Creteil.

Polyclonal antibodies against aFGF were used to perform indirect immunocytochemistry as well as quantifications by a sensitive Enzyme Immunoassay in various tissues. No cross-reactivity against bFGF was found. In the brain, indirect immunofluorescent and immunogold studies indicate that, although aFGF is found all over, its repartition is regular. Very intense specific colorations were detected in the cephalic axis (mostly the hypothalamic area), then the hemisphera. Cervelet were weakly reactive to the antibody indicating a diffuse and low concentration of aFGF. These observations were quantified after extraction of aFGF by EIA, and a good correlation was found with our histological studies.

In ocular tissues, a FGF was found in decreasing order of abundance -optic nerve, cornea (epithelium and stroma), retina, vitreous body, lens, aqueous humor and sclera. No aFGF was found in the pigmented epithelium and choroïd. In human child retina, aFGF was found to be mostly associated with outer segments of photoreceptors and with the axons of the ganglion cells. In bovine retina, cones, horizontal cells and axons of the ganglion cells were heavily stained in the central region, while in peripheral areas a more diffused labelling was seen. However, staining of neuroretina was intense and appeared homogeneous, independently of the region observed.

C 602 POSSIBLE AUTOCRINE MECHANISMS IN THE EVOLUTION OF KAPOSI'S SARCOMA, Peter Biberfeld[1], Shuji Nakamura[2], Barbara Ensoli[2], S. Zaki Salahuddin[2], Flossie Wong-Staal[2] and Robert C. Gallo[2], [1]Department of Pathology, Karolinska Institute, Stockholm, Sweden, [2]Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

By using conditioned media from a T-cell line transformed by HTLV-II (HTLV-II$_{s}$) we were able to culture continuously endothelial cells from Kaposi's sarcoma lesions (KSE). By ultrastructure and by immunphenotype, KSE cells closely resembled the s.c. spindle cells of KS lesions and had also features in common and distinctive from those of in vitro cultured human umbilical vein endothelium (H-UVE). In addition, the KSE cells expressed significant levels of mRNA's for bFGF, IL-1α and ß, TGFß and GM-CSF, whereas H-UVE cells expressed low levels of bFGF and no IL-1. The HTLV-II$_{s}$ KSE stimulatory factor(s) was distinguishable from a,bFGF and IL-1. KSE cells, in contrast to H-UVE elicited a strong angiogenic response, demonstrable by CAM assay and by s.c. injection of KSE cells in nude mice. The present findings could indicate that the KS-lesion may be initiated by a lymphocytokine(s) and subsequently progress by the expression of autocrine growth stimulatory mechanisms by the KSE (spindle) cells.

C 603 GROWTH REGULATION OF SOMATOTROPHS IN GRF-TRANSGENIC MICE, Nils Billestrup, Sylvia Asa, Jenny Price, Larry Swanson and Wylie Vale, The Salk Institute and SIBIA, La Jolla, CA 92037 and St. Michael's Hospital, Toronto, Canada.

Clinical studies of patients with growth hormone releasing factor (GRF) secreting tumors have indicated that GRF stimulates proliferation of somatotrophs and may be involved in events leading to pituitary hyperplasia and subsequent adenoma formation. Recently we have shown that in vitro GRF is able to stimulate proliferation of primary somatotrophs by a cAMP dependent mechanism. GRF also induced transcription of the c-fos proto-oncogene as well as biosynthesis of the fos protein. In order to study the long term effects of GRF in vivo on somatotroph proliferation, GRF-transgenic mice were developed from fertilized eggs microinjected with a mouse metollothionine 1 promotor/human GRF cDNA construct. Plasma levels of human GRF were high (range 18-279 ng/ml) resulting in hypersecretion of GH and accelerated growth rate (growth rate relative to controls 1.78 + 0.20). The size of the anterior pituitary gland in transgenic animals was found to be increased 5-20 fold. Immunohistochemical analysis of anterior pituitaries from transgenic mice showed a marked hyperplasia as well as hypertrophy of the somatotropic cells. The number and size of the other endocrine cell types in the pituitary was not different from controls, indicating a direct role of GRF in stimulating somatotroph growth. However, no signs of adenoma formation was found. Electron microscopy of the pituitaries revealed cells of normal morphology and no transformed cells were observed. These data demonstrate that in vivo, hypersecretion of GRF is associated with somatotroph hyperplasia and hypertrophy. However GRF does not seem to directly induce adenoma formation, but more likely contributes to transformation of somatotrophs by acting concertedly with other growth or transforming factors.

C 604 *Abstract withdrawn*

C 605 GM-CSF AND INTERLEUKIN 1 INCREASE DENDRITIC CELL ACCESSORY ACTIVITY FOR T LYMPHOCYTE PROLIFERATION, William E. Bowers, Mary S. Ruhoff, and Estelle M. Goodell, Bassett Institute for Medical Research, Cooperstown, New York 13326

Our previous work established that rat dendritic cells function as potent accessory cells for responses of T lymphocytes to mitogenic treatment with periodate or Con A. After fractionation of rat lymph node cells in a discontinuous gradient of bovine plasma albumin, dendritic cells are recovered almost entirely in a minor population (5%) of low density cells (LDC). LDC were irradiated and incubated overnight in microtiter wells with silica to get rid of macrophages. When mitogen-treated T lymphocytes were added, responses increased 3-4-fold above those of the control LDC without silica. Responses were measured by ^{3}H-thymidine incorporation during the last 4 hr of a 48 hr culture period. A supernatant from LDC exposed to silica was able to augment to the same extent the accessory activity of LDC not previously incubated with silica. LDC removed from this supernatant also increased the proliferation of mitogen-treated T lymphocytes, indicating that the supernatant affected accessory cells rather than T lymphocytes. A panel of highly purified or recombinant growth factors was tested for the ability to reproduce this effect. Only mouse rGM-CSF and human rIL-1 were active, both to about the same extent as that of the silica-induced supernatant. Human rIL-4, rat rIFN-γ, and L cell-conditioned medium containing G-CSF were not active. The mechanism by which GM-CSF and IL-1 enhance dendritic cell functions is currently under investigation. Supported by grants from NIH AI 17887 and the Stephen C. Clark Research Fund.

C 606 EFFECTS OF CYTOKINES ON HUMAN MONOCYTE FUNCTION, Connie L. Erickson-Miller, Camille N. Abboud and James K. Brennan, University of Rochester School of Medicine and Dentistry, Rochester, NY.

The influence of colony stimulating factors and IL-1 on several properties of human blood monocytes were investigated. G-, GM-, and M-CSF, IL-1 and LPS were examined for their ability to enable human monocytes to survive in culture in the presence of 10% fetal calf serum. Under all conditions there was an initial 40% drop in cell number 17 hours after harvest. After that time, only M-CSF (500 u/ml) was able to maintain a constant number of cells over 10 days. In the absence of M-CSF the cell number continued to decline, reaching 1/7 that observed with M-CSF at day 10. A study of actin polymerization showed that human monocytes are stimulated by IL-1 and fMLP to have a shift from G- to F-actin within 30 sec. No shift has been found to occur when the cells are exposed to recombinant G-, GM-, M-CSF or LPS. When measuring shifts in internal calcium using FURA-2 on monocytes cultured for 1 or 8 days, fMLP was found to stimulate a calcium flux, whereas G-, GM-, M-CSF or LPS did not. In contrast to its stimulation of actin polymerization, IL-1 did not cause a calcium flux. Currently, work is being done to investigate synergism between these factors.

Growth Factors and Their Receptors: Genetic Control and Rational Application

C 607 BIOSYNTHETIC HUMAN EPIDERMAL GROWTH FACTOR ACCELERATES EPIDERMAL REGENERATION OF DONOR SITES IN MAN. G.L. Brown, A. Cramer, G.S. Schultz, L. Holtzin, L. Curtsinger, L. Nanney, M.J. Jurkiewicz, Depts. of Surgery and Biochemistry, University of Louisvillle, Louisville, KY, Emory University, Altlanta, GA, and Vanderbilt University, Nashville, TN.
A clinical trial was conducted to determine if EGF when applied topically to partial-thickness wounds in man would enhance the rate of epidermal regeneration. Twelve patients were enrolled in the prospective, randomized, double-blind trial. Each patient had two donor sites, 5 cm x 15 cm, at 12/1000 inch depth. One donor site of each patient was treated with Silvadene cream and the other donor site was treated with Silvadene cream containing 10 ug hEGF/ml. Healing was measured by planimetry of photographs of wounds. Silvadene-treated control wounds healed in an average of 10.75 days while the wounds treated with Silvadene containing EGF healed in an average of 8.1 days ($p<0.05$, paired T-test). Morphometric analysis of the biopsies revealed significantly more epithelium present on EGF-treated wounds than on control wounds ($p<0.05$ paired T-test). In addition, two patients with chronic nonhealing diabetic ulcers were treated with Silvadene containing hEGF. One patient unhealed for 4 years following transmetatarsal amputation was totally healed after 3 weeks of therapy. A second patient with a diabetic foot ulcer unhealed for 3 months healed totally healed after 2 weeks of treatment with hEGF. These clinical studies support previous animal studies that demonstrated enhanced wound healing with hEGF treatment.

C 608 PURIFIED RECOMBINANT HUMAN-(RH)- G-CSF and -CSF-1 SYNERGIZE IN VIVO TO ENHANCE PROLIFERATION OF GRANULOCYTE-MACROPHAGE (CFU-GM), ERYTHROID (BFU-E) AND MULTIPOTENTIAL (CFU-GEMM) PROGENITOR CELLS IN MICE. H.E. Broxmeyer, D.E. Williams, S. Cooper, G. Hangoc, and P. Ralph. Indiana University School of Medicine, Indianapolis, IN, and Cetus Corporation, Emeryville, CA.
Combinations of murine-CSF-1, -IL-3, and -GM-CSF synergize when administered to mice in vivo to increase cycling rates of hematopoietic progenitor cells (Broxmeyer et al., PNAS 84: 3871, 1987; Williams et al. Blood 70: 401, 1987). Experiments were set up to determine if rhG-CSF and rhCSF-1 synergize in vivo. BDF_1 mice were pretreated i.v. with purified human lactoferrin in order to reduce endogenous mouse CSF levels, and were administered rhG-CSF or rhCSF-1 i.v. at 10000, 5000, 2000 or 1000 units or were given rhG-CSF and rhCSF-1 together at 500, 100, 50 or 10 units each. Mice were sacrificed at 24 hrs and femoral marrrow and spleen evaluated for the % of CFU-GM, BFU-E and CFU-GEMM in S-phase. 10000 units of either rhG-CSF or rhCSF-1 enhanced cycling of all marrow and spleen progenitors to 30-61% from values of <10% in control mice given pyrogen-free saline ($p < 0.001$). 2000-5000 units of either CSF alone had no significant effect. Dosages of rhG-CSF and rhCSF-1 needed to significantly increase progenitor cell cycling ($p < 0.001$) were reduced by 100-200 fold when these CSFs were administered in combination at dosages of each which were not active by themselves. Effects noted were not due to endotoxin. Enhanced proliferative effects of these molecules in vivo may be due to effects directly and/or indirectly on the progenitors and may be of relevance to the efficacy of action of these molecules in clinical trials.

C 609 CYTOSTATIC ACTIVITY OF TGF-β1: IN VITRO AND IN VIVO STUDIES, Joseph J. Catino, William C. Rose, George A. Basler, Lillian J. McRae, Anna M. Casazza, Bristol Myers Company, Wallingford, CT 06492; Daniel R. Twardzik, and A. Purchio, Oncogen, Seattle WA 98121. Several biological effects of TGF-β1 have been reported, and it has been suggested that this growth factor can regulate tumor growth in vivo. To explore this possibility, we have first investigated several in vitro cell lines for their sensitivity to TGF-β1. Material from R&D Systems, or recombinant TGF-β1 was used. In vitro, the cytostatic activity was tested by measuring the inhibition of H^3- thymidine incorporation or of colony formation in soft agar. Similar inhibition was produced on the B16 melanoma and the A549 human lung carcinoma cell lines. At 5 ng/ml the % inhibition was 30% - 44% in the thymidine incorporation assay, and 91 - 69% in the colony assay, for the two cell lines, respectively. These data encouraged in vivo studies. The B16 melanoma was implanted sc into BDF1 mice, and the A549 into nude CD-1 mice. TGF-β1 was administered sc, by Alzet pump or by injection at the site of the tumor implant or a few cm distally. Treatment started on Day 0 (B16 experiments) or on Day 9 (A549 experiments) post-tumor implant. The following dose range was used: 0.01 to 0.8 µg/mouse/day or in repeated daily injections. A transient 45% inhibition of B16 melanoma growth was observed in one of two experiments at the dose of 0.1 µg/mouse/day (Alzet pump, 7 days exposure). In the A549 tumor model, 50% inhibition was seen in mice treated distally with 0.8 µg/mouse/day q2d x 7, and in mice treated locally at 1/10 of the aforementioned dose. These preliminary results suggest that TGF-β1 may have an inhibiting effect on human tumors in an in vivo setting. Further studies are needed to confirm and extend this original observation.

C 610 **INSULIN MEDIATED DECREASE IN THE CONCENTRATION OF CLATHRIN HEAVY CHAIN ASSOCIATED TO RAT ADIPOCYTE PLASMA MEMBRANES.** Silvia Corvera, University of Pennsylvania Medical School, Philadelphia, PA 19104-6082

Treatment of isolated rat adipocytes with insulin causes a rapid increase in the cell surface number of several low abundance membrane proteins, such as the glucose transporter, and the receptors for IGF-II/mannose-6-phosphate, transferrin, and alpha 2 macroglobulin. These three membrane receptors are internalized through clathrin coated pits at the plasma membrane, and continually recycle between the plasma membrane and a low density microsomal membrane pool. One of these receptors, the IGF-II/mannose-6-phosphate receptor is phosphorylated in vivo on serine and threonine residues. The receptors present on the plasma membrane are phosphorylated to a higher stoichiometry than those receptors present in the intracellular membrane pool; within the plasma membrane, a highly phosphorylated subpopulation of receptors can be identified. These receptors are tightly associated to a Triton-X-100 insoluble fraction of the membrane. Treatment of cells with insulin results in a marked decrease in the phosphorylation of these receptors. In recent experiments, immunobloting of plasma membranes with a monoclonal antibody specific toward clathrin heavy chain has shown: a) That all the clathrin associated to the plasma membrane is contained within the Triton X-100 insoluble fraction , and b) a marked decrease in the amount of clathrin heavy chain found associated to purified plasma membranes obtained from insulin treated cells. These data suggest that insulin can modulate the number or the composition of clathrin coated pits at the cell surface, perhaps through changes in the kinases or phosphatases present in these structures.

C 611 ROLE OF INTERLEUKIN 1 IN ACUTE MYELOID LEUKEMIA CELL GROWTH, Federico Cozzolino, Donatella Aldinucci, Maria Torcia, Anna Rubartelli, University of Firenze, Italy.

We studied Interleukin 1 (IL-1) production by leukemic cells from 37 Acute Myeloid Leukemia (AML) patients. We found that AML cells produced and released IL-1 in vitro in 20/37 cases. Immunofluorescence studies demonstrated the presence of intracytoplasmic protein in both releasing and non-releasing cells. In lysates from the latter cell type, however, no IL-1 biologic activity could be recovered. Since the identity between IL-1-α and hemopoietin 1, a cytokine active on normal myeloid precursor cells, has recently been demonstrated, we investigated whether the spontaneous proliferative activity of AML cells could be enhanced by the addition of exogenous IL-1. The results showed that in 6/8 cases a 3-10 fold increase of proliferation could be observed following the addition of either IL-1-α or IL-1-β. This effect was inhibited by monospecific anti-IL-1 antibodies. Fractionation of AML cells on Percoll gradients showed that high density, non-cycling cells were responsive to IL-1. These data suggest that the endogenous IL-1 could be involved, at least as a co-factor, in maintaining the neoplastic growth of AML cells.

C 612 **TRANSFORMING GROWTH FACTOR-β STIMULATES COLLAGEN SYNTHESIS in vitro, AND IS ELEVATED IN AN in vivo MODEL OF HEPATIC FIBROSIS.** MJ Czaja, FR Weiner, M-A Giambrone, and MA Zern. Liver Research Center, Albert Einstein College of Medicine, Bronx, New York

Transforming growth factor-β(TGF-β) is a cytokine produced by mononuclear cells which has been shown to induce collagen formation in fibroblasts. However, to our knowledge no previous study has investigated the association of this cytokine with hepatic fibrosis. Hepatocytes obtained from perfused rat livers were cultured in serum-free, hormonally defined medium. The cells were then treated with TGF-β(provided by Collagen Corporation, Palo Alto, CA) in picogram concentrations for 24 hrs. Total RNA was extracted from the cells and subjected to Northern and dot blot analysis. Hepatocytes treated with TGF-βshowed an increase of greater than thirteen-fold in type I procollagen mRNA levels. There was no significant change in the steady-state mRNA levels of β-actin or albumin in these hepatocytes. Given this cytokine's stimulation of collagen production in hepatocytes and fibroblasts, we determined whether TGF-βexpression was increased in an in vivo model of hepatic fibrosis, murine schistosomiasis. CF-1 mice were infected with 50 cercaria of S. mansoni and then sacrificed at eight weeks after infection, a time of maximal collagen synthesis. Total RNA was extracted from the livers and subjected to Northern blot hybridization with a TGF-β cDNA clone (a gift of Genentech, Inc. So. San Francisco, CA). Steady state levels of TGF-β mRNA were elevated more than forty-fold in the infected mice and nuclear run-on analysis indicated that this change was due to an increase in TGF-β gene transcription. No increase was found in interleukin-1 mRNA levels, another cytokine thought to stimulate fibrogenesis. These findings indicate that TGF-βstimulates collagen synthesis in vitro and that the synthesis of this cytokine becomes markedly increased in an in vivo model of fibrosis. It is tempting to speculate that TGF-β may play a significant role in hepatic fibrogenesis.

Growth Factors and Their Receptors: Genetic Control and Rational Application

C 613 RECOMBINANT GROWTH FACTORS ACCELERATE WOUND HEALING PROCESSES, Jeffrey M.Davidson, Anne Buckley, Gregory S. McGee, and Achilles Demetriou, Vanderbilt University, Nashville, TN 37232

Three major rate-limiting factors in the process of wound repair are the rate of formation of granulation tissue (GT). the accumulation of fibrillar collagen, and the formation of new blood vessels. GT repopulates areas of injury with a unique, myofibroblastic cell population which promotes or supports the neovascularization of these injury sites. Collagen produced by GT cells provides the extracellular architecture and tensile strength required for completion of repair. Polypeptide growth factors such as epidermal growth factor (EGF), basic fibroblast growth factor (bFGF; provided by A.Sommer, Synergen, Inc.), and transforming growth factors (TGFα and TGFß; provided by R.Derynck and B.Bear, Genentech, Inc.) elicited accelerated GT formation in a rat-polyvinyl sponge model of deep wound repair. EGF and TGFα only worked effectively if slowly released. Increased angiogenic activity was particularly apparent with bFGF treatment. Although these proteins, now produced as recombinant molecules, are best characterized as mitogens, they also possess potent chemoattractant properties, suggesting that recruitment of cells may play an important role in stimulation of the repair process. GT cell migration was vigorous to TGF-α and bFGF, but the response to bFGF was attenuated in cells from mature (d28) GT. The influence of these factors on collagen synthesis was usually null, and only TGF-ß under certain in vitro conditions showed any sign of a selective influence . The regulation of collagen deposition and remodeling could be rapidly adjusted in the degradative pathway, either by modulating collagenase or its activation, or by regulating the levels of the collagenase inhibitor, TIMP. For example bFGF, but not EGF, stimulated the production of latent collagenase in GT cells from early wounds, older wound cells having no collagenase activity. The vulnerary potential of these growth factors was further supported by the demonstration of increased tensile strength, breaking energy, and collagen content in an incisional wound model. Single injections into the wound site on day 3 after injury resulted in significant improvement of these parameters using either bFGF or TGFß. Collagen metabolism at the wound site was slightly increased by bFGF and reduced by TGFß at days 7, 14, and 21 after incision. These compounds may be of therapeutic value in cases of compromised or delayed healing.(Supported in part by USPHS, NIH grant AG06528, the Veterans Administration, and Synergen, Inc.)

C 614 LYMPHOKINE-MEDIATED ACTIVATION AND GROWTH OF SEZARY T CELLS FROM CTCL, Elaine DeFreitas, J. Todd Abrams, William Ju, Peter Nowell, and Eric Vonderheid, The Wistar Institute, Univ. of Pennsylvania, and Temple University, Phila., PA 19104

Cutaneous T cell lymphoma (CTCL) is a malignancy of CD4 helper T cells which appears first in the skin then slowly progresses to blood, lymph nodes and visceral organs. This group of diseases includes the leukemic erythroderma known as Sezary syndrome and the nonleukemic disease known as mycosis fungoides. The neoplastic T cell infiltrating skin, lymph nodes and blood are atypical large lymphocytes with a serpentine or cerebriform nucleus which express T cell activation markers and appear to be clonal in origin. Despite several efforts, no one has been successful in growing these cells from blood or skin of CTCL presumably due to functional defects in their activation or clonal expansion. We have been successful in growing, from the blood of 13 out of 15 Sezary Syndrome patients, long-term T cell lines and clones which have the phenotypic, karyotypic, or clonotypic characteristics of neoplastic Sezary cells. The requirement for their activation appears to be a lymphokine (here termed "Sezary activating factor"; SAF), which induces high affinity Interleukin 2 receptors (IL2R) on these cells. Addition of exogenous rec. IL2 results in their sustained proliferation. Neither IL1 alpha or beta, rec IL2, interferon gamma nor tumor necrosis factor substitutes for SAF. The sources, biochemical characterization, and relationship to other lymphokines will be discussed.

C 615 PHENYTOIN AS A PHARMACOLOGIC GROWTH FACTOR FOR CONNECTIVE TISSUE, Russell E. Dill, Walter L. Davis and James H. Martin, Baylor Dental College and Baylor University Medical Center, Dallas, TX 75246.

Accumulating evidence suggests that phenytoin (5,5-diphenylhydantoin), an often prescribed antiseizure drug, has several characteristics of a growth factor for connective tissue (c.t.) or a stimulus for wound healing. For example, phenytoin (PHT) has in vitro mitogenic effects on fibroblasts; in vivo proliferative effects (dose related) on visceral c.t.; is associated with accumulation of macrophages in PHT-induced hyperplastic c.t. [preliminary evidence indicates that PHT stimulates peritoneal macrophages to produce growth factor(s)]; and as presented here, induces differentiation of myofibroblasts in vivo. Four rats were given 100 mg/kg PHT by i.p. injection daily for 9 days and sacrificed on the 10th day. Samples of liver, spleen, and intestinal mesentery were processed for electron microscopy. Four control rats were similarly treated with the drug solvent (10% DMSO). Ultrathin sections of all specimens were examined on a JEOL 100 CX II electron microscope. All PHT-treated hyperplastic tissues were found to contain cells meeting the morphologic criteria for myofibroblasts, as opposed to the controls where the latter cells were infrequently seen. These observations provide further evidence that PHT mimics the stimulus of wounding as well as acting as a mitogen. Supported by a grant from Baylor College of Dentistry Research Fund.

C 616 INTERLEUKIN-1/HEMPOIETIN-1 EFFECTS ON EARLY HEMOPOIETIC CELLS. Peter P. Dukes, Kris M. Zsebo, Jette Wypych, Victoria N. Yuschenkoff, Hsieng Lu, Pam Hunt, Keith E. Langley, Andrew Ma and Curtiss Polk, Childrens Hospital of Los Angeles-USC, Los Angeles, 90027 and AMGEN, Thousand Oaks, CA 91320.

The action of human recombinant interleukin-1α (IL-1α) was investigated in several systems involving the growth of mouse bone marrow hemopoietic progenitor cells. Hemopoietin-1 was purified from the human bladder carcinoma cell line 5637. The preparation contained 2 components which by amino acid sequence comparison were found to be identical to IL-1α and IL-1β respectively. When IL-1α was present during a 2 day suspension culture of bone marrow cells the number of Day-12 CFU-S present at the end of the culture period was significantly higher than in controls with no addition. This effect also took place in an adherent cell depleted system suggesting that the growth factor has a direct survival enhancing effect on Day-12 CFU-S. Experiments utilizing the high proliferative potential colony-forming cell assay showed that the target cells of IL-1α in this system are not accessory cells and are different from the target cells of IL-3 and granulocyte-macrophage colony stimulating factor.

C 617 STRUCTURAL ORGANIZATION OF THE HUMAN ORNITHINE DECARBOXYLASE GENE, Margaret A. Flanagan and Mary C. Fitzgerald, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

An increase in ornithine decarboxylase (ODC) activity is tightly coupled to mitogen stimulation. This is presumably due to the role of ODC in the biosynthesis of polyamines, which are required for protein biosynthesis and DNA replication. This increase in activity has been shown to be regulated at the transcriptional, translational and post-translational levels. In order to better understand the signals leading to increased rates of transcription in response to mitogenic stimulation, we have cloned the human ODC gene using a human ODC cDNA clone as a probe (Hickok et al. (1987) Gene 6: 179). Genomic Southern-blots suggest that there is only a single gene hybridizing at high stringency, unlike the murine multigene family. Hybridizations at moderate stringency showed a small number of additional bands, as previously reported (Winqvist et al. (1986) Cytogenet. Cell Genet. 42: 133). A lambdaphage clone which hybridized to the cDNA probe at high stringency has been isolated from a human leukocyte library. Partial sequence analysis of an 11.1 kb Sal I fragment of this clone showed the presence of at least 12 exons with an average size of about 150 bp. The putative active site lysine (Lys 298) is in the ninth exon. There are several introns of 1 kb or greater which may have regulatory significance.

C 618 **INTERLEUKIN-1 IN PULMONARY HYPERTENSION.** M.N. Gillespie, D.A. Cohen, C.J. McClain, S.E. Goldblum, B. Hennig, and J.W. Olson. Univ. of Kentucky, Lexington, KY 40536.

Monocrotaline (MCT) causes edematous lung injury, pulmonary vascular hyperreactivity, enhanced lung ornithine decarboxylase activity (ODC), and progressive vascular remodeling leading to sustained pulmonary hypertension in rats. Because interleukin 1 (IL-1) exerts pro-inflammatory actions and is mitogenic for fibroblasts, both of which have been been implicated in hypertensive pulmonary vascular disease, we sought to determine if IL-1 could be involved with the adverse effects of MCT in rats. Bronchoalveolar lavage fluid contents of bioassayable IL-1 were increased at day 4 post MCT, had returned to control levels at day 7, and were increased again at days 14-20. These changes were temporally related to MCT-induced increases in lung ODC activity, pulmonary edema, and vascular hyperreactivity (days 4-7) and the later development of right ventricular hypertrophy (RVH) indicative of sustained pulmonary hypertension (days 14-20). Whereas murine monocyte-derived IL-1 administered to intact rats failed to increase lung ODC activity, the monokine caused a 100 fold increase in ODC activity in cultured porcine pulmonary vascular endothelial cells. IL-1 induced pulmonary edema in intact rats as evidenced by increases in both the lung wet-to-dry weight ratio and pulmonary extravasation of ^{125}I-bovine serum albumin and by light microscopic assessment. Lungs isolated from animals treated 3 hours previously with IL-1 exhibited depressed reactivity to angiotensin II while at 24 hours post-treatment, pressor responses to the peptide were markedly enhanced. Administration of IL-1 every other day for 14-16 days was associated with RVH suggestive of sustained pulmonary hypertension. These observations suggest that IL-1 accumulates in the lungs of rats with MCT-induced pneumotoxicity and that IL-1 mimics some of the pathophysiologic and biochemical changes associated with MCT-induced lung injury and pulmonary hypertension.

C 619 THE LEVEL OF SECRETED TGFα- LIKE ACTIVITIES IS CORRELATED WITH THE ONCOGENIC PHENOTYPE OF HUMAN TRANSFORMED CELLS IN NUDE MICE, Hédi Haddada [1], Christian Lavialle [1], Olivier Brison [2], Pierre May [1], and Arthur S. Levine [3], 1 - I.R.S.C. B.P. n° 8, 94802 Villejuif France, 2 - I.G.R., 94805 Villejuif, 3 - N.I.H., N.I.C.H.D., Bethesda, Md 20892 USA.

Transforming growth factors (TGFs) are believed to play an important role in determining many phenotypic characteristics of transformed cells. To determine whether the malignant phenotype is accompanied by high secretion of mitogenic TGFα-like factors, we have examined the activity of conditioned media from two sublines SW 613-S cl.4G and SW 613-Tu2 derived from the human breast cancer cell line SW613-S (Modjtahedi et al., Cancer Res., 45, 1985). SW 613-Tu2 cells had a 60 fold amplification of the c-myc oncogene and were tumorigenic in nude mice, whereas, SW 613-S cl.4G had a 3 fold amplification of c-myc and were not tumorigenic. Our data showed that conditioned media from SW 613-Tu2 cell culture contained a powerful TGFα-like factor which induced the growth of quiescent NRK cells and of human A549 cells and competed with ^{125}I EGF for receptor binding on NRK cells. Conditioned media from non tumorigenic SW 613-S cl.4G cells contained lower mitogenic activity. The mitogenic factor eluted from size exclusion HPLC columns with a molecular weight of 8 to 10 Kd. In addition, by immunoprecipitation, we found that EGF receptor proteins (which also binds TGFα) were more expressed in tumorigenic SW 613-Tu2 cells than in SW 613-S cl.4G cells. However, cells from both sublines bound weakly ^{125}I EGF and were poorly stimulated by addition of EGF in the media. These data suggest that these cells preferentially use, via an "internal" autocrine pathway, growth factors they produce. Overexpression of these factors my contribute to maintenance of cell transformation and to tumor-inducing capacity.

C 620 AUTOCRINE GROWTH STIMULATION OF HUMAN COLORECTAL CARCINOMA CELLS IS MEDIATED BY TRANSFORMING GROWTH FACTOR - ALPHA AND PLATELET-DERIVED GROWTH FACTOR, Meenhard Herlyn and Alban J. Linnenbach, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

Autonomous growth of colorectal carcinoma cells was demonstrated by long-term culture in a protein-free medium. Of eight growth factors tested for mitogenic activity, SW 707 colorectal carcinoma cells responded only to insulin and IGF I, with both factors acting via the IGF I receptor. On optimal concentrations of either IGF I (0.1 ug/ml) or insulin (1.0 ug/ml), the doubling time of cells (48 h) was similar to that of cells incubated in 2% fetal bovine serum although with a slower initial attachment rate. SW 707 cells secrete TGF-alpha, a PDGF-like factor, and TGF-beta, but not IGF I, IGF II, EGF, or FGF. Dissociation of receptor-bound ligand increased the number of EGF/TGF-alpha receptor sites from 3000 to 5000 per cell. Transcripts for the A and B chains of PDGF and for the EGF receptor but not the PDGF receptor were expressed. Antisera to PDGF and synthetic TGF-alpha inhibited growth of cells by 85% and 94%, respectively, indicating that both growth factors are secreted for autostimulation. These studies demonstrate that growth autonomy of colorectal carcinoma cells in culture is controlled by at least two growth factors, alpha-TGF and a PDGF-like factor.

C 621 BINDING, PROCESSING, AND EFFECTS OF IGF-II ON NORMAL AND MALIGNANT MAMMARY EPITHELIAL CELLS, Kenneth S. Hirsch[1], George W. Sledge Jr.[2], Robert A. Swift[1] and Julie F. Falcone[1], [1]Eli Lilly and Company, Indianapolis, IN 46285 and [2]Richard Roudebush V. A. Medical Center and Indiana University, Indianapolis, IN 46202.

The mechanisms through which IGF-II induces cellular proliferation were examined <u>in vitro</u> using two mammary carcinoma cell lines; (ER^+) MCF-7 and (ER^-) MDA-MB-231 (MDA) and a normal mammary epithelial cell line, HMEC-184. Both MDA and MCF-7 cells bind IGF-II in a time- and temperature-dependent fashion with the MCF-7 cells binding approximately 10 times more IGF than MDA cells. IGF-II binding appeared to be through the type-II receptor in both lines. HMEC-184 also exhibits IGF-II binding sites. MCF-7 and MDA cells exhibit both high affinity ($K_D \simeq 0.1$ nM) and low affinity ($K_D \simeq 5$ nM) IGF-II binding sites. HMEC-184, exhibited only the low affinity binding site. Processing of the IGF-II-receptor complex was more rapid in MDA cells as compared with MCF-7 cells. This may be important given the more rapid growth rate exhibited by the MDA cells <u>in vitro</u>. In the cancer lines a majority of the IGF-II binding sites appeared to be occupied by an endogenously produced ligand, suggesting that IGF-II may be acting as an autocrine growth factor. When MCF-7 and MDA cells were plated at different densities IGF-II binding was inversely proportional to cell number. This phenomenon correlates with observed growth patterns. All three cell lines express the mRNA for IGF-II. IGF-II mRNA levels were, however, low in the HMEC-184 cells. Unlike IGF-I which failed to stimulate thymidine incorporation by MDA cells, IGF-II stimulated DNA synthesis in both neoplastic cell lines, reaching a maximum at 25 ng/ml. IGF-II stimulated thymidine incorporation in HMEC-184 cells, though total DNA synthesis was relatively low. These data tend to support the hypothesis that IGF-II acts to regulate cell growth in breast cancer cells.

C 622 RECOMBINANT INTERFERON-γ INHIBITS THE MITOGENIC EFFECT OF PDGF AT A LEVEL DISTAL TO THE GROWTH FACTOR RECEPTOR, Markus Hosang, F.Hoffmann-La Roche & Co. Limited Company, CH-4002 Basle, Switzerland.

Highly purified preparations of recombinant human interferon (rIFNs) αA, β and γ all inhibited platelet-derived growth factor (PDGF)-induced DNA synthesis in normal human dermal fibroblasts. rIFN-γ was the most potent, since it blocked the PDGF response by 50% at about 10 U/ml or 0,3 ng/ml, whereas with rIFN-αA and rIFN-β 4000 U/ml and 600 U/ml, respectively (10 ng/ml in both cases), were required to achieve the same effect. There was a close parallelism between the ability of these rIFNs to inhibit PDGF activity and their capacity to inhibit cell proliferation in serum-containing medium. None of the rIFNs blocked specific binding of ^{125}I-PDGF to fibroblasts. The mechanism of action of rIFN-γ was analyzed further. This rIFN did not inhibit uptake of [^{3}H]-thymidine. However, if shifted the time point of initiation of DNA synthesis from about 14 h after stimulation with PDGF to about 21 h and decreased significantly the rate of DNA synthesis. rIFN-γ could be added up to 6 h following stimulation with PDGF with no loss of its inhibitory effect. rIFN-γ also blocked the mitogenic activity of epidermal growth factor and basic fibroblast growth factor. Taken together these results implicate that rIFN-γ exerts its antimitogenic effect by inhibiting a process that occurs late in the PDGF signalling pathway and onto which the activity pathways of other mitogens converge. Furthermore, they point to a possible regulatory role of IFN-γ in a number of processes in which PDGF is thought to be involved, including woundhealing and the formation of proliferative lesions of arteriosclerosis.

C 623 TWO PROTEIN INHIBITORS OF PHOSPHOTYROSYL-PROTEIN PHOSPHATASE, T. S. Ingebritsen, V.M. Ingebritsen and S.K. Lewis, Iowa State University, Ames, IA 50011.

Two phosphotyrosyl-casein phosphatase inhibitors, termed PTPI-I (Mr >500,000) and PTPI-II (Mr 36,000), have been detected in and partially purified from bovine brain extracts. Both inhibitors are proteins as judged by their inactivation by proteinase K and they exhibited remarkable stability during incubation at 95°. Of the seven phosphotyrosyl-casein phosphatase activities which have been isolated from bovine brain, PTP-4 and PTP-5 were most sensitive to the two inhibitor proteins. Inhibition of the other five phosphotyrosyl-casein phosphatatases was only observed at very high inhibitor concentrations. Inhibition of PTP-5 by PTPI-I or PTPI-II was rapid (maximum effect in <1 min) and readily reversed upon removal of the inhibitors by dilution. PTPI-I and PTPI-II are distinct from the three heat-stable protein inhibitors of protein phosphatase 1. Inhibitor activity with properties similar to PTPI-I and/or PTPI-II was detected in extracts prepared from rabbit brain, spleen, kidney, liver, heart and adipose tissue and from murine T and B cell lines. PTP-4 and/or PTP-5 activity was also detected in extracts from each of the tissues and cells and there was a strong positive correlation between inhibitor and protein phosphatase specific activities in the extracts. The highest activities were detected in spleen and in the T and B cell lines. The results suggest that the two inhibitor proteins may be physiological regulators of PTP-4 and PTP-5 activity and that these proteins may be involved in immune function. The ability of PTPI-I and PTPI-II to preferentially inhibit PTP-4 and PTP-5 provides an important new criteria which can be used to relate PTP-4 and PTP-5 to other phosphotyrosyl-protein phosphatases.

C 624 AUTONOMIC CONTROL OF GROWTH FACTOR SECRETION INTO RAT PROSTATIC FLUID, Stephen C. Jacobs, Michael T. Story and Russell K. Lawson.

We have determined that both EGF and bFGF are present in tissue extracts of rat ventral prostate. With castration both EGF and bFGF decrease as does prostatic weight and tissue acid phosphatase. Testosterone replacement brings both growth factors back to normal tissue concentration in parallel with the restoration of prostatic weight and acid phosphatase.

A model has been developed for collecting rat prostatic secretion. Ligation of the vasa deferentia , seminal vesicles, coagulating glands, and ureters was performed in 400 gm Sprague Dawley rats. Urethral catherization with silastic tubing allowed collection of the prostatic urethral fluid. Peristaltic pump perfusion at the bladder neck through silastic tubing of Tris .15 M NaCl with protease inhibitors provides enough volume to collect the prostatic secretion.

Intravenous epinephrine or phenylephrine stimulates growth factor release into prostatic fluid as measured by incorporation of 3H-thymidine into quiescent human foreskin fibroblasts. IV pilocarpine causes a minimal secretion of growth factor activity into rat prostatic fluid and this can be partially blocked with phentolamine. IV isoproterenol fails to stimulate prostatic secretion of growth factor activity. Using an RIA to mEGF, we have found that phenylephrine stimulates the secretion of EGF into rat prostatic fluid.

It appears that prostatic secretions of EGF and bFGF are primarily under -adrenergic control.

C 625 BASIC FIBROBLAST GROWTH FACTOR BINDING SITES IN OCULAR TISSUES DURING EMBRYONIC AND POST-NATAL MOUSE DEVELOPMENT, Jean Claude Jeanny, Nicole A. Fayein and Yves Courtois, INSERM U. 118, 29 rue Wilhem, 75016 Paris, France.
Fibroblast growth factors (aFGF and bFGF) can be extracted from bovine retina and brain. We have previously shown that they bind specifically to basement membranes of mouse embryonic eye structures such as lens capsule, inner limiting membrane of the retina... This is also true for chick, bovine or human capsules, walls of blood vessels and mouse EHS tumor. In order to investigate bFGF implication during mouse embryonic and post-natal development of the eye, we have studied autoradiographically, its binding to ocular tissues. Frozen sections of embryos (9 to 18 days), newborns and adults (1 day to 6 months) were incubated with iodinated bFGF. Two controls, unlabelled FGF, heparitinase were done to assess the specificity and the nature of the binding. There are two types of FGF binding patterns. One is on the proteoheparan sulphate of the basement membranes, which first appears at day 9 around the neural tube, the optic vesicles and below the head ectoderm and by day 14 of embryonic development is found in all basement membranes of the eye. At day 16, very intensely labelled patches appeared, corresponding to mast cells which have been characterized by metachromatic staining of their heparin rich granulations with Toluidin Blue. Beside the latter binding, which can be a storage form, we have always observed a general diffuse distribution of silver grains on all tissues and preferentially in the ecto- and neuroectodermic tissues. From day 17-18, there is heterogenous labelling inside the retina, localized in four different neural layers and in the pigmented epithelium. This one may correspond to a specific binding on cell receptors. Both types of binding patterns observed suggest a significant role for bFGF in eye development and physiology.

C 626 GENERATION OF TUMOR CYTOTOXIC LYMPHOCYTES IN RENAL CELL CARCINOMA, L. A. Kerr, H. Zincke, D. J. McKean, Mayo Clinic, Rochester, MN 55905.
We are selectively expanding cytotoxic effector lymphocytes with specificity for autologous renal cell cancer (RCC) from peripheral blood and tumor draining lymph nodes. Initial studies with PBLs from normal donors demonstrate after 7 days in culture moderate or significant responses to stimulation with anti-CD3 or IL-2 respectively with highly significant synergy when the two are combined. IL4+anti-CD3 are also significantly synergistic. Proliferation of resting PBLs to IL-2 and IL-4 is lower than with IL-2 alone. The effect on proliferation of IL-3 is small and additive in most cases. In cytolytic assays, IL-2 versus IL-2+anti-CD3 stimulated cells have equivalent functional capacity to mediate LAK-like and CTL killing. IL-4+anti CD3 activates predominantly a CTL response. Cells stimulated with anti CD3+ alone or with IL-2 or IL-4 are activated to coexpress high levels of mature T cell markers and the IL-2 receptor. Three out of five RCC draining lymph nodes proliferate less well than normal PBLs, but with a similar pattern. If the lymph nodes are initially depleted of a postulated suppressor cell population bearing surface OKM1 and Leu 7, proliferation is markedly enhanced. Taken together these results demonstrate the efficacy of employing different combinations of lymphokines to rapidly expand a population of cytolytic lymphocytes from peripheral blood or lymph nodes for immunotherapeutic protocols.

C 627 IN VIVO EFFECTS OF RECOMBINANT HUMAN GM-CSF ON HEMATOPOIESIS IN CYNOMOLGUS MONKEYS D. Krumwieh and F.R. Seiler, Behringwerke AG, Research Laboratories, P.O. Box 11 40, 3550 Marburg/Lahn, FRG
One of the major human hematopoietic growth and differentiation factors, the granulocyte-macrophage colony stimulation factor (GM-CSF)*, has been molecularly cloned from HUT 102**. The gene product has been expressed in E.coli and yeast and is available as a highly purified protein. To study the in vivo effectiveness of the glycosilated versus the non-glycosilated human GM-CSF, cynomolgus monkeys were treated for 10 consecutive days. Administration of 100 µg/kg GM-CSF resulted in a drastic increase in the peripheral white blood cells (WBC) up to 70×10^3 c/µl, a plateau was reached at day 5-6. This result could be achieved with both CSF's indicating that the main increase of WBC with respect to absolute granulocyte number is not dependent on glycosilation of the protein. Increase in different granulocyte subpopulations were studied by staining the cells with granulocyte specific monoclonal antibodies using the APAAP method. The potency of GM-CSF is independent of the administration route (i.v. or s.c.), as could be demonstrated within the same animals. These results indicate an effective way for clinical application of GM-CSF and its role in divers hematological deficiencies.

** 1. Cantrell et al., PNAS, Vol. 82, 6250 ff, 1985

* - GM-CSF is jointly developped by Immunex Corporation Seattle, USA and Behringwerke/ Hoechst AG, FRG

C 628 TRANSFORMING GROWTH FACTOR-α: MUTATION OF ASPARTIC ACID 47 AND LEUCINE 48 RESULTS IN DIFFERENT BIOLOGICAL ACTIVITIES. Eliane LAZAR[1,2], Shinichi WATANABE[1], Stephen DALTON[1], Rik DERYNCK[3] and Michael B. SPORN[1]. 1- NCI, NIH, Bethesda, MD 20892 ; 2 - IRSC, BP 8, 94802 Villejuif, France ; 3 - Genentech, South San Francisco, CA 94080.

To study the relationship between the primary structure of TGF-α and some of its functional properties (competition with EGF for binding to the EGF receptor and induction of anchorage-independent growth), we introduced single amino acid mutations in the sequence for the fully processed, 50 amino acid human TGF-α. The wild type and mutant proteins were expressed in a vector using a yeast α mating pheromone promoter. Mutations of two amino acids that are conserved in the family of the EGF-like peptides and are located in the carboxy terminal part of TGF-α resulted in different biological effects. When aspartic acid 47 is mutated to alanine or asparagine, biological activity is retained ; in contrast, substitutions of this residue with serine or glutamic acid generate mutants with reduced binding and colony forming capacities. When leucine 48 is mutated to alanine, a complete loss of binding and colony-forming abilities results ; mutants of leucine 48 to isoleucine or methionine have very low activities. Our data suggest that these two adjacent conserved amino acids in positions 47 and 48 play different roles in defining the structure and/or biological activity of TGF-α, and that the carboxy terminus of TGF-α is involved in interactions with cellular TGF-α receptors. The side chain of leucine 48 appears to be crucial either indirectly in determining the biologically active conformation of TGF-α or directly in the molecular recognition of TGF-α by its receptor.

C 629 **AUTOGENOUS GROWTH FACTORS: THEIR POSSIBLE ROLE IN THE GENESIS OF MESOTHELIOMA,** **J.F. Lechner, B.I. Gerwin, M. A. LaVeck, R.R. Reddel, A.N. Somers and C.C. Harris, Laboratory of Human Carcinogenesis, NCI, Bethesda, MD 20892.** **The growth factor requirements of normal human mesothelial (NHM) cells and a non-tumorigenic SV-40 T-antigen gene transformed "immortal" variant have been identified. Both cultures need insulin (INS), high density lipids (HDL) and any one of several growth factors, e.g., epidermal or fibroblastic growth factor, or interleukin 1 and 2, or transforming growth factor beta (TGF-β), interferon β and β', or platelet derived growth factor (PDGF). Growth stimulation by the latter 3 factor families was unexpected; interferons and TGF-β usually inhibit epithelial cell growth and epithelial cells were not known to have PDGF receptors. Since in the presence of INS and HDL many different factors are mitogenic, we are testing if putative autocrine growth factors play a role in the asbestos caused neoplastic transformation of NHM cells. We have found that mesothelioma cell conditioned media will support the growth of the same cells when cultured at low cell densities. In addition, whereas NHM cells do not have detectable levels of *sis*, IL-1 or TGF-α mRNAs, many mesothelioma cell lines produce *sis* mRNA (and a growth promoting activity that is neutralized by anti-PDGF antiserum), one expresses IL-1 mRNA, and TGF-α mRNA has been detected in several others. Thus, autogenous growth factor production appears to be a common characteristic of mesothelioma cells.**

C 630 **STIMULATION OF HEMATOPOIESIS IN CANINES BY IN VIVO ADMINISTRATION OF RECOMBINANT HUMAN GM-CSF (rhGM-CSF).** Thomas J. MacVittie, Michele M. D'Alesandro, Rodney L. Monroy, Ann Farese, Myra L. Patchen, Steven Clark, and Robert Donahue. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814 and Genetics Institute, Cambridge, MA. Recombinant human GM-CSF was injected subcutaneously (3X daily) for 4 consecutive days at various doses into normal healthy canines (beagles, 9-11 kg). Marrow-derived granulocyte-macrophage progenitors (GM-CFC) per 10^5 mononuclear cells and peripheral hematologic values [total white cells (WBC), neutrophils (PMN), monocytes, lymphocytes, platelets], were determined at selected intervals during and after the injection period. Fluorescent flow cytometry and chemiluminescence were used to measure transmembrane potential changes, intracellular hydrogen peroxide production and respiratory burst activity, respectively, in isolated peripheral blood PMNs. Total WBC increased gradually peaking at day 6 (2 days after cessation of rhGM-CSF injection, 4 x 10^5u/kg/day) at values 220% of baseline. PMN's (segmented and band forms) accounted for the increase. In contrast, platelets decreased to 30% of baseline after 4 days of the rhGM-CSF injection, remained at that level for 2d and gradually returned to normal levels by day 9. Marrow-derived GM-CFC increased to values 300% of baseline within 4 days, maintained that level of activity through 7 days and decreased to normal levels by day 10-14. Isolated neutrophils showed a variable response relative to increased biochemical activity throughout the administration of rhGM-CSF. Injection of rhGM-CSF into canines elicited significant increases in marrow granulopoiesis and peripheral neutrophils while inducing significant thrombocytopenia.

C 631 ROLE OF GRANULOCYTE MACROPHAGE STIMULATING FACTOR IN THE PROGESSION OF MYELOID LEUKEMIA CELL PROLIFERATION: AN AUTOCRINE PROCESS?, Patrice Mannoni*, Antonio Tabilio, Tom Mueller*, Marie J. Pebusque*, Franca Falzetti, and Francoise Birg*, [* Inserm U.119-Marseille (France) and University of Perugia (Perugia (Italy]. Colony Stimulatin Factor (CSF) are polypeptide hormone-like growth factors which regulate growth and differentiaion of haemopoietic cells comitted to myelo-monocytic patway. Acute myeloid leukemias (AML) can be seen as an expression of clonal population of myeloid cell which express an uncoupling of proliferation and differentiation. Therefore using an in vitro liquid culture method the response of leukemia cells to CSF and more precisely to human recombinant Granulocyte-Macrophage (GM-CSF) was investigated at several levels:
- 1 Biological responses of leukemic cells to the addition of GM-CSF in a liquid culture system. - 2 Expression of mRNA specific for GM-CSF in the leukemic blast cell population. - 3 GM-CSF activity in supenatants of leukemia cell cultures. We conclude from this study that : -1 Most of the myeloid leukemia cells studied (24 cases): respond to the addition of recombinant GM-CSF by an induction or an increase of proliferation without induction of differentiation. -2 The expression of the mRNA specific for GM-CSF was observed in high percentage of tested leukemias. -3 CSF like activity was found in the supernatant of cultured leukemic cells. This activity was assessed by the induction of CFU-GM colonies from normal bone marrow progenitors an by specific activities on GM-CSF responding cells. Thus these observations support the bypothesis that an autocrine mechanism involving GM-CSF is responsable for the clonal expansion of most of human acute myeloid leukemias.

C 632 A COMPARISON OF THE BIOLOGICAL ACTIVITIES OF PDGF-B_2 AND TGF-ß1 IN VIVO, J. McPherson, B. Pratt, Y. Ogawa, S. Sawamura and G. Ksander, Collagen Corporation, Palo Alto, CA 94303; M. Murray and J. Forstrom, Zymogentics Inc., Seattle, WA 98103.
Platelet-derived growth factor (PDGF-B_2) and transforming growth factor beta form 1 (TGF-ß1) were delivered to the guinea pig subcutis, over 8 days, using Alza mini-osmotic pumps. The animals were sacrificed; the pumps and associated fibrous capsules were surgically removed. At doses ranging from 0.1 to 10 ug/day, both factors promoted dose-dependent increases in: 1) wet and dry weights; 2) total protein; 3) collagen; 4) GAGs; and 5) DNA content of the capsules. At equivalent doses, TGF-ß induced signifcantly greater capsular dry weights and GAG content (primarily hyaluronate), whereas PDGF-B_2 promoted a more cellular response as judged by DNA content of the capsules. When normalized to DNA content, TGF-ß caused dose-dependent increases in capsular protein, collagen and GAG levels, while PDGF caused dose-dependent decreases in these same parameters. Histological observations confirmed biochemical analyses and further showed the increased cellularity of PDGF-induced capsules was partly due to increased levels of inflammatory cells in these capsules. Alcian blue stained sections of TGF-ß-induced capsules revealed relatively high levels of neutral GAGs which seemed to be associated with capsular edema. These results are consistent with in vitro activities reported for these factors.

C 633 ADENOSINE IS A GROWTH FACTOR FOR ENDOTHELIAL CELLS, Cynthia J. Meininger, Margaret E. Schelling and Harris J. Granger, Texas A&M Univ, College Station, TX 77843.
The proliferation of bovine aortic or coronary venular endothelial cells (EC) in vitro was stimulated by the addition of adenosine (0.5 µM or 5.0 µM) to the culture medium. Cell counts of adenosine-treated EC were 18-63% greater than non-treated controls. An even greater stimulation of EC proliferation was observed when the poorly metabolizable adenosine agonists, N^6-cyclopentyladenosine and 5'-(N-cyclopropyl)-carboxamido-adenosine (5 nM, 50 nM or 500 nM), were utilized. Cell counts of agonist-treated EC were 18-167% greater than non-treated controls. The catabolic byproducts of adenosine, inosine and hypoxanthine, did not stimulate endothelial cell proliferation when added at the same concentrations as adenosine (0.5 µM and 5.0 µM). The addition of the adenosine receptor blocker 8-phenyltheophylline (10 µM) prevented the stimulation of proliferation caused by adenosine, suggesting that adenosine mediates its effect via an external membrane receptor. A dose-response experiment for adenosine did not demonstrate a dose-dependent response of EC to adenosine stimulation, perhaps indicating that a maximal response had been achieved at the lowest dose. When the concentration of fetal calf serum in the culture medium was reduced to 4% (compared to 20% in the previous experiments) a clear dose-dependency could be seen. These results suggest that adenosine might potentiate other growth factors known to be present in fetal calf serum. The exact mechanism by which adenosine stimulates endothelial cell proliferation remains to be elucidated. (Supported by NIH grant #HL21498)

C 634 INTERNALIZATION AND LIMITED PROTEOLYSIS OF FIBROBLAST GROWTH FACTOR. LONG-TIME LIFE OF THE RESULTING PEPTIDES, Moenner Michel, Zaki LeTla, Badet Josette, and Barritault Denis, Université Paris XII, Dept Biotechnology, Avenue du Général de Gaulle - 94010 Créteil - France.

When incubated at 37 °C on Chinese hamster fibroblast (CCL39), ^{125}I bFGF bound to cells in such a way that this association became progressively insensitive to acidic (pH 2.5) or trypsic treatment. This indicates that the growth factor was internalized by these cells, presumably in accordance with the common schedules described for the interactions between other growth factors and target cells.

However, kinetic analyses of the cell-associated radioactivity showed that a substantial value of the initially internalized radioactivity remained intracellularly associated, even after a prolonged incubation time over 24 hours, and in normal growing conditions for cells. Analyses of this internalized radioactivity by $NaDodSo_4$-PAGE revealed the existence of labeled peptides issued from the processing of the native ^{125}I bFGF form (17 kD) and migrating respectively at 10 kD and 8 kD. Similar data were found using ^{125}I-labeled acidic FGF (15 kD), giving rise to 12 and 8 kD peptides.

In order to determinate the origin of this observation, ^{125}I bFGF was allowed to internalize at 37 °C with increasing concentration of non-labeled bFGF. Results indicated that up to one hour, bFGF was preferentially internalized by high-affinity receptors whose kD closely correlates with the one precedently described for the interaction at 4°C of the growth factor on Bovine Epithelial Lens cells and other cell lines including CCL39. This suggests that part of the ^{125}I bFGF associated with high affinity receptors may be consequently converted to 10 and 8 kD peptide forms. A hypothesis which is found consistent with the facts that i) The degraded forms of ^{125}I bFGF are readily detectable in cells incubated with the growth factor at 0.6 pM, a value 80-fold lower than the high affinity kD (50 pM) ii) the appearance of these peptides is independent of whether cells were or were not incubated with heparin or pretreated with heparinase.

Finally some well-known enzyme inhibitors were successfully tested on the degradation pattern of the bFGF, suggesting a possible way to interact with this processing, and to estimate its potential physiological relevance.

C 635 PHARMACOLOGICAL MODULATION OF RABBIT AND HUMAN CORNEAL ENDOTHELIAL CELL PHENOTYPIC EXPRESSION IN VITRO, Arthur H. Neufeld, Erika D. Matkin, Nancy C. Joyce and Marcia M. Jumblatt, Eye Research Institute, Boston, MA 02114.

A monolayer of endothelial cells on the posterior surface of the cornea is responsible for maintaining corneal transparency. To understand the cellular and molecular mechanisms involved in corneal endothelial function and repair, we studied the effect of epidermal growth factor (EGF) and indomethacin (INDO) on cultured rabbit corneal endothelial cells. Under basal conditions, confluent monolayer cultures exhibited a polygonal shape and polarized distribution of cellular organelles characteristic of cells in vivo. Rh-phallicidin staining of these cells revealed circumferential bands of F-actin at the cell periphery. In the presence of 10 µg/ml EGF and/or 1.0 µM INDO, confluent cells became markedly elongated, lost their polarity and tended to overlap one another - characteristics typical for cells migrating into a wound area. Such treatment also resulted in a redistribution of actin filaments into a diffuse cytoplasmic reticulum. Normal phenotypic expression was maintained in cells treated with EGF, INDO and 0.5 µg/ml PGE_2. Similarly, cultured human corneal endothelial cells elongated when grown in EGF and/or INDO. Cells phenotypically modulated with these agents appeared to produce more Type III collagen and fibronectin than polygonal cells. Some change in the relative production of Type IV collagen and laminin also appeared to occur after EGF or INDO treatment. Growth of human endothelial cells in the presence of EGF, INDO and PGE_2 resulted in retention of normal polygonal shape. Our results indicate that pharmacological agents can modulate the phenotype of cultured human and rabbit corneal endothelial cells and suggest that growth factors and drugs which influence the arachidonic acid pathway may be useful in treating corneal endothelial wounds.

C 636 A MONOCLONAL ANTIBODY INHIBITS EFFECTS OF IL-3 IN VIVO. P. Orban, H.J. Ziltener and J.W. Schrader. The Biomedical Research Centre, The University of British Columbia, Vancouver, BC, Canada.

Murine IL-3 is a hemopoietic growth factor whose only physiological source is the activated T cell. It is, however, unique in its breadth of activity, being capable of supporting the growth of colonies of all hemopoietic cells other than those committed to the lymphoid series. It has not been possible to detect endogenous IL-3 in vivo other than in situations of massive immune stimulation such as graft-versus-host disease, however, the aberrant autostimulatory production of IL-3 by hemopoietic cells has implicated the molecule in the pathogenesis of a number of murine leukemias. It has previously been shown that the presence in peripheral tissues of a localised nodule of the WEHI-3B myelo-monocytic leukemia, results in the stimulation of splenic hemopoiesis with the proliferation of various cells notably "IL-3 dependent" mast cells and their precursors. Here we show that a monoclonal antibody to IL-3 blocks this stimulation. The antibody-mediated inhibition supports the contention that the response is due to IL-3 released by the tumour, and suggests the possibility of the therapeutic potential of monoclonal antibodies in certain myeloid leukemias.

C 637 BIOLOGICAL ACTIVITIES OF NERVE GROWTH FACTOR BOUND TO NITROCELLULOSE PAPER BY WESTERN BLOTTING, B. Pettmann, J.A. Powell, M. Manthorpe and S. Varon, University of California San Diego, La Jolla, CA 92093.

A cell-blot technique was previously developed to visualize, directly in tissue extracts, molecules that display the biological activity of Ciliary Neuronotrophic Factors (CNTFs). This technique involves SDS-PAGE of the tissue extract, Western blotting on nitrocellulose paper, neuronal cell culture on the paper and, using a vital dye, visualization of the neurons which selectively survive on the trophic factor band.

In the present study, we show that 1) the 26 kD Nerve Growth Factor (NGF) dimer either purified or in a crude extract from submaxillary glands, can be successfully recognized using a slightly modified cell-blot technique -- thus, capable of operating after surface anchorage; 2) a variety of ganglionic neurons can be used to probe either NGF or CNTF in precise correspondence with their known responsiveness in more traditional neuronal cultures; 3) while CNTF and NGF both support the survival of their common target cells, only NGF also promotes neuritic extension.

Supported by NINCDS grant NS-16349 and the INSERM to B.P.

C 638 TGF-B INCREASES THE EXPRESSION OF FIBRONECTIN AND OF THE FIBRONECTIN RECEPTOR COMPLEX IN FIBROBLASTS. C.J. Roberts[1], T.M. Birkenmeier[1], J. McQuillan[1], S.S. Yamada[2], S.K. Akiyama[2,3], W.T. Chen[4], K.M. Yamada[2] and J.A. McDonald[1], Washington Univ., St. Louis, MO[1]; NCI/NIH[2]; Howard U.Med.Sch.[3], and Georgetown M.Sch.[4].

TGF-B dramatically increases fibronectin (FN) expression and deposition in matrix. Because we found the dimeric fibronectin receptor complex (FNR) is involved in FN matrix deposition, we hypothesized that TGF-B increases FN matrix assembly by increasing expression of the FNR as well as of FN. Using antibodies and cDNA probes to FN and to the alpha (A) and beta (B) subunits of the FNR, we found that TGF-B increased FNR and FN protein synthesis after 8 and 12 h, respectively. The increases were 10-30 fold compared with serum starved control fibroblasts by 48 h. Increased FN and FNR protein synthesis was accompanied by increases in steady-state mRNA levels. TGF-B increased FN mRNA levels 5, 10 and 15 fold compared to serum-starved control fibroblasts at 12, 24, and 32 hr respectively. The stimulatory effect of TGF-B (100pM) on FNR mRNA levels occurred within 4 h and peaked at 24 h. There were 3-fold increases in FNR-A and FNR-B mRNA levels. Actinomycin D (400ng/ml) blocked the TGF-B induced increases in the FN and FNR mRNAs. Cycloheximide (20mg/ml) also blocked the TGF-B mediated increase in FN mRNA. We conclude that TGF-B, a polypeptide growth factor implicated in embryogenesis and in the fibroproliferative response to tissue injury, increases synthesis of FN and of both subunits of the FNR. This increase is mediated via corresponding increases in their mRNAs and is in part transcriptionally regulated.

C 640 IL2-INDUCED GENES IN A CLONED T LYMPHOCYTE: EXPRESSION OF GLYCOLYTIC ENZYME mRNA FOLLOWING IL2 STIMULATION Daniel E. Sabath and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania

IL2 induced cDNA clones were isolated from a cDNA library by differential hybridization. Partial sequence information was obtained for four different clones. Three of the clones had strong similarity to the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase, triosephosphate isomerase, and non-neuronal enolase. The fourth was weakly similar to pyruvate dehydrogenase. When used as probes in RNA blot analysis, all clones hybridized to mRNA species that were induced by IL2 in the cloned T helper cell L2, with RNA levels increasing from 2 to 11-fold over levels in unstimulated cells. RNA levels were maximal at 24 hours after IL2 stimulation, when the cells were in late G_1 or early S phase of the cell cycle. Given the similar patterns of expression, and the functional relationships between these glycolytic enzymes, these findings suggest possible coordinate regulation of expression of these genes following IL2 stimulation.

C 641 CELL LINES DERIVED FROM HUMAN FIBROSARCOMAS AND HUMAN FIBROBLASTS TRANSFORMED IN CULTURE SPONTANEOUSLY OR AFTER CARCINOGEN TREATMENT EXHIBIT GROWTH FACTOR INDEPENDENCE, Robert J. Schilz, Veronica M. Maher, and J. Justin McCormick, Michigan State University, East Lansing, MI. 48824-1316.

Five cell lines derived from human fibrosarcomas, three cell lines from human fibroblasts transformed by carcinogen treatment and one spontaneously transformed human fibroblast cell line were tested for their ability to form tumors when injected into x-irradiated, athymic mice and for their ability to grow in serum-free medium (Ryan et al., **Exp. Cell. Res.**, 172: 318-328, 1987) containing low concentrations of Ca^{++} (0.1 mM), but no peptide growth factors. Four of the five sarcoma-derived cell lines and three of the other cell lines produced progressively growing tumors at the site of injection, but with variable latent periods. In the presence of a low concentration of calcium, all nine cell lines grew in serum-free medium in the absence of exogenous protein growth factors. Non-transformed cells would not replicate in this medium. Two distinct patterns of growth in response to exogenously added growth factors were observed in the nine cell lines tested. One class replicated as rapidly in the absence of growth factors as in the presence of 10% serum or when PDGF or EGF or insulin or Ca^{++} at 1.0 mM was added to the serum-free medium. The other class replicated in serum-free medium but still showed an increase in their rate of replication in response to exogenously added growth factors. These results suggest that growth factor independence is one of the changes that must be acquired by human fibroblasts in the process of malignant transformation. This work was supported by DOE Grant DE-F602-87ER-60524 and DHHS CA21289.

C 642 ANTIGENIC COMPONENTS OF A RAT C_6 GLIOMA CELL LINE AS REVEALED BY MONOCLONAL ANTIBODIES, Ela Sharma and Shail K. Sharma,All India Institute of Medical Sciences,New Delhi, India.

In an attempt to study the antigenic determinants that modulate with differentiation monoclonal antibodies have been raised to C_6 glioma cells. The ease with which this cell line can be experimently maipulated in culture into two different phases comprising the actively proliferating and the morphologically differentiated cells has been particularly useful in such studies. Indirect immunofluorescence studies revealed that the rapidly proliferating cells showed a uniform staining all over the cell body leaving the nucleus dark. Differentiated cells exhibited a gradient of fluorescence at one end of the cell inferring that differentiation is accompanied by polarization of the antigen. When differentiated cells were reverted back to the rapidly proliferating state the gradient was lost and the antigen redistributed uniformly. Antibodies did not cross react with variuos cell lines and normal rat tissues. A number of rat tissues including brain,kidney and lung when put in primary culture were positive suggesting that the antigen may be either appearing or being unmasked when the tissue is put in culture. The work on the biochemical characterisation of the antigen is in progress.

C 643 THE PRODUCT OF THE *ddd*$^{+}$ GENE OF *DROSOPHILA* IS REGUIRED FOR THE SYNTHESIS OF A FACTOR WHICH REGULATES THE GROWTH OF SPECIFIC IMAGINAL DISCS, Allen Shearn, Amanda Simcox, Cynthia Bennington, and Evelyn Hersperger, Department of Biology, Johns Hopkins University, Baltimore, Maryland,21218.

The lethal mutation of *Drosophila* which originally identified the *defective dorsal discs* gene, *ddd*^{L6}, causes the dorsal thoracic imaginal discs (wing, haltere, and humerus) to be extremely small or absent (Shearn et al., 1971). Mutant wing discs do not develop normally if transplanted into normal larvae. So, the expression of the mutation is disc-autonomous (Simcox et al., 1987). Wing disc mosaics produced by somatic recombination and wing disc chimæras produced by cell or nuclear transplantation do develop normally. So, the expression of the mutation is not cell-autonomous (Wurst et al., 1984; Simcox et al., 1987). These results have led us to propose that the *ddd*$^{+}$ gene product is required for the synthesis of a factor which regulates the growth of wing and other dorsal thoracic discs and that this factor can be transferred between cells of a disc. This poster describes the phenotype of *ddd* mutations, the results of nuclear transplants, and the use of an insertion mutation to clone the gene.

C 644 IDENTIFICATION AND CLONING OF A MURINE cDNA ENCODING PCNA/CYCLIN, AN INTERLEUKIN 2-RESPONSIVE GENE. P.M. Shipman, D.E. Sabath, P.G. Comber and M.B. Prystowsky. Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Proliferating cell nuclear antigen (PCNA)/cyclin is an acidic non-histone nuclear protein whose synthesis is increased and nuclear distribution changes as cells, including IL2-stimulated T cells, proliferate. A cDNA library constructed in λgt10 from polyA RNA of rIL2-stimulated clone murine T helper cells (L2 cells, in late G_1) was probed with a cDNA encoding rat PCNA/cyclin. Plaques (.01%) hybridized with the rat cDNA; these positive plaques were replated and rescreened. Phage DNA from 14 positive plaques was isolated and subjected to Southern blot analysis. Inserts from 6 plaques hybridized with the rat PCNA/cyclin probe. Clone H5.3 was subcloned into phage ml3mpl8, and the nucleotide sequence of 180 bases 5' and 254 bases 3' were determined. The 5' end was 84% and 79% homologous to the 5' end of rat and human PCNA/cyclin cDNA's, respectively, with the exception of a region spanning 30 bases not present in the murine cDNA. The 3' end was not homologous to coding or non-coding regions of either rat or human cDNA's. A Northern blot prepared from rIL2-stimulated L2 cells was probed with H5.3. Relative densitometric analysis demonstrated a 6-fold increase in PCNA/cyclin message 1h after stimulation and a maximal (32-fold) increase at 25h. At 9h, in the presence or absence of cycloheximide, the level of PCNA/cyclin message was stimulated 12-fold and 8-fold, respectively. Southern blot analysis of human genomic DNA, probed with H5.3, provided evidence that the PCNA/cyclin gene may exist as a single copy in the human genome.

C 645 RESPONSE OF PRIMARY HUMAN LUNG CARCINOMAS TO AUTOCRINE GROWTH FACTORS PRODUCED BY A549 LUNG CARCINOMA CELLS, Jill M. Siegfried, Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27711.

The effects of medium conditioned by the lung carcinoma cell line A549 on cells derived from primary lung carcinomas were examined. Conditioned medium (CM) was added to cultures of minced tumor tissue at a concentration of 50%, and used to produce primary colonies of tumor cells. These cells were harvested and used in quantitative assays to demonstrate growth stimulation by CM. Both colony formation in monolayer culture and in agar culture were measured. Using CM, primary cultures were obtained in 20/21 cases (adenocarcinoma, squamous cell carcinoma, and large cell carcinoma). Subcultures were obtained in 12/21 cases to date, and cell lines were established in 5 of these 12. These cultures contained neoplastic epithelial cells as evidenced by production of keratin, presence of desmosomes, tumorigenicity in immunodeficient mice, and expression of tumor markers (altered lactate dehydrogenase isoenzyme patterns, and synthesis of pregnancy-specific beta peptide 1 and ACTH). Addition of CM in colony-forming assays produced a 50-100% increase in colony forming efficiency (CFE) in monolayer culture and a 2-5 fold increase in CFE in soft agar culture. Experiments are in progress to compare the growth-stimulating effects of crude CM to the growth factors TGFα and IGF-1 which have been previously detected in medium conditioned by A549 cells. This is an abstract of a presentation and does not necessarily reflect EPA policy. Performed under contract 68-02-4456 from the U.S. EPA.

C 646 EPIDERMAL GROWTH FACTOR ENHANCES SERUM-FREE GROWTH OF PRIMARY AND METASTATIC HUMAN TUMORS, S. Eva Singletary, Didier Frappaz, Susan L. Tucker, Lily Larry, William A. Brock and Gary Spitzer, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030. The effect of epidermal growth factor (EGF) on the in vitro growth of human malignant tumors was compared in serum-supplemented (n=54) and serum-free (n=41) media at clonal density to determine the true EGF dependency of tumors. In the complete absence of serum at a 2,000 cells/ml seeding inoculation (approximately 200-400 adherent cells), EGF increased growth by greater than 50% in 27 of 41 specimens (66%) and in 18 of these EGF-sensitive tumors, by 100% or more. In 12 serum-free cultures (29%), no in vitro growth occurred without EGF. With 10% serum supplementation and a lower cell density (500 cells/ml), EGF increased growth by greater than 50% in 34 of 54 specimens (63%), of which 25 had more than a 100% increase. The maximum growth induced by EGF in serum was usually seen in those tumors already capable of moderate in vitro growth. No difference in response to EGF was detected between specimens from primary tumors (n=24) or from metastases (n=30). Under these stringent culture conditions of complete absence of serum and with tumors seeded at a low cell number, EGF is required by most primary or metastatic human tumors to successfully establish and sustain short-term in vitro growth.

C 647 EFFECT OF EPIDERMAL GROWTH FACTOR (EGF) AND PLATELET-DERIVED GROWTH FACTOR (PDGF) ON FRESH HUMAN BRAIN TUMORS, Didier Frappaz, S. Eva Singletary, Gary Spitzer and William A. Brock, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

The growth response of fresh human brain tumors (n=20) to EGF (0-100 ng/ml) and PDGF (0-20 ng/ml) was examined at low cell density (250 to 2,000 cells/ml) in both serum-free and serum-supplemented media using the adhesive tumor cell culture assay (1). In serum-free conditions, a 100% growth increase by EGF was observed in 16/20 tumors (80%): 5/8 glial tumors, 2/3 meningiomas, 0/2 neuroectodermal tumors and 7/7 brain metastases. In 10% serum, this EGF effect was less obvious, with a 100% growth increase seen in only 4 tumors (20%). No difference in EGF response was detected based on the tumor's histologic grade. Serum-free, PDGF increased growth by 100% or more in 2/2 glial tumors, 1/2 meningiomas, and 2/2 neuroectodermal tumors. With serum, PDGF's effect was seen only in 2 tumors. The combination of EGF and PDGF did not increase growth more than either factor alone. Most human brain tumors, primary and metastatic, can respond to EGF, and to a lesser extent PDGF, in serum-free short-term culture.

Baker, F.L. et al: Drug and radiation sensitivity measurements of successful primary monolayer culturing of human tumor cells using cell-adhesive matrix and supplemented medium. Cancer Res., 46:1263, 1986.

C 648 GROWTH FACTOR EFFECTS ON HUMAN MAMMARY EPITHELIAL CELLS IN CULTURE, Martha R. Stampfer, Midori Hosobuchi and Myriam Alhadeff, Lawrence Berkeley Laboratory, U. California, Berkeley, CA 94720.

Human mammary epithelial cells (HMEC) in culture can display active, long term growth in a serum-free medium containing several growth factors [insulin (I), EGF, hydrocortisone (HC), bovine pituitary extract (BPE), ethanolamine, phosphoethanolamine, transferrin, and isoproterenol]. HMEC from over 20 individual donors have been examined, and all grow rapidly (doubling times of 18-30 hours) for around 45-70 population doublings (11-22 passages). Normal HMEC, as well as cells transformed to extended life or immortality following *in vitro* exposure to benzo(a)pyrene, have been tested for response to removal of specific growth factors and addition of TGF-β. For normal HMEC, removal of individual factors (I, HC, BPE) results in a gradual increase in doubling times. EGF removal from mass cultures has only a small effect on number of or time for population doubling. However, in clonal assays, EGF removal results in almost no HMEC growth. This difference is likely due to the synthesis of TGF-α mRNA and protein by normal proliferating HMEC. The immortally transformed cell lines show different responses to growth factor removal, and subpopulations can be isolated which grow rapidly in media without one or more factors. In cases where removal of multiple factors results in little or no growth of the immortal cell lines, treatment with ENU has yielded populations which grow well under these more stringent conditions. The different HMEC populations have been tested for their response to TGF-β. While normal HMEC show a reversible growth inhibition, populations of the immortally transformed cells are largely resistant to TGFβ growth effects. The relationship of TGF-β inhibition to cell age in culture, cell cycle, and the presence of other growth factors is currently being explored. Southern blot analysis using probes for specific oncogenes has shown no major genomic differences among these HMEC. The expression of specific oncogenes in these cells is also being assessed; thus far we have found that the normal proliferating cells express mRNA for c-k-ras.

C 649 AUGMENTATION OF RESPONSE TO MELANOMA GROWTH STIMULATORY ACTIVITY (MGSA) WITH INSULIN-LIKE GROWTH FACTOR-1, H. Greg Thomas, Eddie Balentein, Michael P. Kelleher and Ann Richmond, V.A. Medical Center and Emory University, Atlanta, GA. 30033.

Melanoma growth stimulatory activity (MGSA) is an endogenous growth factor which functions through an autocrine mechanism by augmenting the growth of the Hs294T human melanoma cell line. Two bioassays have been developed for MGSA. One monitors stimulation of ^{3}H-thymidine incorporation into DNA of low density Hs294T cells cultured in serum-free medium. The second assay monitors stimulation in cell number in low density serum-free cultures of the Hs294T cell line. Other growth factors do not mimic the effects of MGSA in the ^{3}H-thymidine assay (Richmond et al., Cancer Res. 42, 1982; Richmond and Thomas, J. Cell. Biochem., 1987 in press). Using the standard MGSA ^{3}H-thymidine bioassay, recombinant IGF-1 does not as a single agent stimulate ^{3}H-thymidine incorporation in Hs294T cells. However, ng quantities of IGF-1 were capable of augmenting the response of low density cultures of Hs294T cells to exogenous MGSA. Similarly, IGF-1 is capable of stimulating an increase in Hs294T cell number after a six day exposure, presumably by augmenting the response to endogenous MGSA produced by these cells in culture. Studies with ^{125}I-IGF-1 demonstrate specific binding of this ligand to receptors on Hs294T cells. MGSA does not compete with ^{125}I-IGF-1 for binding to IGF-1 receptors. Neither platelet-derived growth factor nor interleukin-1 demonstrates a capacity to augment the response as IGF-1 does. These data suggest that MGSA and IGF-1 may stimulate the growth of Hs294T human malignant melanoma cells through separate but complementary mechanisms.

C 650 MESODERM INDUCTION BY HEPARIN BINDING GROWTH FACTORS AND TRANSFORMING GROWTH FACTOR-ß, Heinz Tiedemann[1], Horst Grunz[2], Wallace L. McKeehan[3], Walter Knöchel[1], Jochen Born[1], Peter Hoppe[1], Beate Loppnow-Blinde[1] Hildegard Tiedemann[1] and Rüdiger Volk[1], Freie Universität Berlin, D-1000 Berlin 33, FRG[1], Universität GHS Essen, D-4300 Essen 1, FRG[2], W. Alton Jones Cell Science Center, Inc., Lake Placid, NY 12946[3].
A vegetalizing factor inducing mesodermal and endodermal tissues in amphibian gastrula ectoderm has been isolated from chicken embryos employing heparin-sepharose affinity chromatography (Cell Differentiation 21, 131).When acidic and basic heparin binding growth factors were tested, they induced muscle, coelomic endothelia, blood cells and mesenchyme, depending on secondary interactions. Using synthetic oligonucleotides as probe the Xenopus gene encoding a FGF has been isolated from a genomic DNA library and partially sequenced. - TGF-ß from human platelets (2 µg/ml, 97% pure), which in some chemical properties seems to be closely related to the vegetalizing factor, induced in ectoderm of Triturus alpestris mostly smaller areas of endothelia, mesenchyme, blood cells and clusters of cells alike intestine cells. It is likely that factors which in normal development promote the differentiation of mesoderm and endoderm are not identical with, but related to the heparin binding growth factors and TGF-ß. NGF, EGF, crude PDGF, bombesin and insulin did not induce. (Supported by Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie and US Public Health Service).

C 651 STRUCTURE AND REGULATION BY MALIGNANT TRANSFORMATION OF THE GENE FOR MEP: A SECRETED LYSOSOMAL PROTEASE, Bruce R. Troen and Michael M. Gottesman, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
The major excreted protein (MEP) of mouse fibroblast cells is the 39 kDa precursor to a cathepsin induced by malignant transformation, growth factors, and tumor promoters. The MEP cDNA sequence suggests that MEP is the mouse analog of human cathepsin L. We have placed the MEP cDNA in a eukaryotic expression vector and demonstrated the production of the 39 kDA polypeptide form of mouse MEP in monkey CV-1 cells. A cosmid clone from NIH-3T3 cells, containing the complete mouse MEP gene, has been isolated and transfected into monkey kidney (CV-1) cells and human epidermoid carcinoma (A431) cells. Both the transfected CV-1 clones (CVMEP) and A431 clones (A4MEP) produce mouse MEP that is an active cathepsin that is secreted, glycosylated, and processed intracellularly to lower molecular weight forms as in the wild-type NIH-3T3 cells. Both Northern and primer extension analysis demonstrate that the MEP mRNA's from both mouse cells and stably transfected human cells are the same size and have the same single major site for initiation of transcription. These studies indicate that the mouse MEP promoter is intact on the cosmid clone. The sequence of the MEP promoter contains both typical and atypical elements upstream of the site of initiation of transcription. pSVOCAT vectors containing the MEP promoter exhibit CAT activity in both mouse and primate cells. We are presently subcloning different regions of the MEP promoter to isolate those sequences responsible for TPA and PDGF responsiveness. These results demonstrate that we have isolated both a functional mouse gene and an intact promoter for a transformation-sensitive lysosomal protease.

C 652 EPIDERMAL GROWTH FACTOR STIMULATES CALCIUM INFLUX VIA VOLTAGE SENSITIVE CALCIUM CHANNELS IN CULTURED HUMAN FIBROBLASTS. Mitchel L. Villereal and Gordon A. Jamieson, Jr. University of Chicago, Chicago, IL, 60637.
Previous data indicated that epidermal growth factor (EGF) stimulates a rise in intracellular Ca activity in cultured human fibroblasts which is strictly dependent upon the presence of extracellular Ca. We have investigated the possibility that EGF activates voltage-sensitive Ca channels (VSCC) in cultured human fibroblasts. Although VSCC have been studied frequently in excitable tissues, they have been reported only recently to exist in cultured fibroblasts. Patch clamp studies in mouse 3T3 fibroblasts and human foreskin fibroblasts (Chen and Hess, Biophys.J. 51:226a,1987) and ^{45}Ca influx studies in human foreskin and lung fibroblasts (Villereal et al. J.Cell Biol. abstract, 1987) indicate the existence of slowly inactivating, dihydropyridine-sensitive channels activated at positive potentials (L-type channel). We report now that EGF stimulates a ^{45}Ca influx in serum deprived human foreskin fibroblasts. Influx appears to be via an L-type VSCC and EGF stimulates ^{45}Ca influx approximately 4-fold over that observed under basal conditions. This compares favorably to the 3-fold increase of ^{45}Ca influx seen in high K medium, the 2-fold increase seen with the addition of the Ca channel agonist Bay K 8644, and the 5 to 6-fold increase seen with the combination of high K_+ and Bay K 8644. EGF stimulated ^{45}Ca influx, as well as the high K and Bay K 8644 stimulated ^{45}Ca influx, is blocked by the addition of the Ca channel antagonist nitrendipine. The dose response curve for nitredipine inhibition of EGF-stimulated ^{45}Ca influx indicates that half-maximal inhibition occurs at 1 nM. These data suggest that L-type VSCC channels exist in cultured human fibroblasts and can be activated in response to physiological stimuli.

Oncogenes and Cell Growth

C 700 EFFECTS OF X IRRADIATION ON EXPRESSION OF SOME PROTOONCOGENES IN REGENERATING RAT LIVER. Kouichi Asami, Chidori Muraiso and Hiromichi Matsudaira. National Institute of Radiological Sciences, Chiba-shi 260, Japan.

Cells usually show pleiotypic responses when stimulated by their growth factors. Some of the responses will be related to initiation of DNA synthesis, but others may not. Suitable techniques will afford a clue to resolve the relationships among the responses. We have been analyzing inhibition of DNA synthesis in the regenerating liver by X irradiation. X rays with a dose of 4.8 Gy inhibited almost completely incorporation of [^{3}H]thymidine into DNA, while with 1.9 Gy inhibited it partially. These doses of X rays affected in a dose-dependent way, phosphorylation of histone H1 occurring at the onset of DNA synthesis, but not that of HMG 14. Phosphorylation of histone H1, thus, will be closely related to DNA synthesis.

Some oncogenes are expressed during pre-replicative phase of liver regeneration. We hepatectomized partially just after partial-body irradiation and extracted total RNA at various times after partial hepatectomy. Purified RNA was examined on the expression of some proto-oncogenes with Northern blot hybridization. Expression of Ki-*ras* and Ha-*ras* increased markedly from 8 to 15 hrs after hepatectomy, while DNA synthesis started at about 24 hr. The increase in mRNA levels of both oncogenes was inhibited by 4.8 Gy of X rays. Expression of c-*fos* which was maximal just after partial hepatectomy was not not inhibited by X rays but rather enhanced. These results suggest that expression of Ki-*ras* and Ha-*ras* have close relationship with onset of DNA synthesis.

C 701 LOCALIZATION OF *Evi-2* TO CHROMOSOME 11: LINKAGE TO OTHER PROTO-ONCOGENE AND GROWTH FACTOR LOCI USING INTERSPECIFIC BACKCROSS MICE. Arthur M. Buchberg[1], Hendrick G. Bedigian[2], Benjamin A. Taylor[2,1], Elise Brownell[1], James N. Ihle[1], Shigekazu Nagata[3], Nancy A. Jenkins[1] and Neal G. Copeland[1]. [1]BRI-Basic Research Program, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD, 21701. [2]The Jackson Laboratory, Bar Harbor, ME, 04609. [3]Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-Ku, Tokyo 108 Japan. A common site of ecotropic murine leukemia virus integration designated *Evi-2* (ecotropic viral integration site-2) has been identified in BXH-2 myeloid tumors. As part of experiments to determine whether *Evi-2* identified a new proto-oncogene locus involved in myeloid disease, we determined its chromosomal location. We mapped *Evi-2* to mouse Chromosome 11 using standard recombinant inbred strain and genetic backcross analysis. We then determined the location of *Evi-2* relative to other proto-oncogene and growth factor loci located on Chromosome 11 by interspecific backcross analysis. The loci included in this study were the proto-oncogene loci, *Erbb*, *Erba*, and *Rel*, as well as *Il-3* (interleukin-3), *Csfgm* (granulocyte-macrophage colony stimulating factor), and *Trp53-1* (transforming protein p53). All loci except *Erbb* had been previously mapped to Chromosome 11 with the use of somatic cell hybrids and consequently their positions on Chromosome 11 are not known. One proto-oncogene, *Erbb-2* (analogous to the *neu* proto-oncogene), and one growth factor locus, *Csfg* (granulocyte colony-stimulating factor), which had not been mapped in the mouse were also localized on Chromosome 11 using the interspecific backcross mice. This study revealed a number of interesting conserved linkage groups common to mouse and man.

C 702 **CHARACTERIZATION OF GENOMIC CLONES CODING FOR HUMAN LCK PROTEIN TYROSINE KINASE,** Evelyne Rouer, Marie-Claude Lang, Remi Fagard, Siegmund Fischer, Richard Benarous, INSERM U.15, 24 rue du Fg St Jacques, 75014 PARIS, France.

Lck is a member of the src family protein tyrosine kinase, highly expressed in lymphoid cells and in particular in T lymphocytes. Lck cDNA was cloned in mouse and human (1,2,3). It codes for a Protein Tyrosine kinase of 56 KD highly similar to 60 scrc. As was shown by us and others p56 lck is overexpressed in several human and murine lymphoma and leukemia. To study the regulation of the expression of the human lck gene in normal and pathological conditions we have undertaken its genomic cloning. Using a 700 bp fragment located at the 3' end of human Lck cDNA (from A. Veillette) we screened a human genomic library constructed in the phage EMBL4. We have isolated a 17 kb clone and performed a partial restriction map. From these results it appears that the 5' end of this clone maps near the ATG translation start site, while the 3' end is located beyond the 3' end of Lck mRNA. We have isolated a second genomic clone with 5' regulatory sequences upstream from the transcription start site. We are presently mapping the different exons. As expected, preliminary results indicate that the organisation of the human Lck gene is very similar to that of the human c-src gene.

References
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C 703 EFFECTS OF TUMOR NECROSIS FACTOR-α AND RETINOIDS ON VARIOUS MYELOID LEUKEMIC CELLS.

Rune Blomhoff, Marie Botilsrud, Tone Bjerkelund, Karl-Olaf Wathne, Heidi Kiil Blomhoff and Kaare R. Norum. Inst. for Nutr. Res., P.O.Box 1046, University of Oslo, 0316 Oslo 3.

We have studied the effect of TNF-α and retinoids on HL-60 cells, U937 cells and two subclones of these cells (HL-60/OSL and U937/GTB). In the cell lines HL-60 and U937, retinoids cooperated with TNF-α in inducing differentiation as well as growth inhibition. The growth of the subclones HL-60/OSL and U937/GTB was effectivly reduced in the presence of retinoids in the medium. However, retinoids had no effect on the differentiation of these cells. Northern analysis of c-myc showed no downregulation of c-myc in the subclones, although cell proliferation was reduced by retinoids. Furthermore, TNF-α was more than 100 times as effective in inhibiting the growth of HL-60/OSL and U937/GTB when compared to HL-60 and U937.
These results suggest that retinoids might inhibit the proliferation of cells without inducing a downregulation of the mRNA for c-myc. In addition, since two different subclones that did not respond to retinoids by differentiation also was found to be hypersensitive to TNF-α, one might speculate that the mechanism of action of retinoids and TNF-α is integrated.

C 704 RAS GENE MUTATIONS AND HUMAN MALIGNANCIES. Johannes L. Bos, Sylvius Laboratories, P.O.Box 9503, 2300 RA Leiden, The Netherlands

The presence of activated ras genes in various human malignancies was determined by means of synthetic oligonucleotide hybridization after in vitro amplification of relevant ras sequences (PCR). Of each type of malignancy 30-100 samples were screened for mutations in codons 12 and 61 (H-, K- and N-ras) and codon 13 (K- and N-ras). We have found mutated ras genes in colon carcinomas (40%, mostly K-ras), lung adenocarcinomas (20%, mostly K-ras), acute myeloid leukemias (20%, mostly N-ras), seminomas (20%), melanomas (20%) and myelodisplastic and myeloproliferative syndromes (<10%). No ras gene mutations were found in cervical cancer, ovary tumors, glyoblastomas, CML and follicular lymphomas. These results demonstrate that activated ras genes do occur rather frequenctly in certain human malignancies, in particular in colon carcinomas, whereas they are absent in others. Analysis of premalignant precursor tissues such as colon adenomas, indicates that the mutational event can occur relatively early but also late in the course of tumorigenesis. These findings will be discussed in terms of the role activated ras genes can play in the initiation of tumor development as well as in progression.

C 705 DIFFERENTIAL PROMOTER UTILIZATION BY THE c-myc GENE IN CLONED AND FRESHLY ISOLATED T CELLS. H.E. Broome, J.G. Monroe, P.A. Scherle, K. Yui, M.B. Prystowsky, and R.G. Hoover. Department of Pathology, University of Pennsylvania Medical School, Philadelphia, PA 19104-6082.

Transcription of the c-myc gene initiates from two principal promoters, P1 and P2. The relative utilization of the two promoters can be quantified by S1 nuclease analysis of steady-state mRNA levels and expressed as a P1/P2 ratio. We previously reported that a shift in promoter utilization occurred in human peripheral blood mononuclear cells (PBMC) that were stimulated to proliferate with PHA. We now report that a similar shift occurred in murine T cells freshly isolated from splenocytes. The P1/P2 ratio reached a maximum of approximately 1.1 at 4 hours after ConA stimulation and a minimum of 0.35 at 48 hours. At certain times after an initial ConA stimulation, adding IL2 to the T cells increased c-myc mRNA, but the P1/P2 ratio stayed less than 0.35. Cloned, antigen-dependent, and IL2-dependent murine T cell lines responded differently. Stimulation with either antigen + APC, ConA + phorbol ester, or IL2 resulted in increased c-myc mRNA, but the P1/P2 ratio stayed less than 0.35. These data suggest that c-myc gene expression differs between cloned and freshly isolated murine lymphocytes.

C 706 EXPRESSION OF TRANSFECTED PDGF A- AND B-CHAIN cDNA IN RAT-1 CELLS AND HUMAN FIBROBLAST, Margaret Bywater, George Mark, Carl-Henrik Heldin, Bengt Westermark and Christer Betsholtz, Department of Pathology and Ludwig Institute for Cancer Research, Uppsala Sweden and National Cancer Institute, Bethesda, U.S.A..

The role played by PDGF-like molecules in autocrine stimulation of cell growth and neoplastic transformation has still to be defined. Since the two chains of PDGF are frequently independently expressed in human tumour cells, we have attempted to elucidate the effect of the single and constitutive expression of the A and B chain genes under the control of a strong viral promoter in Rat-1 and human fibroblasts. Viral constructs containing a cDNA of the separate genes and including a G-418 resistence gene were transfected into producer cell lines and the supernatent used to infect Rat-1 and human fibroblasts in culture. Cellular clones exhibiting constitutive expression of the genes showed a distinct change in the phenotype. Whereas the morphological change in the B transfectants resembled closely that of Simian Sarcoma Virus transformed cells,the A-chain transfectants were morphologically unaltered. Both grew,however,to a greater terminal cell density.The transformed phenotype of the B-transfectants reverted on the addition of suramin,indicating an autocrine stimulation. Studies on the bio-synthesis of protein molecules in Rat-1 transfectants indicated the production and secretion of a 31K A-chain homodimer in the A-chain transfectants and an intra-cellular dimeric 24K,as well as,a secreted slightly heterogeneous 30K B-chain product. Neither of the PDGF-chain constructs conferred an immortal phenotype or otherwise changed the life-span of the human fibroblasts.

C 707 ABNORMAL EXPRESSION AND REGULATION OF AN ALTERED fos GENE, Gillian S. Charles and Natalie M. Teich, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, England.

A cell line has been established from a spontaneously arising mouse osteosarcoma in which one allele of the proto-oncogene fos has been disrupted by an interchromosomal recombination event. An insertion has occurred in the last exon of fos: this is the same region where the v-fos genes of the osteosarcomagenic viruses FBJ-MSV and FBR-MSV differ from c-fos. These three are the only examples of altered fos genes and interestingly all have occurred in osteosarcomas.
In most cells c-fos is not expressed but is inducible by a variety of mitogenic agents including serum and cell-type specific growth and differentiation factors. However, this osteosarcoma cell line constitutively expresses altered fos mRNA and protein. The unaltered c-fos allele is not expressed but can be induced by serum with the expected kinetics, but the altered allele is not superinduced at the same time, suggesting independent regulation of the normal and altered fos alleles.
As the three examples of altered fos result in immortalized cells with high growth capacity, whereas fos is otherwise constitutively expressed in terminally differentiated cells only, deregulation of fos may play an important role in the modulation of specific requirements for growth factors.

C 708 CELLULAR AND MOLECULAR CHARACTERISTICS OF UV-TDTX CELLS, A. C. Chen and H. R. Herschman, Mol. Biol. Inst., U.C.L.A., Los Angeles, CA 90024

The C3H10T1/2 murine cell line has been used as a major cell culture model for two-stage carcinogenesis. C3H10T1/2 cells are exposed to UV irradiation followed by exposure to the tumor promoter, TPA. This transformation protocol yields UV-TDTx foci whose cells appear transformed in pure culture. When grown in mixed culture with 10T1/2 cells in a secondary focus forming assay, UV-TDTx cells are dependent upon the presence of TPA for transformation. A clonal cell line (UV-TDTx10e), established from one of the UV-TDTx foci, is weakly anchorage independent when grown in the absence of TPA and is non-tumorigenic in nude mice. In contrast, both a one-step methylcholanthrene (MCA) transformed 10T1/2 cell line (MCAC116/39) and an EJ-transfected 10T1/2 cell line with 50 copies of EJ (rasC4) are anchorage independent and highly tumorigenic. A clonal one-step MCA-transformed 10T1/2 cell line isolated in our laboratory (MCATx1e) is anchorage dependent and non-tumorigenic. Thus, the two MCA-transformed cell lines differ in at least two transformation parameters. Somatic cell hybrids formed between parental 10T1/2 and UV-TDTx10e cells show dominant focus formation in a secondary focus forming assay in 28/59 hybrids. The ability of these hybrids to form foci is TPA-dependent. 10T1/2/MCAC116/39 hybrids also exhibit dominant focus formation in 6/10 hybrids while 10T1/2/rasC4 hybrids behave dominantly in 25/25 hybrids. These hybrids behave in a TPA-independent manner. MCAC116/39 cells have been shown to contain one copy of an endogenously activated c-Ki-ras gene. The possible involvement of an activated c-Ki-ras gene in the expression of the MCATx1e and UV-TDTx10e phenotypes is under investigation.

C 709 c-fos AND THE CELLULAR IMMEDIATE-EARLY RESPONSE: A MARKER FOR A FAMILY OF GENES INVOLVED IN SIGNAL TRANSDUCTION. Donna R. Cohen and Tom Curran, Dept. of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The c-fos gene is the cellular homolog of the oncogene (v-fos) carried by the FBJ and FBR murine osteogenic sarcoma viruses. It encodes a nuclear phosphoprotein (Fos) that is associated with chromatin and which displays general and sequence-specific DNA binding properties. The c-fos gene is expressed rapidly and transiently in response to a wide variety of extracellular stimuli, including events leading to mitosis, differentiation and depolarization of neurons. Furthermore, we have recently demonstrated that c-fos is expressed in brain following induction of seizures in mice by a variety of agents. In view of these properties, it has been proposed that c-fos functions as a "cellular immediate-early gene", coupling events at the membrane to long-term adaptive responses of the cell, by influencing the expression of target genes. We have previously shown that Fos protein is co-induced, depending on the circumstances, with some or all of a set of antigenically related proteins that have similar biochemical properties to Fos. We have now cloned genes that encode proteins antigenically related to Fos, but which show considerable nucleotide sequence divergence from the c-fos gene. In addition, the conditions under which they are induced overlap with, but are not identical to, those resulting in the expression of c-fos. These genes show characteristics that have been attributed to immediate-early genes. We propose that c-fos is a marker for a set of genes, some of which encode proteins that are antigenically related to Fos, that form the basis of the cellular immediate-early response of gene expression to extracellular stimuli.

C 710 A NEW ONCOGENE FROM KAPOSI'S SARCOMA BELONGS TO THE FGF GENE FAMILY, Anna Maria Curatola, Pasquale Delli Bovi and Claudio Basilico, Department of Pathology, NYU Medical Center, New York, NY, 10016. A novel human oncogene (KS oncogene) was isolated following transfection of NIH3T3 cells with DNA of Kaposi's sarcoma. The transforming sequences are rearranged with respect to the normal human DNA and encoded two species of mRNA (3.5Kb and 1.2Kb). A cDNA clone (KS3 cDNA) representative of the 1.2 Kb mRNA was able to transform NIH3T3 cells with high efficiency upon insertion into an eucaryotic expression vector. The sequence DNA analysis revealed that the KS3 cDNA encodes a protein of 206 amino acids with significant homology to the basic and acidic FGF and to the int-2 protein. Thus the KS oncogene appears to belong to the same gene family of the FGFs, but it is distinct from them. Conditioned medium from NIH3T3 transformants and the KS protein produced in bacteria and in COS cells, showed a significant growth-promoting activity on NIH3T3. The KS protein expressed in COS cells, has a MW of approximately 25Kb and is glycosylated. Immunoprecipitation and in vitro translation experiments indicated that it is secreted and cleaved. We have also cloned the normal homolog from a human genomic library using the KS3 cDNA as a probe. Comparison of its restriction map to the transforming one as well a partial sequence analysis, allowed us to precisely map one of the rearrangements at the 5' end of the KS oncogene and it is outside of the encoding region. The transforming activity of the normal counterpart of the KS oncogene and the mitogenic activity of the KS protein on different cell lines as well as its ability to induce expression of cell-cycle oncogenes, by stimulation of quiescent 3T3 cells, will be discussed.

C 711 TRANSFORMATION OF AN IL-3 DEPENDENT HEMATOPOIETIC CELL LINE BY THE CML-SPECIFIC P210 bcr/abl PROTEIN, George Q. Daley and David Baltimore, Whitehead Institute and Department of Biology, MIT, Cambridge, MA 02142. The novel hybrid P210 bcr/abl protein is associated with virtually every case of human chronic myelogenous leukemia (CML). Unlike the related P160 gag/v-abl oncogene product of Abelson Murine Leukemia Virus (A-MuLV), P210 bcr/abl does not transform NIH-3T3 fibroblasts (Daley et al., SCIENCE 237:532-535, 1987). To assess whether P210 bcr/abl might transform hematopoietic cell types, retroviral constructs specifying P210 bcr/abl were used to infect the bone-marrow-derived interleukin 3-dependent Ba/F3 cell line. As for P160 gag/v-abl, cell lines expressing P210 bcr/abl proliferated in the absence of IL-3 and were tumorigenic in nude mice. No evidence for autocrine production of IL-3 by IL-3 independent lines was found in control experiments with conditioned media or on Northern blot analysis. These experiments established that P210 bcr/abl can transform hematopoietic cell types in vitro, implicating P210 bcr/abl in the pathogenesis of CML.

C 712 TRANSFORMING POTENTIAL OF THE erbB-2 GENE AND EGF RECEPTOR, P. Paolo Di Fiore, Jacalyn H. Pierce, Oreste Segatto, Matthias H. Kraus, Timothy P. Fleming, C. Richter King[1], Joseph Schlessinger[1], Axel Ullrich[2] and Stuart A. Aaronson, National Cancer Institute, Bethesda, MD 20892, [1]Rorer Biotechnology, Rockville, MD, [2]Genentech Inc., San Francisco, CA

Eukaryotic expression vectors were engineered to study the transforming capability of the erbB-2 gene and of the EGF receptor (EGFR). ErbB-2 cDNA under transcriptional control of the SV40 early promoter lacked transforming ability despite expression of detectable levels of erbB-2 protein. A further 5-10 fold increase in its expression under the influence of the long terminal repeat of Moloney murine leukemia virus was associated with the activation of erbB-2 as a potent oncogene (10^4 ffu/pmol of DNA). The transforming potential of the erbB-2 gene was exerted in the apparent absence of any exogenously added ligand. Conversely, transfection of an LTR-EGFR cDNA into NIH 3T3 cells led to no significant alterations in the growth properties. However, EGF addition (20 ng/ml) led to the appearance of the transformed phenotype (10^2 ffu/pmol). The levels of overexpression of EGFR and erbB-2 associated with malignant transformation were comparable to those detected in human tumors overexpressing either gene. Thus, our studies establish a mechanistic basis for amplification of growth factor receptor genes as representing a causal driving force in the clonal evolution of a tumor cell.

C 713 TRANSFECTION OF NORMAL HUMAN FIBROBLASTS WITH SV40 DNA: RELATIONSHIP BETWEEN LIFESPAN, QUIESCENCE AND SENESCENCE, L.M. Donahue and G.H. Stein, University of Colorado, Boulder, CO 80309. We are interested in determining the regulatory mechanisms underlying arrest of cell growth in normal human diploid fibroblasts (HDF). When HDF are crowded or deprived of serum, they enter a viable, G1-arrested quiescent state (Q^+), which is readily reversible. When HDF reach the end of their finite proliferative lifespan (FPL^+) they enter a viable, G1-arrested senescent state (S^+), which is essentially irreversible. We are investigating the extent to which arrest in the quiescent state and arrest in the senescent state share common regulatory mechanisms. To this end we have transfected HDF (freq. 2.4 x 10^{-3}) with SV40 DNA and examined the effect on the regulation of the Q and S phenotypes (i.e. are all FPL^+ cell types Q^+S^+ or Q^-S^- but not Q^+S^- or Q^-S^+ ?). IMR-90 HDF transfected with pSV3neo (containing both SV40 tumor antigen genes) were T antigen positive but still expressed an FPL^+ phenotype. These clones were incapable of becoming quiescent when crowded (Q^-), and at the end of their lifespan these clones did not cease synthesizing DNA (S^- phenotype, i.e., ^{3}H-thymidine labeling indices of 30-60%). Thus the introduction of only the two SV40 tumor antigens transformed IMR-90 HDF to both Q^- and S^-. We are presently transfecting IMR-90 HDF with the individual SV40 tumor antigens (and other known oncogenes) to test whether a single gene can change a cell with a Q^+S^+ phenotype to a Q^-S^- phenotype.

C 714 INCREASED RESPONSIVENESS TO IL3 OF A MURINE MYELOID CELL LINE (FDCP-2) FOLLOWING INFECTION WITH KIRSTEN RETROVIRAL VECTORS. Dominique Dumenil, Henry Neel, François Dautry, INSERM U.250, Laboratoire d'Oncologie Moléculaire, Institut Gustave-Roussy, 94805 Villejuif - France

To study the role of the ras genes in the response to hematopoietic growth factors, we have used retroviral vectors to introduce a kirsten ras gene into the IL3 dependent murine myeloid cell line FDCP-2. We have used two different constructs containing the neomycin resistance gene and a mutated kirsten gene under the control of either the LTR or of internal promotors.
After a 24 h co-cultivation with high titer ($>10^5$ i.p./ml) producing cell clones, virtually all FDCP-2 cells become resistant to neomycin (G418). This has enabled us to evaluate the proliferative response to IL3 of the uncloned population shortly after infection. Following infection, cells are able to grow in lower amounts of IL3 although they remain absolutely dependent upon IL3 for proliferation. The same growth behaviour was observed for individual cell clones and Northern blot analysis showed they express the expected viral transcripts. Thus expression of an activated ras gene does confer a growth advantage to an IL3 dependent cell line but is not enough to render them growth factor independent. We are currently extending these observations to other ras genes as well as other hemopoietic cell lines.

C 715 EVIDENCE THAT THE VIRAL Ki-RAS PROTEIN OF KSV, BUT NOT THE pp60$^{v\text{-}src}$ PROTEIN OF ASV STIMULATES NRK CELL PROLIFERATION THROUGH ITS PDGF RECEPTOR, Jon P. Durkin and James F. Whitfield, Division of Biological Sciences, National Research Council of Canada, Ottawa CANADA K1A 0R6

NRK cells infected either with a temperature-sensitive mutant of Kirsten sarcoma virus (tsKSV), or of avian sarcoma virus (tsLA23-ASV) are transformed at 36°C, but are non-transformed at 41°C which inactivates the abnormally thermolabile oncogenic products of the viral Ki-ras and v-src genes, respectively. At the nonpermissive 41°C, both tsKSV-NRK and tsASV-NRK cells can be arrested in G_0 phase by incubation in serum-derived medium, but can be induced to start proliferating again by adding serum at 41°C. However, reactivating the viral oncogene products in these quiescent cells *in serum-free medium* is also a potent mitogenic signal which triggers the cells to transit G_1 phase and initiate DNA replication.

Protamine sulfate (PS), a specific blocker of PDGF action, inhibited the proliferative response of tsKSV-NRK cells to a reactivated, viral Ki-RAS protein, but it did not affect the proliferative response of tsASV-NRK cells to a reactivated pp60$^{v\text{-}src}$ protein kinase. The inhibition by PS of the proliferation response of tsKSV-NRK cells to reactivated Ki-RAS protein was overcome by serum growth factors, notably EGF, and concentrated serum-free conditioned medium from cultured NRK cells infected with wild-type KSV, but not by a combination of PDGF and insulin. These observations suggest that the viral Ki-RAS protein, but not pp60$^{v\text{-}src}$, stimulates proliferation exclusively by inducing the host cells to produce PDGF or PDGF-like mitogenic factors.

C 716 STRUCTURALLY DIVERGENT ONCOGENES INDUCE THE METASTATIC PHENOTYPE. S.E. Egan*, J.A. Wright*, L. Jarolim*, K. Yanagihara+, R. H. Bassin+, A.H. Greenberg*, *The Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba, Canada, R3E 0V9, +Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, MD, USA, 20892.

We and others have established that the ras oncogene can transform established rodent fibroblast cell lines into fully metastatic tumors and that the metastatic behavior of these cells is directly regulated by ras. In subsequent work, we found that overexpression of ras as well as mutations effecting GTPase, nucleotide binding or exchange, all transform NIH-3T3 to metastatic tumors. These results support a role for increased ras-GTP active complex in regulating metastatic behavior of ras transformed cells. It is clear, however, that not all human tumors contain activated or greatly overexpressed ras genes. To directly test the hypothesis that other oncogenes may be involved in regulating or inducing the metastatic phenotype we have assessed the ability of NIH-3T3 (clone 7) cells transformed by various oncogenes to form metastases. Cell lines transformed by the serine/threonine kinase oncogenes v-raf, A-raf or v-mos as well as the tyrosine kinase oncogenes v-src, v-fes or v-fms were metastatic. In contrast, cells transformed by either of two nuclear oncogenes, myc or p53, were tumorigenic when injected subcutaneously but were virtually non-metastatic. Control NIH-3T3 or NIH-3T3 infected or transfected with MoMuLV were also non-metastatic. These data demonstrate that, in addition to ras, a structurally divergent group of kinase oncogenes can induce the metastatic phenotype.

C 717 ABERRANT EXPRESSION OF SIS IN ACUTE MYELOGENOUS LEUKEMIA, Mary Jean Evinger-Hodges, Irene Cox, Joel Bresser and Karel A. Dicke, UTSCC, Houston, Texas 77030.

Recent results from our laboratory using a rapid (2-4 hrs) and sensitive (5 copies/cell) RNA in situ procedure point to the potential role abnormal growth factor regulation may play in leukemogenesis. In greater than 70% of human acute leukemia patients studied, the sis oncogene is overexpressed in a variable percentage of bone marrow progenitor cells. By multiple-labelling experiments we find that the same cells which produce high levels of sis mRNA also express myc at unusually high levels and JE, one of the PDGF competence genes. These results raise the possibility that these cells are stimulated by PDGF in an autocrine fashion. We also examined marrow samples from more than 15 acute leukemia patients in remission and find between 0.02% and 20% of cells expressing both myc and sis at a greater than 5-fold increase compared to that found in normal hematopoietic cells.

Several investigators have shown an antagonism between the interferons and cell responsiveness to PDGF as measured by increased myc and JE expression and subsequent cellular proliferation. Interferon production appears to act as a feedback mechanism in normal hematopoietic proliferation. We are currently investigating the possibility that these cells expressing high levels of myc and sis are abnormal in their production and/or response to endogenous interferons explaining their unregulated production of growth factor.

C 718 INSERTIONAL ACTIVATION OF *c-erb B*: RESTORATION OF A SIGNAL SEQUENCE AND ITS EFFECT ON PROTEIN PROCESSING AND TRANSFORMATION POTENTIAL.
T. W. Flickinger, N. J. Maihle, M. Raines, and H.-J. Kung, Dept. of Molecular Biology, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106.

Avian Leukosis Virus (ALV) induces erythroblastosis in chickens by insertion into the middle of the host *c-erb B* locus. We have used cDNA clones corresponding to two alternate forms of the insertionally activated (IA) *c-erb B* transcripts (Nilsen et al., Cell, 1985) to generate stable quail fibroblast transformants expressing high levels of the IA *c-erb B* products. These clonally isolated cell lines have been used for analysis of the biosynthesis of IA *c-erb B* products. We have prepared antisera directed against bacterially produced *trpE* fusion products for these studies. Two protein products are predicted from the deduced amino acid sequence of the cDNA clones. Both initiate with the first six amino acids of *gag* p19, and one (env^+) contains an additional 53 amino acids of *env* gp85. Our results indicate that the two insertionally activated products are processed differently in fibroblasts; the env^+ products undergo proteolytic processing and extensive terminal glycosylation. In contrast, the env^- products are predominatly N-linked glycosylation intermediates, and are not proteolytically processed. The consequence of the addition of amino-terminal *env* sequences to a truncated growth factor receptor is the restoration of a functional "signal sequence", which may allow more efficient membrane association and/or membrane translocation. The influence of the *env* sequence as well as selective mutations on fibroblast transformation will be discussed. (T.W.F. is supported by USPHS training grant HD07104-11).

C 719 MACROPHAGE INDUCED DNA STRAND BREAKS IN TUMOR TARGET CELLS,
Amy Fulton, Leslie Paul, Yen Chong and Gloria Heppner, Michigan Cancer Foundation, Detroit, MI 48201

We have shown that macrophages isolated from murine mammary tumors can induce mutation in the Ames assay. Activated macrophages can also induce the appearance of drug resistant variants of mammary tumor cells. We have proposed that tumor-infiltrating macrophages can, by acting on genetically unstable tumor cells, induce tumor cell diversity that may fuel progression. We now report that macrophages can induce DNA strand breaks in tumor cell targets as detected by fluorometric analysis of DNA unwinding. These breaks occur after brief co-incubation (60 min.), at macrophage-target cell ratios of 1:1, and cause the equivalent damage of 900-1800 rads of irradiation. We found, to our surprise, that the level of macrophage activation correlated negatively with the ability to induce DNA strand breaks. That is, unstimulated resident peritoneal macrophages induced the greatest number of strand breaks, primed (MVE-2) macrophages expressed an intermediate level of activity while fully activated macrophages were least active. Using inhibitors of reactive oxygen species or of arachidonate metabolism, divergent results were again seen for the different macrophage populations. Superoxide dismutase (SOD) or catalase alone each reduced the number of strand breaks induced by resident macrophages. The combination of the two was somewhat more protective. Indomethacin or NDGA were moderately protective in all experiments. In contrast, when MVE-2 macrophages were examined, SOD was either not protective in 2/9 experiments, or led to more strand breaks in 4/9 cases. Catalase was protective in half the tests. Thus, in the case of MVE-2 cells, treatment with SOD may have led to more H_2O_2 production. With these cells NDGA, which inhibits both cyclooxygenase and lipoxygenase metabolism of arachidonate, was never protective. These studies suggest that both oxygen-centered species and arachidonate metabolites may contribute to macrophage-induced DNA strand breaks in tumor target cells.

C 720 A GENE RELATED TO fms EXPRESSED IN TRANSFORMED RAT TRACHEAL EPITHELIAL CELLS,
Tona Gilmer, Paul Nettesheim and Cheryl Walker, National Institute of Environmental Health Sciences, Research Triangle Park, N.C. 27709.

Neoplastic progression of chemically transformed rat tracheal epithelial (RTE) cells in vitro is a multistep process. We have examined oncogene expression in tumor-derived clonal cell lines following transformation of primary RTE cells with MNNG. DNA from the tumor-derived lines gave negative results when tested by the NIH 3T3 assay, suggesting that activation of the ras family of oncogenes did not occur during neoplastic progression in this system. However, when RNA from the tumor-derived lines was hybridized with 15 different oncogene probes, H-ras and a novel gene related to fms were expressed at elevated levels (3-fold and 5 to 20-fold, respectively) in three out of five lines. Analysis of preneoplastic and neoplastic stages of the tumor-derived cell lines indicated that the fms-related gene was not expressed by the transformed cells prior to tumor formation in vivo, suggesting that expression of this gene occurs late in neoplastic progression. We are currently characterizing the biological role of this gene by examining a panel of hematopoietic cell lines which also express this fms-related transcript. In addition, we are screening a cDNA library from one of the tumor-derived lines to identify clones that may be used to further identify this gene and its relevance to cellular transformation.

C 721 MODIFICATION OF C-FOS AND C-MYC EXPRESSION IN MASTOCYTOMA CELLS BY cAMP, Martyn D. Goulding and Raymond K. Ralph, Department of Cell Biology, University of Auckland, New Zealand

Cyclic AMP (cAMP) alters the expression of the c-fos and c-myc proto-oncogenes in P815 mastocytoma cells. Elevation of intracellular cAMP levels in P815 cells causes growth arrest and differentiation into mature mast like cells. The proto-oncogenes c-fos and c-myc are expressed in cycling cells only during late G1 phase/early S phase. Transcription of c-myc is not directly controlled by cAMP and the lowered expression of c-myc in cells where cAMP levels are elevated probably occurs because cells are arrested in a part of G1 phase where c-myc is not normally expressed. Removal of cAMP results in an increase in c-myc expression that coincides with the entry of cells into S phase. In contrast, cAMP rapidly increases c-fos expression resulting in mRNA levels at least 50 fold higher after 15h. Increased expression occurs primarily by increased transcription of c-fos in the presence of cAMP, however c-fos mRNA is slightly more stable in the presence of cAMP. Removal of cAMP results in a rapid decrease in c-fos mRNA to undetectable levels 2h after removing cAMP. Increased c-fos expression in P815 cells is not associated with differentiation as temperature sensitive P815 cells that differentiate at non-permissive temperature show no increase in c-fos expression.

C 722 EFFECT OF C-MYC DOWN-REGULATION ON F9 CELL DIFFERENTIATION. Anne E. Griep and Heiner Westphal. Laboratory of Molecular Genetics. NICHD. NIH. Bethesda, MD. 20892.

The murine teratocarcinoma cell line, F9, differentiates into parietal endoderm when treated with retinoic acid. One of the earliest known changes in gene expression after treatment with retinoic acid is downregulation of the c-myc mRNA level. We asked whether expressing c-myc sense or antisense genes under heterologous control would influence the ability of F9 cells to differentiate either in response to retinoic acid or spontaneously.

We cotransfected F9 cells with plasmid DNAs containing SV40 directed antisense c-myc sequences and SV40 driven neomycin resistance gene. We found that transfection of antisense DNAs specifically lead to spontaneous differentiation of F9 cells as judged by plasminogen activator assays performed approximately one week after transfection. Furthermore, we were unable to establish cell clones which expressed the antisense c-myc constructs. When we cotransfected F9 cells with DNAs containing SV40 directed sense c-myc DNA and SV40 directed neomycin resistance gene, we were able to establish cell clones expressing the transfected myc gene. When treated with retinoic acid these clones exhibited resistance to differentiation as they produced much less plasminogen activator and laminin than did retinoic acid treated control cell clones. Thus our results indicate that differentiation of F9 cells requires downregulation of c-myc expression.

C 723 SPECIFIC INHIBITION OF C-MYC PROTEIN BIOSYNTHESIS USING USING AN ANTISENSE OLIGONUCLEOTIDE IN HUMAN T LYMPHOCYTES, Annick Harel-Bellan, Douglas Ferris and William L. Farrar, National Cancer Institute, Frederick MD 21701.

C-myc gene expression is a strict function of the cell cycle in T lymphocytes, in which it is activated only upon entry of the cells into a proliferative process. In order to gain further insight into the function of c-myc gene, we specifically blocked it's expression using an antisense 15 mer deoxy-oligonucleotide, complementary to the 5' end of the translated sequence. We show that ^{32}P-labeled oligonucleotide penetrates the cells without any treatment. The penetration seems to be a temperature dependent active phenomenon, also inhibited by glutaraldehyde fixation of the cells and resulted in a significant intracellular concentration of undegraded oligonucleotide. Preincubation of human resting T lymphocytes with the antisense oligonucleotide prevented the subsequent induction of c-myc protein synthesis by PHA, as assessed by two dimensional gel analysis of ^{35}S-labeled proteins and immunoblotting using c-myc specific monoclonal antibodies. The antisense oligonucleotide prevented the lymphocytes from entry into S phase, regardless of the activating ligand used, whether PHA on resting T lymphocytes or Interleukin 2 on T lymphoblasts. The inhibition was specific since it was not observed with a control sense oligonucleotide and could be reversed by an excess of sense on antisense strand.

C 724 **ENHANCED EXPRESSION OF THE MYC PROTO-ONCOGENES IN HUMAN FETAL KIDNEY, BRAIN AND RETINA.** Hirvonen H.[1], Sandberg M.[2], Kalimo H.[3], Hukkanen V.[1], Salmi TT.[4], Vuorio E.[2], Alitalo K.[2,5] Departments of Virology[1], Medical Biochemistry[2] , Pathology[3] and Pediatrics[4], University of Turku, Turku, Finland, and Department of Virology[5], University of Helsinki, Helsinki, Finland.

The cellular proto-oncogenes of the myc family are regarded as key regulators of normal cellular proliferation and differentation, and are active in a wide variety of human tumors as well as during normal fetal development. We analysed the expression of the myc genes in human fetal tissues of 16-19 gestational weeks by Northern blotting and by *in situ* hybridization. Enhanced expression of c-myc, N-myc and L-myc mRNA's was detected in the kidney and in the brain. To further localize the expression and to assess eventual coexpression of the genes, *in situ* hybridizations were carried out. In the kidney, mesenchymal blastema-derived structures and cells showed a strong N-myc signal in *in situ* hybridization. N-myc autoradiographic grains were primarily located over cells forming the excretory units and proximal and distal portions of the kidney tubules. In contrast, the collecting tubules reacted much less, and the undifferentiated mesenchymal cells showed mostly background radioactivity. N-myc expression was rather generalized in the brain, spatial differencies largely resulting from variations in cell density, whereas c-myc signal was pronounced in the ependymal zone. In the retina, N-myc was expressed at high levels both in the ganglion and nuclear layers but not in the pigment cell layer. Interestingly, extrinsic ocular musculature showed a low but definite N-myc mRNA signal. We conclude that expression of N-myc in human fetal tissues is pronounced in cells originating from the mesenchymal blastema in addition to cells of neural origin. We are now in the process of localizing the L-myc mRNA's by in situ hybridization.

C 725 ANALYSIS OF THE C-ERB B-2 PROTO-ONCOGENE IN HUMAN BREAST CARCINOMAS: CORRELATION OF GENE AMPLIFICATION; PROTEIN EXPRESSION AND CLINICAL PARAMETERS, N.E. Hynes*, M. Berger+, G. Locher°, W. Gullick§ and Bernd Groner*, Ludwig Institute for Cancer Research (LICR), Bern Branch, Inselspital, 3010-Bern, Switzerland* and LICR, Univ. College Branch, London+, Women's Hospital, Bern°, and Chester Beatty Laboratory, London§.

We have analyzed a series of primary human breast tumors for amplification of the c-erbB-2 proto-oncogene. Twenty-five percent (13/51) of the DNA samples contained multiple gene copies. Paraffin-embedded tumor material was available from 47 of the cases. We used a c-erbB-2 specific antiserum (Gullick et al., 1987, Int. J. Cancer 40:246-254) to immunohistochemically stain the tumor sections. There was a correlation ($\overline{p}$=0.03) between tumors containing amplified c-erbB-2 gene copies and positive staining with the c-erbB-2 specific serum. We also observed that some tumors containing single copy c-erbB-2 sequences were strongly stained with the antiserum. This suggests that mechanisms other than gene amplification may lead to elevated levels of c-erbB-2 protein. Finally, we observed a statistically significant correlation between c-erbB-2 protein expression and parameters used in breast cancer prognosis. Positive staining was associated with the nodal status of the patient (p=0.02) and with the nuclear grade of the tumor (p=0.02). These results suggest that the determination of the level of c-erbB-2 protein may be of prognostic value for the course of human breast cancer.

C 726 ACTIVATING MUTATIONS OF THE C-ABL PROTOONCOGENE. Peter Jackson and David Baltimore. Whitehead Institute, 9 Cambridge Center, Cambridge, MA 02144.
Studies of the two major c-abl protooncogene messages(types I and IV) suggest that retroviral overexpression of the genes coding for these two messages is not sufficient for transformation of murine fibroblasts. The type IV c-abl gene has a consensus sequence for N-terminal myristylation and has an N-terminus homologous to the c-src protooncogene. The lack of transformation by c-abl type IV is reminiscent of the differences between c-src and v-src. We have characterized 3 classes of mutations that activate c-abl IV, expresses in a retroviral vector, to transform murine fibroblasts: 1) addition of gag sequences; 2) N-terminal small deletions and linker insertions; 3) C-terminal point mutations. Lymphoid transformation by these viruses is under examination. These results will be considered in light of a model of regulation of c-abl and by way of comparison to analogous mutations in c-src.

Growth Factors and Their Receptors: Genetic Control and Rational Application

C 727 STRUCTURE AND EXPRESSION OF THE HUMAN L-MYC GENE REVEAL A COMPLEX PATTERN OF mRNA PROCESSING, Frederic Kaye, James Battey, Marion Nau, Burke Brooks, Eric Seifter, Jacques De Greve, Michael Birrer, Edward Sausville, and John Minna, NCI-Navy Oncology Branch, National Cancer Institute and Naval Hospital, Bethesda, MD 20814.

The L-myc gene was first isolated from a human small cell lung cancer (SCLC) cell line where it was detected by the presence of gene amplification and its sequence homology to c-myc and N-myc . We have analyzed in detail the structure of the L-myc gene isolated from human placental DNA and characterized its expression in several SCLC cell lines.The gene is composed of three exons and two introns spanning 6.6 kb in human DNA. Several distinct mRNA species are produced in all SCLC cell lines that express L-myc. These transcripts are generated from a single gene by alternative splicing of either the first or second introns and by use of alternative polyadenylation signals. In some mRNAs there is a long open reading frame with a predicted translated protein of 364 residues. Amino acid sequence comparison of this protein with c- and N-myc demonstrates multiple discrete regions with extensive homology. We have observed that alternative splicing of the first intron may affect the ability to generate a slightly larger protein containing an additional amino-terminal domain. In contrast, other mRNA transcripts, also generated by alternative RNA processing, could encode a truncated protein with a novel carboxy-terminal end.

C 728 ONCOGENE EXPRESSION IN RADIATION-INDUCED LUNG TUMORS, Gregory Kelly[1], and Paul R. Kerkof[2], [1]Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, N. M. 87185, and [2]Dept. of Biology, University of New Mexico, Albuquerque, N. M. 87131.

We are examining the mechanisms responsible for the radiation induced activation of oncogenes in tumors, especially tumors of the respiratory tract. We have examined a set of lung tumors induced in Beagle dogs following exposure to $^{238}PuO_2$ or $^{239}PuO_2$ by probing a battery of 22 oncogene clones with labeled cDNA transcripts from the tumors. We have identified sequences similar to the v-myc oncogene over-expressed relative to the other oncogenes examined in a $^{239}PuO_2$ induced lung tumor (1222T). The alpha dose was 400 rad at the time of death (2800 days) for the $^{239}PuO_2$ induced tumor. In addition, sequences similar to the erb-B oncogene are amplified four fold in this and one other $^{239}PuO_2$ tumor (1070B). RNA dot blot analysis fails to detect erb-B transcripts in the 1222T tumor. We have established cell lines from each of the primary tumors in our initial set of radiation induced lung tumors. The 1222T derived cell line is able to form tumors when injected subcutaneously into nude mice. The 1222T derived cell line forms a solid carcinoma in a nude mouse that is indistinguishable histologically from the primary tumor. (Research sponsored by the U.S. Department of Energy's Office of Health and Environmental Research under Contract No. DE-AC04-76EV01013.)

C 729 MULTIPLE DISCRETE FACTORS INTERACT WITH C-MYC UPSTREAM SEQUENCES, M. Avigan[1], M. Takimoto[1], J. Quinn[1], N. Hay[2], N. Holbrook[3], J.M. Bishop[2], and D. Levens[1], 1. Laboratory of Pathology, National Cancer Institute, 2. G.W. Hooper Foundation, Univ. of California, San Francisco, 3. Laboratory of Molecular Genetics, National Institute on Aging.

We have employed a sensitive exonuclease assay to examine the interactions of numerous, distinct trans-elements with cognate sequences upstream of the c-myc gene. Previous studies have identified several sequence domains influencing c-myc expression, positively or negatively, when assayed with a stable transfection system. Binding studies have demonstrated the interaction of factors with a 57bp region associated with decreased levels of c-myc expression and with a repeated element, probably associated with transcription from P1. We have extended these studies and demonstrated that the negative element can bind at least two chromatographically separable proteins as determined with both exonuclease and gel retardation assays. Comparison of extracts from multiple cell lines indicates that these factors are not ubiquitous. One of these proteins binds tightly to a 22bp sequence of the gibbon ape leukemia virus; this 22bp segment possesses enhancer activity and binds a 40kD protein.

Numerous other loci of protein-DNA interactions have been identified, some occurring more than 2kb upstream of P1. Several of these interactions are cell type specific or are regulated, probably reflecting alterations of c-myc transcription. Other proteins bind to specific sites but have not been observed to vary with growth conditions, cell type, or c-myc expression. The role of these constitutive binding factors in c-myc transcription, if any, remains to be elucidated. A clear description of c-myc regulation will necessitate the elucidation of the mechanisms of interaction of these many factors over several kilobases.

C 730 EXPRESSION OF TRANSIENTLY-INDUCIBILE GENES IN TWO MOUSE EMBRYO CELL LINES AND THEIR CORRESPONDING TPA-NONRESPONSIVE VARIANTS, R. W. Lim, B. Livingston, B. Varnum and H. R. Herschman, Dept. of Biological Chemistry, Univ. of California at Los Angeles, Los Angeles, CA 90024

We have previously cloned a set of TPA inducible (TIS) cDNAs which are transiently expressed when Swiss 3T3 cells are stimulated by other mitogens including FGF and EGF. TPA appears to activate gene expression by a protein kinase C dependent pathway. Pretreatment of cells with TPA leads to a depletion of kinase C, and drastically reduces the ability of TPA to further induce TIS gene expression. Stimulation by EGF and FGF is only partially inhibited. The data suggest that EGF and FGF activate TIS genes through both kinase C dependent and independent pathways. Expression of these TIS genes has also been examined in 2 independently derived TPA non-proliferative variants (TNR2 & TNR9) of Swiss 3T3 cells; as well as in a Balb 3T3 cell line (A31T6) and its variant (A31T6E12A) selected for the inability of TPA to stimulate the sodium/potassium/chloride cotransport system. None of the 3 variants proliferate in response to TPA but all 3 show a mitogenic response to serum. The TIS genes are all inducible by TPA in these TPA nonresponsive variants. Interestingly, expression of one of the TIS genes (TIS 1) in at least two of the variants (TNR2 & TNR9) persists at a high level following TPA induction. Stimulation of the same gene by serum remains transient. Possible involvement of this and the other TIS genes in the mitogen stimulation/response pathway is being investigated.

C 731 REGULATION OF THE pp56^{lck} PROTEIN TYROSINE KINASE: MECHANISMS OF INVOLVEMENT IN ONCOGENESIS. Jamey D. Marth, Jonathan A. Cooper, Connie S. King, Steven F. Ziegler, Donald A. Tinker, Robert W. Overell, Kathryn E. Meier, Edwin G. Krebs, and Roger M. Perlmutter. *Howard Hughes Medical Institute SL-15, the Immunex Corporation,* and the Fred Hutchinson Cancer Research Center, University of Washington, Seattle, WA 98195

The lymphocyte-specific protein tyrosine kinase pp56^{lck} is encoded by a member of the *src* gene family and is implicated in the control of T cell development and oncogenesis. By phosphopeptide mapping, pp56^{lck} is phosphorylated *in vivo* at a carboxy-terminal tyrosine residue (Tyr505) analogous to Tyr527 of pp60$^{c\text{-}src}$. Substitution of phenylalanine for tyrosine at this position results in increased phosphorylation of a second tyrosine (Tyr394) and is associated with a 3-5-fold increase in kinase activity. In addition, this single point mutation unmasks the oncogenic potential of pp56^{lck}. Viewed in the context of similar results obtained with pp60$^{c\text{-}src}$, it is likely that the enzymatic activity and transforming ability of all *src*-family protein tyrosine kinases can be regulated by carboxy-terminal tyrosine phosphorylation. In the murine lymphoma LSTRA, overexpression of *lck* mRNA as a result of retroviral insertion produces a kinase protein that despite wild-type primary structure is nevertheless hypophosphorylated at Tyr505. The level of pp56^{lck} protein is elevated over 50-fold in LSTRA cells compared to the YAC-1 lymphoma. Retroviral insertion at the *lck* locus in LSTRA cells results in only a 7-fold increase in *lck* mRNA levels. However, the LTR-*lck* fusion transcript produced lacks 5' untranslated sequences present in normal thymocytes. Site-directed mutagenesis and expression studies reveal that this region lowers the translational efficiency of the *lck* mRNA transcript by over 7-fold. These observations suggest that the 50-fold increase in pp56^{lck} abundance in LSTRA may titrate out elements that direct the phosphorylation of Tyr505 in lymphoid cells. It thus seems likely that normal lymphocytes can regulate pp56^{lck} activity by controlling both the abundance of pp56^{lck} and its phosphorylation state.

C 732 CONTROL OF ras p21 GTPase ACTIVITY BY A CELLULAR PROTEIN, F. McCormick, M. Trahey, B. Rubinfeld, G. Wong and H. Adari Cetus Corporation, 1400 53rd St, Emeryville Ca 94608

Cytoplasmic extracts from Xenopus oocytes and mammalian cells have been shown to stimulate GTPase activity of normal N-ras p21, but have no effect on oncogenic mutants (Trahey and McCormick, Science, in press). As a result, the normal proto-oncogene product is maintained in the inactive GDP-bound state, whereas oncogenic mutants are GTP-bound, with high biological activity. The protein responsible for GTPase stimulation, referred to as GAP (GTPase activating protein), has a native molecular weight of about 60,000 daltons. Its ability to stimulate p21 GTPase is reduced by relatively low salt concentrations, and enhanced by Mg++. GAP activity has been detected in extracts from all mammalian cells tested so far, as well as in extracts from insect cells. However, extracts from S. cerevisiae and from S. pombe have failed to stimulate ras p21 GTPase activity. The significance of GAP in the regulation of ras p21 function will be discussed.

C 733 ACTIVATION OF ONCOGENE TRANSCRIPTION AFTER IL3 TREATMENT OF IL3 DEPENDENT LYMPHOID AND MYELOID STEM CELL LINES. James A. McCubrey and John P. McKearn, Medical Products Department, E. I. duPont de Nemours, 500 S. Ridgeway Avenue, Glenolden, PA. 19037.

We have compared the levels of nascent (Nuclear runoff) and steady state (northern) transcription of oncogene, growth factor receptor, immunoglobulin, T cell receptor and housekeeping genes in IL3-dependent lymphoid (FL5.12) and myeloid (FDC-P1) cell lines as well as B, T, macrophage, and fibroblast cell lines. The IL3-dependent clones were synchronized in Go and then pulsed with IL3 for various time intervals. The stage of the cell cycle at various time intervals was determined by FACS analysis. Aliquots of the cells were isolated and divided into two fractions, one for nuclear runoff experiments and the other for poly A+ RNA extractions. The patterns of IL3-induced transcription for many oncogenes, Ig, TcR and housekeeping genes observed in the two IL3-dependent cell lines are similar, however, distinct differences in nascent transcription (cell cycle specific) was observed between the two cell lines for a subset of oncogenes. These differences may be related to the hematological origins of the two cell lines and will be discussed.

C 734 REGULATION OF PROTO-ONCOGENE EXPRESSION BY INSULIN, Joseph L. Messina, Dept. of Physiology, SUNY Health Science Center, Syracuse, N.Y. 13210
Insulin is known to affect the cellular levels of many different mRNAs. We have shown that insulin alone stimulates the accumulation of a specific mRNA (termed p33) in serum-deprived rat H4 hepatoma cells (JBC 260: 16418, 1985). There was both a transcriptional and a post-transcriptional effect of insulin on this mRNA. Here we show that insulin can regulate the transcription rate of 2 proto-oncogenes, c-myc and c-fos, in rat hepatoma cells. The control of c-myc by insulin was complex, with an initial insulin-induced decrease in c-myc transcription (to approximately 50% of control) at 15-30 min. However, this was followed by an increase in transcription of about 3-fold by 60-120 min. Insulin's control of c-fos transcription was less complex. There was a rapid, dose-dependent increase in c-fos transcription of 7-fold following 15-30 min of insulin addition (5×10^{-9}M). The increase in c-fos was transitory, returning towards baseline transcription rates within 120 min. The increase in transcription of c-myc and c-fos was dependent on the dose of insulin added to the H4 cells, with a maximal concentration of approximately 1×10^{-8}M. Insulin is known to increase DNA synthesis and cell division in the H4 cell line. Alterations in c-myc and c-fos gene expression have been correlated with changes in cell division and differentiation in several other systems. Thus, insulin's first action in initiating cell division may be to regulate the levels of c-myc and c-fos mRNAs.

C 735 FUNCTIONAL SIGNIFICANCE OF P53 ONCOGENE INACTIVATION IN ERYTHROLEUKEMIA CELL LINES: ESCAPE FROM THE ADAPTIVE IMMUNE RESPONSE. M. Mowat, G. Hicks, D. Chow Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada R3E 0V9.
The objective of these studies is to determine the significance and mechanisms of rearrangement of the p53 oncogene in the multistage erythroleukemia induced by Friend leukemia virus. We have previously described several erythroleukemia cell lines altered in their expression of p53 [Mowat et. al. (1985) Nature, 314:633-636, Chow et. al. (1987) J. of Virol., 61:2777-2781]. Some cell lines do not express p53 whereas others express a truncated p53 protein. Cell lines not expressing p53 sho' reduced tumor forming ability compared to the p53 expressing cell lines. We wanted to know what selective mechanism allows outgrowth of p53 non-expressing cell lines in spite of reduced tumorigenicity. To test the possibility that the loss of p53 expression allows cells to escape the adaptive immune response, we have compared the adaptive stimulation and susceptibility to the immune response between p53 expressing and non-expressing cell lines isolated from the same mouse. We have found the p53 non-expressing cell line, with rearrangement of both alleles of the p53 gene, stimulates poorly as well as being less susceptible to splenic cytolysis compared to the p53 expressing cell line. This cytolysis was due to T-lymphocytes. We will be reporting on other matched sets of cell lines. To understand the mechanism of p53 gene rearrangements, we have cloned both alleles of the p53 gene from one cell line. We have found insertion of sequnces related to the helper virus FMULV into both alleles of p53. We will be presenting detailed molecular analysis of these clones. These results indicate that inactivation of an oncogene may also play a role in tumor progression.

C 736 SIMULTANEOUS GENETIC TRANSFER OF THE RADIORESISTANT AND TRANSFORMING PHENOTYPES, K.F. Pirollo[1], W.A. Blattner[2], and E.H. Chang[1], USUHS, [2]NCI, Bethesda, MD 20814
The non-cancerous skin fibroblasts (NCSF) of members of a cancer prone family displaying the Li-Fraumeni syndrome, which is manifested in a variety of neoplasms, were found to exhibit the unique characteristic of resistance to the killing effects of ionizing radiation (RR). Northern hybridization has revealed a 3-8 fold elevation in the expression of c-myc in the NCSF of several family members but not in those of normal controls. Similar hybridizations with several other oncogenes showed no abnormal levels of expression. The NCSF line with one of the highest levels of c-myc expression also exhibited the most prominent RR phenotype. When HMW DNA from this NCSF was transfected into NIH3T3 cells the transfectants produced tumors in nude mice. Secondary and tertiary (3°) Alu+ tumors were obtained. Of the oncogenes tested by Southern analysis strong homology was shown only between the human transforming sequences and raf. A single cell clonal line derived from one such Alu+, raf+ 3° tumor was found to have an elevated RR level comparable to that of the NCSF line from which the donor DNA was originally obtained. Therefore, it appears that there has been a concominant transfer of both the RR and the transforming phenotypes. Evaluation of the RR levels of a series of cell lines obtained by transfection of various oncogenes into NIH3T3 cells revealed the following results. The c-myc and v-fes transfectants had a normal RR level, comparable to that of the recipient NIH3T3 cells. In contrast, v-mos, MH2 (a v-mil(raf)/c-myc containing virus) and H-ras transfectants showed a markedly increased RR level, similar to that displayed by the 3° tumor and the original NCSF lines. Therefore, there appears to be a relationship between the presence of certain activated oncogenes and the RR phenotype.

C 737 Investigation of bcl-2 Function by Gene Transfer and Anti-Sense Oligonucleotides. Reed J., Tsujimoto, Y.*, Croce, C.*, and Nowell, P. University of Pennsylvania and *Wistar Institute, Philadelphia PA.

The bcl-2 gene is involved in t(14;18) chromosomal translocations in follicular lymphomas, and is transiently transcribed in human lymphocytes when stimulated to proliferate. This gene has thus been postulated to play a role in abnormal and normal lymphocyte growth. To explore the functions of the human bcl-2 gene, we have introduced bcl-2 DNA sequences, under the control of strong constitutive promoters, into lymphoid and fibroblast cells. Transfection of bcl-2 constructs into freshly isolated rat embryo fibroblast (REF) cells demonstrated that bcl-2 can substitute for c-myc in complementing with an activated Ha-c-ras oncogene to morphologically transform these cells. REF cells transformed by bcl-2 plus ras formed colonies in soft agar and tumors in nude mice, thus providing functional evidence for the oncogenic potential of the bcl-2 gene. Electroporation of recombinant bcl-2 sequences into JURKAT cells (human T cell leukemia) reduced accumulation of endogenous bcl-2 mRNAs, indicative of a feedback suppression mechanism. Culturing leukemic B cells and normal peripheral blood lymphocytes with synthetic oligonucleotides (20' mers) complementary (anti-sense) to strategic locations in bcl-2 mRNAs specifically inhibited the growth of these cells. Taken together, our findings provide evidence for a functional role for bcl-2 in the regulation of normal and neoplastic lymphocyte growth.

C 738 NUCLEAR ONCOGENES RESCUE *V-HA-RAS* -INDUCED GROWTH ARREST IN SCHWANN CELLS
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We are using homogenous populations of normal rat Schwann cells as a model system to study the molecular mechanisms that underlie oncogene cooperation. In order to investigate the effect of single or multiple oncogenes, we have sequentially introduced a temperature-sensitive SV40 Large T gene and the viral *Ha-ras* oncogene into these cells.

The effect of the *v-Ha-ras* oncogene on Schwann cell growth is dramatically different in the presence or absence of SV40 Large T. In cells expressing Large T at the permissive temperature *v-Ha-ras* causes transformation, frees the cells from the requirement for exogenous growth factors, induces growth factor secretion and increases the cell growth rate six-fold. In the absence of functional Large T, at the non-permissive temperature, it induces growth arrest without evidence of differentiation. This arrest is fully reversible both on shifting the cells back to the permissive temperature, and on introducing the nuclear oncogenes *v-myc* or adenovirus E1A at the non-permissive temperature. FACS analysis indicates that the cells arrest in both the G1 and G2 stages of the cell cycle. Schwann cells expressing only the ts Large T gene continue to grow normally at the non-permissive temperature.

These data indicate that in this system oncogene cooperation is not simply due to the combined actions of two different oncogenes. The changes in cell growth induced by *v-Ha-ras* are dependent on the cellular and genetic background in which it is expressed.

C 739 **BASIC FIBROBLAST GROWTH FACTOR (bFGF) IS A TRANSFORMING PROTEIN WHEN FUSED TO A SIGNAL PEPTIDE**, Snezna Rogelj, Robert A. Weinberg, Paul Fanning* and Michael Klagsbrun*, The Whitehead Institute, Cambridge, MA and *Children's Hospital, Harvard Medical School, Boston, MA.

To investigate how the apparently intracellular bFGF finds and activates its cognate cell surface receptor, we introduced the bFGF genes without or with an artificial signal sequence into the receptor positive NIH 3T3 fibroblasts. Two mammalian expression constructs were made: one containing the native bovine bFGF cDNA (pbFGF), and another containing the 19 amino acid mouse heavy chain IgG signal sequence fused in frame with the initiation of the bFGF coding sequence (pIg-bFGF). Both constructs were cotransfected into NIH 3T3 cells with a dominant selectable marker, and clonal cell lines expressing high levels of bFGF were selected. Both constructs induced expression of bFGF that is immunologically positive, biologically active, binds to heparin-Sepharose and elutes at 1.5M NaCl. NIH 3T3 cells transfected with pbFGF and expressing the native 18Kd bovine bFGF appear to have only slightly greater saturation density and serum independence in culture and a low frequency of tumorigenicity in the syngeneic NSF mice. In contrast, the cells transfected with the pIg-bFGF construct are focus forming, have transformed morphology and are consistently tumorigenic in NSF mice, although they also do not secrete the growth factor into the extracellular medium. The hybrid, unlike the native protein, appears to be entering an intracellular compartment from where it has a direct access to the receptors and their activating mechanisem. Our cell lines can serve as a model system for studying transformation by a growth factor via an apparently direct, intracellular autocrine loop.

C 740 ANALYSIS OF SPLEEN FOCUS-FORMING VIRUS-INFECTED CELLS FOR EPO PRODUCTION AND EPO RECEPTORS, Sandra Ruscetti[1] and Francis Ruscetti[2], National Cancer Institute, Bethesda, MD 20892[1] and Frederick Cancer Research Facility, Frederick, MD 21701[2]

The polycythemia-inducing strain of the spleen focus-forming virus ($SFFV_p$)induces an acute erythroleukemia in mice. Erythroid cells from these mice are altered in their hormonal requirements for growth and differentiation. Normal erythroid cells require erythropoietin (Epo) for proliferation and differentiation, while erythroid cells infected with $SFFV_p$ proliferate to high levels in the apparent absence of Epo. The unique envelope protein encoded by this virus is responsible for its biological effects. Since this protein appears to interact with a target present only in erythroid cells and since Epo is specific for these cells, it is possible that the virus is exerting its effect through this hormone. In an effort to ascertain if this is the case, we examined cells from $SFFV_p$-infected mice to determine (1) if they produce Epo or other erythroid growth factors and (2) if they express elevated numbers of Epo receptors that may result in a reduced requirement for the level of Epo required for growth and differentiation. Our results indicate that $SFFV_p$-infected cells do not secrete Epo or any other erythroid growth factors that could account for the reduced hormonal requirements of these cells. Also, our studies using iodinated Epo in cell binding assays as well as cross-linking studies indicate that $SFFV_p$-infected cells are not significantly different from normal erythroid cells in the number or quality of their Epo receptors. We are currently investigating the possibility that the virus may be exerting its effect on a postreceptor step in the Epo signal transduction pathway.

C 741 AUTOSTIMULATORY MECHANISMS OF ONCOGENESIS IN MYELOID CELLS, Schrader, J.W., Leslie, K.B., Ziltener, H.J. and Clark-Lewis, I. The Biomedical Research Centre, The University of British Columbia, Vancouver, BC, V6T 1W5.

A series of cytokines stimulate the division or differentiation of lympho-hemopoietic cells. Examples of the autologous production of particular cytokines by lympho hemopoietic cells with resultant autostimulation, are now known to occur both physiologically and as a result of genetic aberrations in the regulation of cytokine production. The pathological production of autostimulatory cytokines can be a critical, oncogenic step in the genesis of lympho-hemopoietic tumors, both in vivo and in in vitro models, including those in which cells have been infected with retroviruses directing the constitutive expression of growth factors. It is not clear whether autostimulation due to the autologous production of a growth factor, whether physiological or pathological, requires secretion of the factor (i.e. an "autocrine" mechanism) or can be mediated by the interaction of the factor and its receptor inside the cell. Experiments on the effects on autostimulatory leukemias of antibodies specific for the growth factor or its receptor will be presented. Evidence for a third "paracrine" oncogenic mechanism will also be discussed. The importance of characterizing the biologically relevant receptors for growth factors and resolving discrepancies between biological data and data generated by studies on the binding of radiolabelled factors will be addressed, together with information on the structure-function relationships of IL-3 and GM-CSF molecules.

C 742 EFFECTS OF GROWTH FACTORS ON DNA SYNTHESIS IN RAS-TRANSFECTED FIBROBLAST LINES DIFFERING IN METASTATIC PROPERTIES, L.C. Schwarz, M.C. Gingras, A.H. Greenberg, J.A. Wright, Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada, R3E 0V9. Lines were obtained by either transfection of 10T1/2 cells with T24 H-ras (NR4 and C3), or by treatment with radiation (MDSR.5). These cells were previously shown to vary in their metastatic and tumorigenic potential (Egan et al., Mol. Cell. Biol. 7:830, 1987). Metastatic potential inversely correlated with serum dependence. Cells were exposed for 24 hrs to 10% FBS or a defined medium (DM) ± EGF. Cells were then plated with one of PDGF, bFGF, or TGF-β for 42.5 hrs. DNA synthesis was determined using ^{3}H-TdR. An index of stimulation (IS) was determined as: dpm in the presence of growth factor/dpm in the absence of growth factor. Cells which were not tumorigenic or metastatic (10T1/2) produced the largest IS in response to PDGF (IS = 27.0), and bFGF (IS = 33.0) in the absence of EGF. EGF increased this response. Cell lines which lacked (MDSR.5) or had low metastatic potential (NR4) and were tumorigenic, were much less sensitive to all growth factors. The most metastatic and tumorigenic cell line, C3, was also less sensitive to PDGF and bFGF (IS = 2.0 and IS = 10.0 respectively) than 10T1/2 cells in DM ± EGF. TGF-β inhibited all cell lines except C3 in the absence of EGF and variable responses occurred in the presence of EGF. C3 cells were stimulated by TGF-β (IS = 3.5) in DM ± EGF. All cell lines produced a lower IS in 10% FBS than in DM. In summary, H-ras transfected cells lost their responsiveness to PDGF, bFGF and EGF as they became tumorigenic and more metastatic. Increased metastatic potential may also be due to a completely altered response to TGF-β and EGF as observed in C3 cells.

C 743 EXPRESSION OF ONCOGENE PRODUCTS IN TRANSFORMED AND NON-TRANSFORMED CELLS: FLUORESCENCE ACTIVATED CELL SORTER ANALYSIS. Doris L. Slate and Nicholas Bruno, Syntex Research, Palo Alto, CA 94304

Since fluorescence activated cell sorter (FACS) analysis provides data at the individual cell level, it should prove useful in studying protein expression in heterogeneous tumor cell populations and can provide information to complement immunoprecipitation and Western blot assays. We have used FACS analysis to study the expression of oncogene products in a variety of cell lines. In pilot experiments, we found great variation in epidermal growth factor (EGF) receptor expression in human carcinoma cell lines; A431 epidermoid carcinoma cells had very high levels of EGF receptor expression, consistent with previous work on EGF binding and receptor mRNA levels in this line.

In order to study intracellular protein expression, we employed a number of different fixation/permeabilization protocols including the use of lysolecithin, paraformaldehyde + various detergents, and ethanol fixation. Results with a series of NIH 3T3 cell lines transfected with v-Hras, EJ-ras, and c-Hras (provided by Dr. Douglas Lowy, National Cancer Institute) indicated that lysolecithin permeabilization resulted in the best specific staining for ras p21, although all the evaluated techniques gave the same relative rank order for expression.

We are continuing our studies with antibodies against other oncogene products and will discuss our results with other antigen/antibody combinations.

C 744 CELL-CYCLE DEPENDENT EXPRESSION OF PROTO-ONCOGENES DURING STIMULATION OF NORMAL HUMAN B-LYMPHOCYTES, Erlend B. Smeland[1], Heidi K. Blomhoff[1], Steinar Funderud[1], Jacques Ghysdael[2], Erik Boye[3], Lab. for Immunology, Institute for Cancer Research, Oslo, Norway; [2]Institut Pasteur, Lille, France; [3]Dept. of Biophysics, Institute for Cancer Research, Oslo, Norway.

Normal B-lymphocytes are suitable for studies of cell cycle dependent expression in different genes. Circulating B lymphocytes in peripheral blood are in a physiological resting state, and they can be stimulated into proliferation in vitro by addition of antibodies against surface molecules and/or factors acting on the B cells. Growth signals can be roughly divided into competence inducing growth factors, which activate resting cells from G0 into G1, and progression factors, which act in G1 and promote further cell cycle progression. By varying the triggering conditions, one can achieve stepwise activation of the cells from G0 into S phase. We have studied in detail the expression of the c-myc gene during B cell stimulation. There is a marked increase of c-myc mRNA levels early after stimulation of resting cells, peaking at approximately 3 hours. This increase is mainly due to a transcriptional activation of the myc gene, and is followed by a corresponding increase in c-myc protein levels. We also found that the c-myc mRNA levels could be upregulated in the G1 phase of the cell cycle by anti-immunoglobulins, but not by B-cell growth factor (BCGF). By using "nuclear run-on" experiments, we have shown that several other oncogenes are cell cycle dependent regulated in B lymphocytes. P53, K-ras and H-ras reach maximal expression in mid to late G1. The C-ets-1 proto-oncogene was also found to be cell cycle dependent expressed in B cells, suggesting that it may play an important role during B cell growth. Data on TGF-β and forskolin mediated inhibition of the cell cycle dependent expression of these oncogenes will also be presented.

C 745 **A NOVEL EARLY GROWTH RESPONSE GENE INDUCED WITH FOS-LIKE KINETICS BY FIBROBLAST, EPITHELIAL CELL AND LYMPHOCYTE MITOGENS ENCODES A POLYPEPTIDE WITH THREE DNA BINDING "FINGER" MOTIFS**, Ximmin Cao, Louise Chang, Chon-Hwa Tsai-Morris, and Vikas P. Sukhatme, University of Chicago, Chicago, Illinois 60637.
Mitogens evoke many alterations in gene expression in eukaryotic cells. Genes activated rapidly and transiently, and whose induction is shared by diverse cell types when exposed to different growth stimuli are likely to be of critical importance in transducing mitogenic signals and regulating cellular proliferation. C-myc and c-fos are the only known genes fulfilling these criteria. Recently we have isolated a novel early growth response (**egr**) gene which also satisfies these conditions. (Sukhatme et al. Oncogene Research, in press, 1987). In response to serum, its mRNA is induced dramatically in mouse fibroblasts reaching a peak level at about 30 minutes that is ten times higher than the maximal value attained by c-fos mRNA. Importantly, the gene is highly induced in insulin stimulated rat hepatoma cells, adenosine diphosphate treated monkey kidney epithelial cells, and PHA stimulated human blood lymphocytes. Sequence of the murine **egr** cDNA indicates that we have identified a novel transcript. The 3' untranslated part contains an AU rich region. The single long open reading frame predicts a polypeptide with homology to a non-homeo box region of the Drosophila engrailed gene and displays three DNA binding "finger" motifs with maximal homology to the Drosophila Kruppel gene "fingers" and to Xenopus TFIIIA. These data suggest that the **egr** gene product is a DNA binding protein whose function like that of the fos protein might be to act as a nuclear switch in signal transduction.

C 746 **HUMAN CELL STUDIES OF THE FUNCTIONAL INTERACTION OF HUMAN ONCOGENES AND SUPPRESSOR GENE** **David Krizman, Paul Chiao, Sun O. Yim and Michael A. Tainsky, DEPT. OF TUMOR BIOLOGY , M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE, UTSCC, HOUSTON, TEXAS**
A human cell culture system has been developed to identify genetic elements (oncogenes) which are involved in the transitions in multistage carcinogenesis. PA-1 human teratocarcinoma cells show progression as they are passaged in culture. Early passage cells are nontumorigenic in athymic nude mice while late passage cells readily form tumors. This transition was induced by an activated N-ras oncogene which activated spontaneously during culturing and can be reproduced by transfection of the cloned oncogene into preneoplastic PA-1 cells thus proving its causal role in the transformation process. However, we have found that contact inhibition of growth, is not released by the ras oncogene in this system while ras and myc transformed cells are somewhat resistant to growth inhibition by retinoic acid. Certain preneoplastic cells in this PA-1 series contain genes which make them susceptible to transformation by single oncogenes while others require 2 oncogenes to induce tumorigenesis. The molecular genetic basis of this susceptibility to single oncogene induced transformation may due to the inactivation of a suppressor gene. We have found that the mechanism by which cells acquire the susceptibility to ras is related to responsiveness to growth factors possibly affecting an autocrine mechanism. This represents a new concept for inactivation of a suppressor gene during a preneoplastic stage progressing toward tumorigenicity. We will discuss differences in the expression oncogenes and growth factors as well as other transformation sensitive genes in cell lines in the PA-1 series representing various stages of tumor progression with regard to loss of a suppressor gene.

C 747 LEVELS OF PROTO-ONCOGENE mRNA IN NORMAL HUMAN BRONCHIAL EPITHELIAL (NHBE) CELLS EXPOSED TO TYPE β TRANSFORMING GROWTH FACTOR (TGF-β) AND/OR ITS ANTAGONIST, EPINEPHRINE. Tohru Masui, Brenda I. Gerwin, John F. Lechner, and Curtis C. Harris, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892

Epinephrine and TGF-β have opposing effects on NHBE cells. While TGF-β is a negative growth factor and plays a positive role in squamous differentiation of NHBE cells, epinephrine stimulates growth of NHBE cells and neutralizes the effects of TGF-β. We examined the steady state levels of the proto-oncogenes c-myc, c-fos, and c-Ha-ras under the influence of these two agents. Specific mRNA was detected by Northern blotting and normalized by probing the constitutive enzyme glyceraldehyde-3-phosphate-dehydrogenase. The level of c-myc mRNA was reduced by TGF-β up to 40% at 1 hr and returned to the control level after 24 hrs. Epinephrine increased the expression of c-myc about 2-3 fold and neutralized the reduction of c-myc mRNA by TGF-β. An increase in the level of c-fos mRNA was induced at 1 hr by TGF-β (1.5 fold) and epinephrine alone (2-3 fold). The level of c-fos returned to the control level after 9 hrs. TGF-β and epinephrine in combination acted synergistically to increase c-fos mRNA levels. The level of c-Ha-ras mRNA was not affected by TGF-β or epinephrine. These data suggest that in NHBE cells a transient alteration in c-myc mRNA levels reflects or is involved in a signal in the proliferative pathway. On the other hand the levels of c-fos and c-Ha-ras mRNA were uncoupled from the process of growth or squamous differentiation of NHBE cells.

C 748 AMPLIFICATION OF PROTOONCOGENE C-FMS IN HUMAN PLACENTA AND A BREAST CANCER CELL LINE MCF-7. Raman Tuli, Maria Zannis-Hadjopoulos, Pierre Major, McGill Cancer Centre, McGill University, Montreal, P.Q., Canada, H3G 1Y6.
The protooncogene c-fms encodes a 170 kd growth factor receptor with associated tyrosine kinase activity. Increased expression of the c-fms in some human breast carcinomas and normal placental tissue has been previously documented. We have demonstrated the c-fms protooncogene to be amplified approximately 20 fold in the human breast tumor cell line MCF-7, and 10 fold in normal human placenta. These observations were made by dot blot hybridization of DNAs obtained from MCF-7, placenta and the human fibroblast cell line, WI38. The amplification was quantitated using a 0.9 kb fragment of the cloned c-fms gene as the probe. Genetic rearrangement was also demonstrated at the c-fms locus in both MCF-7 and placental DNA by restriction enzyme analysis and Southern blot hybridization. In addition to the 16 kb normal band, an EcoRl digest revealed 3 new restriction fragments in MCF-7 DNA and 1 other fragment in placental DNA. These novel bands were of greater intensity than the 16 kb primary band. We believe these additional bands are responsible for the amplification. The increased expression of c-fms in placenta may be associated with the observed amplification and genetic rearrangement at this locus. The observation of amplification and possibly rearrangement in both a tumor cell line and normal embryonic tissue suggests that the tumor cells, subsequent to an activation event, may have acquired a cellular phenotype that is normally associated with embryonic tissue.
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C 749 EXPRESSION OF EGF-RECEPTOR AND TGFα IN NORMAL HUMAN MAMMARY EPITHELIAL CELLS AND ONCOGENE TRANSFORMED SUBLINES. E.M. Valverius, S.E. Bates, M. Stampfer, R.Clark, F. McCormick, M.E. Lippman and R.B. Dickson, Medicine Branch, NCI/NIH, Bethesda, MD 20892, Univ. of California, Berkeley, CA 94720, and Cetus Corp., Emeryville, CA 94608.
TGFα is expressed in 70% of human mammary tumor biopsies and most human breast cancer cell lines. We have examined EGF-receptor expression and production of TGFα in non-neoplastic, immortalized and oncogene transformed human mammary epithelial cells. Immortalized 184A1N4 cells were obtained following benzo-a-pyrene treatment of the 184 mammary epithelial cell line (Stampfer, PNAS 82: 2394, 1985). Oncogenes were introduced into 184A1N4 using retroviral vectors carrying SV40T, v-Ha-ras, or SV40T+Ha-ras. Only the nontumorigenic, anchorage dependent 184 and 184A1N4 required EGF/TGFα supplementation for proliferation. Transformation by T rendered the cells weakly tumorigenic and sensitive to EGF/TGFα stimulation of anchorage independent growth (AIG). Ras transformation made the cells capable of some AIG, not further stimulated by EGF/TGFα, and weakly tumorigenic. Transformation with T and ras appeared to completely substitute for the EGF/TGFα signal, yielding cells that cloned extensively in soft agar, not responsive to EGF/TGFα, and were highly tumorigenic in nude mice. There were no detectable differences among the cells in expression, at high levels, of both the 4.8 kb TGFα mRNA and 10.5 kb EGF-R mRNA. They all produced 0.1-0.4 ng TGFα/ml of conditioned medium and displayed 0.4-1x10^6 EGF-R sites/cell with K_D values in the nanomolar range. Thus, in human mammary epithelial cells we found that SV40T appeared to couple EGF/TGFα to the transformed phenotype, and we could observe no significant association among EGF-R and TGFα expression and malignant progression.

C 750 EMBRYONIC LETHALITIES AND ENDOTHELIAL TUMOURS IN EMBRYONAL STEM CELL CHIMAERAS EXPRESSING POLYOMA MIDDLE T ONCOGENE. R. Lindsay Williams, Sara A. Courtneidge and Erwin F. Wagner. European Molecular Biology Laboratory, 6900 Heidelberg, Germany. The consequence of constitutive expression of the transforming middle T (mT) antigen of polyoma virus in embryos and adult mice is being investigated to study the cell specificity of mT and the role of its associated cellular tyrosine kinases *in vivo*. A replication-defective selectable retrovirus which constitutively expresses mT has been constructed and used to introduce the mT antigen into a wide variety of cell types *in vivo*(1). To determine the effect of mT expression on mouse development, embryonal stem (ES) cell clones were infected with the virus. Several selected clones were established which constitutively express mT and its associated tyrosine kinase activity. The cells appeared morphologically normal and mT expression appeared to relieve the requirement for differentiation inhibiting factor(s) normally necessary to prevent their *in vitro* differentiation. Chimaeric embryos obtained by blastocyst injection of individual ES cell clones initially developed normally but were specifically arrested at mid-gestation, most likely due to the observed disruption of blood vessels by multiple cavernous haemangiomas. Injection of these chimaeric embryos into syngeneic hosts resulted in the formation of endothelial tumours. In a seperate series of experiments direct injection of the virus into newborn and adult mice also resulted in the rapid appearance of multiple haemangiomas due to endothelial cell proliferation. The haemangiomas of chimaeric embryos and infected mice were used to establish endothelial cell lines. The cell lines, which expressed mT, retained expression of the endothelial cell differentiation marker von Willebrand factor, were contact inhibited and failed to grow in soft agar, yet were tumourigenic when injected into syngeneic mice. Analysis of tumour induction in non-syngeneic animals revealed endothelial tumours of host origin suggesting that the introduced mT expressing endothelial cells may release an angiogenesis inducing factor. Recent results concerning the characterisation of mT activity in ES and endothelial cell lines *in vitro* and *in vivo* will be presented. (1) Williams,R.L., Courtneidge, S.A. and Wagner, E.F. Cell submitted.

C 751 EXPRESSION OF ACTIVATED c-Ha-ras OR v-Ha-ras IN MURINE MELANOCYTES OVERCOMES GROWTH DEPENDENCE ON TPA AND INDUCES TUMOURIGENICITY, Rosemary E. Wilson*, Tom Dooley and Ian R. Hart, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, WC2A 3PX, U.K.

An immortal line of non-tumourigenic melanocytes (Mel-ab) dependent upon 12-O-tetra-decanoyl phorbol-13-acetate (TPA) for growth (Bennett et al, Int.J.Cancer, 39, 414, 1987) has been transfected with plasmids harbouring either the activated c-Ha-ras gene or the v-Ha-ras gene, in addition to the gene encoding aminoglycoside phosphotransferase. Transfectant colonies resistant to G418 (800 µg/ml) were isolated and examined for in vitro growth characteristics and tumourigenicity. Those transfectants (Mel-ab pAGT) expressing activated c-Ha-ras from the herpes simplex virus Tk promoter grew equally well in the presence or absence of TPA. Cells expressing v-Ha-ras from a retroviral LTR (Mel-ab LTR ras) also grew well in the absence of TPA, but unlike Mel-ab pAGT cells were actually growth inhibited in the presence of TPA (160 nM). As expected, control transfectants lacking a cellular or viral Ha-ras insert had the same TPA growth requirement (150-200 nM) as the parental Mel-ab line. In Mel-ab cells and control transfectants cholera toxin (CT) at 10^{-9}M acts synergistically with TPA to induce mitogenesis, but on its own has no growth promoting effect. Conversely, in Mel-ab LTR ras transfectants, a strong proliferative response to 10^{-9}M CT alone was observed, although this effect was totally abolished by the inclusion of TPA. Tumourigenicity of the cell lines was monitored by subcutaneous injection into athymic nude mice. All 5 animals injected with 1×10^6 Mel-ab pAGT or Mel-ab LTR ras cells grew tumours >1cm diameter in less than 20 days, whereas 2×10^6 parental Mel-ab cells failed to produce tumours even after 200 days. Histological examination and specific stains revealed that the tumours were malignant melanomas. These results show that ras-induced tumourigenicity is associated with the induction of factor-independent growth. Both TPA and CT promote growth via pathways involving plasma membrane-localized 'G' proteins (Rozengurt, Science 234, 161, 1986). We speculate that in the ras-transformed melanocytes the ras protein is functioning as, or interacting with, one or more G proteins.

C 752 MOMULV-INDUCED PROMONOCYTIC TUMORS IN PRISTANE PRIMED MICE: COMPLETE INHIBITION OF TUMORS BY INDOMETHACIN, Linda Wolff, J. Frederic Mushinski, Grace Shen-Ong, and Herbert C. Morse III *, National Cancer Institute and *National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892.

Recently we have developed a system in pristane-primed BALB/c mice for rapid induction of promonocytic tumors. The incidence of these tumors is 58 percent with an average latency of 71 days. In this system Moloney murine leukemia virus (MoMuLV) is inoculated intravenously into BALB/c mice that have received an intraperitoneal injection of pristane. These tumors, which arise as a consequence of insertional mutagenesis of the c-myb gene, become evident in the peritoneal cavity as an outgrowth of the granuloma. It is clear that their growth and development is dependent on the peritoneal inflammatory response because, in the absense of pristane treatment, tumor incidence drops to 0 percent. The objective of the present study was to test the effects of indomethacin, a nonsteroid anti-inflammatory drug, on the development of these tumors. For this study indomethacin was provided in the drinking water at a concentration of 20ug/ml. It was found that with indomethacin treatment tumors were virtually eliminated. We suggest that the observed effect of indomethacin may be through its inhibition of prostaglandins, either reducing the influx of myeloid cells that may be facilitated by prostaglandin secretion or reversing an immunosuppression caused by prostaglandins.

C 753 MODULATION OF RAS EXPRESSION BY NOVEL ANTI-SENSE ANALOGS, Z.Yu[1], D.Brown[1], R.Black[1], P.Miller[2], P.Ts'o[2], and E.Chang[1], 1:USUHS, Bethesda, MD 20814, 2:Johns Hopkins University, Baltimore, MD 21205

Modified nucleic acid analogs, oligodeoxyribonucleoside methylphosphonates(ONMP) have been examined as selective inhibitors of gene expression. These analogs possess several advantageous properties: nuclease resistance, nontoxicity at reasonable concentration(≤500uM) and ready uptake by cultured cells. Ras genes have been implicated in the initiation and development of numerous human neoplasms. Anti-ras ONMP's specific for the initiation codon(IC) region have been synthesized to explore their efficacy and specificity on ras-p21 translation in vitro. The entire Balb-ras gene inserted into pGEM4 served as a source of ras mRNA. The cell-free translation product was immunoprecipitated by a monoclonal antibody to ras-p21 and analyzed by SDS-PAGE. The oligomer, 3'TACTGACTTAT5'(IC-0) complements the Balb-ras IC region precisely. Oligomer IC-1(3'TACTGCCTTAT5') contains one nucleotide mismatch whereas oligomer IC-2(3'TACTCGCTTAT5') contains two sequential mismatches. At 100uM and with preannealing to ras mRNA, IC-0 inhibits p21 translation up to 97%; IC-1 and IC-2 have considerable less inhibitory effect. At the same concentration, ONMP's not preannealed to the mRNA were considerably less effective. Thus inhibition appears to be a result of the specific complementation of the oligomers to the message. Oligomers complementary to the 12th amino acid codon region have also been shown to inhibit cell-free translation of p21. An oligomer complementary to the splice junction of Intron I and Exon II of the human c-Ha-ras has exhibited a significant inhibitory effect on p21 expression in mouse cells transformed by human c-Ha-ras(containing introns) but not in cells transformed by v-Ha-ras(lacking introns).

C 754 A POINT MUTATION IN THE FIRST INTRON OF THE c-myc LOCUS IN A BURKITT'S LYMPHOMA ABOLISHES BINDING OF A NOVEL NUCLEAR PROTEIN. Maria Zajac-Kaye[1], Edward Gelmann[1], and David Levens[2], Medicine Branch[1] and Laboratory of Pathology[2], NCI, Bethesda, MD 20892. The Burkitt's lymphoma cell line, PA682, carries a t(8:22) chromosomal translocation more than 16kb downstream from exon 3 of the c-myc gene. Sequence analysis of the abnormal c-myc allele from this cell line revealed point mutations scattered throughout the 3' region of exon 1 and the 5' region of intron 1. In addition, there was a 38 bp deletion of a T-rich segment downstream of the exon 1 splice-donor site. Nuclear run-off analysis of PA682 cells demonstrated a strong block in mRNA elongation, indicating that the c-myc activation in PA682 cells is not due to release of this block by deletion of the T-rich region. To determine if any of the point mutations may be important in the deregulation of the c-myc gene, we performed an exonuclease assay which demonstrated specific binding of a novel nuclear protein to normal intron 1 DNA, but not to the same region from the mutated PA682 c-myc allele. Examination of the nucleotide sequence bordered by barriers to exonuclease hydrolysis revealed two base mutations in PA682 as compared to a normal c-myc gene. DNase I footprint analysis defined the same segment of the normal allele. Gel retardation analysis using defined oligonucleotide probes and competitors demonstrated that each of the two mutations diminished the strength of the interaction between the putative factor and this binding site. Examination of abnormal c-myc sequences, cloned from two other t(8:22) Burkitt's lymphoma cell lines, revealed point mutations located precisely within the same binding site identified above. We speculate that specific mutations in this cognate sequence may be a common feature contributing to c-myc activation in a subset of Burkitt's lymphoma.

C 755 ACTIVATED C-HA-RAS STOMACH CANCER, Guoren Deng, Beijing Institute for Cancer Research, Beijing, China. Activated c-Ha-ras was identified and cloned from a cell line, BGC-823, of a Chinese stomach cancer patient by DNA transfection assay and screening of the genomic library of the transformant. By comparing the sequence of the activated c-Has-ras and its normal homologue, the activation lesion of the oncogene was identified as single nucleotide substitution of thymine for guanine in the 12th codon. This results in the substitution of valine for glycine at the 12th amino acid of p21 protein. Further more, the point mutations at the 12th codon of c-Has-ras were also determined in more than 30% of DNA samples from the stomach cancer patients by restriction fragment length polymorphysm (RFLP) assay and hybridization with oligonucleotide probes. The relationship between the transformation potency of the DNA from the tumors and the length of variable tandem repetition (VTR) region downstream of the c-Has-ras were also discussed.

Late Additions

C 756 NMR SOLUTION STRUCTURE OF TGFα, Brian D. Sykes+, James C. Jenson*, and Richard N. Harkins*, +NMR Group in Protein Structure & Function and Department of Biochemistry, University of Alberta, Edmonton, Alberta T6G 2H7 Canada, and *Triton Biosciences Inc., 1501 Harbor Bay Parkway, Alameda, CA 94501.

Two dimensional high resolution ^{1}H Nuclear Magnetic Resonance Spectroscopy (2D-NMR) techniques have been used in conjunction with the computational techniques of energy minimization and molecular dynamics to determine the structure of TGFα in solution. The 2D-NMR approach relies on the assignment of the protein spectrum in H_2O and D_2O solutions (to include the non-exchangeable CH protons and the exchangeable peptide NH protons) using COSY (spin correlated) and RELAY (spin relayed) 2D-NMR techniques followed by the determination of the through space internuclear distances between these assigned protons. The distances are determined via 2D-nuclear Overhauser enhancement spectroscopy (NOESY). Once these distances have been obtained they are used as pseudo potential energy restraints in the energy minimization and molecular dynamics programs so that the structure is determined in part by experimental constraints and in part by theoretically calculated potential energies. The structure of TGFα is composed of antiparallel β-pleated sheet segments, similar to that published for the homologous EGF molecule.

C 757 EXPRESSION OF A SYNTHETIC GENE ENCODING BOVINE BASIC FIBROBLAST GROWTH FACTOR bFGF IN ESCHERICHIA COLI; Wiebke Knoerzer, Klaus Schneider, PROGEN, Heidelberg; Hans-Peter Binder, University of Heidelberg; Peter Gruss, Max Planck Institute, Göttingen; W. Risau, Max Planck Institute, Tübingen, (FRG).

A synthetic gene encoding bovine bFGF was assembled and cloned using established E. coli plasmids. Transformed E. coli cells directed the synthesis of a fusion protein consisting of a truncated ß-Galactosidase and bovine bFGF. The growth factor was purified from E. coli lysates by cationexchange and Heparin sepharose affinity chromatography. The produced recombinant bFGF was biologically active as monitored by the mitogenic activity for BAE cells and the angiogenic capacity of the purified factor in the rabbit cornea. Experiments using the recombinant factor and a model which represents a real wound situation in which repeated quantitative measurements of micro-vascular changes can be evaluated are being investigated.