# GROWTH REGULATION OF CANCER

Marc E. Lippman, Organizer
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*Poster Sessions*

# *Growth Factors as Regulators of Neoplastic Cell Growth - I*

**A 001** ROLE OF PDGF-LIKE GROWTH FACTORS IN AUTOCRINE STIMULATION OF CELL GROWTH
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Platelet-derived growth factor (PDGF) is the major mitogen in serum for connective tissue-derived cells. Structurally it is a dimer of two different but homologous peptides, denoted A and B. Studies on cell transformation by simian sarcoma virus (SSV) have indicated that the transformation is exerted by an externalized PDGF-like growth factor structurally related to a homodimer of PDGF B chains. Purification and structural characterization of a PDGF-like growth factor form a human osteosarcoma cell line, U 2 OS, revealed that this factor is a homodimer of PDGF A chains. Preliminary studies, which will be discussed, indicate that PDGF purified from human platelets is a heterodimer of one A chain and one B chain. Thus, all possible dimers of the PDGF polypeptide chains binds to and activate the PDGF receptor, thereby eliciting a mitogenic response. Whether different dimeric forms of PDGF in addition have functional differences is currently not known..

The A and B chains of PDGF are transcribed as precursors from different mRNA molecules. The expression of PDGF A and B chain mRNA:s are frequent among human transformed cell lines and occurs also in some normal cell types. There seems to be no correlation between the expression of A and B chain mRNA:s, indicating that their expression is independently regulated.

Expression of PDGF-like growth factors occurs in certain cell types that carry PDGF receptors and were one can speculate about autocrine mechanisms. Direct proof that PDGF-like growth factor can participate in autocrine stimulation of growth has, however, so far been obtained only in the case of SSV-transformed cells. There are, in addition, several examples of cell types without PDGF-receptors that express PDGF-like growth factors. The possible importance of a paracrine effect of these PDGF-like growth factors, e.g. in stimulation of stromal cell growth in vivo, will be discussed.

**A 002** **TRANSFORMING GROWTH FACTOR ß: PRODUCTION, PROCESSING AND RESPONSE IN NEOPLASTIC CELLS**, Harold L. Moses, Departments of Cell Biology and Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

Transforming growth factor ß (TGFß) is a 25kD molecule that bears no sequence homology with TGFα or other known growth factors and has biological effects very different from those of TGFα. TGFß binds to specific TGFß membrane receptors which, like the TGFß molecule itself, are highly ubiquitous in adult as well as embryonic tissue. TGFß, originally described as a mitogen for selected fibroblastic cells, has been shown to be a potent growth inhibitor for most cell types, including epithelial cells. Evidence has been presented that the mitogenic effects of TGFß on fibroblastic cells are indirect either through induction of c-sis and autocrine stimulation by PDGF and/or through stimulation of fibronectin synthesis and release. The mechanism of growth inhibition remains unknown. TGFß is released in an inactive form by platelets and virtually all cells in culture and almost all cells have TGFß receptors. This suggests that a major regulatory step in TGFß action is activation of the inactive form. Strong acid and other treatments that would dissociate non-covalent protein-protein bonding give irreversible activation of TGFß. We have recently demonstrated that treatment with selected proteases activates TGFß from conditioned medium and protease activation is proposed as a potential physiological mechanism of control of TGFß action. Changes concerning TGFß that occur in neoplastic transformation differ markedly depending on the cell type. In chemically-transformed mouse embryo-derived fibroblastic cells we have shown that a major change in the transformed cells is an increased proliferative response to TGFß which is produced in roughly equal amounts by the transformed cells and their nontransformed parents. No change in the degree of activation of the TGFß released was detected. The data indicate that the difference in response could not be accounted for by a change in the number or affinity of TGFß receptors suggesting an alteration in a post-receptor mechanism. Transfection of one of the TGFß non-responsive parent lines with c-myc linked to an SV-40 promoter indicated that c-myc expression may account, at least in part, for increased TGFß responsiveness. In a squamous cell carcinoma cell line a lack of inhibition by active TGFß was observed. Other neoplastically transformed epithelial cells appear to have lost the ability to activate the TGFß precursor. It is hypothesized that autocrine stimulation by endogenous TGFß (fibroblastic cells) or loss of sensitivity to the normal autocrine or paracrine inhibitory effect of TGFß (epithelial cells or other cells normally inhibited by TGFß) could lead to an increased proliferative potential and thereby contribute to the transformed phenotype.
(This investigation was supported by PHS grant number CA 42572 awarded by the NCI, DHHS.)

**A 003** THE EGF-RECEPTOR PROTO-ONCOGENE: STRUCTURE, EVOLUTION AND PROPERTIES OF VARIOUS RECEPTOR MUTANTS, J. Schlessinger, Biotechnology Research Center, Meloy Laboratories, Inc., 4 Research Court, Rockville, MD 20850.
The EGF-receptor is a 170 KD membrane glycoprotein which has 3 major functional domains, an extracellular, glycosylated EGF-binding domain, a single hydrophobic transmembrane region and a cytoplasmic kinase domain. The extracellular domain of EGF-receptor contains 2 cysteine rich clusters which reveal internal homology and repetition of the cysteine residues. Questions concerning the mechanism of action and regulation of EGF-receptor were addressed by exploring properties and cellular effects of various EGF-receptor mutants introduced into cultured cell lines. Transient expression of EGF-receptors and various EGF-receptor mutants in COS-1 cells was achieved by using shuttle vector containing the SV-40 origin of replication. The same vector together with the DHFR gene were used to obtain stable cell lines expressing different amounts of EGF-receptor and its various mutants in CHO cells, which are normally devoid of EGF receptor (1). A retroviral shuttle vector was used to express intact EGF-receptor and EGF-receptor mutants in NIH-3T3 cells and for obtaining retroviruses containing sequences coding for the intact receptor and its various mutants (2). Initially we have generated constructs with deletions in the cytoplasmic domain of the EGF-receptor including a receptor mutant which has only 8 amino acids in the cytoplasmic domain (devoid of Thr 654). Subsequently, we have generated various point mutations in the kinase domain, autophosphorylation sites and kinase-C phosphorylation site of the receptor. Using this approach we have explored the role of various receptor domains in the regulation of receptor internalization, endocytosis and transformation (3).

1. Livneh, E., Prywes, R., Kasheles, O., Reiss, N., Sasson, I., Mory, Y., Ullrich, A., and Schlessinger, J. (1986). J. Biol. Chem. 261: 12490-12497.
2. Prywes, R., Livneh, E., Ullrich, A., and Schlessinger, J. (1986). EMBO J. 5: 2179-2190.
3. Schlessinger, J., Honegger, A.M., Livneh, E., Dull, T., and Ullrich A. In preparation.

## *Growth Factors as Regulators of Neoplastic Cell Growth - II*

**A 004** THE GASTRIN RELEASING PEPTIDE GENE PRODUCES MULTIPLE PEPTIDE HORMONES BY ALTERNATIVE RNA PROCESSING, Anne-Marie Lebacq-Verheyden, Edward A. Sausville, Geoffrey Krystal, Sanford Markowitz, Frank Cuttitta, and James Battey, NCI-Navy Medical Oncology Branch, Naval Hospital, Bethesda, Building 8, Rm. 5101, Rockville Pike, Bethesda, MD 20814.
Gastrin releasing peptide (GRP) is a 27 amino acid neuropeptide that represents the mammalian homologue to the amphibian tetradecapeptide bombesin. GRP is found normally in peripheral nerves of the gut lining and in pulmonary endocrine cells, as well as in lung carcinoid tumors and human small cell lung cancer (SCLC). GRP can stimulate the growth of normal human bronchial epithelial cells, mouse Swiss 3T3 fibroblasts, and some human SCLC cells growing in culture. Since GRP is both synthesized and mitogenic for some SCLC cells, it may function as an autocrine growth factor contributing to the transformed growth properties of SCLC. Molecular analysis of pre-pro GRP cDNA clones obtained from several human SCLC cell lines clearly show at least three forms of mature mRNA are synthesized. The translation product of all three forms would make GRP; they differ in the region encoding a GRP-associated peptide synthesized in addition to GRP from the prepro GRP mRNA. The different forms are the result of alternative RNA processing of the primary transcript from a single human GRP gene. Antisera specific for the differing forms of the GRP-associated peptide demonstrate detectable quantities in the pulmonary endocrine cells of developing lung and SCLC. Chromosomal mapping experiments localize the pre-pro GRP gene to 18q21. Nucleotide sequence comparison between the human and rat prepro GRP gene predicts considerable conservation of sequence for both GRP and the GRP-associated peptide, consistent with biological significance for both hormones. The growth promoting effects of a constitutively expressed pre-pro GRP gene are currently being assessed by transfecting GRP gene constructs into various cultured cell hosts.

**A 005** TRANSFORMING GROWTH FACTOR-α: CELLULAR EXPRESSION AND PROCESSING OF THE PRECURSOR AND TISSUE-SPECIFIC DISTRIBUTION, Rik Derynck, Timothy S. Bringman, Patricia B. Lindquist and Josiah N. Wilcox, Molecular Biology Department, Genentech, Inc., South San Francisco, CA 94080.

The structure of the human transforming growth factor (TGF-α) cDNA has previously revealed that the 50 amino acid TGF-α form is encoded as part of a 160 amino acid precursor (Derynck et al., Cell 46, 301-309 [1984]). We have studied the processing of the human TGF-α precursor in cells which exhibit a high level TGF-α synthesis upon transfection with a TGF-α expression vector. Immunoprecipitation studies have revealed that the TGF-α precursor is synthesized as a transmembrane precursor. This precursor is N-glycosylated at a position closely following the signal peptide cleavage site. Proteolytic cleavage releases the 50 amino acid TGF-α and larger glycosylated TGF-α species. The cytoplasmic portion of the precursor is associated with the membrane and is palmitoylated. Immunofluorescence with a TGF-α specific antibody allows the detection of a TGF-α form at the cell surface of TGF-α expressing cells. Incubation of the cells with the antibodies at 37°C results in an internalization of the antibody-antigen complex.

Tissue-specific expression of TGF-α in the mouse was evaluated by in situ hybridization. Murine TGF-α was detected and localized in the adult brain. The same methodology also allowed the localization of the anatomical sites of TGF-α expression in developing mouse fetus.

**A 006** GROWTH FACTOR PRODUCTION BY MAMMARY EPITHELIUM, William R. Kidwell, Lab of Tumor Immunology and Biology, National Cancer Institute, Bethesda MD 20892.
Growth in normal mammary tissues is apparently controlled by growth promoting and growth inhibiting factors produced within and outside the gland. The process is integrated and involves an interplay between cell types and structural elements. Mammary tumors may retain and amplify the pathways that promote cell growth and suppress those that limit it. Additionally, growth regulation of tumors may change during the tumor progression process. Some comparisons of normal mammary epithelium with that of early and late progressed stages of tumors are given to illustrate the thesis. Two growth promoting factors, collagen synthesis stimulating activity (CSSF) and transforming growth factor alpha (TGFα) have been quantitated in normal mammary epithelium, in primary, carcinogen-induced tumors and in transplantable, metastatic tumors derived from the primaries by serial transplantation. CSSF is a 68 KD, acidic growth factor that has been purified from primary tumors. The factor is present in low amounts in normal epithelium, is high in primary tumors and is very low or absent in the metastatic tumors. When the purified factor is added to cultures of normal epithelium it greatly amplifies their production of basement membrane proteins. Responsiveness of the cells is, however, subject to feedback regulation. If the cells are plated on basement membrane coated culture dishes, their sensitivity to CSSF is greatly reduced. Primary tumor cells in culture release substantial amounts of CSSF and they show little responsiveness to exogenously added factor. Transplantable tumor cells neither produce nor respond to CSSF in culture, regardless of the substratum type on which they are plated. Production of and responsiveness to TGFα paralles that of CSSF in the normal and tumor model systems studied to date. Conditions regulating CSSF production are not known at present. However, preliminary studies of rodent mammary tumors have indicated that TGFα production may be regulated by ovarian steroids since ovariectomy causes a sharp drop in the levels of TGFα mRNA in primary rat mammary tumors. Two potential negative growth regulating factors, transforming growth factorβ (TGFβ) and mammary cell growth inhibitor (MCGI) have been assessed in normal mammary gland and in the types of mammary tumors. Levels of TGFβ are similar in all three. Low levels of MCGI are seen in the two types of tumors, whereas high MCGI levels are detectable in non-proliferating normal glands, consistent with the findings of Grosse's laboratory.

**A 007** PROTO-ONCOGENES FOS AND FMS, Inder M. Verma, The Salk Institute, San Diego, CA 92138.

Modulation of expression of proto-oncogenes during growth, differentiation and development is indicative of their crucial role in normal cellular metabolic processes. We are interested in understanding the mechanism by which such normal cellular genes acquire the ability to induce transformation. Proto-oncogene fos is a multifaceted gene whose expression is transiently inducible in a wide variety of cell types. The c-fos gene is unable to induce transformation, but if sustained amounts of c-fos protein can be synthesized, cellular transformation can be observed. To synthesize sustained amounts of c-fos protein, removal of sequences in the non-coding domain of the c-fos mRNA is obligatory. We propose that regulation of c-fos protein synthesis dictates its ability to induce transformation. Analysis of the transcriptional unit of the fos gene indicates that a region upstream of the TATA box is required both for transformation and induction. Regulation of the c-fos transcription is modulated by netagive and positive cellular factors. We propose that regulation of fos expression occurs at both transcriptional and post-transcriptional levels. In contrast,transformation by the fms gene product may be governed by negative signal transduction. The c-fms gene product is likely to be CSF-1 receptor and its sequence comparison with the viral fms gene product indicates extensive homology. However, the c-fms and v-fms gene products differ at their C-termini. In particular, the altered v-fms C-terminus lacks a tyrosine residue. Chimeric constructs between c-fms and v-fms genes are being tested to assess the significance of altered 3' termini. Additionally, transcription of the c-fms gene is being investigated to understand its highly tissue-specific expression.

## *Growth Factor Receptors and Kinases*

**A 008** EGF RECEPTOR REGULATION AND FUNCTION, Gordon N. Gill, Division of Endocrinology and Metabolism, Department of Medicine, University of California, San Diego, School of Medicine, La Jolla CA 92093

The EGF receptor is a transmembrane glycoprotein with intrinsic tyrosine protein kinase activity. It is the proto-oncogene for erb B which is truncated at both the amino and carboxyl termini; proto-oncogene EGF receptor is also amplified and/or over-expressed in a number of human tumors. Structure/function analysis of the EGF receptor is being investigated by use of site-directed mutations in human EGF receptor cDNA with analysis of transfected, expressed protein in vivo and in vitro. Analysis of $Thr^{654}$ reveals this to be the major site of heterologous regulation via protein kinase C. Analysis of $Tyr^{1173}$, the major site of self-phosphorylation, reveals this to function as a competitive inhibitor in regard to exogenous enzyme substrates, serving as a reversible regulatory constraint on enzyme activity. Based on computer modeling, mutations have been placed in the EGF receptor to investigate the mechanisms of ATP and $Mg^{2+}$ binding and to test models of transmembrane signal transduction.

**A 009** THE PROTEIN TYROSINE KINASE DOMAIN OF THE INSULIN RECEPTOR: REGULATION AND ROLE IN INSULIN ACTION, Ora M. Rosen, Roman Herrera, Chen K. Chou, Roberto Gherzi, David S., Russell and David Lebwohl, Department of Molecular Biology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

The insulin receptor is a hormone-dependent protein tyrosine kinase that belongs to the family of tyrosine kinases associated with growth factor receptors and oncogene products (1). Autophosphorylation on tyrosine residues activates the kinase activity (2) whereas phosphorylation on serine and threonine residues, inhibits it (3). Antipeptide antibodies elicited to peptides corresponding to the deduced amino acid sequence of the β subunit of the receptor were used to designate the autophosphorylation site that correlates with the activation of the receptor kinase (4). CHO cells were transfected with cDNA's encoding either the normal human insulin receptor or the human insulin receptor with a lys → ala mutation at the ATP binding site of the kinase domain. Stable cell lines were developed and their sensitivity to insulin was compared. The following conclusions were reached: the kinase activity of the receptor is not necessary for processing of the proreceptor or for insulin binding; it is essential for the ability of insulin to stimulate deoxyglucose uptake, S6 kinase activity, glycogen synthesis, thymidine incorporation into DNA and cellular protein phosphorylation on tyrosine residues.

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2. Rosen, O.M., Herrera, R., Olowe, Y., Petruzzelli, L.M. and Cobb, M.H. Phosphorylation activates the insulin receptor tyrosine protein kinase. Proc. Natl. Acad. Sci. U.S.A. 80:3237-3240 (1983).
3. Stadtmauer, L., and Rosen, O.M. Increasing the cAMP content of IM-9 cells alters the phosphorylation state and protein kinase activity of the insulin receptor. J. Biol. Chem. 261:3402-3407 (1986).
4. Herrera, R. and Rosen, O.M. Autophosphorylation of the the insulin receptor in vitro, designation of the phosphorylation sites and correlation with receptor kinase activation. J. Biol. Chem. 261:11980-11985 (1986).

**A 010** MOLECULAR FEATURES INVOLVED IN CELL SURFACE RECEPTOR FUNCTION AND THEIR ROLE IN ONCOGENESIS, H. Riedel, Y. Yarden, A. Gray, L. Coussens, T. Dull, J. Schlessinger*, and A. Ullrich, Dept. of Developmental Biology, Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080; *Meloy Laboratories, Biotechnology Research Center, 4 Research Court, Rockville, MD 20850.

Growth factors and their receptors are involved in the regulation of cell proliferation, and a variety of recent findings suggest that they may also play a key role in oncogenesis. Of approximately twenty identified oncogenes, the three that have been correlated with known cellular proteins have each been found to be related to either a growth factor or a growth factor receptor. The B chain of platelet-derived growth factor (PDGF) is encoded by the proto-oncogene c-sis, the erb-B oncogene product gp68 appears to be a truncated form of the epidermal growth factor (EGF) receptor, and the proto-oncogene c-fms may be related or identical to the receptor for macrophage colony stimulating factor (CSF-1).

The receptor-related oncogenes are members of a gene family by virtue of the fact that each possess tyrosine-specific protein kinase activity. This characteristic is shared by several other polypeptide hormone receptors, including those for insulin, platelet-derived growth factor (PDGF), and insulin-like growth factor I (IGF-I). To investigate in detail the mechanisms involved in ligand-induced growth stimulation, we characterized as a first step the primary structures of receptors for EGF, insulin, IGF-I, CSF-I and PDGF. Furthermore, we cloned two oncogene-related putative receptors, HER2-neu and c-kit, which also belong to the tyrosine kinase gene family. The overall structural organization of these receptor molecules suggests the existence of three subclasses which may reflect functional differences and possibly independent evolutionary origins.

We have undertaken efforts to functionally characterize the structural domains which we were able to identity on the basis of sequence comparison. For this purpose we prepared point and truncation mutants using cloned receptor cDNA sequences and SV40 promoter based gene expression vectors. Furthermore, we created receptor chimera in an attempt to determine domains that define receptor-specific functions. Insights obtained with these receptor mutants after introduction into mammalian cells and their relevance to normal growth control and oncogenesis will be discussed.

**A 011** EXPRESSION OF THE PDGF RECEPTOR IN NORMAL AND TRANSFORMED CELLS, Lewis T. Williams, Jaime A. Escobedo and Mark T. Keating, Howard Hughes Medical Institute and Cardiovascular Research Institute, University of California, San Francisco, CA. 94143

The mitogenic effects of PDGF-like compounds that act through autocrine mechanisms are mediated by the PDGF receptor. When activated, the receptor rapidly stimulates a diverse group of cellular responses including tyrosine kinase activity, the turnover of membrane phosphatidylinositol, and the enhanced expression of a group of genes including the c-myc and c-fos proto-oncogenes. To correlate these responses with structural domains of the receptor, a full-length cDNA clone was used to predict the receptor amino acid sequences[1]. The tyrosine kinase domain is divided into two parts separated by a 107-amino acid inserted sequence. This region and the unique carboxyl terminus may play a role in some of the intracellular responses to PDGF. The complete receptor sequence, under the transcriptional control of the SV-40 early promotor, was expressed in Chinese hamster ovary (CHO) fibroblasts that normally lack PDGF receptors. Several transfected clones that expressed high levels of PDGF receptor mRNA and protein were isolated. The addition of PDGF to the transfectants stimulated receptor autophosphorylation, tyrosine phosphorylation of a cellular 32 kDa substrate, activation of phosphatidylinositol turnover, receptor down regulation and cell division. These responses to PDGF were not observed in control CHO cells. Thus the receptor cDNA clone clearly encodes functional PDGF receptors that mediate the diverse group of responses to PDGF.

The receptor gene, which is located at bands 5 q31→q32 on human chromosome 5, encodes a 5.2 kb mRNA that is expressed in placenta, kidney, 3T3 cells, smooth muscle cells, and simian sarcoma virus transformed cells, but is notably absent from epithelial cells, lymphoid cells, and endothelial cells. Processing studies, performed with antibodies generated against receptor peptide sequences, showed that receptor precursors were processed to a mature 180 kDa receptor by the covalent addition of carbohydrates and ubiquitin. Similar precursors of the receptor were also found in cells transformed by the simian sarcoma virus. However in these cells the receptor was processed to a broad band (170-190 kDa) that was only transiently expressed. Thus when the receptor is activated by an autocrine mechanism the normal precursors appear to be formed but the half life of the most mature form appears to be altered.

[1]Y. Yardin, J.A. Escobedo, W-J. Kuang, T.L. Yang-Feng, T.O. Daniel, P.M. Tremble, E.Y. Chen, M.E. Ando, R.N. Harkins, U. Francke, V.A. Fried, A. Ullrich, and L.T. Williams, Nature 323:226-232, 1986

## *Angiogenesis and Host Interactions*

**A 012** HOST MICROENVIRONMENT DETERMINANTS IN CANCER METASTASIS, Isaiah J. Fidler and Janet E. Price, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Avenue, Houston, TX 77030.

The process of metastasis consists of a sequence of events during which tumor cells disseminate from a primary site to distant organs. It is generally regarded as a selective process, as only the tumor cells possessing multiple properties will interact with host factors and survive to produce metastases, and these cells represent only a minor subset of the heterogeneous neoplastic population. These findings agree with the concept forwarded by Paget in 1889 that metastasis results only when certain tumor cells ("seeds") can favorably interact with certain organs ("soil"). The purpose of these studies was twofold. First, we wished to determine the extent of metastatic heterogeneity in malignant human neoplasms ("seed") and second, to examine whether host organ microenvironment ("soil") plays a critical role in the outcome of metastasis.

Cells from human melanomas, colorectal carcinomas and renal cell carcinomas were implanted into multiple organ sites of nude mice (subcutis, intravenous, intraperitoneal, spleen, kidney subcapsule). For all tumors studied we observed preferential organ sites for both tumorigenicity and metastasis. Human melanoma cells produced tumors in most organs directly injected and also produced extensive metastasis. In contrast, human colorectal cancer cells were most tumorigenic when implanted into the spleen or kidney. Metastases from human colorectal cancer, however, were mainly produced from tumors growing in the spleen. Conversely, human renal cell carcinoma cells were tumorigenic in the subcutis, kidney and spleen. Maximum metastatic potential was achieved by tumors growing in the kidney and not the spleen.

Passage in nude mice or in vitro cultures has been used to isolate and propagate human tumors and tumor cell lines. To determine whether different methods for the isolation and propagation of human tumor cells influence their biological behavior, a surgical specimen of a human renal carcinoma was enzymatically dissociated. Viable cells were either grown directly in culture or first implanted into the subcutis or kidney of nude mice. Subsequent tumors were dissociated and established in culture. The biologic behavior of these 3 tumor lines varied significantly upon reimplantation into different organs of nude mice, indicating that the method used to isolate the lines affected their properties.

Collectively, our data suggest that both the tumorigenicity and metastatic capability of human tumor cells are influenced by the host organ environment. Maximum tumorigenic and metastatic potential requires implantation of metastatic human tumor cells into an anatomically compatible organ of nude mice. This conclusion supports the "seed and soil" hypothesis and explains the nonrandom pattern of cancer metastasis.

**A 013** BIOCHEMICAL FACTORS INVOLVED IN TUMOR INVASION AND METASTASES, Lance A. Liotta, Ulla M. Wewer, Nageswara C. Rao, Elliott Schiffmann, Mary L. Stracke, Raouf Guirguis, Unnur P. Thorgeirsson, Ruth J. Muschel, and Mark E. Sobel Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20892

Cancer invasion and metastases is a complex multistep process. In order for a tumor cell to successfully traverse all the steps of this process and initiate a metastatic colony, it must express the right combination of gene products. Such gene products may include proteins which regulate cell interaction with the basement membrane and cell motility. Tumor cells attach to the basement membrane glycoprotein laminin via the cell surface laminin receptor. The human laminin receptor was purified and molecularly cloned. The level of laminin receptor mRNA is a variety of human carcinoma cells correlated with the number of laminin receptors on the cell surface of these cells. Following attachment to the basement membrane, the tumor cell next secretes proteases which may degrade type IV collagen. A biochemical linkage between type IV collagenase secretion and metastases was studied using a new genetic system for inducing the metastatic phenotype employing the ras oncogene. Following attachment and local proteolysis, the third step of invasion is tumor cell motility. We have isolated a tumor cell autocrine motility factor (AMF). This factor is secreted by the tumor cells and binds to a cell surface receptor resulting in a profound (>100x) stimulation of cell locomotion. The n terminal sequence of AMF is unique. The mechanism of AMF action may involve G proteins and methylation of membrane phospholipids. AMF is produced in large amounts by ras transfected 3T3 cells but not by the 3T3 parent cells. AMF may play a major role in the autonomous invasive behavior of tumor cells.

## *Negative Signals*

**A 014** HUMAN TUMOR NECROSIS FACTOR AND INTERFERONS: EXPRESSION AND ACTION

Bollon, A.P., Berent, S.L., Torczynski, R., Pichyangkul, S., Jia, F., Pardue, A., Aleman, C., Dickson, J., Hill, J.M., Hilario, R., Hill, N.O. and Khan, A.
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Lymphokines or cytokines are proteins which are synthesized by select cells in response to various stimuli and play a leading role in the implementation of the immune system. Since these proteins which exist in low circulating levels may be of considerable therapeutic value for treating various disease states including cancer, we have established a program which involves: the isolation and manipulation of select human lymphokine genes; analysis of the biological activity of the proteins in vitro; animal testing of the proteins and human clinical trials. Special attention has been given to the coordinate regulation of some of the lymphokine genes and synergy of their biological activity. Several genes have been isolated from a cDNA library made against poly(A)$^+$ RNA isolated from Sendai virus treated human peripheral blood leukocytes (PBLs) obtained from our blood bank. Although we isolated over twelve $\alpha$-IFN, $\beta$-IFN and IL-1 genes from this library we also isolated TNF at a 50-100 fold higher frequency than other investigators using tumor cell lines and induced by bacterially-derived endotoxins. Analysis of the induction of $\alpha$-IFN and TNF by Sendai virus indicates that TNF is maximally induced about two hours prior to maximal $\alpha$-IFN induction and that the levels of $\alpha$-IFN and TNF mRNA are similar, namely, about 0.6% of total PBL mRNA. Analysis of TNF and $\alpha$-IFN produced by the PBL induced with Sendai virus indicated a 200 fold difference in maximal levels. Mixing experiments suggest that the difference between the mRNA and protein levels of TNF compared to $\alpha$-IFN may be a lower efficiency of translation of TNF mRNA. Human TNF cDNA has been engineered for expression using an expression vector containing a $P_L$ promoter and a temperature sensitive repressor. Levels up to 2 X $10^9$ U/liter of E. coli culture have been obtained. The recombinant TNF was purified and was cytotoxic on a range of tumor cell lines and potentiated by $\alpha$-IFN and $\gamma$-IFN. The recombinant TNF caused regression of Sarcoma-180 in BALB/c mice at a dose of 6000 units/mouse and acute toxicity studies indicated an $LD_{50}$ of 6 X $10^7$ U/kg. Phase I clinical trials of recombinant TNF were initiated at our cancer hospital on September 25, 1985, using escalating doses of TNF (i.v.) by a modified Fibonacci scheme. The half life (T 1/2) of TNF was biphasic at 150,000 U/kg - where the first phase T 1/2 was 2 min. and second phase was 31 min. The Phase I clinical trials indicate that the recombinant TNF can be administered (i.v.) up to 150,000 U/kg with tolerable side effects. Phase II clinical trials of recombinant TNF at a dose of 150,000 U/kg are in progress. Clinical trials involving a combination of recombinant TNF and $\alpha$-IFN are also in progress. These studies are of particular interest due to the joint induction of TNF and $\alpha$-IFN and the synergy of their biological activities.

**A 015** THE TGF-β SYSTEM: HORMONES, RECEPTORS AND BIOCHEMICAL TARGETS. Joan Massague, Sela Cheifetz, Ronald A. Ignotz and Fred T. Boyd, Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA 01605

Transforming growth factor-β is prototypic of a family of homologous polypeptides that control the development of tissues in organisms from Drosophila to humans. We have characterized new polypeptides more closely related to TGF-β than any of the other known members of this family. A new homodimer form of TGF-β, TGF-β2, has been identified in porcine blood platelets. TGF-β2 is homologous to but distinct from TGF-β1, the form previously found in platelets and other tissues. TGF-β1.2, the heterodimer consisting of one TGF-β1 chain linked to one TGF-β2 chains has also been isolated.

In addition to altering the rate of cell proliferation, TGF-β1 and TGF-β2 inhibit processes of adipogenesis, myogenesis and hematopoietic differentiation, but stimulate chondroblast attachment and osteogenesis. Elevated expression at the protein and mRNA level of fibronectin, fibronectin receptors and type I collagen is a widespread early response of cells to TGF-β. Available evidence indicates that alterations in the architecture of the extracellular matrix induced by TGF-β could be involved in regulating the expression of specific phenotypes and inducing anchorage-independent proliferation of fibroblasts by this factor.

TGF-β1 and TGF-β2 interact differently with a family of receptors in target cells. We have identified three cell surface glycoproteins of 280 kilodaltons (K), 85 K and 65 K, respectively, that show the properties of high affinity receptors for TGF-β. The 280 K receptor species is the component of a larger (~600 K) receptor complex held together by disulfide bonds. Many cell lines and solid tissues from mammalian and avian origin display all three types of TGF-β receptors simultaneously. The three TGF-β receptor types are distinguished from each other by peptide mapping of their respective ligand binding domains, and by the postranslational modifications required to acquire affinity for the ligand. The three TGF-β receptor types can also be distinguished by their ability to discriminate among different ligands. The 280 K receptor component has high affinity for both TGF-β1 and TGF-β2, while the 65 K and 85 K receptor forms exhibit a 10-fold higher affinity for TGF-β1 than TGF-β2. Occupancy of the 280 K receptor type by TGF-β1 or TGF-β2 correlates with their ability to cause inhibition of cell proliferation, inhibition of adipogenic differentiation, and stimulation of fibronectin and collagen expression. Preliminary evidence suggests that the 65 K receptor or the 85 K receptor are linked to a ligand degradative pathway. The existence of distinct forms of TGF-β that interact differently with a family of TGF-β receptors could provide flexibility to the regulation of tissue growth and differentiation by the TGF-β system.

**A 016** REGULATION OF CYTOKINE PRODUCTION AND CLASS II ANTIGEN EXPRESSION BY TRANSFORMING GROWTH FACTOR-BETA, Michael A. Palladino, Christine W Czarniecki, Henry H. Chiu, Susan M. McCabe, Irene S. Figari and Arthur J. Ammann. Genentech Inc., 460 Point San Bruno Blvd, South San Francisco, CA 94080.

Agents which modulate the production of Tumor Necrosis Factor alpha (TNF-alpha) and Interferon-Gamma (IFN-gamma) may be important therapeutically in suppressing inflammatory reactions and as immunosuppressive agents. Recent reports demonstrate that Transforming Growth Factor-beta (TGF-beta) can inhibit T and B-cell proliferation, IL-2 receptor expression and IFN-alpha but not IL-2 induced NK activity. In this report we demonstrate the inhibitory effects of TGF-beta on the production TNF-alpha and IFN-gamma and additionally our results indicate that TGF-beta also inhibits IFN-gamma induced expression of Class II antigens. Pretreatment of peripheral blood mononuclear cell cultures with 0.01 to 10 ng/ml TGF-beta inhibited the production of TNF-alpha and IFN-gamma with the maximum inhibition after 72 hours of culture reaching 70% for both cytokines. We reported recently that cyclosporine A (CsA) also inhibits the production of TNF-alpha and IFN-gamma (Palladino et. al. 1986 Clinical Immunology Society Symposium). As many cytokines and growth factors act synergistically, we investigated the possibility that CsA and TGF-beta could act synergistically in inhibiting cytokine production. However, in these studies CsA and TGF-beta were additive and not synergistic in inhibiting TNF-alpha production. The investigation of the effects of TGF-beta on Class II antigens expression demonstrated that pretreatment of Hs294T human melanoma cells for four hours with 10 ng/ml TGF-beta inhibited IFN-gamma induced Class II expression with a maximum of 50% at 48 hours. In contrast, constitutive expression of Class II antigen on an Hs294T melanoma subclone could not be inhibited. The results demonstrate additional immunoregulatory effects of TGF-beta.

TGF-BETA---MULTIFUNCTIONAL REGULATOR OF CELL GROWTH AND PHENOTYPE
**A 017** Michael B. Sporn and Anita B. Roberts, Lab. of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

TGF-beta, a homodimeric peptide of 25,000 MW, originally characterized by its ability to phenotypically transform fibroblastic cell lines in vitro, is now known to have many diverse effects on a wide spectrum of both neoplastic and nonneoplastic target cells. Many different cells synthesize TGF-beta and essentially all have high-affinity receptors for TGF-beta; it is thus likely to be a fundamental regulatory molecule, acting by both autocrine and paracrine mechanisms. Depending upon the particular target cell and upon the set of other growth factors simultaneously acting on that cell, TGF-beta can stimulate or inhibit cell proliferation, promote or block cell differentiation, and modulate a variety of cell functions. Examples of the diversity of cellular functions affected by picomolar concentrations of TGF-beta are 1) matrix synthesis, 2) amino acid uptake, 3) EGF receptor synthesis, and 4) activity of cellular enzymes such as aromatase and enzymes involved in steroidogenesis. It is also a potent suppressor of IL-2 action in lymphocytes. It is likely that TGF-beta plays a role in inflammation and repair processes in vivo; it is released from platelets as well as from macrophages and activated lymphocytes and induces both fibrosis and angiogenesis in connective tissue.

It is now apparent that TGF-beta is a prototype for a family of regulatory peptides all sharing the same configuration of disulfide bonds. This family now includes activin, inhibin, Mullerian inhibitory substance, and the product of the decapentaplegic gene complex in Drosophila. In pituitary cells, TGF-beta and activin have similar biological activities, yet each appears to act through distinct cell membrane receptors. TGF-beta, itself, is highly conserved across 4 mammalian species. A second, less abundant, type of TGF-beta peptide (type 2) which has greater than 80 % homology to TGF-beta and which appears to act through the same receptors as TGF-beta and to elicit the identical biological responses on a variety of target cells has recently been characterized from bovine and porcine sources; thus far, type 2 TGF-beta has not been found in human tissues.

## *Oncogenes and Growth Factors - I*

GROWTH FACTOR RECEPTORS AS ONCOGENE PROTEINS, C. Bargmann, D.F. Stern and R.A.
**A 018** Weinberg, Whitehead Institute for Biomedical Research, Cambridge MA 02142 and Dept. of Biology, Massachusetts Institute of Technology, Cambridge MA 02139

Recent work has shown that the neu oncogene (also termed erbB2) encodes a protein of 185 kilodaltons. It can become activated as an oncogene protein by small changes in the DNA sequences that specify the transmembrane domain of the protein. Replacement of a valine residue in this region by either glutamic acid or glutamine suffices to convert the protein into one possessing oncogenic activity. Other amino acid substitutions are either unable to impart activation or do so only weakly. This amino acid substitution continues to drive oncogene activity, even in the absence of the extracellular domain. C-terminal deletions appear to further potentiate the oncogenic activity of certain variants of the protein. The activities of these deleted alleles lead to several models of the mode of action of GF receptors and their role in signal transduction.

**A 019** GENES FOR GROWTH FACTORS AS ONCOGENES, Stuart A. Aaronson, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda MD 20892.
Recent discoveries identifying the products of certain proto-oncogenes as growth factors or their receptors have provided strong evidence that proto-oncogenes play a central role in normal growth regulation. The altered or aberrant expression of such genes appears to be fundamental to steps that convert normal cells along the pathway to malignancy. The human sis proto-oncogene encodes one chain of human platelet-derived growth factor (PDGF-2). Expression of this normal growth factor coding sequence in cells possessing PDGF receptors can induce uncontrolled growth associated with acquisition of the malignant phenotype.

The human sis proto-oncogene is under strict regulation in cells such as normal fibroblasts and glial cells. In contrast, a large percentage of human fibrosarcomas and glioblastomas express sis transcripts, and we have demonstrated sis/PDGF-2 gene products associated with such tumor cells. Thus, deregulation of sis/PDGF-2 expression may play an important role in triggering the uncontrolled growth associated with these tumors. We have defined the transcriptional unit of the sis proto-oncogene and identified its functional promoter. Regulatory factors that affect its expression in human normal and tumor cells will be described.

To further elucidate how growth factor expression may contribute to the neoplastic state, the coding sequences of other human growth factors were overexpressed in cells possessing cognate receptors. Our findings demonstrate that pathways of intracellular processing and secretion may play important roles in determining the oncogenic potential of a given growth factor activated under these conditions. Finally, efforts to link the functions of other human oncogenes under investigation within our laboratory to pathways of growth factor stimulated cell proliferation will be discussed.

## *Steroids and Regulation of Cancer - I (joint session)*

**A 020** pS2, OESTROGEN AND PROGESTIN RECEPTOR GENE EXPRESSION IN BREAST CANCERS. P. Chambon[1], J.P. Bellocq[2], M. Berry[1], J.P. Briand[3], B. Gairard[4], A. Krust[1], S. Jakowlew[1], A.M. Nunez[1], R. Renaud[4], M.C. Rio[1], M. Roberts[1] and J. Wallace[1]. [1]LGME/CNRS and U.184/INSERM, Faculté de Médecine, 67085 Strasbourg; [2]Dept. Anatomie Pathologique, CHU, Strasbourg; [3] IBMC/CNRS, Strasbourg; [4]Dépt. Gynécologie I, CHU, Strasbourg - France.
We have previously reported the cloning and sequencing of the cDNA of the human pS2 gene (1,2) whose transcription is controlled by oestrogens in the human breast cancer cells MCF-7 (3,4,5). Using an antibody against a synthetic peptide whose sequence was deduced from that of the cDNA, we have now found that the pS2 protein is synthesized by MCF-7 cells grown in the presence of oestradiol (but not in the presence of tamoxifen) and secreted in the medium, as expected from the existence of a putative signal sequence in the cDNA-deduced protein sequence. The existence of an oestrogen-responsive element (ERE) in the pS2 gene promoter has been demonstrated by cotransfection in HeLa and MCF-7 cells of a vector expressing the human oestrogen receptor (hER) and recombinants containing the reporter E.coli CAT gene and various segments of the pS2 gene promoter region. Northern blot analyses of human normal and tumoral cells and biopsies have revealed that pS2 mRNA is present exclusively in a subset of breast cancers. These results were confirmed by the selective presence of the pS2 protein in these cancers, as assayed by immunohistochemistry. A large number (> 200) of human breast cancer biopsies have been analyzed by Northern blotting for the presence of pS2, hER and progestin receptor (hPR) mRNAs, as well as by immunohistochemistry for the presence of pS2 and hER proteins. ER and PR levels were also determined in these biopsies by using conventional binding of labelled hormones. It appears from these studies that the pS2 gene is specifically expressed in a subset of the human breast cancers which express the hER gene. Interestingly, the pS2 gene was not expressed in all of the breast cancers which express simultaneously the hER and hPR genes. The possible value of pS2 gene expression as an additional marker for further defining oestrogen-dependent breast cancers will be discussed.

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**A 021** ROLES OF PDGF/SIS-LIKE PROTEINS IN THE AUTOCRINE GROWTH REGULATION OF SSV-TRANSFORMED FIBROBLASTS, T. F. Deuel[1/2], G. F. Pierce[1/3], H.-J. Yeh[1], L. K. Shawver[1], P. G. Milner[1], Departments of Medicine[1], Biological Chemistry[2], Pathology[3], Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110

The platelet-derived growth factor (PDGF) is highly homologous to the transforming protein of the Simian Sarcoma Virus (SSV), $p28^{v\text{-}sis}$. PDGF-like molecules are expressed in SSV-transformed cells and a PDGF-like growth factor is secreted into conditioned media from some but not all SSV-transformed cells. The secreted growth promoting activity interacts with PDGF cell surface receptors, activates the PDGF receptor tyrosine kinase, and appears capable of stimulating the autocrine growth of SSV-transformed cells. The secreted protein thus resembles $p28^{v\text{-}sis}$ but has not been established as the product of the v-sis gene. Immunoelectron microscopy has been used to localize PDGF/sis-like antigens in endoplasmic reticulum/Golgi, suggesting alternate potential sites for the interaction of $p28^{v\text{-}sis}$ with its cell surface receptor as each is processed. PDGF-like antigens of 66, 65, and 44 kDa also have been identified and partially purified from the cell nucleus, suggesting these proteins may represent a family of PDGF/sis-like molecules potentially important in different roles of growth regulation in SSV-transformed cells.

**A 022** GROWTH REGULATION OF HUMAN BREAST CANCER BY SECRETED GROWTH FACTORS. Marc E. Lippman, Robert B. Dickson, Edward P. Gelmann, Cornelius Knabbe, Neal Rosen, Eva Valverius, Diane Bronzert, Susan Bates, Sandra Swain. Medical Breast Cancer Section, Medicine Branch, National Cancer Institute, NIH, Bethesda, MD.

Our laboratory has been investigating the significance of growth factor production by human breast cancers. We have demonstrated that human breast cancer cells produce insulin-like growth factor 1 (IGF-1); transforming growth factor alpha-like activity (TGF alpha); platelet derived growth factor (PDGF); transforming growth factor beta (TGF beta); and an un-named high molecular weight growth factor activity. IGF-1 has the following properties: breast cancer cells produce radio-immunoassayable IGF-I. The IGF-I material co-purifies with authentic human IGF-I. Breast cancer cells contain mRNA which hybridizes to an authentic human cDNA probe for IGF-I. Breast cancer cells express IGF-I cell surface receptors and are growth stimulated by the addition of exogenous IGF-I in vitro. Estrogens stimulate and antiestrogens inhibit IGF-I secretion. Preliminary studies with antisense IGF-I genes suggest an autocrine role for IGF-I. Transforming growth factor alpha-like activity has the following properties. The major secreted species is a 30 KDa peptide. A cDNA for human TGF alpha recognizes a 4.8 Kb and a 1.6 Kb mRNA species. Radioreceptor activity, radioimmunoassay activity, transforming activity and mRNA are all coordinately induced by estrogens. Breast cancer cells express TGF alpha/EGF cell surface receptors; many lines are growth stimulated by TGF alpha/EGF. EGF infusion into the nude mouse stimulates transient tumor development of coimplanted MCF-7 cells in the absence of estrogen. Preliminary experiments with anti EGF receptor and anti TGF antibodies suggest an autocrine role for TGF alpha. PDGF has the following properties: human breast cancer cells produce fibroblast "competency" activity. Immunoprecipitation with PDGF specific antibodies identifies peptides of expected size (28 KDa unreduced, 15 and 16 KDa reduced). Studies with cDNA directed against PDGF A and B chain mRNA reveal that breast cancer cells express a variable pattern of the expected A chain (2.5, 2.9Kb) and/or B chain (4.1Kb) RNA species. PDGF production is estrogen regulated in an estrogen dependent cell line. Since breast cancer cells are not known to have PDGF receptors, PDGF may act as a paracrine growth factor in breast cancer. Infusion of growth factor concentrates into nude mice bearing estrogen dependent tumors is capable of partially replacing estrogen as a proximate stimulator of breast cancer growth. In addition, conversion to the estrogen independent phenotype is associated with increased growth factor production. Taken together, these experiments strongly suggest a critical role of secreted growth factor activities in the regulation of human breast cancer growth and progression.

**A 023** **AUTOCRINE AND PARACRINE ACTIVITIES OF AN ESTROGEN-INDUCED PROTEASE SECRETED BY BREAST CANCER CELLS,** Henri Rochefort , Françoise Capony, Marcel Garcia, Muriel Morisset, Gilles Freiss and Françoise Vignon, Unité d'Endocrinologie Cellulaire et Moléculaire, INSERM U 148, and University of Montpellier, 60 Rue de Navacelles, 34100 Montpellier France.

We have studied estrogen-regulated proteins in an attempt to understand the mechanism by which estrogens stimulate cell proliferation and mammary carcinogenesis. In estrogen receptor positive human breast cancer cell lines (MCF7, ZR75-1) estrogens specifically increase the production into the culture medium of a 52,000 daltons (52K) glycoprotein. Several high affinity monoclonal antibodies to the partially purified secretory 52K protein have allowed to purify to homogeneity this protein and its cellular processed products. The 52K protein has been identified as the secreted precursor of a cathepsin-D like protease bearing mannose-6-phosphate signals. The protein is processed intracellularly into a 48 and 34K forms which accumulate in the lysosomes. The precursor 52K displays an *in vitro* autocrine mitogenic activity on estrogen deprived MCF7 cells, can be taken up by these cells *via* mannose-6-phosphate receptors and is able to degrade basement membrane and proteoglycans following its activation. The cellular related proteins, as detected by immunohistochemistry and immunoassay are more concentrated in proliferative mammary ducts than in resting ducts and their concentration in breast cancer cytosol appears to be more correlated with lymph nodes invasion than to estrogen or progesterone receptors level. The protein is also produced constitutively by ER-negative cell lines, while in some antiestrogen resistant variants, it becomes inducible by tamoxifen, contrary to the wild type MCF7 cells. The structure of the protein from MCF7 cells has been compared to that of cathepsin-D(s) prepared from normal tissues. This estrogen-induced protease, in addition to other estrogen regulated growth factors, may have important autocrine and/or paracrine functions in stimulating the growth and invasion of hormone-dependent and independent breast cancer.

## *Regulation of Transcription (joint session)*

**A 024** CHROMATIN STRUCTURE AND GLOBIN GENE EXPRESSION, G. Felsenfeld, B. Emerson, D. Jackson, T. Kimura, B. Kemper, M. Lieber, J. Hesse and J. Nickol, Lab. of Molecular Biology, NIDDK, NIH, Bethesda MD 20892.

Chromatin structure near transcriptionally active genes is often marked by domains that are hypersensitive to nucleases. We have examined a number of such domains in the 3' and 5' flanking regions of the globin gene family in chicken erythrocytes. The hypersensitive domain in the 5' flanking region of the $\beta^A$-globin gene is lacking a normal nucleosome, but within the nucleosome-free domain (about 200 base pairs long) at least three specific factors are observed to bind both *in vitro* and *in vivo*. Some of these binding sites play a role in regulation of the expression of the gene. In the 3' flanking region of the gene we have identified a sequence with the properties of an enhancer. We have shown that the enhancer is specific both with respect to cell type and developmental stage. It is located within a hypersensitive domain, and we have identified factors that bind to the domain; some of the factors may themselves be developmentally regulated. In other experiments, we have examined the properties of hypersensitive domains in an embryonic $\beta$ gene and an adult $\alpha$ gene. These domains have an architecture similar to those described above; some of the factors binding to the domains are novel, but it appears that some regulatory elements may be shared.

**A 025** CONTROL OF TRANSCRIPTION IN PROKARYOTES AND EUKARYOTES--A COMMON MECHANISM, Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge MA 02138.
Various experiments bearing on the questions of how regulatory proteins recognize specific sequences in DNA and turn genes on and off in prokaryotes and eukaryotes will be described.

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3. Ptashne, M. 1986. Gene regulation by proteins acting nearby and at a distance. Nature 322, 697-701.

4. Hochschild, A., Douhan III, J., and Ptashne, M. 1986. How λ repressor and λ cro distinguish between $O_R1$ and $O_R3$. Cell 47 (in press, December 5).

## *Steroids and Regulation of Cancer - II (joint session)*

**A 026** GLUCOCORTICOOID-INDUCED RESPONSES IN LYMPHOMA AND FIBROSARCOMA CELL LINES, Suzanne Bourgeois, Michel Crepin and Douglas C. Dean, The Salk Institute, Regulatory Biology Laboratory, San Diego, California 92138.

Glucorticoids induce lysis of T-lymphomas and the synthesis of fibronectin in the human fibrosarcoma cell line HT1080.

The mechanism of hormone-induced cytolysis of T-lymphomas is still obscure. We have demonstrated earlier the existence of a "lysis" function required for glucocorticoid-induced T-cell killing (1). The "lysis" gene is not expressed in the glucocorticoid-resistant cell line SAK8, but its expression (in the presence of dexamethasone) is activated by DNA demethylation in those cells (2). Following up on several recent reports that glucocorticoids induce DNA fragmentation in sensitive T-cells, we have examined the possibility that lymphocytolysis might result from glucocorticoid-induced nuclease activity(ies). We have demonstrated that several calcium-dependent nuclease activities begin to appear after 1 h of dexamethasone treatment in a glucocorticoid sensitive cell line, P3-95, derived from SAK8 cells by treatment with the DNA demethylating agent 5-azacytidine, but are not detected in SAK8 cells. We conclude that this induction of nuclease activities precedes the cytolysis process and is directly dependent on the glucocorticoid-sensitive phenotype of the T-cell. Therefore, the gene(s) which encodes them is a possible candidate for the "lysis" gene.

Fibronectin (FN) is a large glycoprotein which serves to anchor cells to a substratum. Synthesis of FN is strongly inhibited upon neoplastic transformation which may account, at least partially, for alterations in morphology and adhesion which are observed in transformed cells. In addition, the synthesis of FN is stimulated by glucocorticoid hormones (3). As a first step in the study of how expression of the FN gene is controlled, we have isolated genomic clones containing the 5'-end and flanking sequences of the human fibronectin gene. The 5'-end of the FN gene is found on a 3.7 kb EcoRI fragment which contains about 2.7 kb of flanking sequence. The first exon is 414 bp long and contains a 5'-untranslated region of 267 bp. Based on the position of the translational initiation codon, FN is synthesized with a 31 amino acid extension on its amino terminus which seems to consist of both a signal peptide and a propeptide. The sequence ATATAA is found at ~25 and the sequence CAAT is present at ~150. Sequences exhibiting homology to the binding sites for the transcription factor SP1 are present.

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2. Gasson, J.C., Ryden, T. and Bourgeois, S., Nature 302, 1983, 621-623.
3. Oliver, N., Newby, R.F., Furcht, L.T. and Bourgeois, S., Cell 33, 1983, 287-296.

**A 027** STEROID EFFECTS ON CULTURED MAMMARY TUMOUR CELL LINES, Roger J.B. King, James F. Glover, Pirkko Harkonen and Philippa D. Darbre, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The biology of steroid action on breast cancer cells indicates that multiple transducing mechanisms must be involved.

Proliferation From studies on the regulation of S115 mouse- and ZR-75 human mammary tumour cell lines by androgen and oestrogen respectively, we formulated the idea that steroids changed the cell phenotype from normal (-hormone) to transformed state (+hormone). With both cell lines, multiple effects of steroid were noted, some of which could be independently regulated. The relevant steroid (testosterone/S115 cells, oestradiol/ZR-75 cells) stimulated proliferation during log-phase growth and increased saturation density in monolayer culture and markedly increased growth as spheroids in suspension culture (anchorage independence). With both cell lines, glucocorticoids (dexamethasone) inhibited log-phase proliferation in monolayer. In S115 cells, but not ZR-75 cells, glucocorticoids also increased saturation density and anchorage independence. Thus by judicious choice of steroid and cell line, one can divorce the different types of response. What is clear is that no simple model of modulation of one oncogene/growth factor can explain these data.

In the mouse system, steroid effects on density regulation and suspension growth could be mediated by a protein from the 3' long terminal repeat of MMTV; both androgens and glucocorticoids induce a 16S mRNA from this region of the virus. On the other hand, the opposite effect of these two classes of steroid on log phase/monolayer growth would tend to rule out an MMTV contribution to this facet of growth regulation.

Transfection experiments have confirmed that androgen, as well as glucocorticoids, can act directly on the long terminal repeat (LTR) of MMTV in S115 cells. The possibility that this important regulatory element of DNA can be under multiple steroid regulation may be relevant in defining biological action.

Transition from hormone responsive to unresponsive state S115 cells lose their androgen sensitivity when cultured in the absence of androgen. Loss of sensitivity is not accompanied by loss of androgen receptor as evidenced by androgen sensitivity of transfected chaemeric genes coupled to MMTV LTR. The loss of sensitivity is a multistage process, the later stages of which involves hypermethylation of at least the MMTV LTR.

**A 028** MOLECULAR AND GENETIC ANALYSIS OF GLUCOCORTICOID ACTIONS IN HUMAN LEUKEMIC CELLS, E. Brad Thompson[a], R. Evans[b], M. G. Rosenfeld[c], Y-S. Yuh[a], B. Gametchu[a], J. Ashraf[a], and J. M. Harmon[d]. [a]Univ. of Tex. Med. Branch, Galveston, TX. 77550, [b]The Salk Insti., LaJolla, CA., [c]Univ. Calif. at San Diego School of Med., and [d]U.S. Univ. of the Health Sciences, Bethesda, MD.

Glucocorticoids inhibit the growth of and even kill many types of human leukemic cells. In CEM cells, grown from childhood acute lymphoblastic leukemia, occupancy of receptors by glucocorticoids causes cell lysis. Unlike results from mouse model systems, selection for resistant CEM cells yields two broad classes of mutant phenotypes: without prior mutagenesis one with "activation-labile" receptors; with prior mutagenic treatment, one with a paucity of receptors ($r^-$). A third, spontaneously resistant phenotype lacks the lysis function ($ly^-$) but has receptors ($r^+$). Somatic cell hybridization between the receptor mutants shows no evidence for trans-active resistance factors and no complementation. However there was complementation between the $r^-$ cells and the $r^+ly^-$ cells, so that complete sensitivity to glucocorticoids was restored in the hybrids. Monoclonal antibodies to glucocorticoid receptors were prepared and used to analyze the normal and resistant CEM cells. Both activation-labile and $r^-$ cells contain high quantities of normal, 95 KDa, immunoreactive receptor. Northern blot analysis of the mRNA from the receptor mutants with several probes derived from the original alpha-human glucocorticoid receptor cDNA showed the same ~7 and 4.5 kb forms of receptor mRNA as did the wild-type cells. Sensitivity of oncogene transcripts to glucocorticoids in hormone-sensitive and -resistant cells is being investigated.

# *Oncogenes and Growth Factors - II*

**A 029** CELL TRANSFORMATION BY ras ONCOGENES. Douglas R. Lowy, Thierry J. Velu, Pierre E. Tambourin, Hsiang-Fu Kung, and Berthe M. Willumsen*. NCI, Bethesda, Maryland; *University Microbiology Institute, Copenhagen, Denmark.

We have studied the in vivo tumorigenesis of the Harvey murine sarcoma virus (MuSV) and carried out structure-function studies of its activated viral oncogene (v-rasH). We have found that the class of tumors induced by the virus in vivo depend upon ras and non-ras sequences in Ha-MuSV. The wild type Ha-MuSV induces fibrosarcomas and splenic erythroleukemia. A version of the virus from which non-ras sequences located outside the viral LTR have been deleted from the viral genome, which encodes wild type v-rasH protein but at lower levels than full length Ha-MuSV, induces lymphoid tumors.

At the biochemical level, ras transforming proteins bind guanosine nucleotides, possess GTPase activity, and undergo post-translational modifications that are associated with their migration from the cytosol to the cytoplasmic surface of the plasma membrane. Linker insertion-deletion mutagenesis of v-rasH has shown: 1) any of at least four large segments of the protein (representing together about 40% of the protein) can be altered without abolishing its transforming activity; 2) C-terminal mutants fail to reach the membrane and are transformation defective; 3) guanosine nucleotide binding activity is distributed over several N-terminal segments of the protein that are essential for transformation; 4) a class of transformation defective mutants whose amino acids are clustered in an N-termial segment that may participate in the interaction between ras and its cellular target has been identified.

**A 030** CHROMOSOME DELETION, ONCOGENE AMPLIFICATION, & AUTROCRINE GROWTH FACTORS IN THE PATHOLOGY OF SMALL CELL LUNG CANCER, J. Minna, M. Nau, J. Battey, M. Birrer, B. Brooks, F. Cuttitta, J. DeGreves, A. Gazdar, B. Johnson, A. Lebacq-Verheyden, I. Linnoila, E. Sausville, E. Seifter, M. Vinoccour, NCI-Navy Medical Oncology Branch, NCI, USUHS, NAVHOSP, and NIH Bethesda, MD 20814

Molecular genetic studies of human small cell lung cancer (SCLC) lines have demonstrated a consistent set of abnormalities that could potentially explain part or all of the malignant phenotype of these cells. Restriction fragment polymorphism analysis (by Dr. S. Naylor, Univ. Texas) has demonstrated that the cytogenetic deletion of chromosome region 3p (14-23) represents a true DNA deletion. This suggests SCLC may have similar genetic mechanisms operating as those in retinoblastoma and Wilm's tumors. Frequently we have found amplification or deregulated expression of the myc family of oncogenes (including c-, N-, L-myc) as well as expression of the p53 proto-oncogene. The structure and expression of L-myc are interesting as alternative processing of L-myc mRNA is seen in both small cell and non-small cell lung cancer groups. This alternative processing generates messages with different combinations of the 2nd or 3rd exon myc family equivalents, a feature not as yet seen in c-myc or N-myc. In addition, expression of cellular proto-oncogenes of the ras and raf (studies by Dr. U. Rapp, NCI) families in these same cells provide for oncogene cooperactivity. Finally, it appears that both SCLC and non-SCLC lines can replicate in serum and hormone free medium for prolonged periods of time indicating their ability either to produce autocrine growth factors (such as gastrin releasing peptide) or use intracellular transducing signals to function in an autocrine fashion.

**A 031** ACTIVATION OF THE $pp60^{c-src}$ PROTO-ONCOGENE IN HUMAN CELLS. Neal Rosen[1] and Joseph B. Bolen[2]. Medical Breast Cancer Section[1] and Laboratory of Tumor Virus Biology[2], National Cancer Institute, Bethesda, Maryland. 20892.

The proto-oncogene c-src encodes a 60 Kd, membrane associated phosphoprotein with endogenous tyrosine kinase activity. The $pp60^{c-src}$ protein is expressed in all vertebrate cells and generally has a low tyrosine protein kinase specific activity; however, in cells transformed with c-src mutants, v-src or polyoma virus, the specific activity is elevated and closely associated with the capacity of the protein to transform. Elevation of tyrosine kinase activity has been related in these systems to loss of phosphorylation of the tyr 527 of $pp60^{c-src}$. $pp60^{c-src}$ tyrosine kinase specific activity is also elevated in platelets and in cells of neuronal phenotype. The latter elevation is differentiation dependent and not sufficient to mediate transformation. In vitro translation studies show that intracellular $pp60^{c-src}$ protein kinase is inhibited post-translationally and that loss of the post-translational modification is associated with activation of the enzyme during neural differentiation. In contrast to $pp60^{c-src}$ kinase elevation in normal neuronal cells, it is selectively activated in breast and colon carcinoma compared to normal mammary or colonic epithelia. This is true in all colon carcinoma cell lines tested with a mean activity 80-fold greater than that in normal colonic cell lines. Elevated activity cannot be explained by overexpression of the protein or by presence of a protein-protein complex. $pp60^{c-src}$ is phosphorylated on tyrosine 527 in the intact cell. The half-life of the tyrosine phosphate is 3.5 hr in the normal colon cell and less than 15 minutes in colon carcinoma cell lines. The relationship between $pp60^{c-src}$ kinase activation and the lability of this phosphorylation, as well as its significance in colon carcinoma, will be discussed.

**A 032** ACTION OF COLONY STIMULATING FACTOR-1, E. Richard Stanley, Yee-Guide Yeung, Paul T. Jubinsky and R. Sacca. Departments of Microbiology and Immunology and of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461.

Colony stimulating factor-1 (CSF-1) is a homodimeric glycoprotein that selectively regulates the survival, proliferation and differentiation of mononuclear phagocytes (CSF-1 dependent precursor→monoblast→promonocyte→monocyte→macrophage) (reviewed in 1). The molecular aspects of the action of CSF-1 have focused on its receptor. An assay for the solubilized CSF-1 receptor (2) was used to monitor its purification from a membrane fraction of the murine macrophage cell line, J774.2. Approximately 240 mg of membrane protein from $10^{10}$ cells yielded approximately 25 ug of purified material (recovery of activity 77%, 7000-fold purification). The purified receptor behaved as a single 165 kd protein band on 7.5% SDS-PAGE and exhibited increased phosphorylation in tyrosine in the presence of CSF-1. Several independent lines of evidence indicate that this receptor is identical to the c-fms proto-oncogene product (3-5). Because of its probable importance in signal transduction, CSF-1 receptor mediated protein phosphorylation has been studied using macrophage membrane preparations. In membranes prepared from macrophages requiring CSF-1 for proliferation, CSF-1 increased the phosphorylation of 14 proteins. The CSF-1 stimulated phosphorylation was specific, saturable at physiological concentrations and qualitatively different from phosphorylation in the absence of CSF-1. Consistent with a role for CSF-1 stimulated phosphorylation in the macrophage proliferative response, only 3 proteins were shown to exhibit CSF-1 stimulated phosphorylation in membranes from a CSF-1 receptor bearing cell line that did not require CSF-1 for growth. Precipitation of solubilized membranes with an anti-phosphotyrosine antiserum indicated that a subset of 6 of the 14 proteins whose phosphorylation was stimulated in CSF-1 requiring cells were phosphorylated in tyrosine.

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# Poster Session

**A 100** *INVASIVE ACTIVITY AND CHEMOTACTIC RESPONSE TO GROWTH FACTORS OF CULTURES OF KAPOSI SARCOMA CELLS*, *A. Albini, C.D. Mitchell, R. Seeman, G.R. Martin and J.V. Quinnan, JR. NIDR and FDA, Bethesda, MD 20892.*

*Kaposi's sarcoma (KS) is a relatively low grade neoplasm of unknown etiology previously observed primarily in elderly men. A virulent invasive form of Kaposi's sarcoma appears in some 30% of AIDS patients. Histological studies suggest that these lesions are composed of proliferating endothelial cells plus some spindle shaped cells probably mesenchymal in nature. The origin and the identification of the tumor cells in KS lesions are controversial. We have developed an in vitro assay which can be used to measure the ability of cells to invade through basement membrane. In this system, an extract of basement membrane is layered onto a porous filter and allowed to polymerize. Malignant cells are able to penetrate through this barrier as they respond to chemoattractants. KS cells were cultured from biopsies specimens in selective medium containing a low serum concentration. Four such cultures were tested for their invasiveness in the assay described above and they all showed invasive activity comparable to that observed with a variety of malignant cells. Control fibroblasts, smooth muscle cells and endothelial cells did not demonstrate invasive behaviour. These observations suggest the presence of malignant elements within the cultures. To further characterize the nature of the KS cells we tested their chemotactic response to a variety of chemotactic growth factors in comparison to normal fibroblasts, smooth muscle and endothelial cells. These studies showed that each type of normal cell has a unique repertoire of factors to which they are attracted. The KS cells most closely resembled smooth muscle cells and were quite distinct from endothelial cells. Taken together these results indicate that the KS derived cultures contain invasive cells and have a smooth muscle cell-like chemotactic phenotype.*

**A 101** MUTATIONAL ANALYSIS OF HUMAN EGF RECEPTOR, Deborah E. Banker, Angelika M. Vollmar, and Harvey R. Herschman, UCLA, Los Angeles, CA 90024.

EGF and mAb 225 (a monoclonal antibody recognizing the human epidermal growth factor receptor) were used to construct ricin A-chain (RTA) conjugates. The two chimeric molecules, EGF-RTA and mAb 225-RTA, were equally toxic to HeLa cells. Rapidity and potency of protein synthesis inhibition of HeLa cells was similar for the two chimeric conjugates, as was the degree to which colony-forming ability was reduced. However, ammonium chloride ($NH_4Cl$) enhanced the toxicity of EGF-RTA but not mAb 225-RTA, suggesting that the two chimeric toxins are processed differently by HeLA cells.

Independent clones of HeLa populations were mutagenized and subjected to stepwise selection in increasing concentrations of EGF-RTA. After selection, cloning, and expansion, presumed mutant clones were assayed in protein synthesis assays for resistance to toxic conjugate. Three clones proved particularly resistant relative to parental HeLa cells: Hb + H22b were 50-fold, and H20e was 100-fold resistant. $NH_4Cl$ enhanced the efficacy of the toxic conjugate on HeLa cells and on the mutant cells. None of the clones was relatively resistant to ricin or mAb 225-RTA toxic conjugate when compared to parental cells. All three clones bound and internalized iodinated EGF, although quantitative differences in rate and extent of EGF processing were noted. EGF pretreatment led to down regulation of the EGF receptor in the mutants, as in the parent HeLa cells. Other aspects of response to EGF are being investigated in these mutants.

**A 103** ANTIMITOTIC EFFECT OF PHENETHANOLAMINE, LY195448, IS PREVENTED BY DIACYLGLYCEROLS, G.B. Boder, F.W. Beasley, R.C. Cook, Lilly Research Laboratories, Indianapolis, IN

The phenethanolamine, LY195448, is being developed as a single agent antitumor compound and for its ability to potentiate other antitumor compounds such as 5-fluorouracil. In combination studies with 5-fluorouracil, LY195448 (10mg/kg, S.C. twice daily x 10) produced about a 100 fold increase in the therapeutic index of 5-FU using the carcinoma 755. Little or no antitumor activity is observed with LY195448 by itself when the compound is administered orally or subcutaneously; however, excellent antitumor activity is observed when the compound is administered by rapid intravenous injection with greater then 80% inhibition of tumor growth in the CA-755, $C_3H$ Mammary, M5 Ovarian, and Lewis Lung Carcinomas. In cell culture studies, the compound inhibits proliferation at the mitotic phase of the cell cycle by a mechanism that appears to be unique. The compound appears to inhibit assembly of newly forming microtuble associated with the centriole (G.B. Boder et al. Microtubules and Microtubule Inhibitors. DeBrabander and DeMey eds. Elsevier Sci. Publishers-Amsterdam 1985). Recent experiments show that less than equimolar amounts of a synthatic diacylglycerol and the phorbol ester 12-O-tetradecanoylphorbol-13-actetate (TPA) completely inhibit the antimitotic activity of LY195448. These experiments suggest that LY195448 may be affecting cell replication by interfering with protein kinase C modulated set points in tumor cells.

**A 104** IDENTIFICATION OF TRANSFORMING GROWTH FACTOR-BETA BINDING PROTEINS IN SOLID TISSUES. Frederick T. Boyd, Jr. and Joan Massague, Department of Biochemistry, University of Massachusetts Medical School, Worcester, MA 01605

Transforming growth factor-β (TGF-β) is a multi-functional modulator of growth and differentiation which has been isolated from many cell lines and is present in high abundance in blood platelets. TGF-β receptors have been identified in many types of cells in culture and three cell surface glycoproteins of 280-330 kDa, 85-95 kDa and 65 kDa are typically affinity labeled with high affinity and specificity by $^{125}$I-TGF-β. The 280 kDa receptor species is the binding subunit of a large (~600 kDa) disulfide linked receptor complex. Binding of TGF-β to these glycoproteins has been correlated with the biological actions of TGF-β. However, TGF-β receptors have not previously been identified from solid tissues. In these studies we have identified specific TGF-β binding to membrane fractions from various solid tissues. All three receptor types labeled in cells in culture are also labeled using membranes prepared from rat liver, muscle, placenta, uterus and whole fetus. TGF-β binds to membranes from whole rat fetuses with an apparent $ED_{50}$ of 0.4-0.5 nM. This indicates a markedly lower affinity than reported for whole cells in culture, however membranes from cultured cells also have an apparent affinity of approximately 0.5 nM. The demonstration of TGF-β binding to membranes from solid tissues indicates that the receptor species previously identified in cells in culture are also present in whole tissue. This suggests that these receptors may mediate the in vivo effects of TGF-β on growth and differentiation.

**A 105** SYNTHESIS AND SECRETION OF PDGF-LIKE GROWTH FACTOR BY HUMAN BREAST CANCER CELL LINES. D. Bronzert, P. Pantazis, H. Antoniades, M. Lippman. Medicine Branch, National Cancer Institute, NIH, Bethesda, MD 20892 and Center for Blood Research, Boston, MA 02115.

We studied human breast cancer cells for production and release of a growth factor that is biologically and immunologically similar to platelet derived growth factor (PDGF). Serum-free media, conditioned for 48 hr from MDA-MB-231 and MCF-7 cells, contains a mitogenic or "competence" activity that is capable of inducing quiescent Swiss 3T3 cells to divide as measured by increased [$^3$H]thymidine incorporation in the presence of platelet poor plasma. Breast cancer conditioned media contains an activity which competes with [$^{125}$I]PDGF for binding to its receptors in normal human fibroblasts. Like authentic PDGF, this activity is acid and heat stable (100C) and inhibited by reducing agents. Immunoprecipitation of proteins extracted from [$^{35}$S]cysteine labeled MDA-MB-231 cells with antiserum to PDGF demonstrates specific immunoprecipitated proteins in the cell lysate ranging from 16 kDa to 120 kDa while the media contains a predominant 30 kDa band. Under reducing conditions, the 30 kDa band is not seen and both a 15 kDa band and 16 kDa band are obtained, suggesting two polypeptides with disulfide linkage. Hybridization studies with cDNA probes for PDGF-1 and PDGF-2 (c-sis) demonstrated the presence of transcripts for both PDGF chains in the MCF-7 cells. The MDA-MB-231 cells contain predominantly the PDGF-1 transcript. Thus, human breast cancer cells produce a PDGF-like growth factor which may be important in mediating autocrine or paracrine stimulation of tumor growth.

**A 106** PROLACTIN (PRL) ALTERS PHORBOL ESTER BINDING IN LIVER, Arthur R. Buckley, Charles W. Putnam, Diane Haddock Russell, University of Arizona and VAMC, Tucson AZ, 85719.
PRL is a key growth regulatory hormone in liver. Its administration to rats induces liver ornithine decarboxylase and plasminogen activator activities, biochemical markers of the $G_1$ phase of cell cycle, and stimulates DNA synthesis. Chronic PRL administration subsequent to diethylnitrosamine stimulates formation of enzyme-altered foci suggesting that PRL may be a tumor-promoting growth factor for initiated hepatocytes. One mechanism by which promoters stimulate tumorigenesis involves activation of protein kinase C (PKC), a $Ca^{2+}$- and phospholipid-dependent kinase which binds tumor promoting phorbol esters (PE) with high affinity. We have investigated hepatic PRL receptor coupling to PKC by assessing [$^3$H]-phorbol-12,13-dibutyrate [$^3$H-PDBU) binding. Both membrane (106,000 x g pellet) and cytosol (supernatant) exhibited specific saturable binding sites of high affinity ($K_d$, 13.6-18.8). $^3$H-PDBU binding was found to be $Ca^{2+}$- and phospholipid-dependent with optimal binding occurring at 5 mM $Ca^{2+}$ and 400 µg/ml phosphatidylserine. Inhibition of $^3$H-PDBU binding by PE closely paralleled their effectiveness as tumor promoters in mouse skin. PRL administration caused a rapid decrease in $^3$H-PDBU binding in both fractions. Specific binding of 20 nM $^3$H-PDBU was decreased by 30% in cytosol and 54% in membranes 15 min after hormone administration. At 30 min $^3$H-PDBU binding declined to 11% and 30% of control in cytosol and membrane fractions, respectively. Scatchard analysis revealed no alteration in $K_d$ values between PRL and control livers. However, decreased binding at 30 min reflected a decrease in the number of receptor sites available subsequent to PRL administration. We conclude (1) the hepatic PRL receptor is coupled to activation of PKC and (2) decreased $^3$H-PDBU binding following PRL administration reflects either PRL-stimulated enzyme turnover or occupation of the PE binding site by the endogenous ligand.

**A 107** DIGLYCERIDE SECOND MESSENGER: FORMATION IN RESPONSE TO PHORBOL DIESTER EXPOSURE AND INTRACELLULAR FATE, Myles C. Cabot, Zu-Chuan Zhang, Clement J. Welsh and Holly Chabbott, W. Alton Jones Cell Science Center, Lake Placid NY 12946.
We have studied the modes by which nontransformed [C3H/10T½ clone 8, REF52 (REF A)] and transformed [C3H/10T½ clone 16, WT6Ag6 nul (REF D)] fibroblasts, labeled with [$^3$H]glycerol, respond to 12-O-tetradecanoylphorbol-13-acetate (TPA). TPA (60 min, 50 ng/ml) provoked diglyceride (DG) formation in clones 8, 16, and REF A cells; REF D cells were not responsive. To measure total DG formed in the responsive cells, DG metabolites also had to be quantitated. Clone 8 cells showed a high increase in DG upon TPA exposure (3-fold over control) with a concomitant increase in triglyceride (TG, 1.7-fold). In clone 16 cells the DG formed was short-lived, being rapidly metabolized to monoglyceride (DG, 1.3-fold increase; MG, 2.3-fold increase), and there was no increase in TG counts. Although REF A cells respond to TPA treatment with a 2.5-fold increase in DG, the majority of radioactivity was recovered as free glycerol, indicating that a complete mode of mediator destruction by lipase is present in these cells. The results of experiments using REF A cells prelabeled with choline, ethanolamine, or inositol, indicate that the DGs are formed (via phospholipase C) primarily from choline-containing glycerophospholipids. These experiments suggest that phosphatidylcholine-derived DG, as opposed to phosphoinositide, is produced by phorbol ester stimulation in fibroblasts, and that the intracellular amount of this DG is controlled by the action of several enzymes. The involvement of protein kinase C and the capacities by which differet cells are able to eliminate the DG mediator may, therefore, be keys to proliferative control.

**A 108** REGULATION AND MODIFICATION OF TOPOISOMERASE II DURING CELL CYCLE PROGRESSION, Kuan-Chih Chow, Daniel M. Sullivan and Warren E. Ross. University of Florida, Gainesville, FL 32610.
Topoisomerase II (topo II) is a primary target in cancer chemotherapy. In the presence of etoposide (VP-16) topo II can be arrested on DNA as "cleavable-complex". We have studied the regulation of topo II activity during cell cycle progression of Balb/c 3T3 cells. Etoposide-mediated DNA break frequency was used as a parameter of topo II activity. Upon serum stimulation, a marked increase in drug sensitivity began during S phase and peaked in the late $G_2$ phase. The increase in drug sensitivity correlated with the increase in the enzyme content as determined by immunoblotting. However, the drug-induced DNA damage does not correlate with sensitivity of cells in response to drug challenge. Maximal cytotoxicity occurred during S phase and prior to the increase in topo II content. These data indicate that topoisomerase-mediated DNA breaks may be necessary but are not sufficient for the cytotoxic effect. The induction of enzyme activity could be blocked by cycloheximide, but not phidicolin. The exposure to cycloheximide prior to etoposide challenge resulted in a loss of cytotoxic effect of etoposide. Our findings indicate that topo II activity fluctuates with cell cycle with peak activity at $G_2$/M. The increase in enzyme activity is protein synthesis dependent. In the presence of cycloheximide, enzyme turnover could be highly regulated.

**A 109** THE ISOLATION AND SEQUENCE OF cDNA CLONES FOR THE mdr1 (P-GLYCOPROTEIN) GENE FROM MULTIDRUG-RESISTANT HUMAN KB CARCINOMA CELLS, D. Clark#, K. Ueda, A. Fojo, C-j. Chen*, J. Chin*, I. Roninson*, I. Pastan and M. Gottesman, NCI, NIH, Bethesda, MD 20892; * University of Illinois, College of Medicine, Chicago, IL 60612; #Howard Hughes Medical Institute Research Scholar.

The development of resistance to multiple chemotherapeutic drugs is a common problem in cancer treatment. We have studied this problem using a series of human KB carcinoma cell lines selected for resistance to either colchicine, vinblastine or adriamycin. Multidrug resistance in these cell lines has been shown to be due to reduced intracellular accumulation of drugs resulting from an energy-dependent increase in drug efflux. Paralleling the development of drug resistance is overexpression of a membrane glycoprotein of approximately 170,000 Mr (P-glycoprotein) and amplification of the P-glycoprotein gene. We have isolated overlapping cDNA clones encompassing the entire mdr1 (P-glycoprotein) gene from a cDNA library prepared from colchicine-selected cells. Hybridization of the mdr1 cDNA to genomic DNAs and cosmids from various multidrug resistant cells confirmed the amplification determined previously with genomic probes and indicated that the mdr1 gene is at least 100 Kb long. The complete primary structure of P-glycoprotein has been determined from the cDNA sequence and has revealed that the protein contains hydrophobic domains compatible with transmembrane regions and internally duplicated regions homologous with peripheral membrane components of bacterial active transport systems including potential nucleotide-binding sites. The isolation of a full-length cDNA for the mdr1 gene has allowed us to examine its expression in normal human tissues and in tumors.

**A 110** MOLECULAR APPROACHES TO UNDERSTANDING EGF-RECEPTOR STRUCTURE - FUNCTION RELATIONSHIPS. Stella Clark, Justin Hsuan, Charley Greenfield, John Haley, Dorothy Cheng, Michael D. Waterfield. Ludwig Institute for Cancer Research at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The EGF receptor (EGF-R) transduces a transmembrane signal resulting from the binding of EGF and leading to a mitogenic response in target cells. The close homology between the EGF-R and the transfroming genes v-erb B and onc-neu imply a potential role for the EGF-R in unregulated cell growth and transfromation. The cloning and sequencing of the EGF-R allow for new experimental approaches to elucidate the above mechanisms.

An expression system has been developed utilizing fluorescent activated cell-sorting to isolate cells expressing elevated levels of EGF-R. The coupling of EGF binding and tyrosine kinase activity to subsequent short and long-term mitogenic events has been investigated.

In addition, to allow the production of large amounts of EGF-R protein for detailed enzymatic and biophysical studies, the baculovirus expression vector system has also been used. Expression of the EGF-R cDNA, in insect cells, under the control of the polyhedrin promoter has been demonstrated by immunofluorescence utilizing the monoclonal antibody R1, a conformation-dependent antibody. Results to date indicate that this may be a viable method for the production of large amounts of EGF-R protein.

**A 111** BRYOSTATIN 1 INHIBITS PHORBOL ESTER-INDUCED BLOCKAGE OF DIFFERENTIATION IN HEXAMETHYLENE BISACETAMIDE-TREATED FRIEND ERYTHROLEUKEMIA CELLS.
Marie L. Dell'Aquila*, Hong T. Nguyen*, Cherry L. Herald#, George R. Pettit#, and Peter M. Blumberg*; *Lab. of Cellular Carcinogenesis and Tumor Promotion, NCI, Bethesda, MD 20892; and #Dept. of Chemistry, Arizona State Univ., Tempe, AZ 85287.

Phorbol esters inhibit differentiation induced by the chemical hexamethylene bisacetamide (HMBA) in Friend erythroleukemia cells (FELC). This study examines the effect of the macrocyclic lactone bryostatin 1 on phorbol ester responses in an FELC clone, PS 7. In several biological systems, bryostatin 1 mimicked phorbol ester action, including activation of protein kinase C, but it paradoxically blocked phorbol ester-induced differentiation in HL-60 cells. We report here that bryostatin 1 blocks phorbol ester action in Friend cells (clone PS 7). Bryostatin 1 blocks PDBu-induced inhibition of differentiation in HMBA-treated FELC ($ED_{50}$ = 15.0 ± 3.5 nM) and also a second phorbol ester response, induction of cellular adherence. The inhibition of erythroid differentiation by dexamethasone, a nonphorbol compound whose action presumably is not protein kinase C-mediated, is unaffected by bryostatin 1. Although bryostatin 1 competes for [$^3$H]PDBu binding in intact FELC PS 7, the mechanism for antagonism of phorbol ester action by bryostatin 1 in the Friend cells appears to be noncompetitive.

**A 112** IGF-I SECRETION IS INDUCED BY ONCOGENES IN HUMAN BREAST CANCER AND MOUSE FIBROBLAST CELL LINES. Dickson, R.B., Huff, K.K., and Lippman, M.E. Medicine Branch, National Cancer Institute, NIH, Bethesda, MD 20892.

We are interested in the possible role of insulin-like growth factor-I (IGF-I, somatomedin C) in malignant trnasformation. To explore this possibility we have utilized retroviral oncogene-induced transformation of breast epithelial and fibroblast cell lines. The human breast cancer cell line MCF-7 responds in vitro to 17B-estradiol ($E_2$) treatment with an increased rate of proliferation and an increased output of mitogenic growth factors. Among these growth factors are TGFa and IGF-I related polypeptides. To further address the relationship between malignant transformation and IGF-I release we have transfected MCF-7 cells with the v-ras$^H$ oncogene. MCF-7ras cells formed tumors in nude mice in the absence of $E_2$ and secreted 3-10 fold more IGF-I than control transfectants (125 ng/mg DNA/day). MCF-7ras, in contrast to controls, was refractory to further growth stimulation in vitro by exogenous IGF-I, but the number of high affinity cell surface receptors was unchanged (11,000 sites/cell, Kd 1nM). In contrast, NIH3T3 mouse fibroblasts transformed with Harvey sarcoma virus had unchanged secretion of IGF-I. Instead, transformation of mouse fibroblasts with Moloney sarcoma virus (v-mos oncogene) or Rous sarcoma virus (v-src oncogene) induced IGF-I secretion 10-fold and 20-fold, respectively. Induction of IGF-I secretion is associated with retroviral oncogene expression in both epithelial and stromal derived cells.

**A 113** AUTOCRINE STIMULATED GROWTH OF A LYMPHOBLASTOID LINE IMMORTALIZED BY INFECTION WITH A PARASITE
D Dobbbelaere, I Baumann, M Carrington, T Coquerelle, A Ehrfeld, A Gill, V Ivanov, I Roditi, R Williams
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We have been studying the reversible lymphoblastoid transformation of bovine lymphocytes induced by infection with the intracellular parasite *Theileria parva* T.p. is a protozoan parasite related to malaria that causes a fatal lymphoproliferative disease in cattle. Infection by this organism commits the infected T cell to rapid uncontrolled growth. In the early stages of infection one observes extensive proliferation and spread of infected cells in the lymphnodes. The infected lymphocytes can invade other tissues, be cloned in soft agar and cause tumors in nude mice. Infected cells very easily establish themselves in tissue culture where they remain immortalized. Experimental drugs (Wellcome Labs) have been developed that specifically eliminate the parasite. The removal of the parasite returns the dividing cell to a resting state. This reversible transformation provides a novel system for the analysis of the molecular events responsible for uncontrolled growth. An initial screening with oncogenes revealed that both the *myc* and *raf* oncogenes are concomitantly expressed in the parasitized cells. The bovine raf cDNA has been cloned from infected cells. In addition to an oncogene analysis, we will present data to show that the infected cells secrete a growth factor and that the cells need this factor for proliferation. Recombinant human IL-2 can substitute for the endogenous factor. We show that the IL-2 receptor is present and that following treatment with the experimental drug the receptor drops to levels of resting cells. TPA treatment of drug cured cells in the presence of added IL-2 stimulates the cells to divide for a further several months after which they die out. The parasite appears to create an immortalized cell that is driven in an autocrine fashion with a growth factor probably bovine IL-2.

**A 114** COUPLING OF THE BOMBESIN RECEPTOR TO A GUANINE NUCLEOTIDE BINDING PROTEIN, Julian Downward and Robert Weinberg, Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142.

Bombesin is known to be a potent mitogen for Swiss 3T3 cells and an activator of phosphatidylinositol (PI) turnover. Recently it has been shown in several systems that the phospholipase C involved in PI turnover is under the control of an uncharacterised guanine nucleotide binding protein, termed $G_p$. We have set out to investigate possible interactions between the bombesin receptor and guanine nucleotide binding proteins in the hope of establishing the nature of $G_p$.

We have demonstrated that in membranes derived from Swiss 3T3 cells bombesin will induce a three fold increase in the level of GTP binding, suggesting that it activates a G protein. Furthermore, in this system non-hydrolysable analogues of GTP, but not ATP, decrease the affinity of the bombesin receptor for its ligand, again suggesting G protein involvement. Using a cross-linkable analogue of GTP it appears that bombesin activates a single G protein in Swiss 3T3 cell membrane preparations that is distinct from all previously characterised GTP binding proteins. This activation occurs very rapidly, with a bombesin concentration dependence similar to that of the ligand binding to the membranes. It appears to be unaffected by bacterial toxins. We are currently attempting to establish whether this protein is involved in the regulation of PI turnover.

**A 115** THE HUMAN TGFα CODING SEQUENCE IS NOT A DIRECT ACTING ONCOGENE WHEN OVER-EXPRESSED IN NIH/3T3 CELLS, E. Finzi*, T. Fleming*, O. Segatto*, C. Y. Pennington*, T. S. Bringmant, R. Derynckt and S. A. Aaronson*, *National Cancer Institute, Bethesda, MD 20892; and †Genentech, Inc., South San Francisco, CA 94080.
Transforming growth factor α (TGFα) is a peptide which is secreted by a variety of tumor cells and binds to the EGF receptor. To investigate the transforming properties of this factor, the human sequence coding for TGFα was placed under the control of either a metallothionein promoter or a retroviral LTR. These constructs failed to induce primary transformed foci upon transfection of NIH/3T3 cells, whereas oncogenes encoding a truncated form of its cognate receptor, the EGF receptor, or another growth factor, sis/PDGF-2, efficiently induced transformed foci. When NIH/3T3 clonal sublines were selected by transfection of TGFα expression vectors in the presence of a dominant selectable marker, they were shown to secrete large amounts of TGFα into the medium, to have down-regulated EGF receptors, and to be growth inhibited by TGFα monoclonal antibody. These results indicated that secreted TGFα interacts with its receptor at a cell surface location. Single cell derived TGFα expressing sublines grew to high saturation density in culture, but the transformed phenotype was not an intrinsic genetic property. When plated as single cells on contact-inhibited monolayers of NIH/3T3 cells, they failed to grow as transformed colonies, while v-sis and v-erbB transformants grew readily under the same conditions. These and other results imply that TGFα exerts its effect on the entire NIH/3T3 cell population after secretion into the medium, but exerts little, if any, direct effect on the individual cell synthesizing this factor.

**A 116** CHARACTERIZATION OF TYROSINE-PROTEIN KINASES AND THEIR SUBSTRATES USING A HIGH-AFFINITY, SPECIFIC MONOCLONAL ANTIBODY, A. Raymond Frackelton, Jr., Marshall R. Posner, and Richard D. Huhn, Roger Williams General Hospital and Brown University, Providence, RI 02908

Much evidence implicates growth-factor receptor- and oncogene-tyrosine kinases in the control of normal and neoplastic cell growth. However, few candidates exist for their physiologically relevant substrates. To facilitate study the tyrosine kinases and their substrates, we have recently developed a new monoclonal antibody (1G2) that binds phosphotyrosyl proteins with a $K_d$ of $\simeq 2 \times 10^{-9}$ M, but demonstrates no affinity for phosphoserine, phosphothreonine, mono-, di- or trinucleotides, or phosphohistidyl, phosphothreonyl or phosphoseryl proteins. Used as a solid-phase immunosorbent, it efficiently purifies the phosphotyrosyl EGF, PDGF, and insulin receptors, the transforming protein of the Abelson MuLV, as well as a multitude of other cellular phosphotyrosyl proteins. The antibody also recognizes phosphotyrosyl proteins by Western blotting and by cell surface and cytoplasmic immunofluorescence. In detailed studies of cells transformed by the Abelson murine leukemia virus, we have found that the Abelson oncogenic protein (p120) is nearly stoichiometrically phosphorylated on tyrosine. In contrast to the case with many of the growth-factor receptors, *in vivo* tyrosine-phosphorylation of the Abelson p120 appears to inhibit its tyrosine kinase activity (measured using exogenous substrates). In chronic myelogenous leukemia, cells of the neoplastic clone contain an aberrant, activated *abl* protein that is believed to be involved in the pathogenesis of this disease. As a first step in studying the involvement of *abl* in this disease, we have purified phosphotyrosyl proteins from CML cell lines (BV173, RWLeu4, and K562) and from peripheral blood leucocytes of CML patients. In addition to the aberrant *abl* protein, 15 phosphotyrosyl proteins were isolated. Remarkably, both the patients' peripheral leukocytes and each of the cultured CML cell lines harbor virtually identical complements of phosphotyrosyl proteins.

**A 117** THE ROLE OF CALCIUM IN PDGF OR TPA INDUCTION OF C-MYC RNA, K.K. Frick, R.B. Womer, and C.D. Scher, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA 19104.
Platelet-derived growth factor (PDGF) causes the rapid, receptor-mediated, turnover of phosphoinositides in responsive cells; the resulting products, DAG and $IP_3$, serve to activate protein kinase C and to increase cytoplasmic $Ca^{++}$. We investigated the roles of these products in c-myc RNA induction in MG63c cells, a clonal isolate of a human osteosarcoma line. MG63c cells showed c-myc induction after addition of either PDGF or a combination of tetradecanoyl phorbol acetate (TPA) and bradykinin. These agents acted through independent pathways. When protein kinase C, the TPA target, was desensitized by extended pretreatment with TPA, the c-myc RNA response to TPA was severely attenuated; however, induction in response to PDGF was not affected. The TPA effect was inhibited by the calcium ionophore A23187 when added 30 min prior to TPA addition, in medium containing 1.8 mM $CaCl_2$. No inhibition was seen when incubation was conducted in calcium-free medium. The ionophore did not affect PDGF induction of c-myc, nor did ionophore induce c-myc by itself. Thus, increased cytoplasmic $Ca^{++}$ is not an adequate stimulus for c-myc induction; rather it ablated TPA induction. When the cells were treated concurrently with PDGF (or TPA) and a combination of A23187 and EGTA to reduce intracellular $Ca^{++}$, there was an increase in the amount of c-myc RNA. This increase occurred only when the final concentration of EGTA was in molar excess of the $Ca^{++}$ in the incubation medium. Hence, either $Ca^{++}$ exit from an intracellular organelle, or reduced cytoplasmic $Ca^{++}$ per se, acts synergistically with PDGF or TPA to enhance c-myc induction.

**A 118** TWO SETS OF mRNA ARE CODED FROM TWO DISTINCT PROMOTERS OF THE RAT IGF+II GENE.
R. Frunzio, L. Chiariotti, A.L. Brown, D.E. Graham, M.M. Rechler and C.B. Bruni
Centro di Endocrinologia ed Oncologia Sperimentale C.N.R. Via S. Pansini 5 80131 Napoli, Italy and MCNEB, NIDDKD, NIH, Bethesda USA.

Rat insulin-like growth factor-II is synthesized in many embryonal and neonatal tissues as well as in a liver cell line, BRL-3A. A precursor protein of 22 kD pre-pro-rIGFII is processed to an active molecule of 7,5 kD.
We have reported the isolation of a cDNA clone for the rIGF+II from the BRL-3A cell line, and the analysis of the mRNAs in BRL-3A cells and in embryonal and neonatal liver. Different species of mRNA were identified, a major specie of 4 kB, not translatable in an in vitro assay, and minor species of 2,2, 1,7 and 1,2 kB, the latter being responsible for most of the actual synthesis of the precursor of the IGF+II.
We now report the cloning of the genomic region of the unique gene of the Rat IGF+II, the identification of two functional promoter regions in a tandem array, located before the three coding exons of the pre-pro-rIGFII mRNA, and the organization of the whole gene. We also show that more distant 3' regions hybridize to the 4 kB mRNA, and may contain elements that are important for the translation or processing of the same RNA.
We show the relative transcriptional strength, regulation and expression of the two promoters in different cell lines and tissues.

**A 119** CHARACTERIZATION OF DIPLOID HUMAN FIBROBLASTS TRANSFORMED FOLLOWING TRANSFECTION WITH THE SSV PROVIRUS. D.G. Fry, V.M. Maher, J.J. McCormick, Carcinogenesis Lab, Michigan State University, East Lansing MI 48824..
Attempts to transform diploid human fibroblasts via transfection techniques have, to date, yielded conflicting results in various laboratories. The oncogene of simian sarcoma virus (SSV), v-sis, is homologous to the gene encoding the PDGF B-chain. We have been able to successfully and reproducibly transform human fibroblasts using a plasmid containing the SSV provirus and the neo gene. This plasmid induces the formation of dense foci in the transfected cell population. Transformed cells,derived either from those foci or from Geneticin-resistant clones,expressed the v-sis mRNA at high levels and grew to high densities and incorporated $^{3}$H-thymidine in serum-free medium without PDGF or EGF. Control cells did not produce sis-homologous mRNA and required the presence of PDGF or EGF for either $^{3}$H-thymidine incorporation or replication in serum-free media. The transformed cells did not exhibit enhanced anchorage independent growth, were non-tumorigenic in athymic mice and have a finite lifespan in vitro. Diploid human cells transfected with a plasmid containing a cDNA copy of the human PDGF gene driven by an SV40 promotor did not produce foci or other evidence of transformation. Transfection of NIH3T3 cells with this plasmid or with the SSV-containing plasmid efficiently induces focus formation and focus derived cells produced by either plasmid are tumorigenic in athymic mice.

This work was supported in part by Department of Energy Contract EV04659, DHHS grant CA 21289 from the NCI, and by the Leukemia Society of America.

**A 120** EGF AND INSULIN RELATED GROWTH FACTORS COMPETE FOR BINDING TO A SINGLE *DROSOPHILA* PROTEIN, J. Victor Garcia, Karol L. Thompson, M. Patrizia Stoppelli, Stuart J. Decker, and Marsha Rich Rosner, Massachusetts Institute of Technology, Cambridge, MA 02139. The identification of a novel protein from *Drosophila melanogaster* that recognizes both mammalian epidermal growth factor (EGF) and insulin has been reported (Thompson *et al.* (1985), Proc. Natl. Acad. Sci. USA *82*, 8443). This 100 kDa *Drosophila* protein (dp100) also crossreacts with antisera against the human EGF receptor. We have now determined the binding spectrum and relative binding affinities of dp100 for growth factors and hormones related and unrelated to EGF or insulin. Our results indicate that dp100 binds to other insulin-like and EGF-like growth factors with dissociation constants ranging from $10^{-6}$M to $10^{-9}$M, and these ligands compete with each other for binding to dp100. All other ligands tested, including platelet-derived growth factor, transforming growth factor-beta, nerve growth factor, and glucagon, either did not bind or bound with a Kd greater than $10^{-6}$ M. In addition, dp100 binds to human synthetic transforming growth factor-alpha (TGF-alpha) and insulin-like growth factor-II with the highest affinity and, unlike the mammalian EGF receptor, has the unique ability to differentiate between EGF and TGF-alpha with a difference in affinity of three orders of magnitude. Dp100 is the only protein from *Drosophila* that binds TGF-alpha and, in contrast to the *Drosophila* insulin receptor, dp100 does not bind to wheat germ agglutinin. The ability of the EGF-like and insulin-like growth factors to specifically compete for binding to a single protein raises the possibility of a structural relationship between the two growth factor families.

**A 121** EPIDERMAL GROWTH FACTOR IN GYNAECOLOGICAL TISSUES AND FLUIDS. Diane G. Godfrey,W. David George, Colin Porteous, Glenys Neagle, Ian Pragnell.,University of Glasgow,Glasgow G12 8QQ.,U.K.

Growth factors are polypeptides which activate cell proliferation by binding to cell bound receptors. There exists a multiplicity of receptors in many cell and tissue types. This reflects the requirement for concomitant stimulation of more than one growth factor on a cell, in order to elicit a co-ordinated and appropriate growth response in a given tissue.

Studies suggest that growth factors, in particular, epidermal growth factor (EGF), are involved in the initiation of neoplastically transformed cells. There may be a relationship between levels of EGF in certain tissues and propensity to develop cancer.

Levels of EGF were measured in breast cyst fluids, ovarian follicle fluids, normal breast tissue, breast tumour and skin as a preliminary study. Results indicated that in some samples e.g. follicular fluids, levels of EGF were present in significantly higher than are normally found in human urine.These findings will be discussed in relation to other endocrine factors.

**A 122** VARYING EFFECTS OF EGF UPON PROLIFERATION OF CULTURED A431 CELLS, Alec Goldenberg, Hironobu Sunada, Jeff Peacock, Jan Castagnola and John Mendelsohn, Memorial Sloan-Kettering Cancer Center, New York NY 10021.

We are studying the proliferative effect of EGF on A431 cells using a monclonal antibody (mAb) 225 IgG1 that blocks the binding of EGF to the EGF receptor (EGFR) and also blocks EGF- or TGFα -stimulated tyrosine phosphorylation of the EGFR. The mAb can inhibit A431 cell proliferation, conceivably by blocking an autocrine loop involving TGFα. Others have shown that EGF can stimulate the growth of A431 cells implanted in nude mice. We reported that 5 pM EGF also stimulates these cells in culture, although growth is inhibited by 5 nM EGF. We have designed experiments to further examine the role that EGFR activation plays in growth regulation. In monolayer culture mAb inhibits the growth rate of A431 cells by approximately 30% during log phase and decreases saturation density by approximately 60% in plateau phase. This inhibition is reversed by EGF. These observations suggest that the degree of dependency upon EGF in monolayer cultures is determined by the cell density or the growth phase. To determine if the environment in which A431 cells grow might alter their dependency on EGF, we seeded A431 cells on 0.15% type I collagen beds and overlayed the cells with a second collagen layer. This permits multilayered growth of A431 cells. mAb 225 inhibits A431 cell growth in these collagen gels by 80% during log phase. This suggests that log phase growth in collagen is more dependent on EGF than log phase monolayer growth, and that the environment in which cells grow may determine the extent of EGF dependency. (Supported by NCI grants.)

**A 123** TPA-ACTIVATED PHOSPHORYLATION OF A Mr 82,000 PROTEIN IS NOT CATALYZED BY THE ORTHODOX CALCIUM-DEPENDENT C-KINASE. Michael Gschwendt, Walter Kittstein and Friedrich Marks, German Cancer Research Center, D-6900 Heidelberg, Germany.

In a Triton X100-extract from the particulate fraction of mouse epidermis but also of other murine tissues, the phosphorylation of a Mr 82,000 protein (p82) is strictly dependent on phosphatidyl serine (PS) and the tumor promoting phorbol ester TPA or diacylglycerol. Unlike protein kinase C (PKC)-catalyzed phosphorylation, p82 phosphorylation is neither observed in the presence of high concentrations of $Ca^{2+}$ and PS alone nor after addition of exogenous PKC. p82 is phosphorylated on serine and/or threonine, but not on tyrosin. Peptide mapping shows that phosphorylated p82 is different from autophosphorylated PKC. The results obtained after inhibition of protein synthesis by cycloheximid indicate a slow turn over of the p82 phosphorylation system (kinase and/or substrate) with a half life of more than 24 hrs. After treatment of the animals with TPA, however, the p82 phosphorylation system disappears rather rapidly from the particulate fraction ($T_{1/2}$:2-4 hrs). This "down-modulation" is also observed with the "incomplete" tumor promoter RPA, but not with the calcium ionophore A23187, and it appears to be inhibited by the immunosuppressive agent cyclosporin A. Our data indicate that the p82-kinase is different from the orthodox calcium-dependent PKC, but is a member of the PKC family.

**A 124** bFGF, A REQUIRED MITOGEN FOR HUMAN MELANOCYTES MIGHT BE EXPRESSED IN MELANOMAS. Ruth Halaban,* Sikha Ghosh* and Andrew Baird,+ *Department of Dermatology, Yale University School of Medicine, New Haven, CT and +Department of Neuroendocrinology, Salk Institute for Biological Studies, La Jolla, CA.

Normal human melanocytes, unlike pigment cells from metastatic melanomas, do not survive in culture in routine, serum-supplemented media. As of now, the only well defined growth promoters for normal human melanocytes and nevus cells are 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and substances that increase intracellular levels of cyclic-adenosine-monophosphate (cAMP) such as cholera toxin and isobutylmethyl xanthine (IBMX). The search for natural growth factors for melanocytes has shown that mitogenic activity is present in bovine brain, certain cell lines including those established from metastatic melanomas, human placenta, bovine pituitary and pineal glands, liver and lung tissues. Because the eventual transformation of melanocytes or nevus cells into metastatic melanomas appears to involve the acquisition of independence from these factors for growth, knowledge of natural growth factors for melanocytes can contribute to the understanding of the processes involved in malignant transformation of these cells. Of several growth factors tested, basic fibroblast growth factor (bFGF) was the only one mitogenic for melanocytes but only in the presence of cAMP stimulators. The mitogenic activity toward melanocytes in tissues and melanoma cell extracts had high affinity for herparin and antibodies to bFGF synthetic peptides. These results suggest that one of the growth factors for melanocytes might be bFGF or a bFGF-like polypeptide and that autocrine production of bFGF-like molecules by melanoma cells may contribute to the malignant phenotype of melanocytes. Because acidic FGF (aFGF) did not stimulate growth, the receptors for bFGF on melanocytes might be significantly different from those for aFGF.

**A 125** CONSTRUCTION OF A NOVEL ONCOGENE USING A SYNTHETIC GENE ENCODING HUMAN EGF, David L. Hare+, David F. Stern*, Marie A. Cecchini+, and Robert A. Weinberg*, +Amgen Development Inc., Boulder, CO 80301, *Whitehead Institute and MIT, Cambridge, MA 02142.

The autocrine model postulates that constitutive production of a mitogenic growth factor can result in uncontrolled cell proliferation and consequent cellular transformation. To test this hypothesis we constructed a vector for expressing secretory proteins in mammalian cells. The expresion vector consists of four functional elements: a Moloney murine leukemia virus long terminal repeat (LTR), sequences encoding a mouse immunoglobulin heavy chain signal peptide, a synthetic gene encoding human EGF and an SV40 polyadenylation site. The vector construct induced foci in monolayer cultures of FR3T3 rat cells; a frameshift mutation of the construct did not induce focus formation. Cells from five foci were cloned and shown to express mRNA of the predicted size. These cells also expressed moderate to high levels of EGF in radioreceptor competition assays. In culture, the transformed cells had spindle-shaped morphology and were highly refractile, but these cells did not grow well in soft agar. The transformed phenotype was reversible by addition of a neutralizing monoclonal antibody specific for human EGF. To test for tumorigenicity, all five cells lines were injected subcutaneously into irradiated nude mice. All five cell lines induced tumors; control cell lines did not induce tumors. These results demonstrate that high level secretion of EGF by this designed constitutive gene expression system is sufficient to render FR3T3 cells tumorigenic.

**A 126** DETECTION OF MULTIPLE FORMS OF THE PDGF RECEPTOR BY A RECEPTOR SPECIFIC MONOCLONAL ANTIBODY, Charles E. Hart#*, Russell Ross* and Daniel F. Bowen-Pope* #ZymoGenetics, Inc.; Seattle, WA 98103; *Dept. of Pathology, University of Washington; Seattle, WA 98195

We have developed a monoclonal antibody, PR7212 (IgG1), which recognizes the platelet-derived growth factor (PDGF) receptor. With the use of this antibody we have identified multiple forms of the PDGF receptor. By western blot analysis, we can detect proteins of approximately 180 KDa, 160 KDa and 125 KDa from membrane extracts of human skin fibroblasts. When these same membrane extracts are incubated with $^{32}$P-ATP in the presence of 70 nM PDGF, then subjected to immunoprecipitation by antibody PR7212, three major protein bands of 180 KDa, 160 KDa and 130 KDa, and several minor bands ranging from 48 KDa to 102 KDa, are precipitated, all of which show PDGF-stimulated $^{32}$P labeling. When membrane extracts from $^{35}$S-cysteine and $^{35}$S-methionine metabolically labeled cells are used for immunoprecipitation by PR7212, two bands of 180 KDa and 160 KDa are precipitated. Pulse chase experiments have shown that the 160 KDa protein is a precursor of the 180 KDa form.

The antibody recognizes an extracellular epitope of the PDGF receptor, but does not interact with the PDGF binding site, demonstrated by the lack of competition between PR7212 and PDGF for binding to the PDGF receptor. Species recognition by the antibody include human, baboon and monkey.

**A 127** DEXAMETHASONE TREATMENT INCREASES EXPRESSION OF CHROMOGRANIN A IN A NEUROBLASTOMA CELL LINE, Lee J. Helman, Thomas G. Ahn, Michael A. Levine, and Mark A. Israel, NCI, Bethesda, MD 20892 and Johns Hopkins University, Baltimore, MD 21205.
In order to identify genes whose expression may be linked to differentiation within the sympathoadrenal lineage, we constructed a human pheochromocytoma (Ph) cDNA library and used differential hybridization to human Ph and human neuroblastoma (Nb) cDNA probes to isolate genes which are highly expressed in the adrenal medullary neuroendocrine tumor Ph, but not in the more immature embryonal tumor of adrenal medulla, Nb. Among the clones we have isolated by this approach is a cDNA encoding chromogranin A, a known marker of neuroendocrine tissue. An early decision within the sympathoadrenal developmental pathway is the choice between neuronal and neuroendocrine phenotypes. Many studies have suggested that environmental factors influence this developmental decision. Glucocorticoids in particular are thought to stimulate development toward a neuroendocrine phenotype. Since Nb is a very primitive tumor of cells arrested early within the sympathoadrenal differentiation pathway, we treated Nb cells in vitro with physiologic concentrations of dexamethasone to mimic the environmental milieu of high glucocorticoid levels within the adrenal medulla. We found that treatment of Nb cells with $10^{-7}$M dexamethasone resulted in a tenfold increase in the expression of chromogranin A. Similar treatment of a human breast cancer cell line known to have glucocorticoid receptors failed to give any detectable expression of chromogranin A. This suggests that high local glucocorticoid concentrations in the adrenal medulla influence differentiation of primitive neural crest cells toward a neuroendocrine phenotype by increasing the expression of genes which mark this phenotype.

**A 128** HORMONAL REGULATION OF INSULIN-LIKE GROWTH FACTOR I (IGF-I) SECRETION FROM MCF-7 HUMAN BREAST CANCER CELLS. K.K Huff, C. Knabbe, D. Kaufman, M.E. Lippman, R.B. Dickson. Medicine Branch, NCI, NIH, Bethesda, MD 20892.
We have previously reported that human breast cancer (BC) cell lines secrete immunoreactive IGF-I (IR-IGF-I) into serum-free conditioned media (CM)(anti-IGF-I kindly provided by J. Van Wyk, UNC). In addition, concentrations of authentic IGF-I which stimulate BC cell growth (1-5nM) are easily achievable in CM of BC cells. This has led us to propose that secreted IGF-I may contribute to the proliferation of cultured human BC cells in an autocrine manner. The growth of the MCF-7 BC cells is responsive to insulin, glucocorticoids, estrogens and antiestrogens. To further examine the relationship between BC cell growth and IGF-I secretion we have studied the effects of these hormones on the secretion of IR-IGF-I. We found that insulin (0.1uM) which stimulated the growth by 2 fold at 4d increased IR-IGF-I by 3.7 fold. Dexamethasone (1uM) treatment resulted in the reduction of secreted IGF-I to 10.9% of control within 48h. The antiestrogens tamoxifen (TAM), OH-TAM and LY117018 (LY), which are growth inhibitory by 4d, all inhibited secretion of IGF-I with potencies of LY>OH-TAM>TAM. In phenol red-free medium, $E_2$ treatment increased IGF-I secretion dramatically. After 24h of $E_2$ (1nM) the rate of IR-IGF-I secreted (as ng IGF-I/mg DNA/hr) was increased 2 fold. By 72h the rate of IR-IGF-I produced was 3 fold higher than controls. We conclude that growth modulating hormones can regulate the quantity of IGF-I secreted from MCF-7 human BC cells and the changes in rates of IGF-I secretion precede the growth effects attributed to these hormones.

**A 129** NEURON LOCALIZATION AND NEUROBLASTOMA EXPRESSION OF BRAIN-DERIVED GROWTH FACTOR, Jung San Huang*, Cheng C. Tsai*, Steven P. Adams**, and Shuan Shian Huang*, *Departments of Biochemistry and Pathology, St. Louis University School of Medicine, St. Louis, MO 63104, **Biological Sciences Department, Monsanto Company, St. Louis, MO 63198.
Bovine brain-derived growth factor (BDGF) is a ∿16-17 kDa polypeptide mitogen with a broad spectrum of cell specificity. BDGF appears to be a potent chemotactic factor for fibroblasts and astroglial cells. The chemical and physical properties of BDGF resemble those reported for endothelial cell growth factor, brain-derived acidic fibroblast growth factor, and heparin-binding growth factor α. BDGF differs from basic fibroblast growth factor (FGF) in chemical and physical properties, although both BDGF and basic FGF interact with the same receptor on the cell surface of responsive cells. To investigate the physiological role of BDGF, a polyclonal antibody to BDGF was raised. Specificity of the antibody was demonstrated by its reaction with BDGF in immunoblot analysis, its ability to block the mitogenic activity of BDGF, and the ability of excess purified BDGF to block the immunofluorescent staining. Using this highly specific mouse polyclonal anti-BDGF antibody for direct immunofluorescent staining, BDGF was found to be specifically localized in the cytoplasm of neurons of bovine and human brain, human spinal cord, and myenteric plexus of human intestine. Human neuroblastoma cells also showed cytoplasmic staining with anti-BDGF antibody, whereas other cell types did not. The cell lysates of neuroblastoma cells elicited a BDGF-like activity. Furthermore, this BDGF-like factor was identified as a 17 kDa protein following immunoprecipitation of $^{35}$S-methionine labeled neuroblastoma cell lysates with anti-BDGF antibody.

**A 130** IMMUNOREGULATORY FACTOR PRODUCTION BY K-562, A HUMAN PLURIPOTENT LEUKEMIA CELL LINE, Albert T. Ichiki, Joe T. Crossno, Jr., and Wahid T. Hanna, University of Tennessee Memorial Research Center and Hospital, Knoxville, TN 37920

Conditioned media (CM) from sublines of the K-562 human pluripotent leukemia cell line were tested to determine whether they included factors which could affect the response of human T lymphocytes to phytohemagglutinin (PHA) stimulation. Confirming observations of others, the CM depressed the response of lymphocytes from healthy individuals. In contrast, K-562 cells produced a factor which enhanced the response of lymphocytes from patients with acquired immunodeficiency syndrome (AIDS), AIDS-related complex, and hemophilia patients considered to be severely immunodeficient. There was more radiolabeled thymidine incorporation when the PBL were incubated with K-562 CM as compared to control cultures and cpm decreased but was still greater than the control with increasing dilutions of the CM. The cells produce more factors when they are cultured for a longer time interval. The factors appear to be present in cultures from 4 different sublines tested. K-562 CM was precipitated with 80% ammonium sulfate and the precipitate was suspended in buffer, and then subjected to molecular sieving on an ACA44 column. The fractions were assayed for immunoregulating activity with PBL from 13 subjects. The results indicated that it was possible to separate an enhancing factor with very high molecular weight from a lower molecular weight suppressing factor. There is evidence for a third factor with low molecular weight which has both activities. The immunopotentiation of PBL from immunodeficient patients indicates that immunodeficiency may not only involve lymphokines produced by stimulated T lymphocytes, i.e., interleukin 2 or interferon-$\gamma$, but may involve factors produced by hematopoietic progenitor cells.

**A 131** DILINOLEOYL PHOSPHATIDIC ACID STIMULATES THE GROWTH OF NORMAL MOUSE MAMMARY EPITHELIAL CELLS IN PRIMARY CELL CULTURE, Walter Imagawa, Gautam Bandyopadhyay and Satyabrata Nandi, Cancer Research Laboratory, University of California, Berkeley, CA 94720

Epithelial cell clumps obtained by collagenase digestion of mammary glands from virgin BALB/c mice were embedded within collagen gels and cultured for 10 days in basal medium (F12:DME (1:1), insulin (10 µg/ml)) to which were added lipids or growth factors. Synthetic phospholipids (PL) were added as liposomes. Dilinoleoyl phosphatidic acid (PA) or epidermal growth factor (EGF) alone stimulated multifold growth. Replacement of linoleate in PA with palmitate and/or oleate greatly reduced its growth-stimulatory effect. Dilinoleoyl phosphatidylcholine alone or combined with dilinoleoyl phosphatidylethanolamine was only slightly growth-stimulatory. Free linoleate stimulated growth only in the presence of EGF. At supoptimal concentrations, dilinoleoyl PA stimulated growth in synergism with EGF. It also stimulated growth in synergism with the phosphodiesterase inhibitor, isobutylmethylxanthine or exogenously added cyclic AMP. These results suggest that PA is a growth factor for mammary epithelial cells and may thus serve as an important intracellular intermediate in growth factor or hormone-mediated growth. The ability of exogenous dilinoleoyl PA to stimulate growth is dependent upon its fatty acyl composition and deacylation to provide linoleate for further metabolism to arachidonate and prostaglandins may contribute to its growth-stimulatory effect.

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**A 132** PURIFICATION AND CHARACTERIZATION OF THREE TYPES OF PROTEIN KINASE C FROM RABBIT BRAIN CYTOSOL, Susan Jaken and Susan C. Kiley, W. Alton Jones Cell Science Center, Lake Placid NY 12946.

Three types of protein kinase C (PKC) have been resolved by hydroxylapatite chromatography. Each enzyme has been purified to near homogeneity by phosphatidylserine (PS) affinity chromatography. All three types of PKC have apparent molecular weights of ~80 kD on SDS-PAGE. Polyclonal antibodies prepared to Type 3 recognize < 1 pmol of Type 3, and react very poorly with 10 pmol of Types 1 and 2. Similarly, polyclonal antibodies to Type 2 are relatively specific for Type 2. All three enzymes are high affinity phorbol ester receptors as measured with [$^3$H]phorbol dibutyrate (PDBu). $Ca^{2+}$ increases PDBu receptor affinity for all three PKCs. In addition, $Ca^{2+}$ increases total PDBu binding to Type 2 but not to Types 1 and 3. Type 2 has a higher $Ca^{2+}$ requirement for increased PDBu binding than Types 1 and 3. With respect to kinase activity, Type 1 is least dependent on $Ca^{2+}$ and PDBu for maximum activity; Type 3 is most dependent. In all three cases, double reciprocal plots of the PS required for PDBu binding were not simple and extrapolated to ~25,000 pmol [$^3$H]PDBu bound/ mg, indicative of 2:1 stoichiometry. Hill plots had slopes ~2. The identification of three apparently distinct proteins with PKC and phorbol ester binding activities correlates with the recent identification of PKC as a gene family. Further characterization of these proteins will help identify functional aspects of primary structures and provide a basis for determining how different types of PKC may be linked to different cell stimuli and different biological responses.

**A 133** THE erbB-2 GENE IS FREQUENTLY OVEREXPRESSED IN HUMAN MAMMARY TUMOR CELL LINES, C. Richter King, Matthias Kraus, and Stuart A. Aaronson, National Cancer Institute, Bethesda, MD 20892.
A second cellular homologue of the v-erbB oncogene has been identified in the human genome. The coding sequences of this gene, c-erb, indicate considerable homology to the EGF receptor. This suggests that the product of the c-erbB-2 gene may be a growth factor receptor. We originally identified the erbB-2 gene as amplified in a human mammary carcinoma. Current studies indicate that overexpression of c-erbB-2 mRNA occurs frequently in human mammary tumor cell lines and that at least two molecular mechanisms are associated with this overexpression.

**A 134** UROKINASE: A SERINE PROTEASE WHICH CAN FUNCTION AS A GROWTH FACTOR J.C. Kirchheimer, J. Wojta, G. Christ, B.R. Binder, Lab. Clin.Exptl. Physiol., University of Vienna, Austria

Malignant cells secrete urokinase type plasminogen activator, a trypsin-like serine protease to a much higher extent as the respective normal counterpart. Tumor spread and metastasation have been attributed to urokinase induced cleavage of proteins of the extracellular matrix but it is still not proven if tumor urokinase contributes to the malignant process. Urokinase plasminogen activator contains, however, not only an active site domain consistent with its serine protease character which is involved in the activation of plasminogen but also a kringle domain and domains which are homologous with the epidermal growth factor. In the present study it could be shown that the human epidermal tumor cell lines (CCL 20.2) which does not produce urokinase but possesses high affinity ($K_D$ 4.68 times $10^{-14}$M) urokinase receptors (8.5 x $10^4$ per cell) responds in vitro to exogenously added urokinase (10ng/ml) with an increased rate of $^3$H-thymidine incorporation in a dose-dependent fashion. Binding of urokinase to CCL 20.2 cells was found to be dependent on the 17,000 Dalton fragment containing the epidermal growth factor domain explaining why active as well as DFP-inactivated urokinase becomes bound. The stimulatory effect of cell proliferation, however, requires the complete molecule also containing the active site. Beside the speculation about the concentration of proteolytic enzymes on the surface of cells it is the first known biological effect mediated by specific adsorbation of the urokinase receptor.

**A 135** PHOSPHOPROTEIN CHANGES ASSOCIATED WITH GROWTH AND DIFFERENTIATION IN RETROVIRUS TRANSFORMED MOUSE EPIDERMAL KERATINOCYTE CELL LINES. Jayne K. Klinger and Bernard E. Weissman, Childrens Hospital of Los Angeles, Los Angeles, CA 90027.
Balb/MK-2 mouse epidermal keratinocytes require epidermal growth factor (EGF) for continued proliferation and terminally differentiate in response to increased extracellular $Ca^{++}$ concentration. The EGF requirement in these cells can be obviated by infection with acute transformation retroviruses with a concomitant alteration in $Ca^{++}$ induced terminal differentiation. Only partial differentiation can be induced in the virally transformed keratinocytes, the degree of which is dependent on the infecting virus. In general, retroviruses whose onc genes are members of the src gene family inhibit differentiation at an earlier step than members of the ras gene family implying a role for protein phosphorylation in the control of differentiation in epidermal keratinocytes. We therefore compared the phosphoproteins expressed in these cell lines by two-dimensional gel electrophoresis. Comparison of Balb/MK-2 and virally transformed Balb/MK-2 proteins under undifferentiated and differentiated conditions showed alterations in proteins at 36-38K, 29K and 48K relative molecular weight. Because terminal differentiation in the virally infected cells is blocked, proteins induced with $Ca^{++}$ in these cells should represent preliminary events in the differentiation pathway. In addition to differentiation specific proteins, other cellular proteins showed altered expression in virally-transformed cells but still responded to increased levels of extracellular $CA^{++}$. Thus, there were phosphoproteins which were altered by the differentiation signal alone as well as other proteins which were sensitive to both differentiation and transformation signals.

**A 136** SPECIFIC GROWTH DEREGULATION IN $Na^+:H^+$ ANTIPORT-LESS FIBROBLAST MUTANTS, Alain E. Lagarde and Jacques M. Pouysségur, Mt. Sinai Hospital Research Institute, Toronto, Canada and Centre de Biochimie, Université de Nice, France.

Cytoplasmic pH (pHi) is abnormally regulated in mutants lacking $Na^+:H^+$ antiport activity (PNAS 81: 4833, 1984). This single defect conferred upon cells a reduced ability to form tumors, to grow without anchorage and to respond to EGF in serum-free, CO2 buffered medium. Mutants responded normally to α-thrombin (± insulin) or serum. Tumorigenic potential was restored in secondary mutants selected in vitro for neoplastic attributes, together with EGF responsiveness. Tumor-derived as well as $ras^H$-transfected antiport-less mutants were highly malignant (metastatic) and coincidentally acquired the capacity to escape Go arrest. The studies reinforce the role played by the $Na^+:H^+$ antiporter in the transduction of signals evoked after growth factor stimulation of fibroblasts. They also reveal that autonomous modes of replication, associated with the expression of malignancy, may develop and operate independently from pHi-mediated signals. Finally, they suggest that the antiport molecule is additionally involved at some level of the mitogenic response by EGF.

**A 137** TRANSFORMING GROWTH FACTOR-β INDUCED PLASMINOGEN ACTIVATOR INHIBITOR REGULATES PROTEOLYTIC ACTIVITY OF HT-1080 TUMOR CELLS. Marikki Laiho, Olli Saksela and Jorma Keski-Oja, Department of Virology, University of Helsinki, Helsinki, Finland.

The effects of TGFβ on the proteolytic activity and composition of growth substratum deposited proteins of malignant cells were studied using cultured HT-1080 human fibrosarcoma cells as a model. TGFβ induced the synthesis and growth substratum deposition of a $M_r$ 47.000 protein. The $M_r$ 47.000 protein was an endothelial-type plasminogen activator inhibitor (e-PAI) as shown with monoclonal antibodies. The deposition occured rapidly after 4 h incubation of the cultures in the presence of TGFβ. e-PAI was also actively degraded by the cells, and it disseappeared totally from the growth substratum after 24 h incubation of the cell cultures. Also, e-PAI was shown to be sensitive for the degradation by added urokinase (u-PA). From the analysed growth factors (EGF, PDGF and TGFα) TGFβ was the only growth factor capable of inducing e-PAI. The results indicate that TGFβ is the major serum derived growth factor that can reduce the proteolytic activity in the microenvironment of cultured malignant cells by enhancing the pericellular deposition of e-PAI.

**A 138** TRANSFORMATION OF PRIMARY AND IMMORTALIZED CELLS BY HPV-16 AND 18 SEQUENCES, L.A. Laimins, M.A. Bedell, K.H. Jones and J.A. Long, Howard Hughes Medical Institute and Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

Subgenomic fragments containing the early regions of either HPV-16 or 18 were found to transform NIH3T3 cells. Independently these sequences did not transform primary rat embryo fibroblasts, although they did provide an extended life for these cells in vitro. However, the addition of an immortalizing gene such as Ad E1a or v-myc to primary cell transfections did allow for transformation. Cell lines which carried both HPV sequences and known immortalizing genes grew to high densities and exhibited anchorage independent growth. These results suggest that activation of a cellular gene may be required in addition to the presence of HPV-16 and 18 sequences for transformation of primary cells.

**A 139** SERTOLI CELL SECRETED GROWTH FACTOR DECREASES $^{3}$H-THYMIDINE INCORPORATION INTO CELLS. D.J. Lamb*, and G. Spotts, Baylor College of Medicine, Scott Department of Urology and Department Cell Biology, Houston, TX 77030.

Sertoli cell secreted growth factor (SCSGF) is a potent mitogen which stimulates the proliferation (7-10 fold) of cell lines of epithelial, mesodermal, and endodermal origin. SCSGF obtained from conditioned medium from Sertoli cells cultured from 35d rat testis (SCCM) was tested for its ability to stimulate cellular proliferation and $^{3}$H-thymidine ($^{3}$H-Thy) incorporation. SCCM greatly decreased the incorporation of $^{3}$H-Thy into A431 cells, Swiss 3T3 cells, and the $TM_4$ testicular cell line. The inhibition was dose-dependent with a maximal effect at 100% SCCM resulting in 85% less $^{3}$H-Thy incorporation into 3T3 cells as compared with controls. The incorporation of $^{3}$H-Thy into confluent 3T3 cells ($67.8 \pm 2.8 \times 10^{3}$ DPM) was stimulated by 1 ng/ml epidermal growth factor (EGF, $126 \pm 5.1 \times 10^{3}$ DPM) whereas 50% SCCM dramatically inhibited this EGF-induced incorporation ($47.2 \pm 3.2 \times 10^{3}$ DPM). In a parallel study of cell growth and $^{3}$H-Thy incorporation, 33% SCCM stimulated A431 cell growth 2-fold, however, the $^{3}$H-Thy/cell was decreased to 48% of control values. Thus, when cell number was increased by SCSGF, the incorporation of $^{3}$H-Thy did not directly correlate with the observed proliferation. SCSGF, the only known mitogen for A431 cells, has unique characteristics when compared with other known growth factors. These results further suggest that the $^{3}$H-Thy incorporation assay may not always accurately reflect the growth promoting activity of a mitogen.

**A 140** THE EFFECT OF BOMBESIN ANTAGONISTS ON THE GROWTH OF SMALL CELL LUNG CANCER (SCLC). Judith E. Layton, Denis Scanlon and George Morstyn. Ludwig Institute for Cancer Research, Melbourne Tumour Biology Branch, Australia 3050.

Bombesin/gastrin-releasing peptide is a neuropeptide that stimulates the proliferation of normal bronchial epithelial cells and murine Swiss 3T3 fibroblasts. SCLC cell lines (CL) produce bombesin and some may be dependent on bombesin for growth because their growth is inhibited by a monoclonal anti-bombesin antibody. Several analogues of substance P have been described that act as bombesin antagonists and may therefore be useful in the treatment of patients with SCLC. We have tested 6 SCLC-CL in a liquid culture proliferation assay and have found no effect of bombesin ($10^{-10}$ - $10^{-5}$M) on proliferation. The antagonists (spantide and [D-$Arg^{1}$, D-$Pro^{2}$, D-$Trp^{7,9}$, $Leu^{11}$] - substance P) inhibited the proliferation of SCLC-CL. Inhibition was not reversed by excess bombesin and thus appeared to be non-specific. On the other hand bombesin did stimulate Swiss 3T3 cell proliferation in the same assay and the stimulation was partially inhibited by both antagonists. This inhibition was reversed by excess bombesin. The antagonists weakly stimulated proliferation when added alone. Since substance P also caused proliferation of Swiss 3T3 cells, the weak agonist activity of the antagonists may be mediated by a receptor separate from the bombesin receptor. We conclude: i. bombesin antagonists are weak agonists for cell proliferation; ii. they specifically inhibit bombesin stimulated proliferation; iii. bombesin does not stimulate all SCLC to proliferate and so antagonists will not prove useful for therapy in every case.

**A 141** MODE OF ESTROGEN ACTION ON CELL PROLIFERATIVE KINETICS IN BREAST CANCER, Benjamin S. Leung, University of Minnesota, Minneapolis, Minnesota 55455

It is known that estrogen is a potent mitogen in breast cancer. However, the biochemical events leading to estrogen-induced cell proliferation have not been elucidated. Previous studies have shown that estrogen alone, in the absence of serum, is unable to induce cell proliferation. The present study aims to investigate the effect of estrogen and serum on cell proliferative kinetics in order to determine precisely the period of the cell cycle where estrogen exerts it's effect. CAMA-1 cells were synchronized at early G1, G1/S, and G2/M phases by 48 hour serum deprivation, 3 mM thymidine block for 40 hours and nacodazole arrest for 16 hours, respectively. The S-phase formation was determined by a 2 hour thymidine labeling. With each synchronized population, only the very early G1 phase was found sensitive to estrogen stimulation and antiestrogen inhibition in respect to 3H-thymidine uptake, mitotic index and cell number. Estrogen and serum were required to promote the G1 phase cells to traverse S phase, that is, a shorter G1 phase duration and a higher proportion of G1 cells per unit time entering into S-phase, contributing to both a shorter generation time and more mitotic cells per cycle. Tamoxifen or progesterone inhibited this process of G1/S transition. These results provide a model for further investigation of estrogen-regulated events leading to G1/S transition.

**A 142** PHOSPHOLIPASE-C ACTIVITY IN MAMMARY TUMOR MEMBRANES: MODULATION BY GTP AND GnRH, Joseph Levy, Zvia Zadok, Yoav Sharoni, Endocrine Laboratory, Clinical Biochemistry Unit, Soroka University Hospital and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

The hydrolysis of phosphatidyl inositol 4,5 biphosphate (PI 4,5-P2) by phospholipase-C to the intracellular messengers, diacylglycerol and inositol trisphophate, appears to be one to the most common mechanisms for the transduction of hormonal signals over the plasma membrane. The activity of phospholipase-C was measured in subcellular fractions from DMBA-induced rat mammary tumors with [$\gamma$-$^{32}$P]PI 4-P and [$\gamma$-$^{32}$P]PI 4,5-P2 as substrates. The water soluble products of the enzymatic reactions were separated by anion exchange chromatography. The enzymatic activity which hydrolizes PI 4-P is located mainly in the cytosolic fraction of the tumors and was not affected by 2,3 diphosphoglycerate and pyrophosphate nor by GTP or its unhydrolysable analog, GTP$\gamma$S. The activity responsible for the hydrolysis of PI 4,5 was present in the cytosols and membranes and was stimulated in a dose-dependent manner by these compounds. Although high activity was present without any hormome, GnRH stimulated membranal PI 4,5-P2 hydrolysos. This effect was enhanced by GTP and by GTP$\gamma$S. These results suggest that the mechanism for the signal transduction of GnRH in mammary tumors is similar to that found in pituitary cells.

**A 143** THE PHOSPHOTYROSINE CONTENT OF A 100 kDa PROTEIN IS INCREASED IN INSULIN-TREATED 3T3-L1 CELLS, M.E. Linder*, J.S. Gregory+, K.L. Latham*, T.B. Gray+, J.G. Burr* and M.H. Cobb+, *Biology Programs, The Univ. of Texas at Dallas, Richardson, TX 75083-0688 and +Dept. of Pharmacology, The Univ. of Texas Health Science Center, Dallas, TX 75236.

Antibodies against phosphotyrosine have proven useful in identifying protein-tyrosine kinases and their substrates. We have prepared affinity-purified antibodies against azobenzene phosphonate (ABP), a phosphotyrosine analog, and used them in Western blots to examine 3T3-L1 cells for proteins that exhibit increased phosphorylation on tyrosine in response to insulin treatment. The antibodies recognize insulin and epidermal growth factor receptors phosphorylated in vitro. In soluble fractions from insulin-treated and untreated 3T3-L1 cells, there are no differences in tyrosine phosphoproteins detected by immunoblotting of one-dimensional SDS-polyacrylamide (PA) gels. Upon subsequent purification of soluble protein by DEAE-cellulose chromatography, immunoblotting of the fractions from insulin-treated cells reveals a tyrosine phosphoprotein that migrates in SDS-PA gels as approximately 100 kDa. The phosphotyrosine blotting signal from this region of the SDS-PA gel is either absent or found at much lower intensity in the immunoblots of the corresponding fractions from control cells. The phosphotyrosine signal can be detected after a two-step purification, i.e., DEAE-cellulose chromatography followed by phosphocellulose chromatography, exhibiting the same insulin-sensitivity. The 100 kDa protein elutes from both DEAE-cellulose and phosphocellulose near an insulin-stimulated protein-serine kinase activity. The 100 kDa protein appears to be a substrate in vivo for the insulin receptor tyrosine kinase.

**A 144** INDUCTION OF EGF AND TGFα GENE TRANSCRIPTION AND SECRETION OF EGF COMPETITIVE ACTIVITY IN ACTIVATED HUMAN ALVEOLAR MACROPHAGES, D.K. Madtes[1], E.W. Raines[1], K. Sakariassen[1], R.K. Assoian[2], M.B. Sporn[2], G.I. Bell[3], R. Ross[1], University of Washington, Seattle, WA 98195[1], National Cancer Institute, Bethesda, MD 20892[2], University of Chicago, Chicago, IL 60637[3].

The macrophage, as a secretory effector cell, is capable of releasing several growth factors, including PDGFc and IL-1. We present evidence that activated human alveolar macrophages transcribe the genes for epidermal growth factor (EGF) and transforming growth factor-alpha (TGFα) and secrete mitogens which compete with $^{125}$I-EGF for binding to the EGF receptor on A431 cells. This conditioned medium also promotes anchorage independent growth and colony formation of normal rat kidney cells in the presence of a constant amount of transforming growth factor-beta. Fractionation of conditioned medium by gel filtration chromatography in 1N acetic acid demonstrates the presence of three different-sized fractions of EGF/TGFα competitive activity of apparent molecular weights ≈ 30,000, 13,000, and 6,000. The low molecular fraction elutes at a position identical to that of mouse $^{125}$I-EGF. The EGF receptor binding activity of this low molecular weight component cannot be inhibited by monospecific polyclonal antibodies to human EGF or TGFα, respectively. Analysis using cDNA probes to hEGF and TGFα demonstrates that activation of macrophages with LPS results in increased transcription of the messages for both hEGF and TGFα. Thus activation of alveolar macrophages induces transcription of the genes for EGF and TGFα and also stimulates secretion of EGF/TGFα-competitive activity. The lowest molecular weight form of the EGF competitive activity secreted by activated macrophages, although similar in molecular weight to EGF and TGFα, appears antigenically distinct from both of these molecules based upon antibodies presently available for each of these factors.

## A 145 PLATELET-DERIVED GROWTH FACTOR (PDGF) INDUCES ANCHORAGE INDEPENDENT (AI) GROWTH OF HUMAN FIBROBLASTS (HF)

V.M. Maher, H. Palmer and J.J. McCormick, Carcinogenesis Laboratory - Fee Hall, Michigan State University and the Department of Food Science and Human Nutrition, Michigan State University, East Lansing MI 48824

Human tumor-derived cells and cells exposed to carcinogen treatment are capable of forming colonies in semi-solid medium. Growth in semi-solid medium (AI growth) is considered a marker of cellular transformation. Normal HF do not form colonies in semi-solid medium. The cells can grow in AI conditions transiently when the concentration of fetal bovine serum (FBS) in the medium is increased. This suggests that some growth factor(s) present in the FBS is responsible for AI growth. We have investigated the ability of epidermal growth factor (EGF), PDGF, and/or $\beta$-transforming growth factor ($\beta$-TGF) to support AI growth of HE. The base medium was a modified form of MCDB 110 supplemented with 9% FBS which had had its protein growth factors inactivated by dithiothreitol and iodoacetamide. The media also contained 0.5% bovine serum albumin, a lipid supplement, insulin (1 ug/ml) and ferrous sulfate (1 ug/ml). Growth factors were added to this media and tested for the ability to support AI growth.

Of the growth factors tested, PDGF was the best inducer of AI growth of normal HF. This work suggests that cellular transformation reduces or eliminates the need for exogenous PDGF. (Supported by NIH/NCI grant CA 21289)

## A 145A TWO INDEPENDENT ATP-DEPENDENT REACTIONS ARE CATALYZED BY PURIFIED HUMAN A431 CELL EPIDERMAL GROWTH FACTOR RECEPTOR.

C. Fred Fox, Shawn P. Fay and David D.-L. Woo. Molecular Biology Institute and Department of Microbiology, UCLA, Los Angeles, CA 90024. Purified EGF receptor catalyzed two independent ATP-dependent receptor phorphorylation reactions. At 0C and optimal receptor concentration (6.5nM and 0.26pmol/reaction), one process had high ATP affinity (Km=0.6μM) and low specific activity (Vmax=0.03pmol/min); the second process had low ATP affinity (Km=15μM) and high specific activity (Vmax=4.1pmol/min). Hill plots for reactions where ATP was varied had slopes of 1.0 at ATP <0.38μM or >0.67μM, but indicated positive cooperativity between these concentrations. High (Km<0.5μM) and low (Km>100μM) affinity reactions for ATP were also observed for phosphorylation of a model tridecapeptide substrate, and 6.5nM receptor supported Vmax values at 30C (expressed as turnover numbers) of 0.8 and 740 $min^{-1}$ respectively. Hill plots of these data had slopes of 1.0 at ATP <0.02 or >2.5μM, and indicated cooperativity between these concentrations. Prior incubation of receptor with ATP to facilitate self-phosphorylation prior to substrate addition had no quantitative or qualitative effect on the area of positive cooperativity. These data do not support an obligatory role for intramolecular receptor phosphorylation in activating receptor for optimal substrate phosphorylation. Intramolecular phosphorylation event must therefore play some other role in EGF receptor functioning, e.g. the initiation of sequestering, leading to internalization. When receptor concentration was decreased from 6.5 to 0.2 nM, the range of ATP concentration over which the high affinity, low specific activity substrate phosphorylation process was observed was increased from <0.02 to <0.2 μM. These data support a model in which both ATP and receptor must act cooperatively to achieve optimal substrate phosphorylation. This caused us to examine in greater detail the requirement of high receptor concentration for optimal activity (see companion Abstract by Fay, *et al.*). Supported by USPHS grant AM-25826. SPF is supported by USPHS, NIRSA HL-07386.

## A 145B THE SPECIFIC ACTIVITY OF EGF RECEPTOR CATALYZED SUBSTRATE PHOSPHORYLATION IS REGULATED BY EGF RECEPTOR CONCENTRATION.

Shawn P. Fay and C. Fred Fox. Molecular Biology Institute and Department of Microbiology, UCLA, Los Angeles, CA 90024. Log-Log plots of EGF receptor (EGF-R) phosphorylation vs. EGF-R concentration reveal a unimolecular process at low, and a trimolecular process at high receptor concentration. Hill plots of these data yielded slopes of 1.0 and 1.8 respectively. Log-log plots of EGF-R vs. tridecapeptide substrate (tdpS) phosphorylation revealed a unimolecular process at 0.3μM ATP and a bimolecular process at 10μM ATP and high EGF-R. Hill plots of these data yielded slopes of 1.0 and 1.9 respectively. In the absence of EGF, the specific activity of tdpS phosphorylation was increased over 400-fold with increasing EGF-R concentration, but only by 3-fold more when EGF was added at optimal EGF-R. At suboptimal EGF-R, EGF activated tdpS phosphorylation specific activity by greater than 30-fold. Optimal tdpS phosphorylation required maintenance of high EGF-R. When EGF-R functioning at optimal concentration was diluted below the range where it acted in a bimolecular process, activity decayed rapidly, ceasing before 10 min post-dilution. At low EGF-R, high ATP and low tdpS, tdpS acted both as substrate and activator (Hill plot slope=1.6), and at low ATP, as substrate only. Higher concentrations of tdpS were inhibitory at low or high ATP. These data support a model in which EGF-R is characterized by both a substrate binding and regulatory site on EGF-R. At low ATP, EGF-R catalyzes substrate phosphorylation by a unimolecular process at low rate. At high ATP and low EGF-R, EGF-R can be activated further by binding of a model substrate to the regulatory site. At high ATP and high EGF-R, EGF-R acts both as activator and enzyme in high specific activity substrate phosphorylation. In this latter process, EGF-R acts cooperatively, and EGF-R concentration is the principal activator of substrate phosphorylation which is largely EGF-independent. Taken collectively, these data support the view that receptor clustering following EGF addition to cells is the principal event in activating EGF-R. EGF has little effect once EGF-R has been incorporated into vesicles to assume a consitutively activated state. Supported by USPHS grant AM-25826. SPF is supported by USPHS, NIRSA HL-07386.

# Poster Session II

**A 146** A TYROSINE KINASE RELATED TO $pp60^{c\text{-}src}$ IS ABUNDANT IN THE ELECTRIC ORGAN OF THE ELECTRIC EEL, P. Maness, University of North Carolina, Chapel Hill NC 27514.

A tyrosine-specific protein kinase structurally and functionally related to $pp60^{c\text{-}src}$ was abundant in acetylcholine receptor-rich membranes purified from the electric organ of *Electrophorus electricus*, the electric eel. The protein kinase was immunoprecipitated from electric organ extracts with a TBR-antiserum that recognizes $pp60^{c\text{-}src}$ in chick neutral retina. The eel src-related kinase phosphorylated TBR-IgG heavy chains at tyrosine residues in immune complexes. The amount of kinase in electric organ was 10-fold greater than in skeletal muscle and comparable to levels found in nervous tissues. Fractionation of electric organ showed that the $pp60^{c\text{-}src}$-related kinase was associated with membranes rich in the acetylcholine receptor, identified by $^{125}$I-α-bungarotoxin binding. Kinase activity was solubilized from electric organ membranes in detergent, and was fractionated according to our reported protocol for purification of the viral transforming protein $pp60^{v\text{-}src}$ (Maness and Levy, Mol. Cell. Biol. 3 (1983) 102-112. At each step, the src-related kinase from electric organ exhibited chromatographic properties similar to $pp60^{v\text{-}src}$.

Incubation of acetylcholine receptor-rich membranes with $\gamma$-$^{32}$P-ATP revealed 3 major phosphotyrosine-containing proteins with relative molecular masses of 63K, 57K, and 45K. TBR antiserum immunoprecipitated only the 57K phosphoprotein. Phosphorylation of the 63K and 45K proteins was blocked by TBR antiserum, suggesting that they were substrates of the electric organ kinase. Two dimensional gel electrophoresis showed that the 63K and 45K proteins were also phosphorylated *in vivo* during incubation of electric organ slices with $^{32}$P.

**A 147** CONTROL OF EXPRESSION OF THE C-MYC AND GRP GENES IN HUMAN SMALL CELL LUNG CANCER CELL LINES, Sanford Markowitz and James Battey, NCI-Navy Medical Oncology Branch, Bethesda Naval Hospital, Bethesda, MD 20814. Cell lines established from human small cell lung carcinoma tissue (sclc) commonly show activation of the gene encoding the peptide mitogen gastrin releasing peptide (grp). We have established in sclc line H209 the initiation site of the grp message and the presence of a TATA box and GC rich region upstream of the initiation site. We find no evidence for gene rearrangement within 10kb of the promoter. We have characterized the patterns of DNA methylation and of DNAse hypersensitive sites in the region of the grp promoter. Comparing various grp producing and non producing sclc lines we find no requirement for a specific methylation state of the DNA in grp expressors. We have established conditions for transfecting constructs expressing the bacterial chloramphenical acetyl transferase gene (CAT) under the control of grp sequences and are attempting to define enhancer like structures. Small cell lines also show a variety of patterns of expression of the c-myc gene, including nonexpression, expression of single copy c-myc and expression associated with amplification of the c-myc gene. We have exmanined the activity of CAT fusion constructs including varying lengths of 5' flank and first exon from c-myc when transfected into cell lines from each of the above classes. We find cells of all three types acquire the same level of CAT activity when transfected with the same myc-CAT fusion construct extending 300 bases 5' and into the first exon 3'. We conclude differential regulation of the myc gene in SCLC occurs by mechanisms that do not involve this sequence.

**A 148** HEPATOCYTE NUCLEAR BINDING OF $^{125}$I-EPIDERMAL GROWTH FACTOR (EGF)
Uli Marti, S.J. Burwen, M.E. Barker, A.M. Feren, S. Huling and A.L. Jones Cell Biology, VAMC and Depts. of Anatomy & Medicine and the Intestinal Immunology Research and Liver Centers, University of California, San Francisco During the pre-S phase of liver regeneration, endocytosed EGF is diverted from its normal degradation pathway and to the hepatocyte nuclei. Analysis of this nuclear-associated EGF by SDS-PAGE and autoradiography indicated it consisted largely of "free" EGF as well as EGF bound to a ~180 kd protein. To further characterize the nuclear binding of EGF, purified hepatocyte nuclei, from 8 hr post-hepatectomized and control rats, were incubated with $^{125}$I-EGF and subjected to SDS-PAGE and autoradiography. Both showed two autoradiographic bands: one at <10 kd representing free EGF and the other at 180 kd. In addition western blots of nuclear proteins from 8 hr post-hepatectomized and control rats showed crossreactivity of the high molecular weight bands with a monoclonal antibody to the cytoplasmic domain of the EGF-receptor. These data demonstrate for the first time that rat liver nuclei contain an EGF binding protein which has a molecular weight similar to that of the plasma membrane EGF receptor and crossreactivity with an anti-EGF-receptor monoclonal antibody. (antibody courtesy of Dr. Allen Wells)

**A 149** QUANTITATION OF PROTEIN KINASE C ACTIVITY FROM LYMPHOCYTES AFTER STIMULATION BY COMPLETE AND INCOMPLETE MITOGENS. A.M. Mastro and D.S. Grove. The Pennsylvania State University, University Park, PA 16802.

Protein Kinase C activity has been reported to increase in the membrane fraction after treatment of various cells with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Because TPA is comitogenic for lymphocytes, PKC, a TPA binding receptor, may have a major role in mediating this event. We have examined changes in PKC activity upon treatment of lymphocytes with TPA, or the mitogenic lectin Con A by using an *in situ* gel assay described previously (J.Leuk.Biol. 38:92,1985). We used a differential extraction procedure to follow translocation of PKC activity from the cytosol to the membrane. The preparations were electrophoresed, phosphorylated *in situ* in the gel, and the activity quantitated. We have found that treatment of lymphocytes with TPA or Con A caused an increase in PKC activity of 3 to 4-fold in the membrane fraction within 5'. There was a concurrent loss of PKC activity of 30 to 50% in the cytosol. PKC was activated within one minute with Con A but there was a protracted activation from 1 to 5 min with TPA. Treatment of lymphocytes with both simulaneously showed an activation pattern similar to that obtained with Con A alone. After macrophages were removed from the lymphocyte preparations there was still an activation of PKC with TPA. However, Con A which exerts its mitogenic effect through macrophages no longer activated PKC. A23187 another incomplete mitogen also increased PKC activity in the membrane 2 to 3-fold similarly to TPA. (Supported by PHS grant CA39891 awarded by the NCI, DHHS. A.M. Mastro has a RCDA CA00705 from the same institute.)

**A 150** CYTOTOXIC EFFECT OF THE CONJUGATE OF ANTI-EGF RECEPTOR MONOCLONAL ANTIBODY TO RICIN A CHAIN ON HUMAN TUMOR CELLS, Hideo Masui, Heidi Kamrath, Gerald Apell*, Lou L. Houston*, and John Mendelsohn, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, *Cetus Corp., Emeryville CA 94608.

Anti-EGF receptor monoclonal antibody (mAb) 528 IgG binds to EGF receptors on human cells, competes with EGF binding, and activates down-regulation and internalization of receptors. 10 mM mAb is cytostatic against cultured A431 cells. Treatment with 2 mg of this mAb twice weekly prevents growth of A431 cell xenografts in athymic mice. In the search to improve the antitumor effect of the anti-EGF receptor mAb, we linked 528 IgG to recombinant ricin A chain (rRTA). The proliferation of cultured A431 cells, which express a large number of EGF receptors on the cell surface membrane, was inhibited 50% by 10 pM 528 IgG-rRTA, and the conjugate was cytocidal for most A431 cells at 100 pM. The antitumor effect of 528 IgG-rRTA on A431 cells was magnified by 200 and 30,000 fold over that of 528 IgG and free rRTA respectively, and was neutralized by the addition of 1000-fold excess 528 IgG. Comparison of the cytotoxic effect of 528 IgG-rRTA on several cell lines demonstrated that the degree of in vitro antitumor activity and the amount of protein synthesis inhibition both correlated with the EGF receptor numbers on these cells. Intraperitoneal administration of 20 μg 528 IgG-rRTA twice weekly inhibited the growth of A431 tumor xenografts far more effectively than unconjugated mAb. We conclude that the antitumor effect of 528 IgG-rRTA on various cells correlates with the EGF receptor number on these cells, and that this conjugate may be a useful immunotoxin in *in vivo* therapy for tumor cells with a large number of EGF receptors. (Supported by NCI Grants.)

**A 151** EFFECTS OF MONENSIN ON PDGF-INDUCED SYNTHESIS OF CPI, CPII, AND ACTIN. Nancy Olashaw, Peter Mitchell and W.J. Pledger, Vanderbilt University, Nashville, TN

Platelet-derived growth factor (PDGF) induces the synthesis of several proteins including a 29 kd nuclear protein (cpI), a 35 kd secreted glycoprotein (cpII) and the cytoskeletal component, actin. We have examined the effects of the carboxylic ionophore monensin (MN) on the PDGF-induced synthesis of these proteins. Confluent BALB/c-3T3 cells were pulsed with [$^{35}$S]methionine at various times after exposure to PDGF or PDGF+MN; cell extracts were prepared and analyzed by SDS-PAGE. MN prevented the decline in cpI synthesis normally occurring 3-4 hours after addition of PDGF to cells; in the presence of MN, cpI was produced for at least 10 hr. Although MN partially inhibited protein synthesis, its effect on cpI production was unrelated to this action as low doses of cycloheximide did not mimic the effect of MN. In both control and MN-treated cultures, cpII was synthesized for at least 10 hr; however, an increased amount of cpII was present in extracts prepared from cells receiving both PDGF and MN. Because MN has been shown to inhibit vesicular traffic through the Golgi, the increase in cell-associated cpII most likely reflects decreased secretion. The synthesis of actin, both in the presence and absence of PDGF, was decreased by MN. Methylamine ($MeNH_2$) also reduced actin synthesis; this lysomotropic amine, however, did not affect the production of cpI or cpII. Thus, the action of MN on cpI and cpII may involve changes in protein trafficking, a process unaffected by $MeNH_2$, whereas its effect on actin may entail events common to both agents, e.g., cytosolic alkalinization. Whether MN and $MeNH_2$ also decrease actin mRNA is, at present, unknown, as is the mechanism by which MN prolongs the production of the nuclear protein, cpI.

**A 152** IGF-1/GROWTH INHIBITOR IN HUMAN PLASMA. Lawrence S. Phillips, Mushtaq Ahmad, and Steven Goldstein, Department of Medicine, Emory University, Atlanta GA 30303.

Poor growth in diabetes, kidney failure, and glucocorticoid excess occurs despite normal levels of GH and IGF-1. However, IGF activity measured by bioassay is uniformly low, suggesting the presence of an antagonist(s) of IGF action. The inhibitor has been isolated from human plasma Cohn fraction IV-1. Combination of selective extraction, cation exchange on CM-Trisacryl at pH 7.0, anion exchange on DEAE-Sephadex at pH 8.5, and size exclusion HPLC on TSK-2000 at pH 3, yielded purification ~100,000x. Active fractions have apparent pI ~8 and exhibit a common band ~30,000 MW on SDS-PAGE, larger than the IGF-1 carrier protein oligomer; binding of $^{125}$I-IGF-1 was not detected.

The inhibitor has wide antianabolic effects on potential target tissues. With cartilage explants, the inhibitor blunts stimulation by insulin as well as IGF-1; 40 ng produces 70% inhibition of serum-stimulated cartilage sulfate uptake. The inhibitor blunts insulin stimulation of glucose oxidation in adipose tissue, and glycogen formation in muscle. With $BC_3H$-1 myocytes, the inhibitor blunts insulin stimulation of AIB uptake but does not affect glucose transport; insulin binding was unaffected, suggesting post-receptor action. The inhibitor blunts AIB uptake by human fibroblasts, and thymidine uptake by HS0294 melanoma cells; since NRK cells were unaffected, these actions reflect individual cell properties rather than nonspecific toxicity.

CONCLUSIONS: A MW ~30,000 "IGF" inhibitor has been purified ~100,000-fold from human plasma Cohn fraction IV-1. Inhibitor preparations can antagonize both IGF-1 and insulin action, suggesting a role as a broad biological regulator.

**A 153** MEASUREMENTS OF PROTEIN TURNOVER IN PROLIFERATING MYOBLASTS AND DIFFERENTIATED MYOTUBES BY DENSITY LABELING, William H. Phillips, Claire E. Kotts, and Gwen G. Krivi, Department of Biological Sciences, Monsanto Company, Chesterfield, MO 63198

We have recently developed a pulse-chase density labeling scheme to measure the stability of proteins in cultured cells. Proliferating L6 myoblasts were pulse labeled in media supplemented with triple-labeled "heavy" amino acids (13C-15N-2H-substituted), 13C-glucose, and 3H-leucine. Proteins translated during this dense pulse were of heavy buoyant density (1.39 gm/ml) and radioactive. A chase was then initiated by transferring the cells over to an unlabeled media comprised of "light," nonradioactive amino acids. It was demonstrated that all proteins synthesized during the chase were essentially of "light," normal buoyant density (1.33 gm/ml), indicating that the light precursor chase was highly effective at diluting out any remaining dense amino acids that were available for reutilization. Dense proteins persisting during the chase were separated from newly synthesized light proteins on high resolution KSCN/CsSCN equilibrium gradients. By measuring the decrease in the proportion of density labeled proteins during the course of the chase, a rate of protein degradation was obtained. We have determined that protein turnover in the myoblast cells is biphasic. At least one subset of proteins decays with a half-life of approximately 2.4 hr, while another longer-lived subset decays with a half-life of approximately 44.5 hr. We are presently using this methodology to identify any changes in the rate of protein turnover which may occur during the differentiation of rat L6 myoblasts into myotubes and of mouse 3T3-L1 fibroblasts into adipocytes.

**A 154** CHANGES IN COLLAGEN AND DNA FROM NICKEL SUBSULFIDE-INJURED PULMONARY ALVEOLAR MACROPHAGES CULTURED WITH LUNG FIBROBLASTS. J. A. Pickrell, D. J. Good, J. M. Benson, A. L. Brooks, and R. F. Henderson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM. 87185.

To study mechanisms underlying the cellular-molecular bases of collagen regulation in the lung, pulmonary alveolar macrophages (AM) from beagle dogs were incubated with control media, and media containing a known fibrogenic agent, nickel subsulfide, at varied concentrations. When unexposed AM or their products were co-cultured for 3 hours with human lung fibroblasts (F) growing on a collagen gel subphase, there was an approximately 40 % reduction in DNA, indicating some cytotoxicity. Neither collagen synthesis nor digestion of the collagen gel were affected on a per cell basis. Exposures of F to the products of nickel-exposed AM resulted in an increased amount of DNA in the cultures (25 to 120 %) when compared to exposure of F to the products of unexposed AM. The products of nickel-exposed AM also decreased collagen synthesis or digestion of collagen gel on a per cell basis. These data suggest that the products of nickel-exposed AM stimulate proliferation of growing lung fibroblasts. [RESEARCH SUPPORTED BY the U. S. Department of Energy Contract No. DE-AC04-76-EV01013.]

**A 155** DETECTION OF IGF-II RECEPTORS ON PRIMARY WILMS' TUMOUR, Michael N. Pollak[1], Kathy A. Baer[1], and James F. Perdue[2], [1]McGill Cancer Centre and Lady Davis Institute, Jewish General Hospital, 3755 Cote St Catherine Rd, Montreal, PQ, Canada H3T 1E2 and [2]Meloy Research Laboratories, Biotechnology Centre, 4 Research Court, Rockville, Maryland 20850

Highly elevated levels of transcripts of the gene encoding insulin-like growth factor II (IGF-II), a fetal mitogen, have recently been detected [Reeve, A.E., et al, Nature 317:258-260 (1985); Scott, J., et al, Nature 317: 260-262 (1985)] in tissue specimens of Wilms' tumour, an embryonal neoplasm arising in the kidney. We studied the binding of highly purified human IGF-II (Perdue, J., submitted for publication) to a plasma membrane-enriched subcellular fraction obtained from a Wilms' tumour. Specific binding of 3 pmoles IGF-II per milligram membrane protein was shown by Scatchard analysis, and competition studies with insulin and IGF-I showed preferential binding of IGF-II, in keeping with the presence of specific IGF-II receptors. The existence of IGF-II receptors on Wilms' tumour cells provides further evidence consistent with the hypothesis that neoplastic proliferation of this tumour is related to autocrine stimulation by IGF-II.

**A 156** PHOSPHOLIPASE ACTIVATION BY EGF, PROTEIN KINASE C ACTIVATORS AND THE CALCIUM IONOPHORE A23187, Bruce E. Rapuano and Richard S. Bockman, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021

The activation of phospholipase by epidermal growth factor (EGF), activators of protein kinase C and the calcium (Ca) ionophore A23187 was studied in the newly described human tumor cell line (Lu65). Phospholipase activation was followed by measuring the release of $^{3}$H-arachidonic acid from Lu65 cell phospholipids. Lu65 phospholipids, principally PC and PI, were shown to be labeled to equilibrium within 2-4 h of incubation with $^{3}$H-arachidonic acid. EGF (16 uM) and the protein kinase C activators OAG (100 uM), dioctanoyl DG (100uM) and phorbol ester PMA (300 uM) were all found to promote the release of arachidonic acid from prelabeled Lu65 cells in the absence of external Ca (230%, 220%, 210% and 160%, respectively, of control release).

The Ca-ionophore, A23187, was used to distinguish between a receptor-mediated activation of phospholipase and nonspecific activation through elevations of cytoslic calcium. A23187 (2uM) caused a 200% increase in arachidonic acid release from cells incubated in 1mM Ca as compared to cells incubated in the absence (ie. uM levels) of external Ca. Ca alone at 1mM had no effect. The PLA inhibitor p-bromophenacylbromide (50-100uM) caused a 30% inhibition of the arachidonate release promoted by Ca plus A23187. Arachidonate release initiated by EGF and protein kinase C activators were additive but not synergistic with that of A23187. Moreover, the release promoted by OAG, dioctanoyl DG or PMA (EGF was not tested) was not inhibited to any extent by p-bromophenacyl bromide (100uM). In conclusion, phospholipase activation in the human cell Lu65 can proceed via a protein kinase C pathway that is independent of external Ca concentration as well as a PLA-pathway that is Ca-dependent.

**A 157** THE DISTRIBUTION OF IMMUNOREACTIVE MELANOMA GROWTH STIMULATORY ACTIVITY (MGSA) IS NOT RESTRICTED TO MELANOMA TISSUE. A. Richmond, V.A. Varma, R.G.B.Roy, H.G. Thomas, C. Engel. Departments of Medicine and Pathology, V.A. Medical Center and Emory University, Atlanta, GA. 30033.

Melanoma growth stimulatory activity (MGSA) is an acid and heat stable, 16 Kd autostimulatory growth factor which was first isolated from culture medium conditioned by the Hs0294 human melanoma cell line (J. Cell. Physiol., in press). The FB2AH7 monoclonal antibody developed against MGSA preparations from melanoma conditioned medium has been used to screen cultured cells. In these studies the majority of newly established malignant melanoma cultures were FB2AH7 positive. In contrast, newly established, chromosomally normal nevus cultures and human fibroblast cultures were negative. One culture established from a basal cell carcinoma lesion from a patient with basal cell nevus syndrome was also strongly positive. However, utilization of the FB2AH7 monoclonal antibody alone has not been useful diagnostically for screening fixed sectioned tissue, since fixed sectioned benign nevus tissue, hepatic tissue and sarcoid tissue also contains the FB2AH7 antigen, as does the epidermis of the skin. These data suggest that MGSA may be a normal regulator of growth and that the normal microenvironment may regulate both production of MGSA and response to MGSA. We further propose that with tumor progression there is deregulation of either MGSA production or MGSA response, leading to abnormal growth properties. Supported by V.A. Merit Award and NCI grant CA 34590.

**A 158** **ACTIVATION OF INSULIN-LIKE GROWTH FACTOR II TRANSCRIPTION IN PRIMARY HCC FROM WHV CARRIERS.** Xi-xian Fu, S.Y. Su, Young I. Lee and Charles E. Rogler
Department of Medicine, Liver Research Center, Albert Einstein College of Medicine, Bronx, New York 10461.

Insulin-like growth factor II (IGF-II) is primarily involved in fetal growth, is produced in higher levels in fetal liver than adult liver, and is a potent mitogen of chick embryo fibroblasts. The IGF-II gene is located on chromosome 11p15 and is transcriptionally activated in Wilm's tumors. Our observation of deletions in chromosome 11p led us to study the expression of IGF-II in hepatocellular carcinoma (HCC). Total cell RNA was isolated from woodchuck HCCs and normal liver. Northern blots of total RNA and poly A mRNA were hybridized with a cDNA clone for prepro human IGF-II. The hIGF-II probe hybridized to two primary transcripts of 3.5 and 1.6 Kbs. These transcripts correspond in size to the rat IGF-II transcripts present in buffalo rat liver cells (BRL 3A) which secrete large amounts of IGF-II. A high steady state level of IGF-II RNA was observed in three HCCs from one animal. The WHV integration pattern in two of these tumors was identical, indicating that they were a metastases. In a survey of woodchuck HCCs, 1/3 had high IGF-II transcripts, 1/3 medium and 1/3 low, whereas none of the normal liver samples contained high levels of IGF-II transcripts and 2/3 contained low or undetectable IGF-II transcripts. Hybridization of Human cDNA probes for IGF-I, insulin, epidermal growth factor and tumor growth factor alpha to Northern blots of total RNA did not detect transcripts of these genes in woodchuck HCCs. High alpha-feto-protein transcription was sometimes associated with high IGF-II transcription. We conclude that IGF-II activation is prevalent and variable in HCC and may reflect, or be responsbile for, the heterogeneity observed in HCC.

**A 159** ANTIPROLIFERATIVE PROPERTIES OF CORTICOSTEROIDS AND THE CYCLOPEPTIDES, CYCLOSPORINE AND DIDEMNIN B, INVOLVE INHIBITION OF PROLACTIN RECEPTOR BINDING IN RAT Nb 2 NODE LYMPHOMA CELLS, Diane Haddock Russell, Arthur R. Buckley, David W. Montgomery, Charles W. Putnam and Charles F. Zukoski, University of Arizona, College of Medicine, Tucson AZ 85719. Prolactin (PRL)-stimulated ornithine decarboxylase activity (ODC) and subsequent proliferation are inhibited by the cyclopeptides cyclosporine (CsA) and didemnin B (DB) and by the corticosteroids methylprednisolone (MP) and dexamethasone (DEX) in rat Nb 2 node lymphoma cells. The extent of the inhibition correlates to some extent with the ability of these compounds to inhibit [$^{125}$I]-PRL binding to PRL receptors. The phorbol ester, TPA, stimulates ODC activity and [$^{3}$H]-thymidine incorporation to an extent 54% and 31% that of a maximal mitogenic concentration of PRL (10 ng/ml), suggesting that mitogenesis in these cells is coupled to some degree to the activation of protein kinase C (PKC). Since corticosteroids inhibited the ability of TPA to elevate ODC, these hormones may modulate PKC activation by physiological stimuli, a possible new site of action for their permissive effects on a variety of regulatory processes. The extent of ODC inhibition was more extensive than their ability to inhibit cell proliferation. Cyclosporine did not affect the ability of TPA to elevate ODC activity, whereas DB was inhibitory in nature. The establishment of PRL as a liver mitogen in rats and its tumor promoter activity in chemically initiated rat liver suggest that further studies of the regulation of prolactin-dependent mitogenesis may have relevance to the understanding of growth regulation in certain human tumors.

**A 160** TRANSDIFFERENTIATION OF HUMAN NEUROBLASTOMA CELLS: EXPRESSION OF NEUROTRANSMITTER RECEPTORS AND MYC ONCOGENES, Wolfgang Sadée,* Victor Yu,* Mark L. Richards,* Manfred R. Schwab,** Peter N. Preis,*** Frances M. Brodsky,****, and June L. Biedler*****; *School of Pharmacy, **Hooper Research Foundation, and ***Brain Tumor Research Center, University of California, San Francisco, CA 94143; ****Becton Dickinson Immunocytometry Systems, Mountain View, CA 94039; *****Laboratory of Cellular and Biochemical Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
Phenotypic changes during transdifferentiation of the human neuroblastoma cell line SK-N-SH were studied with the use of three subclones that interconvert at a slower rate than the parent cell line, i.e., a neuroblast-type subclone (SH-SY5Y), a non-neuronal, strongly substrate adherent subclone (SH-EP), and an intermediate type (SH-IN). Rhodamine-phalloidin staining of actin fibers revealed differences in the cytoskeleton morphology of the three subclones, while the clathrin subunit proteins (HC and $LC_a$), components of coated vesicles, were invariant. Dramatic differences were observed for the expression of neurotransmitter systems, i.e., the $\mu$ and $\delta$ opioid receptor, the muscarinic cholinergic receptor and its effect on phosphatidyl inositol turnover, and the uptake$_1$ transporter for catecholamines. While these systems were strongly expressed in the neuroblast-like clones SH-SY5Y and SH-IN, they were absent or barely detectable in the non-neuronal EP clone. Further, the oncogenes N-myc and c-myc were only expressed in the neuroblast-like subclones, consistent with their growth characteristics of fully transformed cells. The strong c-myc mRNA expression in the absence of c-myc or N-myc amplification in SK-N-SH, adds a new form of high oncogene activity in neuroblastoma cell lines. The combined results document a remarkable degree of coordinate gene expression during neuroblastoma transdifferentiation, which is of interest in the molecular biology of neurotransmitter systems and possibly in the therapy of neuroblastoma.

**A 161** DISTRIBUTION OF ALPHA TGF IN RODENT AND HUMAN MAMMARY TUMOR CELLS, David S. Salomon, William R. Kidwell, Robert Callahan and Rik Derynck, Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, MD 20892, and Department of Molecular Biology, Genetech, Inc., San Francisco, CA 94080.

The present study was undertaken to determine the distribution of alpha TGF mRNA in rodent and human mammary tumor cells by Northern blot analysis of poly A(+) RNA using mouse or human alpha TGF cDNA probes. Normal mouse mammary epithelial cells, NMuMG cells which have been transformed with a point-mutated cHa-ras proto-oncogene, NMuMG/ras$^{H}$ cells, secrete biologically active and immunoreactive alpha TGF and possess a 5.0 Kb alpha TGF mRNA species. In contrast, nontransformed NMuMG cells secrete reduced levels of alpha TGF and possess barely detectable levels of alpha TGF mRNA. Primary DMBA (P-DMBA) and primary NMU (P-NMU) rat mammary adenocarcinomas also synthesize alpha TGF and possess alpha TGF mRNA. P-DMBA and P-NMU tumors are estrogen-dependent for their growth. Following ovariectomy there is a rapid decline in the levels of alpha TGF mRNA in these tumors which is followed by reduction in biologically active alpha TGF. In contrast, transplantable DMBA (DMBA-I) and NMU (NMU-II) tumors which are undifferentiated carcinomas that are estrogen-independent possess low levels of both alpha TGF protein and corresponding mRNA. The human breast cancer cell lines MDA-MB-231, T-47D and MCF-7 secrete immunoreactive alpha TGF and possess alpha TGF mRNA. In MCF-7 cells, estrogen can increase the levels of alpha TGF protein and mRNA by two- to three-fold. In addition, 8/15 infiltrating ductal carcinomas contained alpha TGF mRNA. Within this group, 75% of the tumors (6/8) were found to be estrogen receptor and progesterone receptor positive suggesting that alpha TGF expression in mammary epithelial cells can be controlled by estrogens.

**A 162** STIMULATION OF TUMOR GROWTH IN VIVO BY NUTRIENTS DERIVED FROM THE HOST FAT STORES, Leonard A. Sauer and Robert Dauchy, Bassett Research Inst., Cooperstown, NY 13326

The growth rates of Morris hepatomas, Jensen sarcoma and Walker carcinoma 256 are increased about 3 times during an acute fast or streptozotocin-induced diabetes. This stimulation of tumor growth occurs only in adult (greater than 250g) rats and appears to require lipolysis of host fat stores. No stimulation of tumor growth is observed in immature (less than about 150g) rats or in adult rats that were depleted of their fat stores by diet restriction. In this study we compared the kinetics of increase in the host arterial blood concentrations of the fat store-derived nutrients during the onset of an acute fast or streptozotocin-induced diabetes with increases in the incorporation of $^{3}$H-thymidine into tumor DNA in vivo. The results were as follows:' 1. During an acute fast, the arterial blood glycerol, free fatty acid, ketone body, and triglyceride concentrations and the rate of tumor DNA synthesis start to increase 6-8 hours after removal of food and reach a peak after 18-20 hours. Refeeding decreases the blood nutrient levels and slows the rate of tumor DNA synthesis. 2. During acute diabetes the fat store-derived nutrients start to increase 2-4 hours after streptozotocin injection, reach a first peak at 12-14 hours, decrease to a trough after 18-19 hours, and then increase to a plateau after 22-24 hours. The kinetics of increase in $^{3}$H-thymidine incorporation follow an identical progress curve. Injection of insulin inhibits blood nutrient levels and slows tumor DNA synthesis. Diabetic immature rats do not show these nutrient and tumor growth changes. The result indicates that the growth of rat tumors is rate-limited in fed rats: nutrients derived from host fat stores appear to be the rate-limiting substances. (Support by USPHS CA 27809).

**A 163** PRODUCTION OF IMMUNOREACTIVE TGF-α BY CULTURED BREAST CANCER CELLS AND ACTIVITY OF TGF-α FRAGMENTS, G. Schultz, K. Darlak, G. Franklin, E. Sonnenfeld, D. Rosendahl, and A. Spatola, University of Louisville, Louisville, KY 40292.

Transforming growth factor-α (TGF-α) is a small mitogenic polypeptide produced by cancer cells and by embryonic cells. It is structurally related to EGF and both bind to the same receptor. Hydrophilicity plots of TGF-α indicate three discrete regions, each contained within the three disulfide loops, which are likely to be exposed on the surface of TGF-α and thus likely to interact with the TGF-α/EGF receptor. We chemically synthesized fragments of TGF-α which encompass these three hydrophilic disulfide loop regions (TGF-α: 1-21, 16-32, 33-50, 34-43, 21-32) and tested them for ability to compete for EGF-receptor binding and induction of DNA synthesis of 3T3 cells. None of the fragments showed significant ability to bind to the receptor or to induce DNA synthesis even at 100 μM. Antibodies to TGF-α (34-50) fragment were generated in rabbits and used in a solid phase RIA which had one-fifteen hundredth cross-reactivity to EGF compared with TGF-α. To determine if human breast cells secrete TGF-α in culture, serum-free conditioned medium (CM) was collected from two malignant (MDA-231, T47-D) and one normal (HBL-100) cell line, then concentrated and extracted. Confluent roller bottles of both malignant cell lines produced substantial amounts of TGF-α during 48 hr while no TGF-α was detected from the normal breast cells. These results suggest that two or more of the hydrophilic regions of TGF-α are required to form the receptor binding domain of TGF-α, and that malignant, but not normal, human breast cells in long term culture secrete TGF-α which may play a role in stimulating their growth.

**A 164** MITOGENIC EFFECT OF SUBSTANCE K CORRELATES TO MYC EXPRESSION, Thomas Sejersen, Jan Nilsson, Anna Hultgårdh Nilsson and Carl-Johan Dalsgaard, Karolinska Institutet, S-10401 Stockholm, Sweden.
The neuropeptides substance K (SK) and substance P (SP) were recently shown to stimulate DNA synthesis in fibroblasts and smooth muscle cells at nanomolar concentrations. SK and SP are two mammalian members of the tachykinin family derived from a common precursor molecule. The ability of smooth muscle cells to proliferate in response to treatment with SK is reduced after more than 24 h in serum-free medium. This suggests that the mitogenic effect of SK is dependent on an intracellular "mediator". This potential "mediator" is supposedly present in proliferating cells and reduced in serum deprived cultures. One plausible candidate for which the kinetics and role in cell proliferation agrees with this "mediator" is the myc protein. Comparisons of the ability of SK to induce DNA synthesis in normal and v-myc (OK10) transformed quail fibroblasts showed a positive correlation to the level of myc transcripts. We, therefore, speculate that the intracellular signal of SK-induced DNA synthesis interacts with the myc protein.
In the competence-progression model, both PDGF and c-myc are classified as competence factors. When Swiss 3T3 cells, $G_0$-arrested by serum starvation, were treated with PDGF alone for 24 h, only 1-2% of the cells underwent DNA synthesis. SK alone was also insufficient for induction of DNA synthesis. A combination of PDGF and SK did, however, induce 20% of the cells to enter the S phase of the cell cycle over a 24 h period. The effect of SK, therefore, appears to be complementary also to PDGF. The possible role of SK as progression factor, and its interaction with c-myc, is now under further study.

**A 165** GROWTH FACTORS INTERFERE WITH THE ANTIPROLIFERATIVE EFFECTS OF TUMOR NECROSIS FACTORS. H.M. Shepard, G.D. Lewis, T.E. Eessalu, B.B. Aggarwal, and B.J. Sugarman, Genentech Inc., South San Francisco, California 94080. **Tumor necrosis factors (TNFs) are a class of cytokines secreted by activated effector cells involved in host defense against tumor progression. Since TNFs have growth enhancing as well as antiproliferative activities, we chose to investigate how they interact with other purified growth factors in affecting tumor cell proliferation *in vitro*. Here we report that epidermal growth factor (EGF), transforming growth factor-$\alpha$ (TGF-$\alpha$), and TGF-$\beta$ can interfere with the antiproliferative effects of human recombinant tumor necrosis factors (rTNFs). EGF and TGF-$\alpha$ antagonized the cytotoxic effects of rTNFs on a human cervical carcinoma cell line, ME-180. This effect was not due to down-regulation of TNF-receptor number or alteration of the affinity of rTNF for its receptor. TGF-$\beta$ antagonized the rTNF-mediated killing of NIH 3T3 fibroblasts. Since EGF, platelet-derived growth factor and TGF-$\beta$ all enchanced 3T3 cell proliferation, but only TGF-$\beta$ interfered with rTNF cytotoxicity, then the protective effects of TGF-$\beta$ are not related in a simple manner to enhanced cell proliferation. These observations suggest that growth factors could enhance tumor growth *in vivo* by a combination of distinct mechanisms: (i) by autocrine stimulation of tumor cell growth, and/or (ii) by interfering with normal effector mechanisms of host defense.**

**A 166** CLONING OF A RAT mRNA BEARING HOMOLOGY TO BOVINE BASIC FGF, Supriya Shivakumar, Robert J. Matusik, University of Manitoba, Winnipeg, Manitoba, R3E 0W3.
We have identified a 100 nucleotides cDNA clone (pMSC) from a rat dorsal prostate library that bears homology to bovine basic FGF. The predicted thirty amino acid sequence corresponds to a.a. 49 to 78 in bovine bFGF with 26% of these a.a. being identical. Inclusion of conservatively substituted a.a. raises the degree of homology in this region to 46% . The percentage of conserved a.a. in this divergent area between acidic and basic bovine FGF is 53%. Northern analysis has indicated expression of a 1.2 kb mRNA in the following rat tissues: kidney, testes, ovary, thymus, dorsal, lateral, and ventral prostate. In addition, there are at least two different mRNAs expressed in the brain. The dorsal prostate expresses a low level of the mRNA, while the Dunning tumour which originates from the dorsal prostate, overexpresses this mRNA. Using pMSC, two high molecular weight mRNAs are detected in human tissue. They correspond to the 4.4 and 2.2 kb mRNAs that are detected by bovine bFGF cDNA in human tissue (Abraham et. al. 1986. Science 233:545). In addition, a 1.2 kb band is detected in human tissue which previously has not been reported. This evidence indicates that pMSC is related to members of the FGF gene family. (Supported by National Cancer Institute of Canada).

**A 167** Detection of Human Lung Epithelial Cell Growth Factors Produced by a Lung Carcinoma Cell Line, Jill M. Siegfried, Environmental Health Research and Testing, Inc., Research Triangle Park, NC 27709.
Serum-free medium conditioned for 72 h by a human undifferentiated adenocarcinoma of lung stimulated colony formation of normal human bronchial epithelial cells, newly cultured cells from human solid lung tumors (non-small cell), and established human lung tumor cell lines. The growth-stimulating activity was concentration dependent and was stable to acid. Gel filtration of concentrated conditioned medium on Bio-gel P10 separated the growth-promotiong activity into 4 regions of apparent $M_r$ 70,000, 12,000, 8,000 and 6,000, and 2 broad regions of apparent $M_r$ 3,000-5,000. All but the 12,000 $M_r$ fraction contained activity which competed for specific binding of Epidermal Growth Factor (EGF) to A431 cell membranes. Conditioned medium was superior to both EGF and recombinant TGF-alpha in stimulating colony formation of normal and neoplastic lung epithelial cells. EGF was inhibitory to lung tumor cells while TGF-alpha stimulated both normal and neoplastic cells. Of other substances tested, Insulin-like Growth Factor I also stimulated colony formation of lung epithelial cells and may account for activity found in conditioned medium with an apparent $M_r$ of 12,000. The results suggest that secretion of autocrine factors may be important in the growth of non-small cell carcinoma of the lung. This research was supported by contract # 68-02-4031 from the U.S. Environmental Protection Agency. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

**A 168** CHARACTERISATION AND QUANTIFICATION OF AN EPIDERMAL GROWTH FACTOR RECEPTOR ($EGF_r$) IN HUMAN BLADDER CANCER, Kenneth Smith, Janet A. Fennelly, David E. Neal and Adrian L. Harris, Cancer Research Unit, University of Newcastle upon Tyne, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.
Transforming growth factors can bind to the $EGF_r$ causing phosphorylation of tyrosine residues in the $EGF_r$ and ultimately leading to proliferation of the cell. We have therefore assayed human bladder cancer samples for the presence of $EGF_r$. Bladder tumour membranes were prepared by differential centrifugation and $EGF_r$ binding was assayed using $^{125}I$-labelled mouse EGF. The binding reaction was complete within 2 hours at 26°C. Bombesin, ACTH and $\alpha$-MSH did not compete with $^{125}I$-EGF for binding sites. The cross-linked receptor-EGF complex migrated as 2 bands (150,000 Dalton region) on SDS gels, and in the presence of EGF the $EGF_r$ was autophosphorylated. Using Scatchard analysis, the number of binding sites ($B_{max}$) and the dissociation constant ($K_d$) were assayed in 40 patients with bladder cancer. 18 patients had superficial transitional cell carcinoma and 22 patients had invasive transitional cell carcinoma. 7 of the 18 superficial tumours (38.9%) contained $EGF_r$ ($B_{max}$ range = 8.5 - 32.4 fmol/mg membrane protein with a mean of 15.5 ± 3.2 SEM). The $K_d$ values were in the range 0.23 - 1.45 x $10^{-9}$M with a mean of 0.83 ± 0.21 SEM. 15 of the 22 invasive tumours (68.2%) contained $EGF_r$ ($B_{max}$ range = 8.9 - 1020 fmol/mg membrane protein, with a mean of 149.7 ± 80.2 SEM). Using a radioimmunoassay specific to urogastrone, we have been able to detect small amounts (ng quantities) in the high speed supernatant of several bladders. The association of $EGF_r$ with more invasive tumours suggests that $EGF_r$ expression is related to tumour progression.

**A 169** CALCIUM CHANNEL-CALMODULIN INHIBITORS ARE ANTIESTROGENIC IN HUMAN BREAST CANCER CELLS, Jeannine S. Strobl, West Virginia University, Morgantown WV 26506
Extracellular calcium and calmodulin are required for initiation of DNA synthesis and cell division in normal cultured cells, and brief rises in free intracellular calcium are associated with the mitogenic response to multiple growth factors acting via cell surface receptors. Evidence is now presented for a role of calcium and calmodulin in human breast cancer growth stimulated by a steroid hormone, estradiol. MCF-7 breast cancer cells were cultured in phenol red-free Minimal Essential Medium +2% stripped fetal calf serum ± 2nM estradiol. After 5 days, estradiol-treated cells exhibited a 70% increase in cell number over control cell cultures. The potent calmodulin antagonists and calcium channel entry blockers, thioridazine and pimozide caused a statistically significant, concentration-dependent antagonism of estradiol-stimulated cell number. Estradiol-stimulated growth was completely inhibited by 2.5 uM thioridazine; growth of cells in the absence of estradiol was completely unaffected by this drug concentration. The specificity of thioridazine's action on estradiol-stimulated growth was confirmed in studies with estrogen receptor negative MDA-MB-231 human breast cancer cells. Pimozide, an even more potent calmodulin antagonist and calcium channel entry blocker, specifically inhibited estradiol-stimulated MCF-7 cell growth by 70% at 0.5 uM and 100% at 1 uM. Thus, calcium antagonism may be a novel mechanism of antiestrogen action in human breast cancer cells.

**A 170** URINARY TRANSFORMING GROWTH FACTORS IN NEOPLASIA: TGF-α ACTIVITY IN URINE OF PATIENTS WITH DISSEMINATED BREAST CANCER, Kurt Stromberg[1], W. Robert Hudgins[1], and David N. Orth[2], [1]National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701-1013 and [2]Vanderbilt University, Nashville, TN 37232.
Cancer detection by radioimmunoassay (RIA) of tumor marker(s) in urine is a non-invasive procedure potentially suitable for use in screening programs of high risk cancer-prone individuals. A tumor-associated growth factor was identified in a 22-liter pool of urine from patients with disseminated breast cancer using an isolation scheme (Cancer Research 46: 6004-6010, 1986) which separates transforming growth factor-α (TGF-α) from the high level of human epidermal growth factor (hEGF) normally present in urine. Concentration of urinary proteins by adsorption onto methyl bonded microparticulate silica, selective elution by acetonitrile, and subsequent gel permeation, cation exchange, and high performance liquid chromatography, resolved an EGF-related growth factor which generated a competitive binding curve similar to that of synthetic rat TGF-α in radioimmunoassay. A 26-liter pool of urine from normal subjects, evaluated in the same manner as part of another study, did not contain detectable quantities of this factor. The relationship of urinary TGF-α to tumor burden, as evaluated in pre-operative and post-operative urine samples from patients with primary breast cancer, is currently being examined.

**A 171** A POSSIBLE FUNCTIONAL SIGNIFICANCE OF RECEPTOR PHOSPHORYLATION IN PROCESSING OF INTERNALIZED EGF RECEPTOR, Hironobu Sunada, Jeffrey Peacock and John Mendelsohn, Memorial Sloan-Kettering Cancer Center, New York NY 10021.
It is well documented that EGF stimulates phosphorylation of the EGF receptor (EGFR). However, the functional significance of receptor phosphorylation is still not defined. We have demonstrated that anti-EGFR monoclonal antibody (mAb) 225 IgG1 is internalized without stimulating EGFR phosphorylation, and we hypothesize that receptor phosphorylation may provide a signal determining the intracellular fate of internalized EGFR. To test this hypothesis, we dissociated EGF-mediated early signals from those responsible for EGFR phosphorylation and devised a system in which EGFR phosphorylation can be modulated. $Ca^{2+}$ influx and EGFR clustering/internalization were not required signals for EGFR phosphorylation. mAb 225 in 15-fold excess above EGF carcinomas blocked EGF-induced Tyr-phosphorylation in A431 cells but only slightly inhibited EGF-stimulated Ser/Thr phosphorylation of EGFR. Using this system, the turnover rate of $^{35}$S-Met labeled EGFR was measured in the presence or absence of methylamine, which inhibits lysosomal function but does not affect phosphorylation and internalization of EGFR. The results showed that under the conditions where EGFR Tyr-kinase was activated, EGFR catabolism was greatly inhibited by methylamine, suggesting the lysosomal pathway as the main intracellular route. In contrast, when EGFR Tyr-kinase activity was impaired, internalized EGFR was translocated into a methlamine insensitive (non-lysosomal) pathway. These observations suggest a functional association of EGFR Tyr-phosphorylation with the intracellular fate of internalized EGFR, independent of the level of serine and threonine phosphorylation. (Supported by NCI Grants.)

**A 172** IN VITRO TRANSLATION OF preproTGF-α mRNA. J. Teixido, R. Gilmore, D. C. Lee and J. Massague, University of Massachusetts Medical Center, Worcester, MA. 01605 and Cancer Research Center, University of North Carolina, Chapel Hill, NC 27514
Transforming growth factor-α (TGF-α) is a highly mitogenic 6K polypeptide expressed by transformed and embryonic cells. Analysis of cDNAs corresponding to rat and human TGF-α has indicated that TGF-α is synthesized as part of a precursor, preproTGF-α, whose C-terminal portion contains a hydrophobic sequence that could act as a transmembrane domain. We find that in addition to mature 6K TGF-α, certain retrovirally-transformed rat cells release into the medium a 17-19K intermediate precursor for TGF-α, which is heterogeneously glycosylated and has full biological activity. However, this intermediate appears to lack the C-terminal portion unique to proTGF-α. To determine whether de novo synthesized preproTGF-α behaves as a transmembrane protein or as a secretory protein fully translocated across the endoplasmic reticulum, we have translated preproTGF-α mRNA in vitro in the presence or absence of rough microsomes. Rat TGF-α cDNA was subcloned into a pGEM vector and was transcribed using T7 RNA-polymerase. The mRNA was translated in a wheat germ translation system. A 17.5K translation product obtained in the absence of microsomes was specifically immunoprecipitated by antibodies against synthetic peptides corresponding to sequences present in mature TGF-α and in the the C-terminus of preproTGF-α. The presence of rough microsomes during translation generated a 20K translation product with proTGF-α immunoreactivity. This species probably corresponds to the primary glycosylation product generated in the lumen of the endoplasmic reticulum. We are currently carrying out protease protection experiments of the translation products to determine the membrane topology of the 20K proTGF-α species.

**A 173** TGF-BETA AND RETINOIDS INCREASE EGF RECEPTOR mRNA LEVELS IN MRK FIBROBLASTS. K.L. THOMPSON[1], R.K. ASSOIAN[2], AND M.R. ROSNER[1]. [1]Massachusetts Institute of Technology, Cambridge, MA and [2]Columbia University, NY, NY.
The growth stimulatory effect of EGF on NRK fibroblasts is enhanced by type beta transforming growth factor (TGF-beta) and retinoids. Both compounds increase the extent of EGF binding to NRK cells by an elevation in receptor number. To examine the potential regulation of EGF receptor gene expression by these agents, we have developed a mouse genomic probe that contains 3' sequences of the receptor and specifically recognizes the 9.5 kb rat EGF receptor transcript. When this probe was used, an increase in EGF receptor mRNA levels was detected after treatment of NRK cells with TGF-beta or retinic Acid. Both compounds induced a dose-related increase in EGF receptor mRNA that was detectable four hrs after addition. Maximum enhancement by retinoic acid (four fold at 1 uM) occurs by 12 hrs and continues up to 18 hrs. In contrast. peak levels of EGF receptor transcripts induced by 100 pM TGF-beta are seen by 4-8 hrs and decline thereafter. Thus, the increase in EGF receptor cells by TGF-beta or retinoids.

**A 174** REGULATION OF ORNITHINE DECARBOXYLASE mRNA LEVELS BY TUMOR PROMOTERS IN MOUSE KERATINOCYTES, Rune Toftgård, Carl Morath and Kaija Hyvönen, Center for Biotechnology, Karolinska Institute, Huddinge University Hospital, F82, S-141 86 Huddinge, Sweden.

Induction of ornithine decarboxylase (ODC) activity is an early and possibly obligatory response to tumor promoters during mouse skin carcinogenesis. Increased ODC activity has in addition been shown in mouse skin papillomas and carcinomas. Recent studies have implicated activation of protein kinase C as a key event in the increased expression of ODC caused by tumor promoting phorbol esters and growth factors such as PDGF and FGF. Both transcriptional and post-transcriptional mechanisms have been suggested to be involved in increasing the ODC activity. Topical treatment of mouse skin with 10 nmol TPA results within 2-4 hr in the coordinated accumulation in epidermal RNA of two mRNAs with molecular sizes of approximately 2.7 and 2.2 kb. Similar results are obtained using primary mouse keratinocytes grown under low $Ca^{2+}$ (0.05 mM) conditions. New protein synthesis is not required for the increase of ODC mRNA after TPA treatment suggesting that protein kinase C may directly act upon an intracellular messenger(s) which regulates ODC gene transcription. When keratinocytes are induced to differentiate by raising the extracellular $Ca^{2+}$ (1.2 mM) level their ability to respond to TPA with induction of ODC activity is rapidly lost. The loss of responsiveness is reflected in the lack of accumulation of ODC mRNA possibly due to either the presence of a repressor of ODC gene transcription or to an alteration in the signal transduced.

**A 175** CLONING OF THE A-CHAIN OF PDGF AND A-CHAIN EXPRESSION IN HUMAN ENDOTHELIAL CELLS, B.D. Tong[1], D.E. Auer[1], M. Jaye[2], E. McConathy[2], G. Ricca[2], W. Drohan[2] and T.F. Deuel[1], Jewish Hospital[1] at Washington University Medical Center, St. Louis, MO 63110, and Meloy Laboratories, Inc.[2], Springfield, VA 22151.
Three clones coding for the A-chain (non-sis homologous) of platelet-derived growth factor (PDGF) were isolated from a lambda gt 11 cDNA library constructed from mRNA isolated from human umbilical vein endothelial (HUVE) cells. The entire nucleotide sequence of one clone and the partial sequences of the other two clones have been completed and compared with the human glioma cell line U-343 MGa C12:6 as reported (Betsholtz, et. al., Nature V. 320, 695-699). Using one of the A-chain clones as a probe, "Northern" blot analysis of mRNA isolated from endothelial cells at the monolayer, organizing, or tube stage of development demonstrated that the three mRNAs previously associated with the A-chain of PDGF are modulated in a manner quite different from that previously reported for the B-chain of PDGF (M. Jaye, et. al., Science, V. 228, 882-885). These results indicate that both the A-chain and B-chain of PDGF are transcribed in HUVE cells and that the mRNA steady state levels are modulated differently in response to growth regulatory stimuli.

**A 176** CALCIUM RESPONSE TO GASTRIN RELEASING PEPTIDE IN SMALL CELL LUNG CARCINOMA Jane B. Trepel, Reino Heikkila*, Frank Cuttitta, John D. Minna, Leonard M. Neckers*, and Edward A. Sausville. NCI-NMOB and *LP, NCI, NIH, Bethesda, MD 20892

Gastrin releasing peptide (GRP) is a mammalian 27 amino acid peptide whose carboxyl terminal portion is highly homologous to the amphibian peptide bombesin (BN). A large proportion of small cell lung cancer (SCLC) specimens, especially those of the classic phenotype with neuroendocrine features express GRP, in both biopsy material and in cell culture. Utilizing the bombesin analog $Tyr^4$ BN, we induced a dose-dependent increase in free cytosolic $Ca^{2+}$ ($[Ca^{2+}]_i$) in three SCLC cell lines, as determined by the quin2 detection system. The increase in $[Ca^{2+}]_i$ was detectable at 1 nM $Tyr^4$ BN and was maximal at 100 nM. Treatment with 100 nM $Tyr^4$ BN induced a 70% increase in $[Ca^{2+}]_i$. This increase was maximal by 30-60 sec, and persisted for 3-5 min. GRP 1-27 and GRP 20-27 the C-terminal octapeptide that shares homology with BN were as potent as $Tyr^4$ BN in inducing the calcium response, while the N-terminal GRP 1-16 fragment which does not share homology with BN was inactive. The $[Ca^{2+}]_i$ increase was maximal in $Ca^{2+}$ containing medium and was incompletely inhibited by EGTA suggesting an induced $Ca^{2+}$ influx and a release of $Ca^{2+}$ from intracellular stores. GRP 1-27, GRP 20-27, and $Tyr^4$ BN induced rapid and complete homologous and heterologous desensitization, while the inactive peptide GRP 1-16 and the 11 amino acid peptide physalaemin, which shares a C-terminal Leu-Met with BN/GRP but had no effect on $[Ca^{2+}]_i$, did not induce heterologous desensitization. These data demonstrate an immediate physiologic response to GRP in three independently derived human SCLC cell lines. These data suggest that $Ca^{2+}$ acts as a transducer of GRP-stimulated cell regulatory events in SCLC.

**A 177** DISSECTION OF SIGNAL TRANSDUCTION AND HIGH AFFINITY BINDING CAPACITY OF HUMAN IFN-RECEPTORS BY USE OF SOMATIC CELL HYBRIDS. Ugur Ücer*, Dinko Berkovic*, Christian Ertel*, Peter Scheurich*, Barbara B. Knowles+, and Klaus Pfizenmaier. *Clinical Research Group of the Max-Planck-Society, 3400 Göttingen, FR Germany, and +Wistar Institute, Philadelphia, PA 19104, USA

Biological responses of human tumor cells to IFN-gamma are initiated by high affinity binding (Kd 1-3 x 10 M) to specific membrane receptors. While the type of IFN-gamma response of a given tumor cell is apparently determined at a post-receptor level, the magnitude of response in sensitive cells is proportional to the quantity of membrane signals. This was evident from a correlation analysis of the number of occupied membrane receptors and the height of the induced response. Thus, at limiting concentrations of IFN-gamma, tumor cells with large numbers of expressed receptors have an apparent higher sensitivity to IFN-gamma as compared to tumor cells with low receptor numbers. Analysis of IFN-gamma binding capacity and induction of response in human-mouse somatic cell hybrids revealed correlation of binding capacity of human IFN-gamma with presence of human chromosome 6. Scatchard analysis indicated high affinity binding with a Kd of 2 x 10 M of all chromosome 6 positive hybrids, and 1500 - 3500 receptors/cell. Despite this normal binding capacity, the hybrids completely failed to respond to human IFN-gamma by enhanced expression of mouse or human MHC-antigens. In contrast, recombinant mouse-IFN-gamma did enhance both H2- and HLA-membrane expression in these cells. These data suggest that the IFN-gamma binding sites coded for by human chromosome 6 are functionally deficient, probably because of lack of a specific signal transducing molecule distal of the IFN-gamma binding proteins and not coded for by chromosome 6.

**A 178** THE TUMORIGENIC POXVIRUSES SHOPE FIBROMA VIRUS AND MYXOMA VIRUS POSSESS GENES RELATED TO EPIDERMAL GROWTH FACTOR, Chris Upton, Joanne L. Macen and Grant McFadden. University of Alberta, Edmonton, Alta, Canada, T6G 2H7.

Although poxvirus replication and assembly occurs outside the host cell nucleus within virosomes found in the cytoplasm of infected cells, a number of poxviruses are known to be responsible for proliferative diseases. Examples of such tumorigenic poxviruses are Shope fibroma virus (SFV) which induces benign fibromas in rabbits and Molluscum contagiosum which causes benign tumor-like epidermal lesions in man. Vaccinia virus, a cytolytic orthopoxvirus, and SFV, a tumorigenic *leporipoxvirus*, both encode gene products, designated vaccinia growth factor (VGF) and Shope fibroma growth factor (SFGF) respectively, which share significant amino acid homology with epidermal growth factor (EGF) and transforming growth factor alpha. The role(s) of such growth factors in poxviral replication and virus-specific cytopathology is still undefined, but two possibilities are that such gene products could [1] facilitate S phase independence of viral replication by stimulating cellular functions such as nucleotide pool levels and ribosome availability, and [2] mediate the cellular proliferation often observed concomitantly with poxviral infections, albeit to markedly different extents with different poxviruses. Poxviral growth factors may play a central role in virus/cell interactions since the genome of malignant rabbit virus (MRV), a natural recombinant between SFV and myxoma virus, induces SFV-like fibromas during the initial stages of infection of domestic rabbits. MRV contains only a few kb of SFV DNA sequences with the replacement of a comparable extent of myxoma virus sequences. This region of the SFV genome includes four intact major open reading frames plus the intact SFGF gene. Because of the unusual pathology of MRV infections and the fact that myxoma virus itself induces fibromatous dermal lesions in its natural host (rabbits of the genus *Sylvilagus*) and a proliferation of endothelial cells in the myxoma lesions in *Oryctolagus cuniculus* it was of interest to determine that myxoma virus also possesses an EGF-like growth factor gene and examine its relationship to SFGF and VGF.

**A 179** EPIDERMAL GROWTH FACTOR ALTERS CELL RESPONSE TO THROMBOSPONDIN, James Varani, Suzanne Fligiel and Vishva Dixit, Univ. of Michigan, Ann Arbor, MI 48109 and VAMC-Wayne State Univ., Allen Park, MI 48101

Thrombospondin induces attachment and spreading of human squamous carcinoma cells but does not promote adhesion of melanoma or glioma cells. Squamous carcinoma cells also synthesize thrombospondin and may use the endogenously produced molecule to attach and spread on plastic culture dishes and dishes coated with type IV collagen. There is a direct relationship between the amount of thrombospondin synthesized by a given cell line and its level of basal adhesiveness. In addition, polyclonal and monoclonal antibodies to thrombospondin inhibit basal attachment and spreading. When squamous carcinoma cells are treated for one hour with epidermal growth factor (EGF), attachment and spreading under unstimulated conditions and in the presence of thrombospondin is increased. Platelet-derived growth factor and fibroblast growth factor do not alter adhesiveness. Treatment of squamous carcinoma cells for one hour with EGF has no effect on the biosynthesis of thrombospondin as indicated by the incorporation of [$^{35}$S]-methionine into forms precipitable with antibodies to thrombospondin. In contrast, there is an alteration in the distribution of the synthesized thrombospondin. A lower percentage is found in the culture fluid and a higher percentage is found in the pericellular matrix. These findings suggest that the effect of EGF on cell attachment and spreading may be mediated by an effect on the expression of endogenous factors (such as thrombospondin) which are synthesized by the cells and used as adhesion factors. (Supported by grants CA 36132 and 2 S07 RR05384 from the USPHS).

**A 180**

REGULATION OF THE PROTO-ONCOGENE FMS, J. Visvader[1], C. Van Beveren[2] and I.M. Verma[1], [1]Molecular Biology and Virology Laboratory, The Salk Institute, Post Office Box 85800, San Diego, CA 92138; [2]La Jolla Cancer Research Foundation, 10901 North Torrey Pines Road, La Jolla, CA 92037.

Proto-oncogenes have been implicated in the regulation of cell growth and differentiation. Proto-oncogene fms (c-fms) probably encodes the receptor (CSF-1 R) for the macrophage colony stimulating factor, CSF-1. Comparison of the human c-fms cDNA (Coussens et al., 1986) and feline v-fms sequences has revealed that the proteins share extensive homology but have different carboxy-termini. Chimeric clones between v- and c-fms have been constructed to establish whether a v-fms gene encoding a protein with the c-fms C-terminus is capable of transforming cells. The results have shown that the fms protein, with either the viral or human C-terminus, can alter the growth properties of murine fibroblasts.

Expression of the c-fms gene has been identified in placental tissue and cells of the monocytic lineage. To investigate the regulation of transcription of the c-fms gene in normal cells, a human placental genomic library was screened to isolate the promoter region of the c-fms gene. A large intron has been identified in the 5' untranslated region of the gene. Oligonucleotide probes complementary to a region 5' of this intron are currently being used to locate the promoter for transcriptional studies.

Coussens, L., Van Beveren, C., Smith, D., Chen, E., Mitchell, R.L., Isacke, C.M., Verma, I.M. and Ullrich, A. Nature 320:277-281.

**A 181** CHARACTERIZATION OF A LATENT FORM OF TRANSFORMING GROWTH FACTOR-$\beta$ SECRETED BY HUMAN PLATELETS, Lalage M. Wakefield, Diane M. Smith, Kathleen C. Flanders, Michael B. Sporn, Lab. of Chemoprevention, National Cancer Institute, Bethesda MD 20892.

Human platelets induced to degranulate by thrombin secrete large quantities of transforming growth factor-$\beta$ (TGF-$\beta$) ($\leq$ 2000 molecules/platelet), of which >95% is in a biologically latent form. This form is unable to bind to the TGF-$\beta$ receptor on A549 lung carcinoma or other cells without prior acidification in vitro. A polyclonal antiserum to TGF-$\beta$ cannot immunoprecipitate the latent form, suggesting platelet TGF-$\beta$ is both immunologically and biologically unavailable. When platelet secreted material is chemically cross-linked and analysed on Western blots, affinity-purified antibodies to TGF-$\beta$ detect two bands under non-reducing conditions; one at 25 KDa characteristic of mature TGF-$\beta$, and one at 220-240 KDa. The same high molecular weight band is also detected by antibodies raised against synthetic peptides corresponding to amino acids 46-56, 91-102 and 267-276 of the predicted TGF-$\beta$ precursor sequence. In the absence of chemical cross-linking, the 220-240 KDa band is no longer detected by anti-TGF-$\beta$ antibodies, and the anti-precursor antibodies now detect a 210 KDa band under non-reducing conditions, and a 39 KDa band after sample reduction. Analysis of platelet secreted material by gel filtration shows a major acid-activatable TGF-$\beta$ peak at >700 KDa, partially overlapping the $\alpha$-2-macroglobulin peak, and a minor peak at ~200 KDa. We propose the latent form of TGF-$\beta$ comprises mature TGF-$\beta$ dimer (25 KDa) non-covalently associated with precursor sequences (78 KDa dimer) which are disulfide-bonded to a third unknown entity to give the 220-240 KDa form. The whole complex may further associate non-covalently with $\alpha$-2-macroglobulin.

**A 182** BRYOSTATIN 1 and 9 INDUCE THE PHOSPHORYLATION OF A UNIQUE SERIES OF 70 KDA PROTEINS IN HL-60 CELLS.

Barbour S. Warren*, Cherry L. Herald#, George R. Pettit#, and Peter M. Blumberg*; *Lab. of Cellular Carcinogenesis and Tumor Promotion, NCI, Bethesda, MD 20892; and #Dept. of Chemistry, Arizona State Univ., Tempe, AZ 85287.

The bryostatins are a group of macrocyclic lactones isolated from the marine bryozoan Bugula neritina. Bryostatin 1, like the phorbol esters, activates protein kinase C; however, it inhibits the phorbol ester induced differentiation of the human promyelocytic leukemic cell line, HL-60. Treatment of HL-60 cells with 200 nM phorbol 12,13-dibutyrate (PDBu) for 30 min increased the phosphorylation of 7 different proteins. Treatment with 200 nM bryostatin 1 under the same conditions increased the phosphorylation of the same 7 proteins. An additional series of three phosphorylated proteins was observed in response to bryostatin 1, however. These proteins, having a molecular weight of 70 kDa and pIs ranging from approximately 6.3 to 6.5, are most probably three species of a multiphosphorylated protein. A 2 min exposure to bryostatin 1 at doses greater than 50 nM was sufficient to produce these unique phosphorylations. With the exception of these protein species, the dose response and time course of phosphorylation by PDBu and bryostatin 1 were similar. Bryostatin 9 was also examined and had a time course and dose response which was analogous to bryostatin 1.

**A 183** TRANSFORMATION OF NRK CELLS BY AN INFECTIOUS RETROVIRUS CARRYING A SYNTHETIC RAT TGF-$\alpha$ GENE, Shinichi Watanabe, Eliane Lazar, and Michael B. Sporn, National Cancer Institute, Bethesda, MD 20892.

We synthesized a gene for rat type alpha transforming growth factor (TGF-$\alpha$), consisting of the leader sequence and the sequence coding for the mature 50 amino acid peptide, without the C-terminal processed region. This gene was inserted into the retrovirus vector pSW272, derived from spleen necrosis virus, to obtain an infectious recombinant virus carrying the rat TGF-$\alpha$ gene. This recombinant virus can infect NRK cells and allow these cells to grow in soft agar in the presence of TGF-$\beta$. Transformed cells isolated from colonies growing in soft agar contain an integrated form of the recombinant virus and secrete biologically active TGF-$\alpha$ into their medium. These results show that a biologically active TGF-$\alpha$ can be produced by a gene synthesized from only part of the coding region, and that the infectious retrovirus carrying the TGF-$\alpha$ gene (SW355) can function as a transforming agent in NRK cells in the presence of TGF-$\beta$.

**A 184** CHARACTERIZATION OF AN ABERRANT EPIDERMAL GROWTH FACTOR RECEPTOR IN HUMAN GLIOBLASTOMA CELL LINE SF268, Alan Wells, Daiga M. Helmeste, J. Michael Bishop, University of California, San Francisco CA 94143.

SF268 cells have been found to express an epidermal growth factor receptor (EGFR) which differs from that seen on A431 epidermoid carcinoma cells (BBRC 132,284, 1985). This receptor has a higher binding affinity for ligand, and when complexed with EGF presents a higher and more heterogeneous molecular weight as determined by both molecular exclusion chromatography and non-reducing SDS-PAGE. Additionally, ligand binding does not elicit a mitogenic response.

We have investigated the molecular basis of these differences. Southern blot analyses of the entire EGFR coding domain revealed approximately 3-fold amplification. All fragments were amplified equally, with no alterations in size. The level of the poly-adenylated RNA was approximately 10-15% of that in A431 cells. Two species were detected at 10.5 and 5.8 kb at a ratio of 5:1. These messages comigrated with A431 and placental transcripts. Monospecific antibodies to the intracellular domains of the EGFR precipitated a major protein at 170 kDa and a minor one at 150 kDa from both SF268 and A431 cells. A monoclonal antibody directed against the carbohydrate moieties on the receptor on A431 cells failed to recognize anything on the glioblastoma cells. EGFR-associated protein kinase activity could not be elicited in SF268 cells, neither in vivo nor in vitro.

These findings suggest that while the gene is amplified, the coding region and transcript unit remain grossly intact. There is a difference in the glycosylated portion of the molecule that alters the extracellular presentation. Interestingly, there seems to be a diminution or abolition of the inherent enzymatic activity. Whether these changes are the results of a subtle mutation in the polypeptide backbone, or are due to cellular parameters requires further investigation.

**A 185** MANOALIDE INHIBITS EPIDERMAL GROWTH FACTOR (EGF) INDUCED CALCIUM MOBILIZATION IN A431 CELLS, Larry A. Wheeler, George Sachs, Gerald W. De Vries, Danon Goodrum and Shmuel Muallem. Allergan Pharmaceuticals Inc./Herbert Labs, Irvine, CA 92715

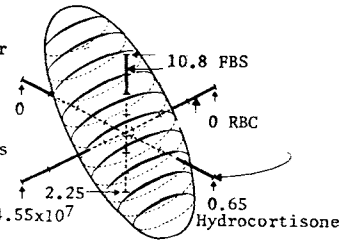
Polypeptide mitogens like EGF induce rapid biochemical, metabolic and early transcriptional changes in responsive cells such as A431. Calcium ($Ca^{2+}$) has been postulated to play a second messenger role in mediating many of these changes. We used A431 cells to study the effect of manoalide (MLD) on EGF-induced $Ca^{2+}$ mobilization. MLD is a novel marine natural product that was shown to be a potent inhibitor of bee venom phospholipase $A_2$ ($IC_{50}$=.05uM; Glaser & Jacobs, Biochem. Pharm. 35:449, 1986). Using quin-2 dye techniques EGF induces a rapid transient $Ca^{2+}$ mobilization in A431 cells that is dependent on extracellular $Ca^{2+}$. Preincubation of A431 cells with 1.5uM MLD completely inhibited the EGF-induced rise in free intracellular cytosolic $Ca^{2+}$ ($[Ca^{2+}]_i$). The inhibition was time and temperature dependent as well as irreversible. Using fura-2 as the $Ca^{2+}$ sensitive dye, a new intracellular $Ca^{2+}$ mobilization component was observed. Treatment of the cells with MLD completely inhibited the $Ca^{2+}$ mobilization from intracellular stores ($IC_{50}$=0.4uM). At a MLD concentration of 1.5uM, which totally inhibited $Ca^{2+}$ mobilization, no effect upon phosphotidylinositol (PI) turnover (release of IP, $IP_2$, $IP_3$) could be detected in $H^3$-myoinositol labeled cells. These results suggest that MLD is a potent inhibitor of EGF-induced $Ca^{2+}$ mobilization whether released from intracellular stores or through the opening of a plasma membrane $Ca^{2+}$ channel. The ability to alter $Ca^{2+}$ without affecting PI hydrolysis should make MLD a unique tool for studying polypeptide induced signal transduction mechanisms as well as help define the physiological role of increased $[Ca^{2+}]_i$ as a second messenger.

**A 186** INACTIVE INSULIN RECEPTORS PREDOMINATE IN INSULIN RESISTANT CELLS. Morris F. White, C. Ronald Kahn, Joslin Diabetes Center, Boston, Massachusetts 02215.

In the intact cell, insulin receptors (IR) undergo tyrosine autophosphorylation immediately after insulin binding. Autophosphorylation activates the phosphotransferase in the purified IR so that it catalyzes phosphorylation of tyrosyl residues in other proteins. Thus, autophosphorylation may be a critical step for transmission of the insulin signal by a cascade of tyrosine phosphorylation. In the well-differentiated insulin-sensitive rat hepatoma cell, Fao, and insulin resistant human fibroblasts, we have identified by sequential immunoprecipitation with anti-phosphotyrosine ($\alpha$PY) and anti-insulin receptor ($\alpha$IR) antibodies, an active ($IR^a$) subset of IR that undergoes tyrosine autophosphorylation during insulin binding and an inactive ($IR^i$) subset which is only precipitated by $\alpha$IR. By analysis of [$^{32}$P]Pi- and [$^{35}$S]methionine-labeled cells, about 75% of the IR is active and 25% is inactive. The same ratio was measured for surface iodinated receptors suggesting that both $IR^a$ and $IR^i$ were exposed at the external face of the plasma membrane. Covalent labeling with [$^{125}$I]insulin showed that both $IR^a$ and $IR^i$ bound insulin. A similar analysis made with insulin-resistant fibroblasts obtained from patients with type II diabetes indicated that 90% of the IR is inactive. $IR^a$ and $IR^i$ are composed of identical oligomeric forms and yield identical [$^{35}$S]methionine-peptide maps; however, the $IR^i$ labeled in-vivo lacks tyrosine phosphorylation at a major site. $IR^i$ was not activated after solubilization and purification. TPA stimulated serine phosphorylation of the IR but did not alter the ratio of $IR^a$ to $IR^i$. Thus, tyrosine autophosphorylation may be important for insulin signaling and a predominance of $IR^i$ may cause some forms of diabetes.

**A 187** OPTIMIZATION OF HUMAN PRIMARY BREAST CANCER CLONAL GROWTH, William H. Wolberg, Greta J. Besch, Martin A. Tanner, Steve P. Howard, and Michael N. Gould, University of Wisconsin-Madison, Madison, Wisconsin 53792.

Statistically designed experiments were used to determine additives required for optimal clonal culture of monodispersed primary human breast cancer cells in an anchorage-independent agar system. The only two way interaction found was between estradiol and insulin with estradiol tending to negate the inhibitory effect of insulin. Hydrocortisone, fetal bovine serum, and red blood cells, but not epithelial growth factor, improved both plating efficiency and median colony size. Concentrations of the three stimulatory ingredients were simultaneously idealized using response surface methodology. The optimal hydrocortisone concentration was 0.35 ug/ml, fetal bovine serum was 6.5%, and red blood cells (RBC) was 2.1 x $10^7$ cells/ml. Plating efficiences of 0.39% for colonies larger than 50 cells diameter and of 0.19% for colonies larger than 130 cells diameter were achieved. RBC's from humans and from several rat strains but not from mice were stimulatory. Clonal growth was the same in medium supplemented with intact or lysed RBC's. Less stimulation was obtained from RBC ghosts or from the soluble fraction but these were stimulatory compared to the non-RBC enhanced medium. The RBC growth factors are being characterized.



**A 188** TGFα AND TGFß EXPRESSION IN HUMAN COLON CARCINOMA CELL LINES: IMPLICATIONS FOR AN AUTOCRINE MODEL. By J. Wolfshohl, R. J. Coffey, H. L. Moses - Vanderbilt U.

Three human colon cancer lines (SW480, SW620, WIDR) produce TGFß-like and EGF-like molecules as measured by competing activity in the TGFß and EGF radioreceptor assay (Cancer Res 46: 1164, 1986). The amounts produced differ; and SW480 cells, the highest producers of TGFß-like activity, lack detectable TGFß receptors while SW620 cells, the highest producers of EGF-like activity, lack EGF receptors. This study investigated these events at a transcriptional level and attempted to determine the mechanism of loss of detectable receptors. Using cDNA probes for TGFß and TGFα, it was demonstrated that mRNA levels correlated with protein production; TGFß expression was most intense in SW480 cells and TGFα expression was most intense in SW620 cells. Mild acid washing of the SW480 cells prior to performing the TGFß binding assay resulted in the appearance of specific binding. Neither mild acid washing or preincubation with Suramin uncovered EGF receptors in SW620 cells. Further, in contrast to the other two lines, SW620 showed neither expression of transcripts homologous to an EGF receptor cDNA probe or immunoprecipitation using a polyclonal antibody to the EGF receptor. Thus two distinct mechanisms (endogenous occupation of TGFß receptor in SW480 cells, and absence of EGF receptor in SW620 cells) explain the lack of detectable TGFß and EGF receptors in the binding assays. The autocrine hypothesis remains viable for TGFß in SW480 cells but not for TGFα in SW620 cells; this would not discount a paracrine role in this latter case.

**A 189** CELL CYCLE DEPENDENT PHOSPHOPROTEINS 78kD AND 80kD DIFFERENTLY REGULATED IN NORMAL AND TRANSFORMED MOUSE FIBROBLASTS, Henry C. Yang and Arthur B. Pardee, Dana-Farber Cancer Institute and Department of Pharmacology, Harvard Medical School, Boston, MA. 02115
The induction of cell cycle dependent phosphoproteins by serum in normal (A31) and benzo(a)pyrene transformed (BPA31) BALB/c 3T3 mouse fibroblasts was investigated. 18kD, 78kD and 80 kD phosphoproteins (pp18, pp78, pp80) were found to be cycle dependent. Pp80 was induced 7-10 fold by serum or phorbol ester within the first 3 hours in A31 cells but was absent and minimally inducible in BPA31 cells. Pp80 appears to be identical to a previously described 80kD phosphoprotein which is a substrate for protein kinase C (1). Pp78 was constitutively present in low serum arrested BPA31 cells and induced by serum in A31 cells by 6 hours before DNA synthesis. The level of pp78 fell during S phase in both BPA31 and A31 cells. Pp78 was inducible by insulin-like growth factor I in BPA31 cells and by epidermal growth factor, platelet derived growth factor and phorbol ester in A31 cells. The results suggest that pp78 serves as a key transducing signal for growth factors and phorbol ester during the $G_0$ to S transition and is abnormally expressed in transformed cells.

1. Rozengurt, E., M. Rodriguez-Pena, and K.A. Smith. (1983) Phorbol esters, phospholipase C, and growth factors rapidly stimulate the phosphorylation of a $M_T$ 80,000 protein in intact quiescent 3T3 cells. Proc. Natl. Acad. Sci. U.S.A. 80:7244-7248.

**A 190** MODULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) TYROSINE KINASE ACTIVITY INDEPENDENT OF LIGAND AFFINITY STATE, Peter P. Yu, Hironobu Sunada, Jeffrey Peacock and John Mendelsohn, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
Phosphorylation of specific threonine and tyrosine amino acids has been demonstrated to modulate such critical properties of the EGFR as EGF binding affinity and capacity to interact with potential EGFR tyrosine kinase substrates. The phenothiazine compound, chlorpromazine, inhibits calmodulin activated pathways as well as protein kinase C activity. Treatment of A431 cells with chlorpromazine (100 μ M) resulted in a 1.5 and 1.8 fold increase in threonine and serine phosphorylation of EGFR compared with control cells grown in the absence of this agent. This was not accompanied by significant loss of high affinity EGFR. EGF (20 nM) resulted in a 7.1, 2.2, and 3.3 fold increase in Tyr, Thr and Ser phosphorylation of EGFR. Simultaneous exposure of A431 cells to EGF and chlorpromazine appeared to completely inhibit EGF-induced phosphorylation of EGFR tyrosine, threonine and serine residues. This effect is metabolic, since at 4° C chlorpromazine did not block EGF-induced tyrosine phosphorylation. Others have shown that phosphroylation of threonine 654 results in loss of high affinity receptors and may account for decreased tyrosine kinase activity. Chlorpromazine blocks EGF-induced activation of EGFR tyrosine kinase activity, but this effect is not mediated by loss of high affinity receptors for EGF. These results suggest that tyrosine kinase activity can be regulated independent of the status of high affinity receptors, and therefore presumably independent of phosphorylation at threonine 654, although phosphorylation of other threonine or of serine residues may be required. (Supported by NCI grants.)

# Poster Session III

**A 191** CLONING AND CHARACTERIZATION OF THE GENES FOR THE ANGIOGENIC PROTEINS, BASIC AND ACIDIC FIBROBLAST GROWTH FACTOR, Judith Abraham, Jacqueline Whang, Annette Tumolo, Ayalew Mergia, Lothar Schweigerer[1], Denis Gospodarowicz[1], and John Fiddes, California Biotechnology, Inc., Mountain View, CA, 94043, and [1]Cancer Research Institute, University of California, San Francisco, CA, 94143.

The two endothelial cell mitogens, basic and acidic fibroblast growth factor (FGF) have both been shown to stimulate the growth of new capillaries (angiogenesis) in model systems. The actual physiological roles of these two proteins in vivo are unknown, but both factors have been postulated as playing a part in processes such as tissue repair. Basic FGF has recently been isolated from several different tumor cell types, raising the possibility that this factor may also be involved in the angiogenesis associated with solid tumor growth.

We have isolated human clones for both basic and acidic FGF. We find that, along with the strong amino acid sequence homology, the two factors share a strong homology in gene structure. Each factor is encoded by a single gene, with the coding region split by two large introns. Both factors appear to be synthesized initially as 155-residue proteins, but neither appears to have a classical signal sequence. Despite the homology between the two genes, they lie on different chromosomes: basic FGF maps to chromosome 4, while acidic FGF resides on chromosome 5. Northern blot analyses have shown that basic FGF sequences are found on two mRNAs of approximately 7.0 and 3.7 kb, and that these RNAs are present in primary bovine capillary endothelial cells as well as cells derived from a human hepatoma, a rhabdomyosarcoma, a melanoma, and a retinoblastoma.

**A 192** **AGONIST AND ANTAGONIST ACTIVITIES OF SYNTHETIC PEPTIDES DERIVED FROM BASIC FGF**

Andrew Baird, David Schubert and Nicholas Ling

Acidic and basic fibroblast growth factors (aFGF, BFGF) are potent mitogens for several cell types and are characterized by their capacity to stimulate angiogenesis in vivo. Because both molecules exist in identical molecular forms (i.e. ECGF/γFGF; aFGF/bFGF; βECGF/βFGF) and have a very high affinity for heparin, they have been recently referred to as α(acidic) and β(basic) heparin-binding growth factors. Synthetic fragments of βHBGFs have identified two functional domains in the primary structure of basic FGF that bind to both heparin and to the bFGF receptor. Peptides derived from the sequence of bFGF(24-68) and bFGF(106-120) can bind, in a dose dependent fashion, radiolabelled heparin and displace $^{125}$I-FGF binding to FGF target cells (BHK cells, endothelial cells, PC-12 cells and $GH_3$ cells). Peptides derived from these two functional domains possess partial agonistic activity and can stimulate the incorporation of $^{3}$H-thymidine into 3T3 cells. Because they occupy the FGF receptor, they also possess partial antagonist activity and can reduce the cellular response to FGF. Thus peptides derived from FGF(24-68)$NH_2$ can reduce endothelial cell growth and peptides derived from both functional domains can inhibit the effects of FGF on differentiated cell function (i.e. prolactin release by $GH_3$ cells). The peptides have no effect on the cellular response to PDGF or the binding of $^{125}$I-EGF to its receptor.The identification of peptidic sequences with partial antagonist activity to FGF provides the first step towards the potential development of FGF specific anti-angiogenic factors.

**A 193** DIFFERENTIAL THROMBIN BINDING AND GROWTH STIMULATION OF VARIOUS ORGAN-DERIVED VASCULAR ENDOTHELIAL CELLS P.N. Belloni, G.L. Nicolson and D.H. Carney, Univ. of Texas Cancer Center, Houston, TX and Univ. of Texas Medical Branch, Galveston, TX.
Endothelial cells which line microvessels of different tissues are highly specialized and may selectively participate in a range of normal and pathological processes including: hemostasis, wound healing, metastasis and nutrient transport. We have previously demonstrated that endothelial cells derived from murine brain (MBE), lung (LE) and liver (HSE) express patterns of cell surface glycoproteins unique to each organ. To further characterize these organ-derived endothelial cells we measured $^{125}$I-thrombin binding to high-affinity receptors and determined the mitogenic responsiveness of each population to thrombin + ECGF or EGF in cells maintained in 2% FBS. Up to 92% of the $^{125}$I-thrombin specific binding to LE cells was inhibited by TR-9 monoclonal antibody to the fibroblast thrombin receptor indicating a high number of thrombin receptors on these cells. MBE and HSE also bound $^{125}$I-thrombin specifically to these receptors, but to a lesser extent than LE cells. The mitogenic response of MBE, LE and HSE to thrombin, ECGF or EGF alone, or most combinations were minimal relative to that seen with complete media. The combination of thrombin ECGF, however, stimulated proliferation 50 to 150% over that of ECGF alone approaching the maximal growth rate of these cells. Differences were observed in the pattern of responses to different factors and in morphology following thrombin addition to endothelial cells from different organs. Thus, endothelial cells in particular organs may have unique patterns of responsiveness when exposed to angiogenic signals. (Supported by CA-29571 to GLN and AM-25807 to DHC.)

## A 194 STIMULATION OF NEOVASCULARIZATION AND REGENERATION OF THE RAT SCIATIC NERVE BY BASIC FIBROBLAST GROWTH FACTOR

Pedro Cuevas, Andrew Baird and Roger Guillemin

Basic fibroblast growth factor is a potent polypeptide mitogen for several cell types and stimulates angiogenesis in vitro and in vivo. Although angiogenic factors are currently being intensively studied, there are nonetheless gaps in our in fine understanding of this process in vivo. In order to evaluate the role of FGF in regeneration of the rat sciatic nerve following transection, the proximal stump of the transected nerve was connected to a osmotic minipump that was filled with a sterile saline solution containing basic FGF (1 µg/ml), and the growth factor was delivered at a rate of 1 µl/hr. Nerve specimens were processed at various times for light and electron microscopy studies. In ultrathin sections many capillaries appear in the regenerating proximal nerve stumps. Endothelial cells are joined with tight and gap junctions. Pinocytotic vesicles are detected in the endothelial plasmalemma, principally on the luminal side. Mitochondria, rough surfaced endoplasmic reticulum, free ribosomes and microfilaments are easily identified and specific endothelial organelles such as Weibel-Palade bodies, are located throughout the endothelial cytoplasm. In some sections these organelles are closely adhered to the luminal endothelial plasmalemma, suggesting an exocytotic process. A basal membrane encloses the capillary cylinder, and pericytes and their processes are located adhered to the endothelial abluminal side in an unfolding of the pericapillary basal membrane. Concomittant analyses of the neuronal segments of the transected nerve show progressive rem lenation of axons. The results demonstrate that basic FGF can promote neocapillary formation in the proximal rat sciatic stump, show that the newly synthesized endothelial cells exhibit normal cytoplasmic organelles and a substructure required for a normal function, and demonstrate a possible role for FGF in nerve regeneration.

## A 195 PRODUCTION OF BIOLOGICALLY ACTIVE RECOMBINANT FIBROBLAST GROWTH FACTOR IN E.COLI

Gary M. Fox, Tsutomu Arakawa, and Allen Banks, Amgen Inc., Thousand Oaks, CA 91320

Basic fibroblast growth factor (FGF) is one of a family of endothelial cell mitogens known to bind strongly to heparin. These factors have been isolated from a variety of sources including both tumor and normal tissues. The angiogenic properties of these molecules suggest that they may be important in the regulation of tumor growth. FGF has not only been detected in tumors, but antiserum against FGF has been successfully used to slow tumor growth in mice (Baird et.al, J Cell Biochem 1986;30(1):79-85).

We have chemically synthesized and cloned the gene encoding basic FGF into a plasmid vector. Expression of the recombinant FGF in E.coli and subsequent purification yields biologically active material of greater than 95% purity. Using the large amounts of highly purified FGF obtainable by these methods, we are currently undertaking further biochemical and biological characterization of this important growth factor.

## A 196 GROWTH FACTOR REQUIREMENTS OF NORMAL AND FIBROSARCOMA-DERIVED HUMAN FIBROBLASTS

J.J. McCormick, R. Schilz, V.M. Maher, Carcinogenesis Laboratory, Michigan State University, East Lansing MI 48824

We have found that human foreskin fibroblasts in a serum-free medium (Ryan et al. Exp. Cell Res. In Press) are quiescent in 0.1 mM calcium, but grow when stimulated with EGF, PDGF, or 1.0 mM calcium. The human fibrosarcoma-derived cell line (HT1080) grows in this same medium in 0.1 mM calcium and growth is not enhanced by adding EGF or PDGF or changing the medium to one containing 1.0 mM calcium. Their growth rate in medium containing 0.1 mM calcium was one population doubling per day. A second human fibrosarcoma cell line (NCI) also grows in 0.1 mM calcium medium. However, these cells show an enhanced growth when EGF or PDGF is added or when the medium is changed to one containing 1.0 mM calcium. Four additional human fibrosarcoma cell lines also tested grow in the same medium containing 0.1 mM calcium without addition of EGF or PDGF. A similar result was found with CT-1 cells, a human fibroblast-derived, unlimited-lifespan cell line.

Just how the transformed cells obviate the need for exogenous growth factors is not yet clear. It is known, however, that HT1080 cells synthesize the mRNA for the B chain of PDGF, carry a mutant n-ras gene and synthesize an EGF related peptide at relatively high levels. Normal human fibroblasts do not synthesize PDGF, have a normal n-ras gene and make very low levels of EGF related peptides, too little to drive replication. Such changes in tranformed cell lines are sufficient to account for their growth factor autonomy is under study.

(Supported by NIH/NCI grant CA 21289)

**A 198** CLONING AND CHARACTERIZATION OF THE HUMAN PIM-1 GENE: A PUTATIVE ONCOGENE RELATED TO THE PROTEIN KINASES, Timothy C. Meeker, Lalitha Nagarajan, Abbas ar-Rushdi, Giovanni Rovera, Kay Huebner, and Carlo Croce, Wistar Institute, Philadelphia PA 19104.

The mouse Pim-1 gene is involved in the pathogenesis of mouse lymphomas (Cell: 46, 603-611, 1986). We have cloned and analyzed the human Pim-1 gene to investigate its role in human lymphoma and leukemia. Overlapping cDNA clones from a K562 (human erythrileukemia cell line) library were isolated and sequenced. These clones contained 289 bp of 5' untranslated sequence, an open reading frame coding for a protein of 313 amino acids and 1297 bp of 3' untranslated sequence. The Pim-1 protein showed significant homology to a number of the protein kinases. The amino acid corresponding to tyrosine-416 of c-src is a tyrosine (position 198) suggesting that Pim-1 may be a tyrosine kinase rather than a serine-threonine kinase. No transmembrane region was identified. An analysis of a genomic clone from the 380 cell line (human B cell leukemia) identified 6 exons and 5 introns derived from .5 kb. The promoter region had no TATA or CAAT boxes, but did have multiple Sp1 binding sites (CCGCCC). Northern blots of human cell lines showed that Pim-1 is transcribed primarily in B lymphoid and myeloid cell lines. This initial characterization of the human Pim-1 gene will allow us to define 1) its role as a human oncogene, 2) its role in hematolymphoid differentiation, 3) the relationship of its structure to its kinase activity, expecially compared to the other protein kinases, 4) the nature of the transcriptional regulation conferred by the GC-rich promoter region.

**A 199** ANTIVIRAL AND ANTITUMORAL XANTHATES INHIBIT PROTEIN KINASE C ACTIVATION, Karin Müller-Decker, Eberhard Amtmann and Gerhard Sauer, Institute for Virus Research, German Cancer Research Center, D-6900 Heidelberg.

The tricyclodecane-9-yl-xanthate (D609) inhibits the growth of DNA- and RNA viruses. The replication of the negative strand RNA virus vesicular stomatitis virus is blocked by the inhibition of the phosphorylation of the regulatory NS protein. Furthermore the compound reverted the growth kinetics of bovine papilloma virus transformed hamster embryo fibroblasts (HEF-BPV) to the growth rate of the normal parental cells (HEF) which remained almost unaffected by such treatment. Since protein kinase C (pkC) plays a fundamental role in the regulation of fibroblast proliferation, the effect of D609 on the stimulation of the phosphorylation of proteins was studied using activators of pkC. In quiescent HEF the xanthate suppressed the stimulation of the phosphorylation of proteins induced by TPA, dioctanoylglycerol and epidermal growth factor. Rather than affecting the activity of pkC directly, the synthesis of phospholipids such as phosphatidic acid and phosphatidylinositol was blocked by D609 in a time and concentration dependent manner. There was a clear-cut difference between transformed and normal cells in that the former reacted already to a lower concentration of the xanthate.

**A 200** THE INVOLVEMENT OF PROTEIN KINASE C IN PROLIFERATION AND DIFFERENTIATION OF LEUKEMIC HL-60 CELLS, Ilana Nathan, Miriam Aharon, Lina Atlas and Alexander Dvilansky, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

The effect of protein kinase C (PKC) inhibitors on the proliferation of human promyelocytic leukemia cells was studied. The growth of HL-60 was inhibited by polymyxin B in a dose dependent manner. Tamoxifen, a non steroidal antiestrogen which is also a PKC inhibitor, suppressed cell proliferation but was far less effective than polymyxin B.

In order to study the role of PKC in the induction of differentiation, the activity of the enzyme was investigated in cytosolic and particulate fractions. 12-O tetradecanoyl phorbol-13-acetate (TPA) at concentrations of $10^{-7}$M at which it induced differentiation of HL-60 cells into monocytes-macrophages, caused a rapid translocation of PKC from the cytosolic to the particulate fraction. In untreated HL-60 cells, about two-thirds of PKC activity occurred in the cytosolic fraction. However, following TPA treatment, only 15-20% of the activity remained in the cytosol. When γ-Interferon, another inducer causing differentiation along the monocytic pathway, was added, a slight decrease in PKC activity of both cytosolic and particulate fractions was observed. However no change in subcellular distribution occurred. These findings suggest the involvement of PKC in the growth regulation of HL-60 cells and in the differentiation induced by TPA.

**A 201** INDIRECT GROWTH REGULATION OF TUMORS BY MODULATING IMMUNO-MECHANISMS OF THE DRUG UKRAIN, J.W. Nowicky, M. Greif, F.Hamler, W. Hiesmayr, W. Staub, Ukrainian Anti- Cancer Institute, Laimgrubengasse 19/5, A - 1060 Vienna, Austria.
Laboratory research work and clinical studies on Ukrain have shown different regulative and stimulating action on the immunocompetent net work concerning tumor growth control. In vitro higher stimulating activity was recognized in the lymphocyte transformation test in animal and human test systems as compared to phytohemagglutinin. Clinical studies revealed an increasing effect on the number and activity of T-Helper cells. T-Suppressor cells are diminished in number and so, an increase in the T-Helper/T-Suppressor quotient is established in primarly immune deficient oncological patients. Beyond NK-cell activity other phagocytic blood elements became effective. The immune profil parameters showed signs of increased reactivity. These phenomena were demonstrated in different malign diseases like mammacarcinomas, osteosarcomas, lung carcinomas and malignant melanomas with very high statistical significance ($p = 0.001$). Other tumor growth controlling mechanisms may be found in the inhibitory actions of Ukrain on certain parts of the respiratory-chain in malignantly transformed cells and in the ability of the drug to be actively accumulated and concentrated in and/or on tumor tissue.

**A 202** ISOLATION OF THREE FORMS OF THE MAJOR HUMAN PLACENTAL SUBSTRATE FOR THE EPIDERMAL GROWTH FACTOR KINASE: SEQUENCE HOMOLOGY WITH LIPOCORTIN AND DISTINCTION FROM CALPACTIN, M.D. O'Connor-McCourt, K.A. Valentine-Braun, D.F. Michiel, J.K. Northup and M.D. Hollenberg, University of Calgary, Calgary, Alberta, Canada T2N 4N1
We have isolated from human placental membranes a major substrate for the EGF receptor kinase. Two independent purification methods were developed; one isolating the soluble substrates after their elution from the membrane by calcium chelation, and the second isolating the cholate-extracted, chelator-resistant substrates. The first procedure yielded pure substrates of MW 38kD and 35kD (p38,p35) while the second yielded a pure substrate of MW 38kD (p38-chol). Immunological cross-reactivity and proteolytic maps demonstrated that p38, p38-chol and p35 were distinct forms of the same protein, with p35 being a proteolytic derivative of the 38kD forms. Since p38-chol was indistinguishable from p38 and p35 by both antibody reactivity and peptide maps it may be that its $Ca^{++}$-independent membrane association results from different post-translational modifications. The amino-terminal sequence of p35 matched exactly residues 13-37 predicted for human lymphoma lipocortin (Wallner, B.P. *et al.*, Nature *320*, 77 (1986)). The src kinase substrate p36 (calpactin) which was well resolved from p38 and p35, was not a substrate for the EGF receptor kinase and was immunologically distinct from p38 and p35. Although p38 and p36 are substrates for two different tyrosine kinases, both can be phosphorylated by protein kinase C *in vitro*.

**A 203** ACCELERATION OF WOUND HEALING BY TGFβ IN A RAT LINEAR INCISION WOUND MODEL, G. F. Pierce[1,2], T. A. Mustoe[3], A. Thomason[5], M. Sporn[6], A. Roberts [6], T. F. Deuel[1,4], Departments of Medicine[1], Pathology[2], Surgery[3], and Biological Chemistry[4], Washington University Medical Center, St. Louis, MO 63110, AMGEN[5], Thousand Oaks, CA 91320 and the Laboratory for Chemoprevention, National Cancer Institute, NIH, Bethesda, MD 20892
A linear incision wound healing model was developed in young adult male Sprague-Dawley rats. Tensometer measurements revealed a linear increase in breaking strength over a two week post-wounding observation period. Initial experiments using bovine collagen applied directly to wound edges before coaptation revealed a statistically insignificant inhibition of wound healing but otherwise no detrimental effects to the wound. Human platelet derived transforming growth factor β (TGFβ) was added to the collagen vehicle and wounds were harvested for breaking strength measurements beginning two days post-wounding. A highly significant increase in breaking strength (2.93 gms on day 5) compared to matched collagen control wounds (1.32 gms on day 5) on each rat was observed using analysis of variance ($p < 0.009$). The effect of TGFβ was linear beginning at 10 pmole/wound. Histologic stains indicated an increased organization of granulation tissue in TGFβ treated wounds. Thus TGFβ, which has been previously shown to increase granulation tissue formation in animal models, has for the first time been shown to increase breaking strength and granulation tissue in experimental wounds made in rats.

**A 204** SPECIFIC RECEPTORS FOR HEPARIN-BINDING GROWTH FACTOR, TYPE-2, ON NORMAL HUMAN CELLS. Gary D. Shipley, James T. Rosenbaum, Jill E. Hendrickson, Winifred W. Keeble, Jeanne Tsai, Joseph E. Robertson and Mark D. Sternfeld. Departments of Cell Biology and Anatomy, and Ophthalmology (JTR, JER), The Oregon Health Sciences University, Portland, OR 97201.

Purified heparin-binding growth factor, type-2 (HGBF-2) [basic fibroblast growth factor] is a potent mitogen for fibroblasts and endothelial cells. In the development of a tumor, it has been hypothesized that the endogenous production of HBGF-2 by the tumor cells may act in an autocrine fashion to stimulate the proliferation of the tumor cells themselves or in a paracrine manner causing the proliferation and invasion of vascular beds. We have purified HBGF-2 from bovine pituitary glands and have developed a specific receptor-binding assay for the growth factor using $^{125}$I-labeled HBGF-2. Utilizing this assay, specific receptors for HBGF-2 were detected on normal human foreskin fibroblasts, human proliferative keratinocytes and human retinal pigment epithelial cells. Scatchard-plot analysis of the binding of increasing concentrations of $^{125}$I-HBGF-2 to these cells at 4 degrees C revealed similar receptor number and affinity on all three cell types with approximately 200,000 receptors/cell and a dissociation constant of $3 \times 10^{-11}$ M. In contrast, no specific binding could be demonstrated to peripheral blood mononuclear cells. We have also performed competition-binding experiments with serum-free culture medium conditioned by several human tumor cell lines. We have found that medium conditioned by two colon carcinoma lines (SW480 and WiDR), a glioblastoma (HTB-14) and a lung carcinoma (A549) contain large amounts of material that competes with $^{125}$I-HBGF-2 for binding to the HBGF-2 receptor on human fibroblasts. These results suggest that the receptor for HBGF-2 is present on normal cell types of diverse embryonic origin, and that the mitogen may be produced by human tumor cells. This work supported by USPHS grants CA42409 (GDS) and EYO6477 and EYO6484 (JTR).

**A 205** FUNCTION AND REGULATION OF MEP: AN ACID PROTEASE SECRETED BY MALIGNANT CELLS, Bruce R. Troen, Susannah Gal, and Michael M. Gottesman, NIH, Bethesda, MD 20892

We have been studying the biochemical properties and the regulation of expression of the major excreted protein (MEP) of mouse fibroblasts in an effort to understand how tumors exert distant humoral effects. MEP is a secreted lysosomal acid protease induced 10 to 50 fold by malignant transformation, growth factors, and tumor promoters. MEP is a 39 kD glycoprotein that contains mannose 6-phosphate and binds to the phospho-mannosyl receptor and is processed to two lower molecular weight forms of 29 and 20 kD. The acid-activated form of MEP cleaves BSA, oxidized insulin B chain, the synthetic peptide CBZ-Phe-Arg-NMec, and a number of extracellular matrix proteins including fibronectin, collagen, and laminin. This protease activity is inhibited by leupeptin. The secreted radiolabeled 39 kD form of MEP can be taken up by Chinese hamster ovary cells (CHO) in a time-dependent manner and its uptake can be inhibited by mannose 6-phosphate in the medium. The CHO cells then process the radiolabeled MEP to the two lower molecular weight forms normally found in mouse cells. We have isolated and sequenced the MEP cDNA and this information along with its enzymatic activity suggests that MEP is cathepsin L. We have also used the cDNA to isolate a cosmid genomic clone for MEP and have subcloned a putative promoter region. We are presently sequencing the promoter region and constructing a plasmid containing both the MEP promoter and the CAT reporter gene. Given the spectrum of activity of MEP and its transformation-sensitive regulation, these results suggest that MEP may be involved in mediating tumor-associated phenomena such as invasiveness, metastasis, or paraneoplastic syndromes.

## *Poster Session IV*

**A 206** TRANSFORMING GROWTH FACTOR ß (TGFß) IS A BIFUNCTIONAL MODULATOR OF THE GROWTH OF NORMAL AND LEUKEMIC PROGENITORS. Massimo Aglietta, Wanda Piacibello, M. Luisa Ferrando, Andrea Perina, Felice Gavosto. Clinica Medica A dell'Università, Torino, Italy.

TGFß, a two chain polipeptide of 25,000 daltons, is able to both stimulate and inhibit cell proliferation with the response obtained depending largely on cell type. We have studied the effect of TGFß on the growth of normal anf leukemic hemopoietic progenitors. In the presence of conditioned medium from the 5637 cell line, TGFß (2.5ng/ml, gift of dr. M. Sporn) enhances the growth of a subclass of normal granulo-monocyte progenitors, the day 7 CFU-GM whilst is ineffective on the growth of their immediate precursors, the day 14 CFU-GM. TGFß is ineffective or inhibitory for the growth of day 7 and day 14 CFU-GM from chronic myelogenous leukemia patients. Leukemic cell line clonal growth is affected in a variable way. KG1 cells are inhibited while U937 cells are slightly stimulated. The growth of other cell lines (HL-60, I937, Jurkatt) is not significantly affected by the molecule. These data show that TGFß is a powerful enhancer of the growth of a subpopulation of normal myeloid progenitors (although it can not substitute for CSFs).Moreover they demonstrate a bifunctional action of the molecule since inside myelopoiesis it can act as an enhancer or as an inhibitor.

**A 207** INHIBITION OF EGF-STIMULATED DNA SYNTHESIS BY A NOVEL CELL-SURFACE SIALOGLYCOPEPTIDE GROWTH INHIBITOR. C.C. Bascom, B.G. Sharifi, and T.C. Johnson. Division of Biology, Kansas State University, Manhattan, KS, 66506.
An 18,000 dalton, pI 3.0 sialoglycopeptide has been isolated and purified to homogeneity from bovine cerebral cortex cell surfaces that reversibly inhibits protein synthesis and DNA synthesis in a number of target cell lines. Preincubation of cells with the calcium ionophore A23187 blocks the inhibitory activity of the sialoglycopeptide. The ability of the purified sialoglycopeptide to antagonize the mitogenic effect of EGF was studied using quiescent Swiss 3T3 cells. The sialoglycopeptide was a potent inhibitor of EGF-stimulated DNA synthesis; 0.20 nM of the sialoglycopeptide completely abolished the stimulatory effect of 1.60 nM EGF. Binding studies showed that neither the mixed affinities (0.11 and 1.9 nM) nor the total number of specific receptors (50,000/cell) for EGF were altered by the sialoglycopeptide, suggesting that the inhibitor mediated its effect at a post-receptor event. Competition experiments for the binding of the sialoglycopeptide to its specific cell surface receptor also demonstrated that EGF and the inhibitor did not compete for the same cell surface receptors. The bovine sialoglycopeptide could be added within 2.5 hr of the addition of EGF and still block the stimulation of DNA synthesis by the growth factor. These data suggest that the sialoglycopeptide blocks EGF stimulation at a relatively early step in the signal transduction pathway. The sialoglycopeptide is also a potent inhibitor of both TPA- and bombesin-induced DNA synthesis in 3T3 cells.

**A 208** INTERRELATIONSHIP OF TRANSFORMING GROWTH FACTOR-β AND A STEROID IN FIBROBLASTS AND LYMPHOMA CELLS. Jean P. Beck, Jean B. Dietrich, Sylvette Chasserot and David A. Lawrence*. IBMC- CNRS, Strasbourg F67084 and *UA532-CNRS Institut Curie, Orsay F91405.
Transforming growth factor β (TGF-β) is a multifunctional peptide involved in the control of proliferation, differentiation and other functions in many cell types. The anchorage-independent growth of untransformed fibroblasts in soft agar is induced by TGF-β and requires in addition exogenous EGF for certain target cells. The formation of colonies of NRK-49 cells is completely inhibited by the synthetic 11-β substituted nor-steroid R38486 added at a final concentration of $1.3 \times 10^{-5}$M.
We also explored the effect of TGF-β on Daudi lymphoma cells. These cells are induced by glucocorticoid hormones for the production of Epstein Barr Virus early antigens (EA) and the induction is inhibited by the antiglucocorticoid R38486. By testing the effect of TGF-β on the Daudi cells an induction capacity varying between 10 and 16% positive EA-cells is observed. This induction is also inhibited by R38486.
Thus, R38486 does not only antagonize the glucocorticoid hormone action (Chasserot et coll. J. Steroid Biochem 1984, 21 p.585) but inhibits also the effect of TGF-β in fibroblasts and in Daudi lymphoma cells. The molecular basis of the interactions observed is currently under investigation.

**A 209** COORDINATE INDUCTION OF ACTIN AND FIBRONECTIN MESSENGER RNA IN EGF-STIMULATED AKR-2B CELLS, Stanley P. Blatti and Michael J. Getz, Department of Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester MN 55905.
A λgtII complementary DNA (cDNA) library, prepared from poly(A)$^+$ RNA isolated from quiescent AKR-2B cells 4 hrs following stimulation with epidermal growth factor in the presence of cycloheximide was differentially screened to identify RNA transcripts whose abundance is specifically increased as a primary response to EGF. Approximately 40% of the inducible clones detected by this procedure corresponded to either β or γ cytoskeletal actin genes, a class of mRNA sequences which are induced within 30 min of stimulation. One non-actin clone, designated c99, was subsequently found to be derived from an approximately 8.5 Kb RNA whose abundance was also increased as early as 30 min following EGF stimulation. DNA sequencing of c99 established the identity of this RNA as fibronectin. These data demonstrate that RNA sequences encoding two proteins whose trans-membrane interaction is required for cell adhesion, actin and fibronectin, can be similarly induced as a primary response to EGF stimulation. Dissociation of this response from the mitogenic effects of EGF could lead to the altered morphology and disorganized growth patterns characteristic of transformed cells. Supported by NIH grant GM 25510 and by the Mayo Foundation.

**A 210** EPSTEIN-BARR MEDIATES A SWITCH IN RESPONSIVENESS TO TRANSFORMING GROWTH FACTOR, TYPE BETA, IN CELLS OF THE B CELL LINEAGE, Heidi Kiil Blomhoff[1], Erlend Smeland[1], Abu Salim Mustafa[1], Tore Godal[1] and Rolf Ohlsson[2] [1]Laboratory for Immunology, Inst. for Cancer Res., The Norwegian Radium Hospital, Montebello, N-0310 OSLO 3, Norway, [2]Centre for Biotechnology, Karolinska Institute, Fâ2 Huddinge Hospital, S-141 48 HUDDINGE, Sweden.

The functional performance of TGFβ appears to depend on the target cell phenotype as well as in vitro culture conditions. In general, TGFβ promotes fibroblast growth but inhibits epithelial cell proliferation. We here present evidence that a switch in responsiveness to TGFβ may operate in B-cells, mediated by Epstein-Barr virus (EBV) infection. TGFβ inhibits traverse of the cell cycle of activated normal human B cells, but promotes cell proliferation of EBV-positive Burkitt's lymphoma cell lines as well as EBV-infected B-lymphocytes. The switch in responsiveness to TGFβ seems independent of the proliferative status of target cells, and thus may contribute to the initiation as well as the maintenance of certain B cell neoplasias.

**A 211** INBRED MOUSE STRAINS WITH DIFFERENT SUSCEPTIBILITY TO THE COLON CARCINOGEN DMH:TARGET TISSUE DNA DAMAGE AND REPAIR. Lidia C. Boffa,Claudia Bolognesi and Maria Rita Mariani Istituto Nazionale per la Ricerca sul Cancro,IST,Viale Benedetto XV n.10,16132 Genova,ITALY.

We are interested in the problem of genetic specificity of carcinogens,particularly of the colon carcinogen DMH (1,2-dimethylhydrazine). This aspect of carcinogenesis could have relevance in human cancer.
In our studies we have used six inbred strains of mouse: two resistant(DBA/1;AKR/J) ,two of intermediate susceptibility(CD1:60%;C57 BL/6N:80%)and one extremely resistant to DMH carcinogenesis(SWR/J). We have found a pretty good correlation between carcinogen susceptibility and extent of single strand DNA breaks in colon epithelial cells at short times after administration(4 hours). Conversely,at this time, there was a substantial and comparable damage in liver and kidney of all strains examined.At longer times(24 hours) after exposure colon DNA of susceptible strains still presented a high degree of damage(maximum for SWR/J).At 48 hours only the very susceptible mouse strains maintained a percentage of colon DNA damage.At this time colon DNA of non susceptible strains and non target tissues was completely repaired.
We have preliminary results indicating that one or more components of the microsomal fraction (S9)could be involved in DMH activation and therefore,when absent or inactive,responsable of the strain specificity of colon carcinogenesis.

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**A 212** CONTROL OF HEPATOCYTE TGFβ RECEPTORS DURING GROWTH STIMULATION, Brian I. Carr, Department of Medical Oncology, City of Hope Medical Center, Duarte, CA 91010.
TGFβ is a potent, non-toxic inhibitor of mitogen-induced DNA synthesis for normal rat hepatocytes in vitro (Cancer Res. 46: 2330, 1986). To investigate the physiological significance of this inhibition, TGFβ serum levels and $^{125}$I-TGFβ cell binding were measured in quiescent and stimulated adult rat hepatocytes using fresh primary monolayer cultures, and TGFβ purified from outdated human platelets, radiolabeled with $^{125}$I cell. Hepatocytes obtained at various times after a two-thirds partial hepatectomy (PH) in vivo rapidly lost high affinity TGFβ receptors, with a nadir at 5 hr post PH and a gradual recovery by 24 hr. At 5 hr post PH, the cells also lost sensitivity to the mito-inhibitory actions of TGFβ. Normal and PH hepatocytes were similar with respect to efflux and degradation of bound $^{125}$TGFβ, and the receptor half-life was 3 hrs for both, as judged by loss of binding upon incubation with cycloheximide. Serum did not contain detectable TGFβ. However, normal but not PH rat portal serum increased the binding of TGFβ to normal hepatocytes by 600% after a 3-hr preincubation in vitro at 37°C. Insulin was also capable of up-regulating the TGFβ receptor. The results suggest that the response to inhibition by TGFβ in hepatocytes is controlled in part by serum factors that regulate the hepatocyte receptor number. It is hypothesized that an important growth-regulatory event in rat liver is the loss of a serum factor after PH which controls the hepatocyte TGFβ receptor levels. The decrease in hepatic TGFβ receptors results in a loss of mito-inhibition by endogenous TGFβ, allowing the cells to respond to ambient mitogens.

**A 213** **FUNCTION OF THE LOW DENSITY LIPOPROTEIN RECEPTOR IN GROWTH OF RAT OSTEOBLAST-LIKE CELLS.** **B.D. Catherwood; Dept of Medicine, VA Medical Center and Emory University, Atlanta GA.**

We undertook studies to characterize the mechanisms of low density lipoprotein (hLDL) stimulation of the growth of ROS 17/2.8 cells and normal rat osteoblasts (ROB). Binding and degradation of [$^{125}$I]-hLDL (50-200 cpm/µg) were assayed after incubation with lipoprotein-starved cells for 1 hr at 4C or 5-24 h at 37C respectively. Receptor-mediated internalization and degradation of [$^{125}$I]-hLDL were blocked by 10X unlabeled hLDL or by the B47 monoclonal antibody to the LDL receptor-binding domain of apoprotein B. Cholesterol utilization was measured by the incorporation of [$^{3}$H]-oleic acid into cholesterol ester (ACAT assay). hLDL in the presence of only transferrin and selenium stimulated ROS cell growth at rates >80% of those in serum. LDL stimulated 2 fold the ROB growth response to platelet-derived growth factor (PDGF) and epidermal growth factor. ROS cells possess a classical LDL receptor, saturable at 150 µg/ml [$^{125}$I]-hLDL with half maximal competition for binding by LDL seen at 40 µg/ml; Apoprotein E-bearing HDL showed weak competition. Preincubation of ROS cells with LDL but not HDL down-regulated receptor number by 70%, with an $EC_{50}$ of 15 µg/ml. Stimulation of ACAT activity to >20X basal by LDL but not HDL demonstrated LDL receptor function. Internalization and degradation of [$^{125}$I]-LDL was 6X higher in ROS cells than in rat fibroblasts or ROB. However, treatment of ROB with PDGF increased high-affinity degradation of LDL 2X and NRK or 208F cells transformed with v-sis, v-fos, or ras oncogenes had LDL receptor levels or degradation rates 3-14X higher than nontransformed cells. We conclude that rat osteoblasts and other rat cells express an LDL receptor during rapid proliferation and this receptor functions to internalize cholesterol, coordinating cell lipid metabolism with cell growth and enhancing the response to polypeptide mitogens. Preliminary results indicate that chronic parathyroid hormone exposure, which inhibits growth, decreases LDL binding and receptor function.

**A 214** THE ROLE OF TRANSFORMING GROWTH FACTORS IN THE GROWTH REGULATION OF MURINE KERATINOCYTES, R.J. Coffey, N.J. Sipes, R.L. Graves-Deal, B. Weissman, and H.L. Moses, Vanderbilt University School of Medicine, Nashville, TN 37323 and Children's Hospital of Los Angeles, Los Angeles, CA 90027.

Balb/MK cells provide a model system to study growth and differentiation control mechanisms of nontransformed epithelial cells. In contrast to the epidermal growth factor (EGF)-dependent MK cells, the Kirsten ras transformed derivative cell line, KC, is distinguished by it's EGF-independence (Mol. and Cell. Biol. 5:3386, 1985). We examined the role of transforming growth factors type alpha and beta (TGF$\alpha$ and TGF$\beta$ ) in the growth of these cells. At equimolar concentration, TGF$\alpha$ supplants the EGF requirement for MK cells. Furthermore, the addition of KC serum-free conditioned medium to cultures of EGF-depleted MK cells was mitogenic in $^{3}$H-thymidine assays, suggesting that the production of EGF-like molecules by KC cells is a potential mechanism of their EGF-independent growth. TGF$\beta$ inhibits rapidly growing MK and KC cells in $^{3}$H-thymidine studies with 50% inhibition at TGF$\beta$ doses of 160pM and 240pM, respectively. In addition, autoradiographic studies with quiescent MK cells restimulated with EGF demonstrate that TGF$\beta$ can override this proliferative signal and reversibly inhibits the growth of these cells. KC cells are morphologically transformed by TGF$\beta$ while MK cells are not. These studies suggest that growth regulation of nontransformed keratinocytes, in part, involves the interaction of positive and negative signals provided by TGFs, and transformation of these cells results in altered responses to TGFs.

**A 215** THE EGF RECEPTOR AS A TARGET FOR GROWTH INHIBITION OF BREAST CANCER CELLS Deborah A. Eppstein[1], Y. Vivienne Marsh[1], Rik Derynck[2], Sherry R. Newman[1], and John J. Nestor, Jr.[1], [1]Syntex, Palo Alto, CA 94304; [2]Genentech Inc., So.San Francisco, CA 94080

Reports from several groups have suggested that the epidermal growth factor (EGF) receptor/ligand system may be important in the progression of breast cancer. Sainsbury et al. (Lancet 1:364, 1985) found an inverse correlation between EGF-receptors vs. estrogen-receptors in primary breast tumors, as well as a greater presence of EGF-receptors on metastases vs. primary tumors. Dickson et al. (Endocrinology 118:138; 1986) demonstrated that MCF-7 cells, an estrogen responsive human mammary adenocarcinoma cell line, secreted an EGF-receptor-binding growth factor upon treatment with 17-β-estradiol. In the murine system, Kurachi et al. (Proc. Natl. Acad. Sci. USA 82:5940; 1985) demonstrated that EGF contributed to the in vitro growth of spontaneous (MMTV related) mammary tumors. Our laboratory has been studying structure-activity relationships of polypeptide ligand/receptor interactions, and we have begun applying these techniques to the study of the EGF receptor system in breast cancer cells. The growth of MCF-7 cells is stimulated by $10^{-11}$M 17-β-estradiol, as well as by exogenously added EGF or transforming growth factor-α (TGF-α). Monoclonal antibodies to the EGF receptor partially inhibit the growth of these cells and inhibit EGF or TGF-α stimulated growth. The above results imply that a degree of growth inhibition can be obtained in vitro by interfering with the EGF-receptor/ligand system in these breast cancer cells. Accordingly, we have studied the effects of a small synthetic peptide encompassing the third S-S loop of TGF-α , and find a small but reproducible inhibition of both estradiol- and EGF-stimulated growth. Studies are in progress to define the mechanism of this peptide-induced growth inhibition.

**A 216** CHARACTERIZATION OF ANTIBODIES AGAINST SYNTHETIC PEPTIDES CORRESPONDING TO REGIONS OF TGF-β AND ITS PRECURSOR, K. Flanders, N. Ling*, N. Thompson, A. Roberts, B. Fleurdelys, U. Heine, R. Derynck+, and M. Sporn, National Cancer Institute, Bethesda, MD 20892, *Salk Institute, San Diego, CA 92138, and +Genentech Inc., South San Francisco, CA 94080

The cDNA analysis of TGF-β suggests that the 112 amino acid TGF-β monomer is synthesized as the carboxyl terminal portion of a 391 amino acid precursor. The formation of the biologically active TGF-β dimer then involves both disulfide bonding of the monomers and proteolytic cleavage from the precursor. Presumably due to the highly conserved nature of the processed peptide (human and murine sequences differ by only one amino acid), generation of antibodies against the TGF-β dimer has been difficult. We have prepared polyclonal antibodies against synthetic peptides corresponding to various regions of the TGF-β monomer and its precursor to be used in biosynthetic studies. The antisera have been characterized by their abilities to react in enzyme-linked immunosorbent assays, radioimmunoassays, immunocytochemical experiments and Western blots. These antisera also have been tested for their abilities to immunoprecipitate radiolabelled cell media and to block receptor binding of TGF-β. An evaluation of these results and their potential application to various types of studies will be presented.

**A 217** RESPONSE TO GROWTH FACTORS BY NORMAL HUMAN MESOTHELIAL CELLS AND THEIR AUTOCRINE PRODUCTION BY HUMAN MESOTHELIOMA CELL LINES, Brenda Gerwin, Edward Gabrielson, John Lechner, Roger Reddel, Anita Roberts, Keith Robbins and Curtis C. Harris, National Cancer Institute, Bethesda,MD 20892

We have reported previously that EGF, TGF-β and PDGF are mitogens for normal human mesothelial cells. Both response-time kinetics and Northern analysis indicate that the induction of DNA synthesis in response to TGF-β does not involve intermediate induction of PDGF as reported in murine AKR-2B cells. Preliminary experiments indicate that medium conditioned by mesothelioma cell lines is mitogenic for normal mesothelial cells. Northern analyses of mesothelioma cell lines indicate that they express TGF-β and PDGF A and B chain mRNAs. In the case of PDGF, the tumors produce significantly more mRNA than either normal or SV40 large-T-antigen transformed human mesothelial cells. When conditioned medium from normal and mesothelioma cells was assayed for PDGF, the tumor but not the normal cells displayed mitogenic activity inhibitable by anti-PDGF antiserum. TGF-β activity was detected in acid-activated medium conditioned by normal and T-antigen transformed mesothelial cells and by mesotheliomas. An assessment of the biological significance of these results awaits determination of whether these three classes of cells secrete TGF-β in an active or latent form. Thus, mesothelioma cell lines produce both PDGF A and B chain mRNA and secrete PDGF activity. In addition, they secrete TGF-β. Since both of these growth factors have been shown to be mitogenic for normal mesothelial cells, these results suggest that an autocrine selection mechanism may operate in the generation of human mesothelioma.

**A 218** TRANSFORMATION OF HUMAN AIRWAY EPITHELIAL CELLS, Dieter C. Gruenert, Andrea M. Wilson, Jonathan H. Widdicombe, and Jay A. Nadel, University of California, San Francisco, California 94143.

Human cell culture has played an integral role in our understanding of biochemical and genetic mechanisms underlying carcinogenesis. Since most cancers are epithelial in origin, analysis of carcinogenic transformation is necessary for a comprehensive understanding of carcinogenesis. Advances in epithelial cell culture have made it possible to address this question. Human tracheal epithelial cell cultures were initiated on a collagen/fibronectin containing matrix following enzymatic dissociation from biopsy material. Pure cultures of tracheal epithelial cells were generated by a selective detachment protocol which removes contaminating fibroblasts. Epithelial cultures are transfected with a plasmid containing a defective SV40 genome (pSVori$^-$) by calcium phosphate precipitation. After transfection, cultures were grown in serum-free medium and then sub-cultured. A portion of the cells were then grown in serum-containing medium to induce squamous differentiation in non-transformed cells. Treated cells were also tested for SV40-T-antigen expression by immunofluorescence. Modulation of the transformed phenotype and measurements of gene expression are in progress. This work is supported in part by a grant from the Cystic Fibrosis Foundation.

**A 219** DENSITY DEPENDENT INHIBITION OF GROWTH : POSSIBLE INVOLVEMENT OF INHIBITORY DIFFUSIBLE FACTOR (IDF45), [+]L. Harel, [+]C. Blat, [+]G. Chatelain, [*]J. Villaudy and [*]A. Goldé, [+]I. R. S. C., B.P. n° 8 - 94800 Villejuif - [*]Institut Curie, 75005 PARIS (France).

Density-dependent inhibition (DDI) of growth has been assumed to be under the control of inhibitory molecules diffusing from dense cell culture. We purified a growth inhibitory factor (IDF45) from medium conditioned by 3T3 cells. IDF45, at the concentration of about 1 nM, inhibited 50% of DNA synthesis in sparse culture of 3T3 cells. It acted in G1 phase of the cell cycle and was able to inhibit the early stimulation of RNA synthesis induced by growth factors (1). It was also an inhibitor of chick embryo fibroblasts (CEF) growth ; this inhibition was reversible.

We compared the inhibitory effect of IDF45 upon the stimulation of DNA synthesis induced either by serum or by src expression in CEF infected by Ny68 virus (a mutant of Rous sarcoma virus ts for transformation). The stimulation by serum at 41°C was 94% inhibited by IDF45. The same Ny68-infected cells were also stimulated by transfer to 37°C (the permissive temperature for src gene expression) in the absence or presence or serum. This stimulation was poorly inhibited by IDF45. From our results, it appears that oncogene expression in CEF induces a loss in their sensitivity to IDF45. This suggests an hypothesis concerning the release of DDI of growth when v-src is expressed.

(1) FEBS Letters, 203 n° 2, p. 175, 1986.

**A 220** **MODULATION OF NUCLEAR PROTO-ONCOGENE EXPRESSION IN MYELOID LEUKEMIC CELLS BY INTERFERON.** **John Hiscott and Adele Marshall. Lady Davis Institute for Medical Research, and Dept. of Microbiology and Immunology, McGill University, Montreal, Canada.**

Recent studies indicate that interferons (IFNs) and interferon-induced enzyme activities play a critical role in limiting cellular growth and may act via a negative feedback mechanism to control nuclear proto-oncogene RNA production during hemopoietic differentiation. We have used the human myeloid cell lines U937 and KG-1 to examine the transcriptional activation and metabolism of the c-fos and c-myc genes following induction of differentiation by tumor promoting agents, growth stimulation by serum, and/or treatment of cells with exogenously supplied α-interferon (rIFNα2, Schering Corp.). Production of fos and myc RNA was measured by S1 mapping using fos DNA probes which would detect either primary, unspliced transcripts or steady state spliced mRNA levels and using a myc probe which spanned the first exon and the two mRNA start sites, P1 and P2. Pretreatment of quiescent cells with IFN for 18 hr before serum stimulation of growth did not abolish the activation of the fos gene but down-regulated fos expression post- transcriptionally by increasing the rate of mRNA turnover. IFN treatment of KG-1 cells also decreased the serum induced stimulation of myc RNA production. In control KG-1 cells, serum addition resulted in a rapid 50 fold increase in myc RNA transcripts initiated at both P1 and P2 promoters in equimolar amounts; serum stimulation increased myc RNA transcripts only four-fold in IFN-treated cells. These results demonstrate that exogenous IFNα2 treatment of quiescent myeloid cells antagonizes the effect of growth factors by altering the expression and metabolism of nuclear proto-oncogenes.

**A 221** PURIFICATION AND CHARACTERIZATION OF AN HEPATIC PROLIFERATION INHIBITOR, Anthony C. Huggett, Henry C. Krutzsch and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, Bethesda, MD 20892.

The purification of a growth inhibitory protein for liver cells, hepatic proliferation inhibitor (HPI), isolated from rat liver has been previously reported (McMahon et al., PNAS USA 79: 456-460, 1982). A number of improvements in the purification scheme for this inhibitor have yielded a product that has an inhibitory activity 1000-fold greater than that previously reported. The growth inhibitor was labile to heat, low pH and organic solvents. In comparison with transforming growth factor-beta (TBF-β) it was relatively resistant to reducing agents and proteases but was sensitive to treatment with trypsin under conditions which favored protein unfolding. Gel filtration data indicated a molecular weight for HPI of about 25 Kd although inhibitory activity was eluted from SDS-PAGE at 17-19 Kd. The $ID_{50}$ (dose for 50% growth inhibition) of HPI for Fischer neonatal rat liver epithelial cells was 52 pg/ml (2.5 pM), compared to a value of 260 pg/ml (10.4 pM) obtained for TGF-β. Also, in contrast to TGF-β, the growth inhibitory activity was unaffected by the addition of an antibody raised against TGF-β. Thus HPI is a liver-derived growth inhibitory protein that is different from TGF-β. The effect of HPI on the in vitro proliferation of primary hepatocytes and liver epithelial cells will be described.

**A 222** TGF-β: A MODULATOR OF EXTRACELLULAR MATRIX PROTEINS AND THEIR RECEPTORS. Ronald A. Ignotz and Joan Massagué, University of Massachusetts Medical Center, Worcester, MA.

Transforming growth factor-β stimulates the production of the extracellular matrix proteins fibronectin and collagen in cell culture. Cells treated with TGF-β also appear to bind more fibronectin and indeed, increased production of the fibronectin receptor has been observed. Using cloned fragments of fibronectin and α2(I) collagen cDNAs and Northern gel analysis, we find that TGF-β induces an increase in the levels of mRNA for these proteins. The increase in mRNA levels is rapid, preceding the increase in protein synthesis, and persists for at least 24 h. Increases in mRNA levels can be blocked by Actinomycin D but not by cycloheximide. Thus, the increased expression of these proteins correlates with increased mRNA levels, probably due to increased transcription of the respective genes. Further, the changes in mRNA levels appear to be independent of protein synthesis. In systems in which TGF-β inhibits differentiation (3T3-L1 preadipocytes and rat $L_6$ myoblasts) the mRNA levels for fibronectin and collagen are elevated in response to TGF-β. Thus, the inhibition of differentiation correlates with increased expression of genes for extracellular matrix proteins. The addition of exogenous fibronectin or collagen can mimic the effects of TGF-β on the inhibition of differentiation of $L_6$ myoblasts and 3T3-L1 preadipocytes. The available evidence suggests that TGF-β may alter cellular activities through modulation of the extracellular matrix.

**A 223** THE GC BOX AS A SILENCER, Jacek M. Jankowski, Eva Walczyk and Gordon H. Dixon, University of Calgary, Calgary, Alberta T2N4N1.

In a study of a series of plasmid constructs in which the chloramphenicol acetyltransferase gene (CAT) is placed under the control of the trout protamine promoter region and truncated forms of this promoter, it has been shown by transfection experiments with COS-1 cells that the presence of a GC box 3' to the TATA down- regulates the expression of the CAT gene. This is in marked contrast to the stimulating effect of GC boxes located upstream of the TATA signal. We, therefore, propose a new silencing role for the GGGCGG sequence known as the "GC box" which activates initiation of RNA transcription from certain eukaryotic promoters by RNA polymerase II. A repressor role of the SP1 factor which binds to GC boxes in active chromatin is also discussed.

This work was supported by the M.R.C. of Canada and the AHFMR.

**A 224** INACTIVE SECRETED FORM(S) OF TRANSFORMING GROWTH FACTOR-ß (TGFß): ACTIVATION BY PROTEOLYSIS. J. Keski-Oja, R.M. Lyons and H.L. Moses. Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

TGFß is secreted from cultured cells usually in inactive form. We have used NRK-49F cells as a model, and studied by immunoprecipitation and affinity chromatography techniques the activation of latent TGFß. Antibodies to porcine TGFß (pTGFß) were raised in rabbits. Detection of anti-pTGFß -immunoreactive proteins was carried out by immunoprecipitation and polypeptide analyses. The cultures were labeled with [$^{35}$S]cysteine for 24-72 hrs, and the conditioned medium was collected. Immunoprecipitation analysis of acid-treated medium showed a specific 25 kDa protein. Plasmin and cathepsin D treatment of conditioned medium followed by immunoprecipitation yielded a 25 kDa protein as well. Studies using different affinity matrices indicated that [$^{125}$I]TGFß bound efficiently to ConA, and to some extent to heparin and gelatin. Affinity chromatography of radiolabeled medium over ConA-Sepharose, followed by treatment with plasmin, also generated a 25 kDa polypeptide that comigrated with purified TGFß. Plasmin affected several ConA-binding proteins of higher mol.wt., while the generated 25 kDa protein appeared to be resistant. An immunoreactive $M_r$ 12,500 polypeptide was frequently detected in the medium of NRK-49F cells. Plasmin treatment of conditioned culture medium resulted in activity that induced soft agar growth of AKR-2B indicator cells. Inactive forms of TGFß appear to bind to ConA like active TGFß. The activation of latent high mol.wt. form(s) of TGFß in cultured cells apparently takes place via dissociation or proteolytic cleavage.

**A 225** SUPPRESSION OF c-myc BY INTERFERON AND TUMOR NECROSIS FACTOR AND ITS DEREGULATION IN RESISTANT CELLS, A. Kimchi, A. Yarden, D. Resnitzky and M. Einat, Department of Virology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Interferon ($\alpha, \beta$ or $\gamma$) and tumor necrosis factor (TNF) reduce within 1 to 3 hrs the c-myc mRNA levels in some target cells as a result of transcriptional inhibition. Adding interferon-$\gamma$ (IFN-$\gamma$) together with TNF to HeLa cells results in enhanced inhibition of c-myc transcription. The inhibitory effect of TNF is not abrogated, but rather potentiated by cycloheximide while the effect of IFN-$\gamma$ depends on continous protein synthesis[1]. The cooperativity between the two cytokines is being analyzed with respect to the cis-acting DNA elements associated with the effect of each factor, and according to DNA binding proteins studied by DNaseI protection assays. A $\beta$-related IFN functions as an autocrine growth inhibitor during terminal differentiation of hematopoietic cells and autoregulated c-myc expression[2]. In many leukemic cells however, the mechanism of the IFN mediated c-myc inhibition has been impaired[3]. Resistant mutants of myeloid cells were isolated in vitro and analyzed in model systems of differentiation. We find that the IFN resistant cells display abnormal growth behaviour following the exposure to the differentiating stimulus and fail to stop proliferating during differentiation. Analysis of somatic cell hybrids reveals that resistance to c-myc suppression by IFN is recessive and can be overridden by fusion with sensitive cells. This type of c-myc deregulation does not result from changes in the cis-acting elements of c-myc but rather from inactivation or complete loss of other gene/s coding for the putative IFN-mediated dominant suppressive elements. References: (1) Yarden A. & Kimchi A.Science, in press; (2) Resnitzky D, Yarden A, Zipori D. & Kimchi A. Cell 46:31-39 (1986);(3) Einat M, Resnitzky D. & Kimchi,A. Nature 313:597-600 (1985).

**A 226** 1,2-DIOCTANOYLGLYCEROL ($DiC_8$) STIMULATES PHOSPHATIDYLCHOLINE METABOLISM IN $GH_3$ PITUITARY CELLS, Richard N. Kolesnick, Memorial Sloan-Kettering Cancer Ctr., NYC 10021

Phorbol esters have recently been shown to cause degradation of phosphatidylcholine (PC) via a phospholipase C and synthesis via the CDP-choline pathway. It has been suggested that these events describe a new signal transduction pathway. The present studies were performed to determine whether a "physiologic" activator of protein kinase C might produce similar effects in $GH_3$ pituitary cells. The synthetic diacylglycerol, dioctanoylglycerol ($DiC_8$) caused concentration and time dependent incorporation of $^{32}P_i$ and ($^3$H)choline into PC. In cells prelabeled for 1h, $DiC_8$ (100$\mu$g/ml) increased ($^{32}$P)PC and ($^3$H)PC to 164±9 and 150±11% of control, respectively, after 30 min. of stimulation. In subcellular fractions derived from stimulated cells, $DiC_8$ activated CTP: phosphocholine cytidylyltransferase (EC 2.7.7.15), the regulatory enzyme for the CDP-choline biosynthetic pathway. $DiC_8$ increased the particulate activity from 2.12±0.20 to 3.20±0.19 nMol/min/mg protein. Additionally, $DiC_8$ caused specific degradation of PC. In cells labeled to equilibrium with ($^3$H)choline, $DiC_8$(100$\mu$g/ml)caused time dependent reduction of PC to 70% of control and its precursor phosphocholine to 65% of control after 4h and ½h,respectively. Concomitantly, $DiC_8$ enhanced degradation products of phosphatidylcholine -- phosphocholine and choline -- in the medium to 140% of control by 1h, and 180% of control by 2h, respectively. Smaller changes were detected within minutes. These studies suggest that 1,2-diacylglycerol, like phorbol esters, causes degradation of PC by a phospholipase C and synthesis via the CDP-choline pathway. The importance of these events to signal transduction remains to be elucidated.

**A 227** ACTIVATION OF PROTEIN KINASE C IS NOT SUFFICIENT TO INDUCE DIFFERENTIATION OF HL-60 CELLS, A.S. Kraft, V.V. Baker, W.S. May, Divisions of Hematology/Oncology and Gynecologic Oncology, University of Alabama at Birmingham, AL., 35294, and the Division of Hematology, The Johns Hopkins Oncology Center, Baltimore, MD. 21205

Bryostatin (BS), a macrocyclic lactone isolated from a marine bryozoan, binds and activates protein kinase C in vitro in a similar manner to phorbol esters (PEs). However, unlike PEs, BS when added to human promyelocytic leukemia cell line (HL-60) does not induce cellular differentiation (PNAS 83:1334). Now we demonstrate that treatment of HL-60 cells with either BS of PEs causes the down-regulation and stimulates the phosphorylation of the transferrin receptor, as well as five identical cytosolic proteins. In contrast, addition of PEs to HL-60 cells causes a marked decrease in myc RNA, whereas BS treatment over a wide range of concentrations has no effect on myc levels. We investigated whether this effect might be secondary to enhanced degradation of protein kinase C by BS. Treatment of HL-60 cells with .01 $\mu$M BA causes the loss of total cellular protein C activity within 24 hours. In comparison, a 100-fold increased concentration, 1 $\mu$M PE is necessary to induce the same rate of cellular loss in protein kinase C activity. This data suggests that bryostatin may mimic phorbol ester effects in the cytoplasm and not the nucleus. In addition, the lack of ability of BS to induce differentiation may be secondary to the rapid down-regulation of protein kinase C induced by bryostatin.

**A 228** MODULATION OF TRANSFORMED CI27 AND NIH 3T3 CELL PHENOTYPES BY GLUCOCORTICOIDS: DEXAMETHASONE REVERSES TRANSFORMATION BY BOVINE PAPILLOMAVIRUS-1 AND ENHANCES TRANSFORMATION BY V-RAS, V-MOS AND V-FES. Richard M. Levenson, Ute G. Brinckmann and Donald A. Young. Departments of Medicine and Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, New York 14642.
The influence of dexamethasone on the phenotypic behavior of a number of transformed cell lines was examined using three criteria: cell number, morphology and degree of acidification of the medium. fes- and mos-transformed C127 cells and v-Ha-ras-transformed C127 and NIH 3T3 cells exhibit enhanced growth and acid production, and little or no change in morphology when grown in the presence of the synthetic glucocorticoid, dexamethasone (DEX) at $10^{-7}$ M. In these cells, DEX increased maximum cell number from 3 to 62%, accompanied by acidification of the medium by 0.10 to 0.36 pH units. A similar influence of the glucocorticoid was evident in C127 cells transformed by the cottontail-rabbit papilloma virus (CRPV) and by a Bgl II-fragment of human papillomavirus type 5 (HPV-5). In contrast, DEX inhibited transformed behavior in almost all of more than 30 C127 and NIH 3T3 cell lines transformed by bovine papillomavirus-1 (BPV-1), its cloned DNA or subgenomic fragments thereof. Cell number was decreased as much as 68% (average 30%), and the pH of the medium generally increased by 0.30 to 1.00 pH units. Furthermore, these cultures exhibited partial or full reversion to the flat, polygonal appearance of their untransformed parental lines. Glucocorticoid inhibition is fully reversible, since removal of the hormone from the medium allows DEX-treated ID13 cells to resume exponential growth and to reach the same final cell density as that reached by untreated ID13 cells. Supported by grants from the J.P. Wilmot Foundation, German Research Soc., Council for Tobacco Research., USA, (1774) and NIH (AM16177).

**A 229** PLATELET DERIVED GROWTH FACTOR LIKE ACTIVITY IN THE VITREOUS OF PATIENTS WITH UVEAL MELANOMA AND PROLIFERATIVE VITREORETINOPATHY, P.E. Liggett, M.D., *T. Deuel, M.D., *G. Pierce, M.D., W.R. Freeman, M.D., N.A. Rao, M.D., S.J. Ryan, M.D., University of Southern California, Los Angeles, CA 90033, and *Washington University, St. Louis, MO 63119.

We measured growth factor activity in samples of vitreous taken from 12 patients: 3 with uveal melanoma, 1 with metastatic pancreatic carcinoma, and 8 from patients with proliferative vitreoretinopathy. Growth factor activity was measured by tritiated thymidine incorporation of DNA in NIH 3T3 cells when compared to known concentrations of purified PDGF. An ELISA assay using rabbit anti-human PDGF was used to measure the specificity of the growth factor. Vitreous from patients with uveal melanoma, metastatic disease and proliferative vitreoretinopathy showed significant elevation of PDGF-like growth factor activity over control samples. This finding suggests the interesting possibility that a PDGF-like growth factor may play a role in the pathogenesis of ocular neoplastic processes and proliferative vitreoretinopathy.

**A 230** RELEASE OF PHORBOL ESTER-INDUCED MITOGENIC SIGNAL BLOCK BY MUTATION AT Thr 654 OF EGF-RECEPTOR. E.Livneh, T.Dull, R.Prywes, A.Ullrich and J.Schlessinger, Department of Chemical Immunology, The Weizmann Institute of Science Israel.
The mitogenic polypeptide epidermal growth factor (EGF) mediates its biological responses by activating an integral membrane glycoprotein denoted as the EGF-receptor. Analysis of binding experiments of $^{125}$I-EGF to intact cells reveals two affinity classes of EGF towards the receptor. The addition of the phorbol ester (TPA) to cells abolishes the "high affinity" binding sites and reduces the protein-tyrosine kinase activity of the receptor molecule. It was suggested that the phosphorylation of Thr654 may regulate the ligand binding-affinity and the kinase activity of the EGF-receptor. To examine the possible regulatory role of Thr654 we have prepared a Tyr654 EGF-receptor cDNA construct by in vitro site directed mutagenesis. Tyr654 - although being a potential phosphate acceptor site - cannot be phosphorylated by protein kinase C. Regulation of the affinity and the internalization process by EGF and TPA in NIH-3T3 and CHO cell lines expressing the "wild type" receptor and this Tyr654 mutant will be discussed. We could also show that the "wild type" human EGF-receptor construct and the Tyr654 EGF-receptor mutant expressed on the surface of NIH-3T3 cells were both able to stimulate these cells to undergo DNA synthesis in response to EGF. The addition of TPA to the cells expressing the "wild type" receptor blocked the mitogenic capacity of EGF. However, this inhibition did not occur in the NIH-3T3 cells expressing the Tyr654 EGF-receptor mutant. Hence, we propose that the phosphorylation of Thr654 by kinase-C may provide a negative control mechanism for EGF-induced mitogenesis in mouse NIH-3T3 fibroblasts.

A 231 CYCLIC AMP REVERSES THE TRANSFORMATION OF 3T3 CELLS BY THE *SIS* ONCOGENE. A.H. Lockwood, A. Lazarus, S.K. Murphy and M. Pendergast. Albert Einstein Medical Center, Philadelphia, PA 19141

The *sis* oncogene encodes a protein virtually identical to human platelet-derived growth factor (PDGF). NIH3T3 fibroblasts transfected with the cloned *sis* oncogene display a malignant phenotype in culture. We have found that elevation of intracellular cyclic AMP (cAMP) can restore many aspects of normal cell growth and morphology to *sis* transformed cells. In the presence of 8-Br-cAMP, cells rapidly become less refractile, flatten on the substratum, develop actomyosin bundles and acquire a more tranquil membrane. Growth rate and saturation density are reduced. Cultures become contact inhibited and, at confluence, assume a normal fibroblastic morphology. The ability of the *sis* transformed cells to grow in reduced serum is lost. SDS-polyacryamide gel analysis of cultures labelled with $^{32}PO_4$ reveals specific alterations in cellular phosphoproteins induced by cAMP. These results demonstrate that cAMP, presumably acting through protein kinase A, can attenuate the function of the *sis* oncogene. They suggest that, in normal cells, the cAMP signal transduction system can modulate physiological response to PDGF (supported by NIH grant CA39232).

A 232 **RETINOID AND PHORBOL ESTER ANTAGONISM OF EGF ACTION IN T24 HUMAN BLADDER CARCINOMA CELLS,** Anne M. McCormick and Matt Knight, UTHSCD, Dallas, TX 75235. The synthetic retinoids 4-hydroxyphenylretinamide (HPR, $ED_{50}$ 5 μM) and 13-*cis*-N-ethylretinamide (13ER; $ED_{50}$ 16 μM) reversibly inhibited the anchorage-dependent and the anchorage-independent proliferation of T24 human bladder carcinoma cells ($ED_{50}$ 5μM). To gain insight into retinoid mechanism of action in the control of transformed cell proliferation, HPR effects on EGF-stimulated T24 cell proliferation were examined. The mitogenic response of T24 cells to EGF was reversibly inhibited by HPR ($ED_{50}$ of 5 μM) indicating that the antiproliferative activity of HPR could be related to antagonism of EGF action. HPR treatment did not alter the number or EGF-binding affinity of cell surface EGF receptors. HPR decreased the rate, but not the overall magnitude, of EGF-receptor complex internalization (80-90% internalization within 2 hr) in T24 cells. In contrast, the protein kinase C activator 12-O-tetradecanoyl-phorbol-13-acetate (TPA) caused an irreversible inhibition of anchorage-dependent T24 cell proliferation. Furthermore, the antiproliferative activities of HPR and TPA were additive. TPA treatment resulted in a loss of high affinity EGF receptors ($K_d$ 0.1 nM) paralleled by an increase in total surface EGF receptors (low affinity; $K_d$ 1 nM). The available data indicate that the observed antagonism of EGF action by HPR is not exerted by direct effects on EGF binding and may not be mediated by a protein kinase C-dependent mechanism. HPR effects on EGF receptor tyrosine kinase and protein kinase C activity in T24 cells are currently under investigation.

A 233 CLONAL ANALYSIS OF THE COLONIZING ABILITY OF SPONTANEOUSLY TRANSFORMED MALIGNANT CELL. Peter T. Mora and Christopher A. Dale Smith, NCI, NIH, Bethesda, MD 20892, and David Winterbourne, Dept. of Surgery, St. George's Hospital Medical School, London SW 17 ORE, England.

A clonal cell line of low tumorigenicity, derived from a mouse embryo mass culture, was injected into syngeneic animals in a sufficient dose ($10^7$ cells) to produce tumors (sarcomas). Cell lines and clones produced from several such tumors were found to have acquired a $10^4$ to $10^5$ higher level of tumorigenicity. Anchorage-independent growth did not co-select with tumorigenicity. There was no evidence for overexpression of dominantly acting cellular oncogenes in the highly tumorigenic clones; p53 protein and mRNA levels were also essentially equal and low. However, in all the tumor cell lines (10/10) when compared with their parent clone there was a specific structural difference in the O' sulfate residues in oligosaccharides of heparan sulfates. The results imply that the more highly sulfated heparan sulfates relate to the growth suppression *in vivo* of the normal cells, while this phenotype in the sarcoma cells is recessive and could relate to the acquired high colonizing ability in the host.

**A 234** GROWTH REGULATION OF THE HUMAN β-ACTIN GENE PROMOTER, Sun-Yu Ng*, Peter Gunning†, Larry Kedes† and John Leavitt*, *Linus Pauling Institute, Palo Alto CA 94306 and †Stanford Medical School and †Veterans Administration Medical Center, Palo Alto CA 94305.

The human β-actin gene promoter is one of the most active cellular promoters. We have shown previously that exogenous β-actin genes are expressed at high levels in transfected cell lines. We now use the expression of β-actin promoter directed chloramphenicol acetyl-transferase (CAT) as an *in vivo* promoter assay to map the positions of various regulatory elements. Full promoter activity requires the presence of about 2 kilobases of 5′ flanking sequences. Moreover, enhancer element(s) seem to be located in the more distal region. The longest intron, containing evolutionarily conserved sequences, interrupts the 5′ untranslated region of the β-actin gene. Additional enhancer activities, though not as strong as those in the 5′ flanking region, are located in the conserved sequences. However, deletion of this intron, surprisingly, does not lead to lower CAT activity. In stable cell lines, the expression of intron bearing CAT genes, after serum starvation, are inducible by serum or growth factors. In contrast, the expression of CAT genes lacking this intron are constitutive and serum independent. Therefore, the serum responsive element(s) of the human β-actin gene appears to be located in this first intron.

**A 235** BOVINE IGF'S: GENOME ORGANIZATION, SEQUENCES, AND EXPRESSION IN BOVINE TISSUES, SHARON D. OGDEN, JOHN C. MINNERLY, KATHERINE W. PRITCHARD AND GWEN G. KRIVI

The insulin-like growth factors (somatomedins) are primary mediators of somatotropin action throughout mammalian development and are also known to be potent mitogens for many types of cells in culture. According to the somatomedin hypothesis, IGF's, especially IGF-1, are released from the liver in response to somatotropin action at hepatic receptors. The focus of our research is the elucidation of the molecular mechanisms underlying the regulation of IGF gene expression in animal cells. In order to investigate IGF gene expression in the cow, we have cloned and sequenced the genes for bIGF-1 and bIGF-2 from genomic libraries. We find that the genomic structure, including the number, size, and position of introns, is highly conserved cross-species. The nucleotide sequence of coding as well as non-coding exons is also highly conserved. Surprisingly, when we assayed the expression of bIGF-1 in a variety of tissue types in the calf and adult cow, we found levels comparable to those found in the liver. This finding suggests that the liver may not be the sole source of somatomedin production in the cow.

**A 236** LEUKOTRIENES AS LATE SIGNALS FOR GROWTH FACTOR STIMULATION OF DNA SYNTHESIS, Ted W. Reid and Valerie Hamill, Univ. Calif. Davis Med. Centr., Sacramento CA 95816

Using mouse lens epithelial cells we find that the inhibitors of arachidonic acid metabolism effect the ability of growth factors to stimulate these cells to progress from G1/Go to S phase. Aspirin, which inhibits $PGH_2$ synthetase (cyclo-oxygenase, the first step in the prostaglandin pathway), caused an increase in growth factor stimulation of DNA synthesis. Indomethacin inhibits cyclo-oxygenase at low concentrations and phospholipase $A_2$ at high concentrations. At low concentrations (0.6 uM) indomethacin shows stimulation of DNA synthesis, in the presence of growth factors, while at higher concentrations (>1.0 uM) indomethacin shows inhibition of DNA synthesis. The half maximal concentrations of indomethacin for inhibition of DNA synthesis were: 4 uM for insulin; 3.4 uM for EGF; and 30 uM for RDGF (retinoblastoma derived growth factor). On the other hand, nordihydroguaiaretic acid (NDGA), which inhibits the lipoxygenase pathways, showed only inhibition of DNA synthesis and was half maximal at 90 ng/ml. More importantly we found that NDGA worked best 6 hours after the addition of the growth factor. NDGA also inhibited DNA synthesis late in the cycle (12-15 hours) while DNA synthesis starts at approximately 14.5 hours. This was consistant with the finding that arachidonic acid would overcome the inhibition of indomethacin only if added after 7-9 hours. These results imply that products of the lipoxygenase pathway are necessary for the stimulation of DNA synthesis by growth factors. The fact that arachidonic acid by itself or inhibitors which stimulate leukotriene synthesis by sparing arachidonic acid do not stimulate DNA synthesis, shows that leukotrienes may be necessary for DNA synthesis but they are not sufficient.

**A 237** RESPONSES OF NBT-II BLADDER CARCINOMA CELLS TO CONDITIONED MEDIUM FROM NORMAL FETAL UROGENITAL SINUS, David R. Rowley and Donald J. Tindall
Baylor College of Medicine, Houston, Texas 77030.

Previous *in vivo* tissue recombinant studies have shown that fetal rat urogenital sinus (UGS) stroma can induce a prostate-specific phenotype in normal bladder epithelium. To address possible mechanisms we have conducted *in vitro* studies to determine whether conditioned medium from UGS organ explants would affect phenotypic characteristics of NBT-II urinary bladder carcinoma cells in culture. NBT-II cells were exposed to medium (30% v/v) conditioned for 48 hours by intact UGS explants derived from 18-day fetal rats. Upon exposure for 23 hours the [$^{3}$H]thymidine incorporation (2 hr pulse) by NBT-II cells was decreased by 40.3% relative to control cultures. This effect was paralleled by a similar decrease in proliferation. NBT-II cultures decreased in cell number by 32.1% and 45.8% on days 2 and 4 respectively after exposure to conditioned medium. UGS explant conditioned medium was capable of inducing an increase in the secretion of newly synthesized proteins. An increase of 18.6% was observed in the incorporation of [$^{35}$S] methionine into newly synthesized, secreted proteins by NBT-II cells exposed to UGS conditioned medium for 23 hours. Morphologically the NBT-II cells exposed to UGS conditioned medium were more spread out, larger, and exhibited a greater array of lamellipodia and filopodia, although [$^{35}$S] methionine incorporation into cellular proteins was decreased by 11.1%. These results suggest that diffusable factors produced by UGS organ explants can induce phenotypic changes in proliferation, protein synthesis, protein secretion, and morphology of NBT-II carcinoma cells in culture. Supported by NIH grants RR05425, AM3597, AM37688, CA32387 and NSF DCB8416979.

**A 238** REGULATION OF C-FOS TRANSCRIPTION BY NEGATIVE AND POSITIVE CELLULAR FACTORS.
Paolo Sassone-Corsi and Inder M. Verma, The Salk Institute, San Diego, CA 92138

Proto-oncogene fos is a multifaceted gene whose expression is induced in response to a variety of growth factors and differentiation-specific agents. The c-fos gene promoter region has recently been delineated. In particular, an element essential for transcription activation has been localized between nucleotides -332 and -276 relative to the mRNA cap site. It contains a region of hyphenated dyad symmetry and has properties similar to those described for a transcriptional enhancer. To further understand the regulation of the c-fos expression, we designed in vivo experiments which could identify the presence of putative trans-acting cellular factors responsible for c-fos activation upon serum induction.

Recent results indicate that c-fos gene transcription is very rapidly stimulated by serum, even when the cells have been previously treated with protein synthesis inhibitors like cycloheximide or anisomycin. To explain this result and the dramatic kinetics of the c-fos induction, we considered the possibility that the trans-acting factor(s) required for the c-fos gene transcription must be present prior to serum treatment.

The results indicate that the c-fos gene expression is regulated by both positive and negative trans-acting cellular factors. We show that the positive cellular factor(s) is present in fibroblasts, both when they are starved or stimulated with serum. The negative factor(s) is responsible for the c-fos transcriptional block in serum-starved fibroblasts, and is perhaps modified upon serum stimulation to allow efficient expression.

**A 239** TUMOR NECROSIS FACTOR-ALPHA STIMULATES BIOLOGICAL ACTIVITIES OF ACTIVATED HUMAN T LYMPHOCYTES, Peter Scheurich, Beate Maxeiner, U. Ücer, and K. Pfizenmaier, Clinical Research Group "BRHTI", Max-Planck-Society, University of Goettingen, FR Germany.

Binding studies using iodinated recombinant tumour necrosis factor alpha (TNF-alpha) revealed that membrane receptors specific for TNF-alpha are induced on human peripheral T lymphocytes upon primary activation. When activated interleukin 2 (IL 2) dependent T cells were treated with high doses of TNF-alpha, stimulatory effects on cell growth were found. These findings are more likely related to enhanced IL 2 receptor expression as to IL 2 production, as non-limiting concentrations of IL 2 had been added exogenously. Immunofluorescence analysis by flow cytometry confirmed that TNF treatment not only elevated membrane expression of HLA, but also of Tac antigens. These data indicate that TNF is involved in the control of IL 2 receptor expression and thus shares this biological activity with IL 1. In addition to growth stimulatory effects, TNF-alpha enhanced the IL 2 dependent production of IFN-gamma by activated T cells. This is in contrast to recombinant IL 1, which under the same conditions failed to influence IFN-gamma production. Neither, endogeneously nor exogeneously produced IFN did interfere with T cell proliferation, suggesting that IFN-gamma plays no dominant role in regulation growth of activated T cells. This might be due to the low numbers of IFN-gamma receptors (about 300/cell), thereby decreasing the sensitivity of T cells to direct effects of IFN-gamma.

**A 240** A TEMPERATURE SENSITIVE CHINESE HAMSTER CELL LINE WITH A DEFECT THAT AFFECTS TRANSCRIPTION BY RNA POLYMERASE I AND II. Thelma C. Slezynger and Immo E. Scheffler, U.C. San Diego, Dept. of Biology, La Jolla, CA 92093.

The cause of defective transcription in a temperature sensitive Chinese hamster cell line is currently being investigated. After 12 hours at 40°C, there is a 40-50% decrease in RNA synthesis in mutant cells.

Run-on transcription in isolated nuclei shows that after 6 hours at 40°C, there is a decrease in the rates of transcription of rRNA and actin genes, while transcription of 5S genes is not changed. This result suggests that the mutation affects transcription by RNA polymerases I and II but not III. We would like to know which RNA polymerase is primarily affectd by this mutation. Quantitative differences in transcription rates by either RNA polymerase I or II at early times after a shift to 40°C are now being studied.

**A 241** HIGH-RESOLUTION MAPPING OF DNaseI HYPERSENSITIVE SITE IN THE PROMOTER REGION OF THE TRANSFECTED CHICKEN β-TUBULIN GENE, Yu-lin Sun, Ching-I Pao, Subrata Sen and Tien Kuo, U T System Cancer Center, Houston, Texas 77030.

A new method for high-resolution mapping of nuclease hypersensitive site (HS) at the promoter region of the chicken $\beta_2$-tubulin gene which has been transfected into monkey Cos-7 cells will be presented. We first determined the HS by the indirect end-labeling method of Wu, and a tissue-specific nuclease HS site around 100 bp upstream of the transcription start site has been located. High-resolution mapping of the HS in the transfected DNA isolated from nuclease-treated Cos-7 nuclei was determined by subcloning a DNA fragment of about 900 bp containing the promoter region into M13 phage. A single-stranded DNA probe with a defined restriction enzyme cleavage site at one was prepared from the M13 phage DNA. The single=stranded probe, after being annealed back to the template, was ligated with the restriction enzyme-digested transfected DNA, which had been similarly reannealed onto the template in a ternary complex. The HS was mapped with precision at the single nucleotide level in a DNA sequencing gel using a dideoxynucleotide sequencing ladders as reference. We mapped a major HS at the -107 nucleotide of the $\beta_2$-tubulin gene, where a CACCC-like sequence is present. This method will be applicable to determine fine structure of chromatin containing high copy numbers of specific gene.

**A 242** THE HUMAN PROSTATE TUMOR CELL LINE LNCaP : ANDROGEN RECEPTORS, GROWTH CHARACTERISTICS AND ANDROGEN STIMULATED PROTEIN SECRETION. D.van Loon, E.Berns, A.O.Brinkmann and E. Mulder. Dept.Biochemistry 2, Erasmus University, 3000 DR Rotterdam, THE NETHERLANDS.

In search for a possible autocrine regulatory function in androgen dependent tumors, we have studied synthesis and release of androgen stimulated proteins in androgen sensitive LNCaP cells. The nuclear extract of the LNCaP cells contained androgen receptors, corresponding to 17,000 ± 2,500 receptor sites/cell(n=5). The receptor sedimented at 4.5 S and showed a molecular mass of approximately 100 kD by photoaffinity labelling with R1881, followed by SDS-PAGE.Estrogen and progesterone receptors were not detectable in the nuclear extracts nor in the cytosol. The growth of the cells was stimulated by androgens in a dose-dependent way. Under optimal conditions, the nonmetabolizable androgen R1881 (0.1nM) stimulated cell growth 2-10 times, depending on the concentration of the fetal calf serum present in the medium. Cells grown in media containing charcoal treated fetal calf serum released significantly lower amounts of several ($^{35}$S)-methionine labelled proteins, especially of a protein with a molecular mass of approximately 40 kD. The release of this protein could be restored from cells cultured in the presence of dihydrotestosterone (DHT, 0.1 - 1 μM) or R1881 (0.1 nM-0.1 μM),whereas estrogens, corticoids and progesterone had no effect. Anti-androgens, which inhibit cell growth, also exerted inhibitory effects on the release of the 40 kD protein. The observed correlation between the effects of (anti)-androgens on the growth of the LNCap cells and the release of the 40 kD protein could be related to the regulation of malignant prostate cell growth.

**A 243** **ANTIPROLIFERATIVE EFFECT OF ANTIESTROGENS, PROGESTINS AND ANTIPROGESTINS IN HUMAN BREAST CANCER CELLS**, Françoise Vignon, Sylvie Bardon, Dany Chalbos, Peter G. Gill, Danielle Derocq, and Henri Rochefort. Unité d'Endocrinologie Cellulaire et Moléculaire, U148 INSERM, 60 Rue de Navacelles, 34100 Montpellier France.
The growth of breast cancer cells is inhibited in vitro and in vivo by antiestrogens such as tamoxifen, progestins such as R5020 and antiprogestins such as RU486. However, the mechanism by which such hormones and antihormones prevent cell growth is presently unknown. We have shown that the antiproliferative effect of antiestrogens was mediated by the estrogen receptor while that of both progestins and antiprogestins required the presence of the progesterone receptor.
The antiproliferative effect of these 3 types of compounds was always correlated with and preceded by a marked decrease in the production of proteins secreted by breast cancer cells. The estrogen regulated 52K protein was more specifically inhibited by antiestrogens and progestins but not by RU486. This common inhibitory effect on secreted proteins may lead to a decrease of the production of autocrine mitogens such as growth factors and proteases, eventhough the initial mechanisms responsible for this inhibition may be different for the three drugs. Progestins stimulate also the expression of several specific proteins suggesting that they may also act by inducing specific inhibitory factors.
We conclude that the selectivity of action of these three drugs is due to their affinity for their hormone receptors and suggest that the decreased production of autocrine mitogens may be a common mechanism.

**A 244** INTERNALIZATION OF INTERFERON-αA AND ITS RECEPTOR IN HUMAN EPITHELIAL TUMOR CELL LINES. S. Vuk-Pavlović, B.T. Vroman, J.S. Kovach. Division of Developmental Oncology Research, Mayo Foundation, Rochester, MN 55905.
Recombinant human interferon-αA (IFN) inhibited colony formation on plastic of lung alveolar carcinoma A549 more than hundred times more efficiently than of melanoma A375 (Proc. AACR, 27:342, 1986). Scatchard analysis of IFN binding indicated that numbers of Type I-IFN receptors and affinities of these receptors for IFN did not differ significantly between these two cell lines. Therefore, we analyzed whether different susceptibility to antiproliferative activity of IFN was correlated to differences in trafficking of IFN-receptor complex in these cell lines. We measured concentrations of surface associated and internalized $^{125}$I-IFN as a function of time at 37 °C. Using a computer program developed in our laboratory we computed association and dissociation rate constants for IFN-receptor interaction, the rate of new receptor insertion into plasma-membrane ($V_r$), rate constants for internalization of free-receptor ($k_t$) and of receptor-ligand complex ($k_e$), the rate constant of radiolabel elimination ($k_h$) and concentrations of free, occupied and internalized receptors at the beginning of experiment and at steady state. The $V_r$ value for IFN-insensitive A375 cells was seven times larger than for A549 cells resulting in proportionally higher concentrations of all receptor species at any time. The $k_e/k_t$ ratio was approximately 40 for both cell lines. The $k_h$ value could not be computed for A375 cells, but was significantly larger than for A549 cells. Supported by Mayo Cancer Center Grant CA15083D2, NCI, and by Hoffmann-La Roche, Inc.

**A 244A** HEAT SHOCK AND MITOGENS ACTIVATE PHOSPHOLIPASE C AND PHOSPHOLIPASE $A_2$ IN MAMMALIAN CELLS. S.K. Calderwood and M.A. Stevenson, Dana Farber Cancer Institute, 50 Binney Street, Boston, MA 02115. Treatment of cells with heat shock or mitogens induces overlapping responses; mitogenic stimulation induces expression of heat shock genes and heat shock in some circumstances may be mitogenic. We have compared the effects of heat shock and mitogens on the activity of phospholipase C and phospholipase $A_2$, two enzymes important in signal transduction. Heat shock activates phospholipase C in CHO Balb c 3T3 and Pc-12 cells; increases in inositol trisphosphate after heat are similar in magnitude to changes induced by serum. Heat shock also increases levels of diacylglycerol and intracellular-free $Ca^{2+}$. In addition, preliminary evidence indicates that chelation of $Ca^{2+}$ with EGTA inhibits the induction of heat-shock proteins by a 37°C → 43°C shift. This may indicate a role for $Ca^{2+}$ in hep induction. Heat shock also induces arachidonic acid release by phospholipase $A_2$ stimulation. Similar degrees of arachidonic acid release are also induced in CHO cells by mitogens thrombin and bradykinin. Changes in arachidonate release appear to be correlated with heat and agonist-induced alterations in protein phosphorylation. Arachidonate release induced by each of the agonists and heat shock is inhibited by glucocorticoids. The data indicates that heat shock and growth factors lead to some common patterns of signal induction which may be involved in their overlapping cellular effects.

# Poster Session V

**A 245** RAS p21 PROTEINS MODULATE PHOSPHORYLATION OF SPECIFIC PROTEINS IN PURIFIED RAT LIVER PLASMA MEMBRANE. Joseph Backer, Columbia University, New York, N.Y. 10032.

We have found that when rat liver plasma membrane, purified via centrifugation through Percoll gradient, are incubated with [$\gamma$-$^{32}$P]ATP and SDS-lysed samples are analyzed by SDS-PAGE there is efficient phosphorylation of two proteins with $M_r$ 17.5 and 18.5kD. Addition of the bacterially expressed human Ha-ras p21 proteins to the reaction mixtures alters phosphorylation of the plasma membrane proteins in two ways. Firstly, both normal and transforming ($val^{12}$) ras p21 proteins strongly inhibit phosphorylation of the 17.5 and 18.5kD proteins. Secondly, addition of the ras p21 proteins to the reaction mixture induces phosphorylation of a new protein with $M_r$ 36kD. Normal ras p21 protein is 5-10 times more effective in this assay than transforming ras p21 ($val^{12}$) protein.

We have found that phosphorylation of the 17.5 and 18.5kD proteins occurs in mitochondrial and microsomal fractions, as well as in 100S fraction. Bacterially expressed human Ha-ras p21 proteins inhibit phosphorylation of the 17.5 and 18.5kD proteins in all subcellular fractions and induce phosphorylation of the 36kD protein in the mitochondrial and microsomal fractions. We have previously reported that phosphorylation of similar 17 and 36kD proteins occurs in the soluble protein fraction dissociated from mitochondrial membrane (10K pellet) by physiological concentrations of glucose-6-phosphate, inositol phosphate and inositol triphosphate (PNAS, 83, 6357, 1986). Taken together, these results suggest that plasma membrane associated ras p21 proteins may modulate the extent of phosphorylation of the specific proteins which are redistributed between various intracellular loci.

**A 246** ACTIVATION OF THE NEU ONCOGENE. Cornelia I. Bargmann and Robert A. Weinberg, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, 9 Cambridge Center, Cambridge, MA 02142.

The neu oncogene is a transforming oncogene frequently isolated from neuro- and glioblastomas of the rat. neu is related to, but distinct from, the gene which encodes the EGF receptor. The sequence of cDNA clones of neu has been determined. Neu encodes a predicted transmembrane protein product which is 50% identical to the EGF receptor at the amino acid level, with over 80% identity in the putative tyrosine kinase domain. By analogy with the EGF receptor, neu appears to encode the receptor to an unidentified growth factor. cDNA clones from normal and transforming alleles of the neu gene have been isolated. The transforming cDNA yields foci on an NIH 3T3 monolayer when inserted into expression vectors, but the normal cDNA does not, although it directs the synthesis of comparable amounts of p185, the neu gene product. The functional difference between the two cDNAs has been localized to a single nucleotide change altering a valine residue in the normal allele to a glutamic acid residue in the transforming allele. This residue, valine 664, falls within the single membrane-spanning domain of p185. Mutagenesis has been performed on the normal and transforming alleles of neu to delineate the domains important for protein function. Virtually all of the extracellular sequences of neu can be deleted without loss of the transforming activity of the activated allele. However, mutations which destroy the tyrosine kinase activity of p185 or prevent proper localization of p185 at the cell surface are no longer transforming. The normal neu cDNA can be activated by a single truncation of its extracellular sequences, although high expression of this protein is necessary for transformation. Various replacements of valine 664 have been constructed. Replacement of this residue by glutamine leads to a neu protein comparable in transforming potential to the naturally arising glutamic acid residue. Aspartic acid, lysine, and glycine residues have also been generated; these mutants are much less active in a focus assay.

**A 247** ESTROGEN REGULATION OF TGF$\alpha$ mRNA IN HUMAN BREAST CANCER, Susan E. Bates, Rik Derynck, and Marc E. Lippman, National Cancer Institute, Bethesda, MD 20892. We have studied the role of estrogen regulation of human breast cancer in the development and progression of malignancy. We have examined the regulation of several polypeptide growth factors by estrogen, including TGF$\alpha$, TGF$\beta$, and IGF-1. TGF$\alpha$ is induced 2-8 fold by estrogen treatment of the estrogen-receptor positive human breast cancer cell line, MCF-7. To learn whether this induction occurred at the level of gene expression, we analyzed RNA from MCF-7 cells and observed a 2-8 fold increase with estrogen in both poly-A selected and total RNA in the level of a 4.8kb and a 1.6kb message, as detected by a synthetic riboprobe made from a cDNA encoding the TGF$\alpha$ precursor. The induction is present 6 hr after estrogen treatment, and remains stable for at least 6 days of continued estrogen treatment. Insulin, another hormone which promotes the growth of MCF-7 cells in culture but unlike estrogen does not support tumorigenesis in animals, fails to induce TGF$\alpha$ mRNA or protein. To learn whether the presence of estrogen receptor correlated with TGF$\alpha$ mRNA production in human breast cancer, we analyzed 36 human tumors. By Northern analysis, 50% of these tumors contained TGF$\alpha$ mRNA, but there was no correlation with receptor status. Thus, although TGF$\alpha$ appears to be related to the growth promoting effects of estrogen in human breast cancer, its presence is not limited to tumors responsive to estrogen.

**A 248** INDUCTION OF CLONAL MONOCYTE/MACROPHAGE TUMORS BY A MOUSE C-MYC RETROVIRUS: EVIDENCE FOR SECONDARY TRANSFORMING EVENTS. W. R. Baumbach, E. R. Stanley* and M. D. Cole, Department of Molecular Biology, Princeton University, Princeton, NJ 08544;*Albert Einstein College of Medicine, Bronx, NY.

A mouse retrovirus containing the c-myc oncogene was found to induce tumors of mononuclear phagocytic cells in vivo. All tumors expressed the c-myc retroviral gene, but not the endogenous c-myc gene, and virtually all tumors were clonal with a unique proviral integration. This observation, coupled with a lag-time in tumor formation, suggests that a second event, in addition to c-myc proviral integration, is necessary for the generation of neoplastic cells in vivo. The tumor cells expressed high levels of both c-fos and c-fms proto-oncogene RNAs (the latter probably encodes the monocyte-specific CSF-1 growth factor receptor). The tumor cells proliferate in culture without the addition of the mononuclear phagocytic growth factor CSF-1, which is required for the proliferation of primary macrophages partially transformed by the same c-myc retrovirus. One tumor was found to secrete high levels of CSF-1, to express altered CSF-1 mRNA and to have a DNA rearrangement at the CSF-1 locus. Conditioned medium from this tumor line stimulated growth, which could be mimicked by CSF-1 and blocked by specific anti-CSF-1 antiserum. The clonality and autocrine growth properties of this tumor suggest that the continual activation of the CSF-1 receptor signal pathway may be the second event required for in vivo tumor formation.

**A 249** STEROID INDUCTION OF GRANULOSA CELL TUMORS IN SWR MICE, W.G. Beamer and B. J.Tennent, Jackson Laboratory, Bar Harbor, ME 04609. Ovarian granulosa cell (GC) tumors in SWR and in SWXJ recombinant inbred mice are strikingly similar to human juvenile GC tumors. In both species, GC tumors are spontaneous, appear prior to adulthood, display similar histopathology, are associated with disturbed endocrine activity, and have malignant properties (Can.Res.45:5575, 1985). Recent reports that adrenal dehydroepiandrosterone (DHEA) ameliorates obesity, diabetes, mammary tumors, and carcinogen-induced tumors in mice contrasts sharply with its effects on GC tumor incidence. We found that DHEA promotes GC tumors in pubertal SWR and SWXJ strains with spontaneous tumors and induces tumors in some previously tumor-free strains. In SWXJ49 mice, feeding testosterone (T) also promotes GC tumors, whereas feeding estradiol (E2) suppresses GC tumorigenesis. Serum steroid analyses of SWXJ49 mice fed DHEA, T, or E2 showed that: 1) DHEA increased all androgen and estrogen levels; 2) T increased all androgen but not estrogen levels; and 3) E2 suppressed DHEA without affecting other androgen levels and markedly increased estrogen levels. These data suggest that DHEA or its androgen metabolites can induce GC tumors in genetically predisposed mice. We hypothesize that inappropriate sequencing or levels of natural steroids during pubertal ovarian development combined with a specific genotype results in GC tumorigenesis.

**A 250** FUNCTIONAL ACTIVATION OF THE c-src PROTO-ONCOGENE IN HUMAN COLON CARCINOMA. Joseph B. Bolen[1], Andre Veillette[2], Virginia DeSeau[1], Arnold M. Schwartz[3], and Neal Rosen[2], [1]Laboratory of Tumor Virus Biology, [2]Medical Breast Cancer Section, National Cancer Institute, Bethesda, MD 20892, and [3]Department of Pathology, The George Washington University Medical Center, Washington, D.C. 20037.

We demonstrate that the tyrosine-specific protein kinase activity of $pp60^{c\text{-}src}$ molecules obtained from human colon tumor tissues and tumor-derived cell lines is consistently and markedly elevated over that found in normal colon mucosa tissues and culture of normal colon mucosa cells. The elevation of $pp60^{c\text{-}src}$ kinase activity in tumor tissues and cultured colon tumor cells is not the result of increased expression of the c-src encoded protein suggesting that the specific activity of the $pp60^{c\text{-}src}$ phosphotransferase is enhanced in these tumor tissues and tumor-derived cell lines. In vitro translation of c-src mRNA from colon tumor cells and normal colon mucosa cells yielded $pp60^{c\text{-}src}$ molecules with similar levels of protein kinase activity suggesting that the regulation of $pp60^{c\text{-}src}$ kinase activity in these cells is regulated differently at the post-translational level. Analysis of the $pp60^{c\text{-}src}$ molecules from normal colon and colon carcinoma cells revealed that despite the differences in protein kinase activity, both possessed an equal number of phosphorylated tyrosine residues in the carboxy-terminal end of the molecules. The biochemical basis for the activation of $pp60^{c\text{-}src}$ kinase activity from colon tumor cells is, therefore, thought to be related to the observed increase in the turnover rate of tyrosine-phosphates within the carboxy-terminal portion of these tumor cell-derived $pp60^{c\text{-}src}$ molecules.

**A 251** C-MYC AMPLIFICATION CORRELATES WITH TUMORIGENICITY IN A HUMAN BREAST CARCINOMA CELL LINE. O. Brison[1], N. Modjtahedi[1], C. Lavialle[2], M.-F. Poupon[2], R.-M. Landin[2], R. Cassingena[2], and R. Monier[1]. 1) I.G.R., rue Camille Desmoulins, 94805 Villejuif Cedex. 2) I.R.S.C., BP 8, 94802 Villejuif Cedex, France. Cell line SW 613-S was derived from a human breast carcinoma and is heterogeneous: some cells have a 5-fold amplification of the c-myc gene and the additional copies are integrated into one chromosome; others have a 15-fold amplification and the copies are localized in double minute chromosomes (DM). When the line was grown in vitro, cells with DM were gradually lost but when it was grown in vivo, as tumors in nude mice cells with a high DM content and a high level of c-myc amplification were selected. Subclones of SW 613-S were isolated. Some contained only cells with no DM and had a low level of amplification. The others contained DM and had various levels of amplification. Subclones with a low c-myc genes content were not tumorigenic whereas those with a high level of amplification were very tumorigenic. Our results indicate that c-myc expression must reach a threshold level in order for these cells to be tumorigenic. The in vitro growth rates of both types of subclones were similar in medium with a high or a low concentration of serum but growing SW 613-S cells in vitro in the presence of mouse fibroblasts selected cells with a high DM content.

**A 252** DIFFERENTIAL PROMOTER UTILIZATION BY THE C-MYC GENE IN MITOGEN AND INTERLEUKIN-2 STIMULATED HUMAN LYMPHOCYTES. H.E. Broome, J.C. Reed, E.P. Godillot, R.G. Hoover. Department of Pathology, University of Pennsylvania Medical School, Philadelphia, PA 19104-6082.

Transcription of the c-myc gene is initiated 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 the relative levels of the transcripts are usually expressed as a P1/P2 ratio. Certain Burkitt lymphoma and murine plasmacytoma cell lines with translocations 5' of the c-myc promoters have P1/P2 ratios greater than 1.0. All other lymphoid cell lines and tissues studied have P1/P2 ratios less than 0.5. We demonstrated that a shift in promoter utilization occurred in human peripheral blood mononuclear cells (PBMC) that were stimulated to proliferate. The P1/P2 ratio reached a maximum of approximately 1.6 at 8 hours after phytohemagglutinin (PHA) stimulation and a minimum of 0.30 at 48 hours. At certain times after the initial PHA stimulation, addition of recombinant interleukin 2 (IL2) to PHA-stimulated PBMC increased c-myc mRNA, predominantly from P2, decreasing the P1/P2 ratio. Our findings suggest that P1 is involved in the initial activation step of PBMC with PHA, which is required for PBMC to become responsive to other growth signals. In contrast, P2 may be involved with the response of PBMC to the secondary signal, IL2, which is required for cell cycle progression.

**A 253** PHOBOL ESTER-INDUCIBLE DNA ELEMENTS IN SV-40 ENHANCER, Robert Chiu, Masayoshi Imagawa, Richard Imbra and Michael Karin, University of California, San Diego, La Jolla, CA.92093.

It has been reported that phobol ester (TPA) specifically induces the Simian virus 40 (SV40) transcriptional activity in a human hepatoma cell line (HepG2). To delineate the control elements required for response to TPA, we have examined the regulation of human $\beta$-globin gene expression by SV40 enhancer. HepG2 cells were transfected with the $\pi$SVHS$\beta\Delta$128 series of plasmids containing the various mutated SV40 enhancer elements. The results indicated that TPA regulation of SV40 enhancer-human $\beta$-globin fusion gene required the presence of the certain cis-acting transcriptional control elements. These serve as a target sites for the inducible factor(s). To further define the sequences of the responsive elements, we obtained plasmids with synthetic enhancer that composed of multiple copies of the control elements. When transiently expressed in tissue culture cells, a similar fashion of TPA regulation of fusion gene was observed. In vitro competition and footprinting experiments indicated that one or more trans-acting TPA inducible factors are specifically involved in SV40 enhancer function. It is possible that binding of factors to the primary element which induces binding to other appropriately funtional elements.

**A 254** C-*FOS* EXPRESSION IN DIFFERENTIATED MACROPHAGES : EARLY CHANGES DURING MODULATION OF FUNCTIONAL ACTIVITY, Martine A. Collart, Dominique Belin, Bernard Thorens, Sylvie de Kossodo, Jean-Dominique Vassalli and Pierre Vassalli, University of Geneva, CH-1211 Geneva, Switzerland.

The transcriptional activity of the urokinase (uPA), tumor necrosis factor (TNFα) and interleukin 1 (IL1) genes provides a sensitive index of the functional activity of macrophages : we have found that phagocytosis and exposure to a variety of cellular effectors (including γ-interferon, dexamethasone, cholera toxin, endotoxin and concanavalin A) induce changes in the transcription and mRNA levels of these genes in primary cultures of both resident and elicited macrophages. For each agent, the pattern of response was unique. In addition to their effects on macrophage secretory products, all these agents also caused rapid and transient changes in the expression of the proto-oncogene c-*fos*. In an attempt to determine which of these effects were direct, we analyzed macrophage gene transcription in presence of cycloheximide or emetine. Surprisingly, arrest of protein synthesis alone dramatically and selectively induced c-*fos*, uPA, TNFα and IL1 gene transcription, as determined in *nuclear run-on experiments*. These and other data suggest that these genes may be under the control of short-lived protein repressors. They offer a model system for testing gene-specific effects of the nuclear proto-oncogene c-*fos*.

**A 255** ONCOGENE AND GROWTH FACTOR INVOLVEMENT IN PROSTATIC DISEASE, Peter Davies, M. Elizabeth A. Phillips and Colby L. Eaton, Tenovus Institute for Cancer Research, Cardiff CF4 4XX, U.K. Expression of cellular oncogenes has been analysed in benign prostatic hypertrophy (BPH) and human prostate cancer specimens. Transcripts of c-myc, c-Ha-ras, c-Ki-ras, c-sis, c-fos, c-myb, c-erbA, c-erbB, c-src and the p53 gene have been found in BPH and carcinoma tissue, the first five most consistently. Elevated expression of c-myc was observed in all specimens of carcinoma compared to BPH. Increased expression of c-Ha-ras was correlated with loss of differentiation. Expression of c-Ki-ras and c-sis was elevated above BPH levels only in poorly differentiated carcinoma specimens. Although c-fos was expressed no differently in BPH and carcinoma, a significant correlation (r=0.923) was found between c-fos expression and nuclear androgen receptor content. The majority of BPH and carcinoma specimens contained receptors for epidermal growth factor (EGF). High-affinity binding of radioiodinated EGF to both androgen-receptor-positive and -negative specimens was observed. These interrelationships were more easily studied in normal epithelial and cancer (both receptor-positive and -negative) cell lines. Similar patterns of oncogene expression have been found in prostate cell lines as observed in surgical samples. Most significantly, androgen-independent prostate cells secrete a powerful novel growth promoting activity affecting normal epithelial cells. The presence of novel growth promoters in androgen-independent cells has implications of the development of an autoregulatory function and the profound effects in normal prostatic epithelial cells may have etiological implications for prostatic cancer. The growth factor has been characterized and its interinvolvement with other cellular growth mechanisms is currently under investigation.

**A 256** SUPPRESSION OF THE EGF-DEPENDENT INDUCTION OF C-MYC ONCOGENE EXPRESSION BY TGFβ IN HUMAN MAMMARY CARCINOMA CELLS IN CULTURE, J. A. Fernandez-Pol*⁺, D. J. Klos*, P. D. Hamilton* and V. Talkad*⁺, *V. A. Medical Center and ⁺Department of Medicine, St. Louis University, St. Louis, Mo 63106.
Alterations in c-myc oncogene expression after treatment of human mammary carcinoma cells MDA-MB-231 with Epidermal Growth Factor (EGF) and/or Transforming Growth Factor β (TGFβ) have been investigated. An stimulation of c-myc messenger(m) RNA was detected within 60 to 120 minutes after treatment with EGF. This induction was inhibited by the presence of TGFβ. This effect was specific as very little change was observed in the levels of c-fos and c-ras$^{Ha}$ mRNA's. In the presence of cycloheximide (CH), the c-myc mRNA was superinduced in response to EGF. Treatment of the cells with TGFβ failed to reduce the EGF-dependent superinduction of c-myc produced by CH, suggesting that inhibition of protein synthesis interferes with the ability of TGFβ to generate the signal(s) required to inhibit the expression of this gene. Under the conditions used here, EGF induces both stimulation of growth in monolayer and in soft agar cultures while TGFβ treatement ultimately results in inhibition of the EGF-dependent stimulation of cell growth. Thus, the early suppression of the EGF-mediated increase in c-myc mRNA expression appeared to be associated with subsequent inhibition of growth by TGFβ in MDA-231 cells.

**A 257** DETECTION OF MUTANT C-KI-RAS GENES IN HUMAN TUMORS Kathleen Forrester, C.Almoguera, K. Han and M. Perucho. Dept. of Biochemistry, SUNY at Stony Brook, New York 11794.

We have previously developed a method to detect and characterize single point mutations in transcribed genes which is based in the ability of RNase A to recognize and cleave RNA heteroduplexes containing single base mismatches (Winter et al, PNAS 82:7575, 1985). Radioactively labeled anti RNA probes are prepared with the SP6 RNA Polymerase, hybridized to total cellular RNA, digested with RNase A and analyzed in denaturing polyacrylamide gels.

We have applied this method for the screening of human primary tumors using an anti RNA probe specific for the first coding exon of the c-Ki-ras gene. About 20% of the tumors (20 out of 103 total samples) contain c-Ki-ras genes with mutations at or near codon 12 of the encoded p21 protein. In the particular case of colon carcinomas, about 30% (13 out 46 total samples) scored positive in our assay. Some of these mutations have been characterized as cysteine and valine substitutions at codon 12 by using the corresponding anti mutant RNA probes. This has been confirmed by cloning and sequencing the first coding exon of the gene from some of these tumors. We have found no evidence for mutations other than those at or near codon 12. Moreover, we have found a colon tumor that contains a mutant N-ras oncogene (detected by the NIH3T3 transfection assay) and a mutant c-Ki-ras gene (detected by our assay). The mutation in the c-Ki-ras gene was confirmed and characterized as a serine substitution at codon 12 by cloning and sequencing its first coding exon.

Our results establish the validity of the RNase A method as a screening assay for the search of mutant ras genes in human primary tumors and indicate that the percentage of human tumors containing mutant ras genes is significantly higher than previously estimated.

**A 258** IMMUNOCYTOCHEMICAL (ICC) DETECTION OF c-Ha-ras p21 IN PRENEOPLASTIC AND NEOPLASTIC LIVER LESIONS DURING HEPATOCARCINOGENESIS IN RATS, Paul A. Galand and Danielle Jacobovitz, Free University of Brussels, Brussels - Belgium.

The expression of genes of the c-ras family and of c-myc has been repeatedly reported to increase in rat hepatoma cell-lines and experimental liver tumors and transiently, during liver regeneration. It remains unclear whether the observations made during hepatocarcinogenesis (HC-genesis) relate to malignancy or to the associated proliferative activity and what cell population is involved. This study addresses these questions using the Scherer-Emmelot model of HC-genesis (diethylnitrosamine-DENA-administration to partially hepatectomized rats). A purified antibody, raised against a synthetic peptide homologous to an a-acid sequence of Ha-ras p21 (Triton Biosciences, Affipure TM) was used and this was detected by indirect streptavidin-biotin-peroxidase (Amersham-UK) labeling. Controls consisted in omission of primary AB or adsorption by EJ cells extract. Positive ICC staining of hepatocytes was observed in the following instances: 1. the hepatocarcinomas, associated nodules and ± 80% of associated foci of alterations, 13-20 months after full HC-genic treatment. 2. all the nodules and ± 80% of associated foci, 5-9 months after such treatment. 3. persistent small foci found 9 months after non-carcinogenic treatment with DENA only. 4. EJ cells. Negative ICC staining was observed in: normal and regenerating liver and benign tumor. Increased c-Ha-ras expression in hepatocytes thus seems an early and stable event in HC-genic process, unsufficient (if at all contributing) for full transformation.

**A 259** THE met ONCOGENE IS A NEW MEMBER OF THE TYROSINE KINASE FAMILY. Mary Gonzatti-Haces, Morag Park, Michael Dean, Arun Seth, Terry Copeland, Stephen Oroszlan and George F. Vande Woude. BRI-Basic Research Program, NCI-Frederick Cancer Research Facility. Frederick, MD 21701

The met oncogene was isolated from a chemically treated human osteosarcoma cell line, MNNG-HOS, using the NIH/3T3 cell transfection assay. Activation of the met oncogene occurred via a chromosomal DNA rearrangement that resulted in a chimeric tpr-met oncogene, containing 5'sequences from the tpr locus (translocated promoter region) on chromosome 1 and 3' sequences from the met protooncogene locus on chromosome 7q 21-31. Analysis of the 3' portion of the met oncogene revealed sequence homology with the tyrosine kinase family of genes. Peptide antibodies directed against the putative 28 C-terminal amino acids of the met oncogene specifically immunoprecipitated a 65 kd polypeptide (p65) only in cells containing the activated oncogene. In addition, three met polypeptides of 160, 140 and 110 kd were identified in epithelial and fibroblast cell lines, which express the met proto-oncogene. These data suggest that p65 is the met oncogene product, encoded by the 5.0 kb hybrid tpr-met transcript and that p160, p140 and p110 are proteins encoded by the met-proto-oncogene 9.0 kb mRNA. Both p140 and p65 have in vitro kinase activity following immunoprecipitation using either the met antipeptide antibody, or sera against the carboxy terminal portion of the met protein expressed in bacteria. Autophosphorylation of p140 and p65 in vitro was on tyrosine residues. Both p65 and p140 were also phosphorylated in vivo and this phosphorylation of p65 was on serine and tyrosine residues, while only serine and threonine were detected on p140. Research sponsored by the National Cancer Institute, DHHS, under Contract NO. NO1-CO-23909 with Bionetics Research, Inc.

**A 260** LOSS OF PDGF-STIMULATED PHOSPHOLIPASE ACTIVITY IN NIH-3T3 CELLS EXPRESSING THE EJ-RAS ONGOGENE, Robert R. Gorman, Christopher W. Benjamin, and W. Gary Tarpley. Departments of Cell Biology & Cancer, The Upjohn Co., Kalamazoo, MI 49001.

The Harvey-ras gene product (p21) shares sequence homology with guanine nucleotide binding proteins (G-proteins) (Hurley et al., Science 226:860-862). Our previous work demonstrated that NIH-3T3 cells expressing the cellular ras oncogene from the EJ human bladder carcinoma (EJ-ras) exhibited reduced hormone-stimulated adenylate cyclase activities (Tarpley et al., Proc. Natl. Acad. Sci., June 1986). We now report that in these cells another enzyme system thought to be regulated by G-proteins is inhibited, namely phospholipase $A_2$/C. NIH-3T3 cells release prostaglandin $E_2$ ($PGE_2$) when exposed to platelet derived growth factor (PDGF); the levels of $PGE_2$ released from EJ-ras DNA transfected cells are only 3% those of controls despite a similar basal (unstimulated) release from control and EJ-ras transfected cells. The lack of PDGF-stimulated $PGE_2$ release from EJ-ras transfected cells is not due to a defect in the prostaglandin cyclooxygenase since incubation of control and EJ-ras transfected cells with arachidonate resulted in identical levels of $PGE_2$ release. The lack of PDGF-stimulated $PGE_2$ release from EJ-ras transfected cells also does not result from the loss of functional PDGF receptors. EJ-ras transformed cells bind 70% as much [$^{125}$I]PDGF as control cells, and are stimulated to incorporate [$^3$H]thymidine and proliferate following exposure to PDGF. This inhibition is not the result of a secondary cellular effect related to the transformed phenotype since NIH-3T3 cells transformed by v-src released wild type levels of $PGE_2$ following exposure to PDGF. Determination of total water soluble phosphoinositide levels and changes in the specific activity of phosphatidylcholine in control and EJ-ras transfected cells demonstrated that PDGF-stimulated phospholipase C/$A_2$ activities are inhibited in the EJ-ras transfected cells.

**A 261** THE V-MOS AND H-RAS ONCOGENE EXPRESSION REPRESSES GLUCOCORTICOID HORMONE-DEPENDENT TRANSCRIPTION FROM THE MOUSE MAMMARY TUMOR VIRUS LTR, Bernd Groner, Brian Salmons and Rolf Jaggi, Ludwig Institute for Cancer Research, Bern Branch, CH-3010 Bern, Switzerland.

We have subjected the viral mos oncogene (v-mos), the activated human H-ras oncogene [H-ras(A)] and the normal human H-ras protooncogene [H-ras(N)] to the transcriptional regulation of glucocorticoid hormones by in vitro recombination with the promoter region of the mouse mammary tumor virus long terminal repeat (MMTV LTR) and transfection into NIH 3T3 cells. Cell clones were selected which exhibit a transformed phenotype strictly dependent on the presence of hormone in the growth medium. The expression of the chimeric genes as a function of time after hormone stimulation was studied at the level of transcriptional rate, mRNA and protein accumulation. Oncogene expression was stimulated rapidly to high levels, after hormone addition, but declined in the continuous presence of hormone. Measurements of the transcriptional rates in nuclei from LTR v-mos and LTR H-ras (A) transfected cells showed a repression of LTR v-mos and LTR H-ras (A) transcription after the initial stimulation by hormone. LTR H-ras (N) transcription was not affected. An independently transfected LTR H-2L$^0$ construct in LTR v-mos or LTR H-ras (A) containing cells is also transcriptionally repressed. These experiments demonstrated a transcriptional repression effect of the oncogene products on the glucocorticoid hormone-dependent MMTV LTR transcription.

**A 262** ANDROGEN-REGULATED EXPRESSION OF MOUSE MAMMARY TUMOUR VIRUS (MMTV) RNA IN S115 MOUSE MAMMARY TUMOUR CELLS, Pirkko L. Härkönen, James F. Glover and Philippa D. Darbre, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX. U.K.

The Shionogi mouse mammary tumour cell line (S115) exhibits a positive proliferative response to androgen in tissue culture. Androgens also cause a transformation-like behavioural change in these cells, especially in terms of morphology, density regulation and ability to grow in suspension culture. Mouse mammary tumour virus (MMTV)-related sequences are expressed in the S115 cells and the accumulation of the MMTV-specific RNAs is regulated by androgens. The major species of RNA produced is 16S in size, contains predominantly sequences from the long terminal repeat (LTR) of the proviral MMTV and may encode the putative 'orf' protein, of unknown function. Our studies aim to evaluate any potential role of this 16S RNA in the androgen regulation of cell morphology/growth.

We have now cloned and sequenced the cDNA specific for the 16S RNA. The cDNA was prepared from the poly A+ RNA fraction which was purified from other MMTV messages by sucrose gradient centrifugation and cloned into the vector pUC 18. Four MMTV-specific inserts (size 1.4-1.8 Kb) were further analyzed by restriction enzymes. The Eco RI-PstI fragments of one of these were then cloned into the vectors M13(18) and M13(19) for sequencing. The structural and functional results will be discussed.

**A 263** CHROMOSOMAL LOCATION OF HUMAN GENES ENCODING THE MAJOR HEAT SHOCK PROTEIN HSP70, G.S. Harrison, H. Drabkin and F.-T. Kao (Univ. of Colorado, Denver, Colo.) and R. Morimoto and B. Wu (Northwestern Univ., Evanston, Il.).

An intriguing relation has been established between expression of heat shock proteins (HSPs) and transformation in mammalian cells. Elevated levels of HSP70 are found in HeLa cells and several other tumor cell lines at normal temperatures. Expression of HSP70 is stimulated by the adenovirus Ela 13s product, and an exogenous c-myc gene can stimulate expression from a Drosophila HSP70 promotor in transfected CHO cells. Since HSP70 is regulated in the cell cycle, induction of HSP70 by these immortalizing gene products may be related to changes in growth control. Multiple copies of the highly conserved HSP70 gene family exist in most organisms. To examine the organization and location of functional human HSP70 genes we used Southern hybridization, in situ hybridization and protein analysis. We assigned genes coding for functional HSP70 proteins to chromosomes 6, 14 and 21, and show that homologous sequences reside on at least one other chromosome as well. In situ hybridization localized the regions on chromosomes 6 and 14 as 6p21-23 and 14q22-23. Interestingly, both these sites correspond to known fragile sites. Furthermore, this region of chromosome 6, where at least two HSP70 genes reside, is involved in translocations and alterations seen in acute leukemia and melanoma, and the HLA locus also maps here. Thus, it is of interest to determine if HSP70 genes are altered as a result of these chromosome changes, thereby being implicated more directly in the process of malignant transformation. This is Eleanor Roosevelt Cancer Institute contribution #694.

**A 264** CHARACTERIZATION OF THE 5' REGULATORY REGION OF THE MOUSE cKi-ras GENE
Eric K. Hoffman, Stephen P. Trusko, Nancyanne Freeman and Donna L. George, University of Pennsylvania, Philadelphia, Pa. 19104

We have examined the structural and functional properties of the 5' region of the cKi-ras proto-oncogene utilizing material obtained from Y1 mouse adrenal tumor cells. In the Y1 cells, there is a 30 to 60 fold amplification and overexpression of the cKi-ras gene. Rnase protection and primer extension studies provided evidence for the presence of multiple transcriptional start sites. Sequence analysis of a primer extended cDNA clone revealed the nucleotide sequence composition and genomic location of a 5' untranslated exon. The nucleotide sequence in the region of transcriptional initiation is very high in G+C content (80%) and contains several copies of the repeat CCGCCC or its inverted complement GGGCGG which may act as potential binding sites for the cellular transcription factor Sp1. We have analyzed genomic fragments from this 5' region for potential promoter activity using the chloramphenicol acetyl transferase gene in transient expression assays. These studies have identified a DNA segment with strong promoter/ enhancer activity. Further characterization of this regulatory region should promote a better understanding of how the expression of this gene is controlled in normal and neoplastic cells.

**A 265** THE ABELSON PROTO-ONCOGENE OF DROSOPHILA, F. Michael Hoffmann, Mark Henkemeyer, and William Goodman, University of Wisconsin, Madison, WI 53706.

DNA sequence analysis of genomic and cDNA clones was used to define a Drosophila gene homologous to the Abelson proto-oncogene. The predicted amino acid sequence of the Drosophila gene is homologous to the mouse gene across the amino terminal half of the protein, which includes the tyrosine kinase domain. The 5' exon is most homologous to the mouse Type IV 5' exon.

Mutations were induced in and mapped to the Drosophila gene. Mutant animals survive through development and many eclose from the pupal case as adult flies. The mutant adults are severely reduced in longevity and are sterile. The Abelson gene does not appear to be required for general cell viability or proliferation during development.
(Supported by Grant no. NP-483B from the American Cancer Society.)

**A 266** RADIATION INDUCED RAT THYROID CARCINOMA: ACTIVATION OF $ras^k$, myc AND abl ONCOGENES. P.Kim, S. Ghosh, G. Siegal, R.Watkins, T. Mulcahy, and S. Zain. Cancer Center, University of Rochester, NY 14642.

We are investigating the involvement and role of various oncogenes in the development of carcinomas in a radiation induced rat thyroid carcinogenesis model system. This system was chosen for its sensitivity to the carcinogenic effects of ionizing radiation, and its pathobiological similarities with human neoplasias. Normal thyroid tissue, tissue from spontaneously developed rat thyroid carcinomas and undifferentiated and highly differentiated radiation induced rat thyroid carcinomas was used for this study. Comparison between the genomic DNA, mRNA and proteins from the above mentioned tissues revealed the involvement of abl, myc, and $ras^k$ oncogenes. The abl oncogene is overexpressed in the radiation induced tumors by a factor of >40 fold compared to normal thyroid. The myc transcriptional patterns are very complex, and there are indications of genomic rearrangements in all the seven tumor DNAs compared to normal thyroid gene. The 2.2 Kb long $ras^k$ specific mRNA is (2-5X) overexpressed but there are no indications of genomic alterations in the $ras^k$ gene. DNA mediated gene transfer assays using NIH 3T3 cells gave transformed foci with tumor DNA. Based on these results there are indications of $ras^k$ oncogene activation. The involvement of oncogene expression in TSH induced proliferation is being investigated using a transformed FRTL-5 rat thyroid cell line mutant. Based on this data the role of radiation as a carcinogenic agent and TSH as a tumor promoting agent during the development of thyroid carcinomas will be discussed.

**A 267** TRANSCRIPTS HYBRIDIZING WITH THE HUMAN 8-LYM ONCOGENE DETECTED IN NORMAL HUMAN EPIDERMIS, Lyne Lemieux, Paolo Giacomoni and Michel Darmon, Cell Biology Department, C.I.R.D. (Centre International de Recherches Dermatologiques), Sophia Antipolis, Valbonne, France.

A screening of different human epidermal cell types, both normal and transformed, for oncogene transcripts has been performed by hybridizing cloned oncogenes to $^{32}P$ labeled cDNAs made out from total cellular polyadenylated RNAs. These experiments revealed the presence of transcripts hybridizing with human c-myc and N-ras clones in all epidermal cell types. However, transcripts hybridizing with the human 8-lym clone were detected only in normal epidermis obtained from surgery. They were not detected in cultured human keratinocytes, whether normal or transformed.

These results suggest that transcripts hybridizing to the 8-lym clone are specific for differentiated epidermis. Although the 8-lym clone used in these experiments contains a repetitive sequence, it seems unlikely that it might be responsible for the hybridization signal since it is only 50 nucleotides long. However, since the detection of transcripts hybridizing to the 8-lym clone in normal epidermis was quite unexpected, we isolated several hybridizing clones from a lambda gt10 cDNA epidermal library in order to compare their sequence to that of the human 8-lym clone.

**A 268** GENES INDUCED BY ONCOGENES FALL INTO TWO CLASSES: THOSE INDUCED BY ACTIVATION OF A SPECIFIC INTRACELLULAR PATHWAY AND THOSE INDUCED AS A CONSEQUENCE OF THE TRANSFORMED PHENOTYPE. L.M.Matrisian*, G. Rautmann and R. Breathnach,*Dept. of Cell Biology, Vanderbilt Univ, Nashville TN 37232 and Institut de Chimie Biologique, 11 rue Humann, 67085 Strasbourg FRANCE.

We have isolated two cDNA clones corresponding to mRNAs that are differentially expressed in FR3T3 cells transformed by polyoma middle T oncogene and not present in the parental FR3T3 cells. One clone, transin RNA, is induced by transformation of FR3T3 or Rat-1 cells with polyoma, Rous sarcoma, and bovine papilloma viruses and H-ras oncogenes. In contrast, the other clone is induced only in FR3T3 cells transformed by polyoma middle T or ras oncogenes. This clone corresponds to a 1.5kB transcript that encodes rat preproenkephalin (ENK). Transfection of FR3T3 cells with polyoma or ras sequences under control of an inducible promoter demonstrated that ENK induction was a direct effect of oncogene expression and not a tissue culture artifact. Treatment of FR3T3 cells with an analogue of the second messenger cAMP also induces ENK RNA. We conclude that there are two classes of oncogene-inducible genes, those that are a consequence of the transformed phenotype and are therefore induced by a large number of oncogenes (ie transin), and those that are specific for certain oncogenes and are probably connected to the intracellular pathway used by these oncogenes to elicit a biological response. Further studies using ENK induction in FR3T3 cells may elucidate a common pathway used by ras, polyoma middle T and cAMP to alter gene expression and elicit a biological response.

**A 269** LOSS OF CELL CONTACT-MEDIATED C-MYC REGULATION IN CHEMICALLY TRANSFORMED FIBROBLASTS IS RESTORED BY N,N-DIMETHYLFORMAMIDE (DMF) AND RETINOIC ACID (RA), Kathleen M. Mulder and Michael G. Brattain, Baylor College of Medicine, Houston, TX 77030.

We have now identified a malignant cell model in which the c-myc gene is overexpressed only at confluency, as mRNA for the gene is barely detectable during log phase growth in either the chemically transformed fibroblasts (AKR-MCA) or the untransformed cells from which they were derived (AKR-2B). We also show that DMF and RA repress c-myc expression in AKR-MCA cells in a manner which correlates with their ability to restore contact inhibition. DMF (1.0%) and RA (1.0 uM) reduced saturation densities to approximately 10% and 40% of control values, respectively. DMF (1.0%) decreased c-myc expression to undetectable levels within 60 minutes following its addition, whereas c-myc expression was reduced, but still detectable after a 4-hour exposure to 1.0 uM RA. The effects of both of these agents on saturation densities and c-myc expression in AKR-MCA cells were reversible and dose-dependent. Cycloheximide blocked the DMF and RA effects on c-myc expression, suggesting that new protein synthesis was required for the effects on c-myc. The transient loss of contact inhibition in serum-stimulated confluent AKR-2B cells was associated with an induction of the c-myc gene. Our results support a regulatory role for cell contact in negatively controlling c-myc expression and suggest an inducible role for c-myc in overcoming contact inhibition in a malignant cell line.

**A 270** AMPLIFICATION AND INCREASED EXPRESSION OF C-MYC PROTOONCOGENE IN THE HUMAN PROSTATIC CARCINOMA CELL LINE - LNCaP. Abhijit Nag and Roy G. Smith, Baylor College of Medicine, Houston, Texas 77030.

We have investigated the structure and expression of the c-myc protooncogene in the LNCaP cells. This cell line was established from a metastatic lesion of the human prostatic adenocarcinoma and maintains the malignant and functional properties, i.e., gives rise to tumor in athymic nude mice and both the cells and tumor produce acid phosphatase. Msp I digest of the LNCaP DNA when hybridized to a human c-myc probe showed a 1.45 kb band that is amplified 2-3 fold in comparison to DNA from lymphocyte of a "normal" individual. In addition the LNCaP contains some rearranged c-myc structures which are not present in the control cells. Both these structures (0.77 and 0.42 kb) were at least 10-fold amplified compared to the normal 1.45 kb band. To determine whether this amplification was accompanied by increased expression, RNA was isolated from these cells and compared to RNA isolated from a model cell line in which c-myc was not amplified. In both cases Northern blot analysis showed three transcripts (4, 2 and 1 kb), however the concentration of each transcript was at least 10-fold higher in RNA isolated from LNCaP cells. Though androgens modulate the cell growth of LNCaP, there was no change in the level of c-myc RNA transcripts in the presence or absence of androgens. It is hypothesized that constitutive expression and amplification of c-myc is associated with metastatic human prostate cancer. (Supported by NIH Grant #36264)

**A 271** ROLE OF C-MYC IN LYMPHOCYTE MITOGENESIS: STUDIES WITH AN ANTI-SENSE OLIGODEOXYRIBONUCLEOTIDE. Leonard M. Neckers, Gisela Schwab, Eric Wickstrom*, Dov Pluznik and Reino Heikkila. NIH, Bethesda, MD 20205 and *Univ. of South Florida, Tampa, FL 33620

We constructed a c-myc antisense oligodeoxynucleotide to study the role of c-myc in lymphoid mitogenesis. An N-terminal 15mer complementary to c-myc mRNA, constructed with a nuclease resistant 3'-5' methylphosphonate group, was added at 30uM to resting ($G_0$) human PBL 4 hours prior to PHA stimulation. Intranuclear c-myc protein was detected with a murine monoclonal antibody. No c-myc protein was detectable in the c-myc anti-sense pretreated cells at 6 and 24 h following PHA while in PHA controls c-myc protein reached its highest levels at 6 h and was still detectable at 24 h. DNA histograms taken at 72 h showed 78% of untreated PBL in $G_0/G_1$; 52% of PHA treated PBL in $G_0/G_1$; and 73% of c-myc antisense pretreated, PHA treated PBL in $G_0/G_1$. A control 15mer antisense to the N-terminal of vesicular stomatitis virus M protein mRNA had no effect on c-myc protein induction or DNA synthesis. To examine the role of c-myc in $G_0$-S traversal, we studied the expression of two growth-related genes, the IL-2 receptor (IL-2R) and the transferrin receptor(TFR). Both genes are transcriptionally silent in $G_0$ lymphocytes, but are activated during $G_1$ in mitogen or antigen stimulated PBL. Flow cytometric analysis showed induction of IL-2R and TFR protein expression in PHA-treated PBL and there was no inhibition of this expression in c-myc antisense pretreated/PHA activated cells. These data suggest that (1)the c-myc gene product is necessary for S phase entry,but not for $G_0$-$G_1$ traversal,(2)c-myc expression isn't required for transcriptional activation of the IL-2R or TFR, and (3)expression of IL-2R and TFR is not sufficient to induce DNA synthesis in PHA-activated PBL when c-myc gene expression is blocked.

**A 272** MULTIPLE FUNCTIONS OF THE V-myc ONCOGENE WITHIN A SINGLE CELL CLONE OF OK10 RETROVIRUS-TRANSFORMED QUAIL FIBROBLASTS, Rolf I. Ohlsson and Susan B. Pfeifer-Ohlsson, Center for Biotechnology, Karolinska Institute, S-141 86 Huddinge, Sweden.

We have studied the relation between myc oncogene-induced transformed status and increased proliferative potentials, by using the OK10 QDP 9c cell line, cloned in soft agar. Thus, in vitro propagation of the transformed OK10 QDP 9c cell line, results in a selective amplification of the OK10 proviral genome to yield maximally 50-fold higher levels of v-myc oncogene products. In addition, increased v-myc oncogene dosage selects for proliferative phenotypes of already transformed cells by relieving dependence of high serum growth conditions for optimal cell growth. The increased rate of cell proliferation is reflected by increased rates of progression through all parts of the cell cycle. These results suggest that the attainment of transformed status and advanced eclipse of growth factor dependence, are initiated from differential myc protein abundances in a single cell and may therefore be regarded as per se separate functions within this cell line.

**A 273** CSF-1-INDUCED GENE EXPRESSION IN MACROPHAGE: DISSOCIATION FROM THE MITOGENIC RESPONSE, A.Orlofsky and E.R.Stanley,Albert Einstein Coll. of Med.,New YorkNY 10461
Colony stimulating factor-1 (CSF-1) is a growth factor specific for target cells of the monocytic lineage. 2F5, a clone of the murine macrophage cell line BAC1, requires CSF-1 for survival and growth. We have examined early expression of four genes in 2F5 in response to CSF-1: the protooncogenes c-fos and c-myc, and the JE and KC genes, which are induced by platelet-derived growth factor (PDGF) in 3T3 cells. Two kinds of protocol were used. In the first, CSF-1 is added to partly arrested cultures and has three detected growth effects: mitogenesis of arrested cells; survival of all cells; and doubling-time reduction in growing cells. In this protocol, CSF-1 strongly induces all four genes in the temporal order: c-fos; c-myc; JE/KC. The kinetics of induction and deinduction are similar to those of PDGF-stimulated 3T3, implying a program of gene induction common to PDGF and CSF-1. The shared response to the "unbiased" genes JE and KC suggests that the common program includes many genes. Granulocyte-macrophage CSF (GM-CSF) can substitute for CSF-1 to support 2F5. In the above protocol, it induces three of the four genes; however, KC is not induced. In the second type of protocol, CSF-1 is added to cells cycling uniformly in its absence. These cells are either GM-CSF supported 2F5, or an autonomous variant subclone of 2F5. In either case, the only detected effect of CSF-1 is doubling-time reduction. Nevertheless, all four "program" genes are strongly induced with the same kinetics as above. We conclude that the growth factor actions mediated by the early-gene common program are not restricted to, and may perhaps not include, mitogenesis or survival.

**A 274** IDENTIFICATION OF A DNA-BINDING DOMAIN IN CHICKEN AND DROSOPHILA MYB PROTEINS
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ZMBH, University of Heidelberg, D-6900 Heidelberg, West Germany

The oncogene v-myb of Avian myeloblastosis virus encodes a 45.000-$M_r$ nuclear DNA-binding protein $p45^{v\text{-}myb}$. $p45^{v\text{-}myb}$ is a truncated version of the protein encoded by the c-myb proto-oncogene, $p75^{c\text{-}myb}$, which also binds to DNA and is located in the nucleus. The aminoterminal region of $p45^{v\text{-}myb}$ and the corresponding region of the c-myb protein appear to be highly conserved in evolution.
To investigate the role of the conserved domain, which presumably is essential to myb protein function, we have expressed the aminoterminal portion of chicken v-myb and the corresponding region of the Drosophila melanogaster analog of c-myb in a bacterial host. The resulting bacterial proteins were in both cases active in DNA-binding. Removal of the aminoterminal domain from a full length v-myb protein abolishes DNA-binding of the protein. This activity could be restored by fusing the homologous region of the drosophila myb gene to the carboxyterminal portion of v-myb.
Our experiments indicate that the region shared by chicken and drosophila myb genes encodes a DNA-binding domain and suggest that DNA-binding may be essential to myb protein function. To identify and characterize the authentic drosophila c-myb protein we have, as a first step, started to isolate and characterize cDNA clones of the drosophila gene.

**A 275** CONSTRUCTION AND ANALYSIS OF A CHIMERIC V-ERB B/SRC ONCOGENE, Martin L. Privalsky, Department of Bacteriology, University of California at Davis, Davis, CA. 95616.
We wish to better understand the relationship of structure to function among the different tyrosine-specific protein kinase oncogenes. Two members of the tyrosine kinase family of viral oncogenes, v-erb B and v-src, display both similar and divergent biochemical and biological properties. The v-erb B encoded polypeptide is a transmembrane glycoprotein synthesized on rough endoplasmic reticulum, and induces principally erythroid leukemias in susceptible host animals, although fibrosarcomas also occur. The v-src protein is synthesized on soluble polysomes and post-translationally associates with the plasma membrane due to a covalent linkage of myristic acid to its N-terminus; v-src retroviruses induce primarily fibrosarcomas in animals. Both oncogene polypeptides contain a conserved region of amino acid sequence, the "kinase domain," thought to define the enzyme active site.

To explore the interrelationship of v-erb B and v-src, we created a chimeric oncogene which joins the N-terminal glycosylated and transmembrane domains of v-erb B to the C-terminal kinase domain of v-src. The chimera, when transfected into avian cells, is expressed as a glycosylated, membrane-associated protein demonstrating in vitro kinase activity. The v-erb B/src chimera is fully capable of the oncogenic transformation of fibroblasts. Fibroblasts transformed by the chimeric construct closely resemble cells infected by the v-erb B parent, and are distinct from fibroblasts transformed by v-src, suggesting that the N-terminal v-erb B sequences are more important in establishing certain aspects of the transformed phenotype than is the C-terminal v-src catalytic domain.

**A 276** DETECTION OF A TRANSFORMING GENE IN SPONTANEOUS RETICULUM CELL SARCOMA OF SJL/J MICE: A GENETICALLY LINKED AND HOST DEPENDENT NEOPLASIA, S. Pulciani, T. Sakano, K. Ohnishi, A.M. Anastasi, A. Percorelli, G. Fiorucci, C. Oppi, G.B. Rossi, and Benjamin Bonavida, Istituto Superiore di Sanita, Viale Regina Elena, 299-00161 Roma, and the Dept. of Microbiology & Immunology, UCLA School of Medicine, Los Angeles, CA 90024.
Spontaneous reticulum cell sarcoma (RCS) tumor induction occurs in 90% of SJL/J mice of 8-13 months of age. Tumor induction and growth has been shown to be under the influence of both H-2 and non H-2 genes as well as the presence of an intact host T cell system. We postulated that cellular oncogenes may play a role in the induction, growth and characteristics of RCS. DNA mediated gene transfer protocols were adopted to investigate the presence of transforming genes in DNA from RCS of SJL/J mice. High molecular weight DNA was isolated from these tumors as well as from brains and livers of control tumor free SJL/J mice and transfected into NIH-3T3 mouse and F2408 rat fibroblast cell lines. Foci of transformed cells with a peculiar round morphology were scored in both rat and mouse cultures given tumor DNA, but not in those receiving DNA from normal tissues. DNA from first cycle transformants were transfected in further cycles of transfection, giving rise to foci with similar morphology appearances and growth properties. These experiments suggest that a transforming gene, present in RCS spontaneous tumors, is involved in the malignant conversion of the transfected normal fibroblasts. The implication of these results with respect to the induction and growth properties of RCS is discussed.

**A 277** C-MYC ANTISENSE RNA INHIBITION OF 3T3 CELL GROWTH. Robert L. Redner, Jeffrey T. Holt and Arthur W. Nienhuis, Clinical Hematology Branch, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892
Though the c-myc proto-oncogene is expressed in most growing cells, and appears to contribute in its mutant forms to cell transformation, the specific function of c-myc protein has yet to be delineated. We are investigating the role of c-myc in normal cell proliferation by down regulating its expression through the use of anti-sense RNA. A plasmid was constructed containing an MMTV dexamethasone inducible promoter driving the non-coding (anti-sense) strand of a 558 base pair c-myc exon 1-exon 2 fragment linked to globin RNA processing signals. A gene that confers G418 resistance to cells was included in the plasmid. Transfection of this plasmid into a dexamethasone resistant 3T3 fibroblast cell line with G418 selection was accomplished by standard $CaPO_4$ precipitation. Addition of the inducing agent did not appreciably decrease transfection efficiency to G418 resistance although colony size was smaller. Growth of clones was inhibited up to 50% upon induction of the c-myc antisense RNA. Removal of dexamethasone from medium allowed subsequent normal growth. Transfection of 3T3 cells with a similar plasmid containing an RSV constitutive promoter (rather than the weaker inducible MMTV promoter) yielded a marked diminution in G418 resistant colonies. These data suggest that (1) c-myc protein production is required for cell growth and (2) antisense inhibition is dependent upon the ratio of antisense:sense RNA.

**A 278** AN ACTIVATED HARVEY RAS ONCOGENE PRODUCES BENIGN TUMORS IN MOUSE SKIN, Dennis R. Roop, Douglas R. Lowy, Dean S. Rosenthal and Stuart H. Yuspa, NCI, Bethesda MD 20892

Based on the mutagenic action required for specific chemicals to produce benign or malignant tumors, at least two genetic events occur prior to carcinoma formation in mouse shin. The isolation of a activated form of the c-ras$^H$ gene from skin papillomas has provided presumptive evidence that this gene may be a target for the first mutation, which could constitute the initiating mutation in skin carcinogenesis. We have recently been able to show that an activated Harvey ras oncogene produces benign tumors when introduced into cultured keratinocytes by a defective retroviral vector and grafted on athymic nude mice recipients. In situ hybridization experiments indicate that expression of the exogenous oncogene is regulated at the transcriptional level in the differentiated portions of the benign tumor even though it is under the control of the MoMuLV LTR. In keratinocyte culture, exogenous v-ras$^H$ gene expression is not suppressed in differentiating cells, but the oncogene blocks cultured keratinocytes in a late basal cell phenotype. When identical cells are allowed to produce benign tumors in vivo, they progress to the later stages of epidermal differentiation where transcripts for the exogeneous v-ras$^H$ gene are diminshed either by a decrease in gene expression or a selective reduction of message stability. It is not clear if suppression of v-ras$^H$ transcription occurs as a primary or secondary event in cells receiving a differentiation signal. The regulation of a mutated c-ras oncogene by the differentiated portions of benign tumors could account for the benign phenotype in chemically induced tumors where ras gene activation is the initiating event. This hypothesis is currently being tested by analysis of the mutated gene under the control of its own as well as other regulatory sequences.

**A 279** ROLE OF 3' UNTRANSLATED SEQUENCES IN THE CONTROL OF C-FOS EXPRESSION. Kathleen Rubino and Tom Curran, Department of Molecular Oncology, Roche Institute of Molecular Biology, Nutley, NJ 07110

Two alterations are required to activate the transforming potential of the fos proto-oncogene (c-fos): a transcriptional enhancer element must be provided and regulatory sequences in the 3' untranslated region must be deleted. Thus, c-fos expression is rigorously controlled. In general, the basal level of c-fos expression is very low; however, treatment of cells with polypeptide growth factors and other agents leads to a dramatic but transient induction. After reaching peak levels at 30 to 45 minutes post stimulation, mRNA is rapidly degraded and returns to basal levels within 2 hours. We have investigated the expression and stability of fos transcripts, derived from SP6 vectors, using the rabbit reticulocyte lysate translation system. Vectors have been constructed that encode portions of the v-fos and c-fos genes. Translation of v-fos mRNA yields a 55 kDa protein as expected. Interestingly, translation of c-fos mRNA (derived from a full-length cDNA clone) results in synthesis of the highly modified 62 kDa form of the c-fos protein that is present in serum-stimulated fibroblasts. The v-fos transcript exhibits a simple dose-dependent translational activity. In contrast, although the c-fos transcript is translated extremely efficiently at low concentrations, very little expression is detected at high concentrations. Analysis of RNAs recovered from lysates suggests that, at high concentrations, c-fos mRNA is rapidly degraded. Experiments using v-fos/c-fos chimeric transcripts indicate that the inhibitory activity is provided by the 3' untranslated region of the c-fos gene. In addition, pre-incubation of lysates with c-fos 3' sequences causes an inhibition of expression of exogenous globin mRNA. The data are consistent with the hypothesis that the 3' end of the c-fos gene activates a nuclease in reticulocyte lysates.

**A 280** SOMATIC ALTERATIONS INVOLVING THE Ha-RAS PROTO-ONCOGENE IN HUMAN BREAST CANCER. Giuseppe Saglio, Piero Sismondi, Maurizia Giai, Anna Serra, Paolo Gasparini, Bruno Peirone, Angelo Guerrasio, Clara Camaschella, Umberto Mazza and Helice Gavosto. University of Turin, Italy.

Human Ha-ras proto-oncogene is characterized by the presence of repeated sequences (VTR) in the 3' flanking region, whose variable length allows to identify different Ha-ras alleles. Recent data suggest an "enhacer" role of the VTRs on Ha-ras transcription, that seems to vary from case to case. It has recently been reported that Ha-ras alleles of specific length have a significantly higher incidence in breast cancer patients than in normal controls. These data seem to suggest a potential pathogenetic role for the Ha-ras proto-oncogene in breast cancer. To further investigate this point, we have studied the structure of the Ha-ras proto-oncogene in 28 DNAs extracted from breast cancers and in the leucocyte DNAs of the same subjects. Out of 16 informative cases, 3 showed a different pattern in the tumor DNA in respect to the germ-line configuration. Deletions, somatic crossing-overs and gene conversion mechanisms may account for these events, which further support a Ha-ras role in breast cancer.

**A 281** POST-TRANSCRIPTIONAL CONTROL OF FOS, SIS AND TNF EXPRESSION DURING ACTIVATION OF HUMAN MONOCYTES, Eric Sariban, Tom Mitchell and Donald Kufe. Dana-Farber Cancer Institute, Boston, MA 02115.

Upon exposure to 12-O-tetradecanoylphorbol-13-acetate (TPA) or gamma interferon (gamma-IFN), human monocytes are activated and acquire the capacity to perform various effector responses. In resting (nonactivated) monocytes, c-fos is expressed, while c-sis and tumor necrosis factor (TNF) mRNAs are undetectable. Here we report that c-fos expression is down-regulated during activation of human monocytes with either TPA or gamma-IFN. In contrast, activation by these agents is associated with an up-regulation of c-sis and TNF expression. The differential expression of these 3 genes appears to be controlled at a post-transcriptional level. Thus, using an in-vitro nuclear run-off assay, we have found that transcription rates of the c-fos, c-sis and TNF genes remains constant during the activation process. In concert with this finding, the level of c-fos mRNA remained stable in activated monocytes exposed to cycloheximide (CHX). Furthermore, c-sis and TNF transcripts became detectable in resting monocytes treated with CHX. Finally, these increases in c-fos, c-sis and TNF mRNA levels after exposure to CHX were not accompanied by detectable changes in transcription rates. Taken together, these results support a model of post-transcriptional regulation of c-fos, c-sis and TNF expression during activation of human monocytes.

**A 282** OLIGONUCLEOTIDE SITE DIRECTED MUTAGENESIS OF THE 8 CYS CODONS IN THE MINIMAL TRANSFORMING REGION OF v-SIS Monica K. Sauer and Daniel J. Donoghue, UCSD, La Jolla, CA 92093

The *v-sis* gene is the transforming gene of Simian Sarcoma Virus (SSV). It is homologous to the gene for the B chain of Platelet Derived Growth Factor (PDGF). The *v-sis* gene product is glycosylated, dimerized and proteolytically processed to form a dimer of about 56 or 64 kD, with monomers of 28 or 32 kD. Our previous work has delimited the minimal transforming region to residues 127 through 214. Within this region are 8 Cys residues which, interestingly, occur at the same positions in the PDGF A chain. Some of these Cys residues are likely to be involved in holding together the monomers of the dimeric protein ie. in intermolecular disulfide bonds, while others must be involved in intramolecular disulfide bonds. It has been shown that reduction abolishes the mitogenic activity of PDGF, and our earlier results suggest a strong correlation between dimerization and transformation. We have used the technique of oligonucleotide site directed mutagenesis to change the codons for the 8 Cys residues to Ser codons. Focus assays have shown that 3 of these single mutants retain full biological activity and are also capable of dimerization. We have also constructed mutants with 2 or 3 of those altered sites. Two of these mutants were still transforming and capable of dimerization, while the others were no longer biologically active. Our data identify two Cys residues that participate in a single disulfide bond. We are undertaking further analysis by peptide mapping which will identify other pairs of Cys residues involved in each disulfide bond. From such results one can begin to assess the importance of individual Cys residues in intermolecular and intramolecular disulfide bonds, as well as the effect of secondary and tertiary structure on the biological activity of the v-*sis* gene product.

**A 283** ESTROGEN REGULATED ONCOGENES IN ENDOMETRIAL CANCER, Beth Schachter and Teresa Garcia, Mount Sinai Medical School, New York NY 10029.

Estrogen unapposed by progesterone has been implicated as a causative agent of human endometrial cancer. Therefore we have begun analyses of estrogen (E) regulated genes in endometrial cell lines, to determine whether there are mutations or altered expression patterns among this set of genes. The first to be examined was the gene for the epidermal growth factor receptor (EGF-R). This gene was selected for study because EGF-R production is regulated by E in rodent uterus, and selective amplification of the gene has been found in several human adenocarcinoma cell lines including those from a vulval carcinoma (A431) and from head and neck tumors (HN5, HN6). Moreover, EGF and E have synergistic biological effects in one of the endometrial cell lines we are studying. Our Southern blot analysis of genomic DNA from one E-responsive and two E-nonresponsive Human Endometrial Cancer lines (HEC10, HEC1, HEC50) showed no apparent gross mutations in the EGF-R gene when compared to normal human placental DNA. More precise quantitation of gene copy number in the DNAs and in A431 DNA showed no amplification of the EGF-R gene in any of the HEC lines. Gel blot analysis of RNA isolated from HEC10 cells showed a much lower level of EGF-R mRNA in these cells than in the A431 line, again suggesting that expression of this gene is not grossly elevated in the HEC10 cells. Similar analysis of other putative cellular oncogenes is currently being pursued.

**A 284** MITOGENS OR ACTIVATED ONCOGENES CAN BLOCK EXPRESSION OF VOLTAGE-GATED CALCIUM CHANNELS. Michael D. Schneider, John M. Caffrey and Arthur M. Brown. Baylor College of Medicine, Houston, Texas.

We have examined the properties of $BC_3H1$ muscle cells, a model of muscle differentiation and its modulation by growth factors, after transfection with activated alleles of c-myc, erbB, both genes, or Ha-ras. We previously found that neither myc nor erbB prevented induction of muscle creatine kinase (mck) mRNA; synergistic inhibition resulted from myc plus erbB; the Val12 ras allele, by itself, suppressed mck mRNA induction. We recently examined whether expression of voltage-gated ion channels in $BC_3H1$ myocytes also would be contingent on mitogen withdrawal or affected by the cellular oncogenes, using the whole-cell clamp method. In differentiated $BC_3H1$ cells, we identified a delayed outwardly rectifying K current; an inward, tetrodotoxin-insensitive Na current; a "fast", low-threshold Ca current and a "slow", higher-threshold Ca current, whose properties corresponded to those of skeletal muscle channels. Ca currents were not detected until 4-5 days of mitogen withdrawal. Ca current density doubled every two days through days 15-20 ($\sim 10^4$ functional "slow" Ca channels per cell). In myc- or erbB-transfectants the induction of Ca channels was delayed by 1-2 days, but was equivalent to control cells at > 15 days. The EJ ras allele, by itself, or co-transfection of erbB with myc suppressed the induction of functional Ca channels at up to 40 days. Corresponding results were obtained for Na currents; in contrast, K currents were not altered during proliferative growth or by the transfected oncogenes. The results presented here comprise the first biophysical studies of the effects of individual oncogenes on voltage-gated ion channels after gene transfer.

**A 285** PROGRAMMED c-ras-H EXPRESSION DURING EMBRYONIC EPITHELIAL-MESENCHYMAL INTERACTIONS, Stephen A. Schwartz and Ross Couwenhoven, The Chicago Medical School, North Chicago, Illinois 60064.

Although much study has been focused on the role of oncogenes in the initiation and progression of cancer, less effort has been applied to resolve the physiologic role of cellular proto-oncogenes during specific developmental events. We selected the mammalian odontogenic organ as a representative model to evaluate the non-neoplastic role of proto-oncogenes inasmuch as the initial proliferative and "invasive" behavior of normal odontogenic epithelium is reminiscent of neoplasia, and because several potential growth factors have been proposed to be elaborated by the primitive tissues during epithelial-mesenchymal interactions. The expression of a variety of proto-oncogenes was first screened by "in-situ" hybridization and immunohistochemical methods. Only c-ras-H expression was observed to be differentially regulated within finite cell populations during programmed odontogenesis in prenatal rats 13-19 days "in-utero". At the earliest stage of odontogenesis, c-ras-H expression was evident along the entire primitive epithelial-mesenchymal interface. Interestingly, ras expression was minimal in the migratory and "invasive" projection of highly mitotic epithelial cells into the underlying mesenchyme, where the extensive biosynthesis of p21 was evident in condensing osteoblasts at days 15 and 16. Thereafter, ras expression was especially greatest among post-mitotic, terminally differentiating secretory cells undergoing extensive cell-cell interactions. These preliminary findings suggest that c-ras-H expression was primarily confined to post-mitotic cells undergoing extensive histo- and biochemical differentiation and inter-cellular induction during programmed odontogenic epithelial-mesenchymal interactions.

**A 286** PRESENCE OF sis-RELATED PROTEINS IN THE NUCLEUS OF SSV-TRANSFORMED CELLS, L. K. Shawver[1], G. F. Pierce[1], H-J. Yeh[1], P. G. Milner[1], A. Thomason[2], and T. F. Deuel[1], Jewish Hospital[1] at Washington University Medical Center, St. Louis, MO 63110, and AMGEN[2], Thousand Oaks, CA 91320.

Subcellular localization by immunofluorescence and protein A-gold labeling using antibodies to platelet-derived growth factor (PDGF) have identified sis-related antigens in the nucleus of SSV-transformed cells and in subcellular organelles associated with protein processing (Yeh, et. al., PNAS, in press). SSV-transformed cells were labeled with $^{35}$S-cysteine in vivo, the nuclei isolated, and nuclear protein immunoprecipitated using antibodies against PDGF/sis or nonimmune serum. The immune complex was analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. Immunoprecipitation of nuclear proteins using antibody against PDGF showed several consistently labeled bands that were not present when normal serum was used, nor were they present in the cytoplasmic fraction of these cells. At least two of these proteins were immunoprecipitated with antibody from several different sources, including an antiserum derived from a recombinant sis protein. In addition, the proteins were not present when the antibody was competed out with either purified PDGF or recombinant sis protein. These data show the presence of PDGF/sis-like proteins in the nuclei of SSV-transformed cells and raise the question that these proteins may function within the nucleus.

Characterisation of the human homologue of the sea oncogene

**A 287** Douglas R. Smith; ICRF Labs., St. Bartholomew's Hospital, Dominion House, Bartholomew Close, London EC1A 7BE, UK.

The sea oncogene is a new member of the tyrosine protein kinase family of oncogenes which was discovered as an env linked gene in S13 avian retrovirus and which bears a close relationship to the human met oncogene (about 70% homology in the region of the kinase domain). Using a v-sea probe, a number of lambda clones corresponding to the human c-sea gene were isolated. So far, these extend over a region of about 60 kb of genomic DNA and include at least 5 exons from the kinase domain and 3' end of the gene. The restriction maps of these regions are different from the corresponding parts of the met gene, indicating that met and sea are two distinct loci which code for related proteins. Work is in progress to further characterize and extend the cloned region, and to determine the location of c-sea on the human genetic map. The results of these studies will be reported together with any available data on the sequence and expression of the gene.

**A 288** TRANSFORMATION OF RAT LIVER EPITHELIAL CELLS BY RAF/MYC RECOMBINANT MURINE RETROVIRUSES. S. Sorrell[1], B. Huber[1], H. C. Morse, III[2], S. P. Klinken[2], and S. S. Thorgeirsson[1], [1]Laboratory of Experimental Carcinogenesis, NCI and [2]Laboratory of Immunopathology, NIAID, Bethesda, MD 20892.
Combining v-myc and v-raf into an infectious retrovirus establishes a powerful transforming agent (J. Virol. 55: 23, 1985; PNAS 83: 6868, 1986), capable of inducing hemopoietic, pancreatic, and liver neoplasms in the mouse. Expression of both myc and raf is also increased during chemical hepatocarcinogenesis in the rat. To determine the possible contribution of these oncogenes in hepatocarcinogenesis, we have used normal rat liver epithelial cells (NRLF) (In Vitro 19: 576, 1985) to study the transforming ability of 3611 MSV, a raf containing retrovirus, J5, a v-myc containing retrovirus and J2, a retrovirus containing both the raf and myc oncogenes. The NRLF cells were infected in culture with 3611, J5 and J2 according to Miller et al. (Mol. Cell. Biol. 6: 2895, 1986). -Glutamyl transpeptidase positive foci of altered cells developed within 2 weeks post infection with J2 and 3611, but no foci were induced by J5. The J2 virus was 4-5 times more efficient in transforming the NRLF cells than the 3611 virus. Cells isolated from J2 and 3611 induced foci and from cultures infected with J5 were injected s.c. in nude mice. Both J2 and 3611 induced focal cells developed radiply growing tumors whereas the J5 infected cells did not form tumors. J2 and 3611 induced tumors were morphologically characterized as poorly differentiated hepatocellular carcinomas. These findings demonstrate that v-raf and in particular a combination of v-raf and v-myc are efficient transforming agents of the NRLF cells, whereas v-myc alone is apparently incapable of transforming these cells.

**A 289** EXPRESSION OF THE HUMAN mdr 1 (MULTIDRUG-RESISTANCE) GENE, K. Ueda, D. Clark, I.B. Roninson*, M.M. Gottesman, and I. Pastan, NIH, NCI, Bethesda, MD 20892 and *University of Illinois College of Medicine at Chicago, Chicago, IL 60612.
Acquisition of resistance to multiple drugs is an important clinical problem in cancer therapy. We have isolated an overlapping set of cDNA clones for the mdr1 gene which is amplified and overexpressed in multidrug-resistant (MDR) sublines of human KB carcinoma cells selected for resistance to either colchicine, vinblastine or adriamycin. The nucleotide sequence of the mdr1 cDNA clones and their cross-hybridization with cDNAs encoding the 170,000 dalton membrane P-glycoprotein (Riordan et al. Nature 316: 817, 1985) indicate that this glycoprotein is the product of the mdr1 gene. We determined transcription initiation sites of the mdr1 gene by primer extension, S1 nuclease protection and RNase protection experiments, and found that the human mdr1 gene has two promoters. The downstream promoter has two major and several minor transcription initiation sites. Vinblastine-or adriamycin-selected human KB MDR cells, human hepatoma cells (HepG2), and human normal kidney cells use mainly the downstream promoter. Colchicine-selected human KB MDR cells use the upstream promoter as well as the downstream promoter. We have isolated a genomic 1 Kb fragment which includes the downstream promoter from a cosmid library made from DNA from vinblastine-selected human KB MDR cells. This genomic fragment linked to the CAT gene has promoter activity in CV-1 cells. This downstream promoter has a CAAT box and two GC box-like sequences, but no TATA box.

**A 290** MITOGEN-INDUCED MESSAGES IN MOUSE 3T3 CELLS, Brian C. Varnum, Robert W. Lim and Harvey R. Herschman, Department of Biological Chemistry, UCLA Center for the Health Sciences, Los Angeles, CA 90024. Tetradecanoyl phorbol acetate (TPA) is a potent tumor promoter, and a mitogen for 3T3 cells. We have identified and isolated a family of recombinant cDNA clones that recognize distinct mRNA species induced in density-arrested mouse 3T3 cells by TPA. Cross-hybridization studies indicate that one of these clones is homologous to v-fos. Nick-translated probes generated from this clone detect the same mRNA and genomic DNA blot patterns as the v-fos probe, suggesting that the clone contains a fragment of the cDNA for proto-oncogene c-fos. All members of this family of TPA-inducible cDNA clones are induced also by serum and purified peptide mitogens. Induction of all these messages is transient; the mRNA levels reach peak value between 30 minutes and 2 hours, then decline. Stimulation in the presence of cycloheximide leads to superinduction of these messages, thus indicating that protein synthesis is not required for induction. Expression of these messages in 3T3 variants defective in their mitogenic response to TPA has been examined.

**A 291** MULTIPLE src RELATED TYROSINE KINASES ARE OVEREXPRESSED IN HUMAN COLON CARCINOMA, André Veillette[1], Francine M. Foss[1], Douglas Yee[1], Arnold M. Schwartz[2], Joseph B. Bolen[3], and Neal Rosen[1], [1]Medical Breast Cancer Section, Medicine Branch, and [3]Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892 and [2]Department of Pathology, The George Washington University Medical Center, Washington, D.C. 20037. We have previously observed that $pp60^{c\text{-}src}$ tyrosine kinase activity is markedly elevated in all colon carcinoma tissues and cell lines examined over that found in normal colon mucosa tissues and cell lines. This is associated with a modest (3 to 5-fold) increase in c-src specific mRNA and protein. Since the increase in protein tyrosine kinase activity is much greater, it suggests the presence of an additional mechanism of activation, possibly post-translational. The presence of other closely related tyrosine kinases in colon carcinoma cell lines was suggested by Northern blot analysis, which revealed other mRNA species hybridizing to a v-src probe under high stringency. Using identical hybridization conditions, we have screened a cDNA library made from a human colon carcinoma cell line. We cloned two different src-related tyrosine kinases. The first is a partial cDNA identical to the 3' end of the syn sequence recently published by Semba and Toyoshima (PNAS, 1986). This tyrosine kinase is markedly overexpressed (5 to 100-fold) in all colon carcinoma cell lines examined. Low levels of mRNA can also be observed in normal colon mucosal cells. The second detects an mRNA species not present in normal colon cell lines but selectively overexpressed in several colon carcinoma cells. The nature of this tyrosine kinase, its normal pattern of expression, and the mechanisms involved in its specific overexpression in colon carcinoma will be discussed.

**A 292** TRANSMEMBRANE SIGNALS AND GENE EXPRESSION DURING T LYMPHOCYTE MITOGENESIS; TWO INDEPENDENT REGULATORY PATHWAYS, M. W. White and D. R. Morris, Department of Biochemistry, University of Washington, Seattle, WA 98195

Within 1-2 h after stimulation of T-lymphocytes with the mitogenic lectin concanavalin A (ConA), both ornithine decarboxylase (ODC) and c-myc mRNAs are elevated. The phorbol esters, 12-O-tetradecanoylphorbol 13-acetate (TPA) or phorbol 12,13-dibutyrate, increased ODC mRNA in the same time frame and to the same magnitude as ConA, but the phorbol esters by themselves were not mitogenic. In contrast to ConA, the phorbol esters alone were without effect on the resting level of c-myc mRNA. Thus, these results suggest that elevation of ODC mRNA by TPA is through activation of a protein kinase C linked pathway only, while that of c-myc is not. The addition of either cadmium chloride or trifluorperazine at levels, which inhibit both mitogenesis and calmodulin-dependent reactions, blocked the normal increase in c-myc message but had no inhibitory effect on ODC mRNA induction. The data suggest that the ConA-induction of c-myc mRNA is linked to a calmodulin-dependent pathway. In a similar experiment the $K^+$ channel blocker, quinine was found to inhibit the ConA-induction of c-myc mRNA without effect on the increase in ODC mRNA levels. Thus we have shown that distinct agents have differing effects on the induction of c-myc and ODC mRNA levels indicating that there are at least two pathways linking recepter-mediated transmembrane signals and early changes in gene expression. One pathway is linked to the activation of protein kinase C, ODC being an example of this class of genes. The other pathway, represented here by c-myc, is linked to the activation of calmodulin via the mitogenic increase in intracellular calcium and requires the activation of voltage-dependent $K^+$ potassium channels.

**A 293** CELLULAR POLYPEPTIDE PATTERNS OF MCF-7 AND TRANSFECTED MCF-7(RAS) HUMAN BREAST CANCER CELLS, Peter J. Worland, Diane A. Bronzert, Robert B. Dickson, Marc E. Lippman, Snorri S. Thorgeirsson and Peter J. Wirth, Lab. of Experimental Carcinogenesis and Medical Branch, National Cancer Institute, Bethesda, MD 20892.
Transfection of MCF-7 cells with the v-ras$^{H}$ oncogene results in attenuation of estrogen dependence for both in vivo tumor formation and in vitro growth stimulation providing a very valuable model for the study of hormone responsive cancers. This study was undertaken to identify polypeptide alterations after ras transfection. Polypeptide patterns of MCF-7, MCF-7(gpt) (transfection control) and MCF-7(ras) cells were determined using 2D-PAGE, separating total cellular proteins ($^{14}$C-labelled) over pH range 5-8 and molecular weight range 20 kDa-200 kDa. Polypeptide patterns were analyzed using a digital image processing system. Most of the polypeptide groups (544) were common to all cell lines. MCF-7(ras) produced 3 polypeptides not detected in either MCF-7 or MCF-7(gpt) and 14 polypeptides constitutively expressed in MCF-7 were not detected in MCF-7(gpt) or MCF-7(ras). Both MCF-7(gpt) and MCF-7(ras) expressed 8 polypeptides not detected in MCF-7 cells. MCF-7(gpt) produced 5 polypeptides not detected in either MCF-7 or MCF-7(ras). Quantifiable differences (> 2-fold) were MCF-7(ras) and MCF-7(gpt) incorporating greater amounts of radioactivity in 14 polypeptides, but only 7 of these were common to both. Conversely 22 and 19 polypeptides were expressed at lower amounts of radioactivity in MCF-7(gpt) and MCF-7(ras) respectively, compared to MCF-7. These data show that there are several additional alterations in polypeptide expression as a consequence of the transfection process and the additional insertion of the ras oncogene.

**A 294** REGULATION OF PDGF GENE EXPRESSION DURING DIFFERENTIATION OF MYELOID LEUKEMIA CELLS Riitta Alitalo, Christer Betsholtz*, Kenneth Nilsson* and Leif Andersson Transplantation Laboratory, University of Helsinki, 00290 Helsinki, Finland, *Department of Pathology, University of Uppsala, Uppsala, Sweden

Platelet-derived growth factor (PDGF) is a polypeptide mitogen stored in platelets, but secreted also by a variety of cells including monocyte-macrophages. PDGF consists of two polypeptide chains, A and B, linked by disulphide bonds. The polypeptides are encoded in distinct chromosomal loci, and share about 40 % amino acid homology. Numerous studies have addressed the regulation of the PDGF-B (v-sis) chain expression in a variety of cellular systems, including myeloid cells. Thus, v-sis is known to be expressed by monocytes differentiating to macrophages, as well as during megakaryocytic differentiation of pluripotent hematopoietic cell lines. However, the expression of the PDGF-A chain has not been studied to the same extent.

We have analyzed PDGF-A gene expression in leukemic cell lines. We find the typical 2.8, 2.3, 1.9 kb mRNA for PDGF-A in the histiocytic lymphoma U937, promyelocytic leukemia HL60 and T-cell leukemia MOLT4 cell lines, whereas a variety of other leukemia cell lines do not show significant expression. PDGF-A expression seems to be stimulated by TGF-beta and also regulated by agents inducing differentiation of the U937 and HL-60 cell lines. - A detailed analysis of PDGF-A expression and its regulation will be reported.

**A 295** CHARACTERIZATION AND SEQUENCE OF THE PROMOTER REGION OF THE HUMAN PROTOONCOGENE HER2 (neu). M. Tal, R. King, J. Schlessinger, A. Ullrich and D. Givol, Department of Chemical Immunology, The Weizmann Institute of Science Israel

HER2 belongs to the tyrosine kinase gene family, it is highly homologous to the human EGF-receptor (HER1) and is probably the human counterpart of the rat protooncogene neu. We have localized the 5' region of the human gene of HER2 in a cloned genomic DNA. This clone contained exons 1-4 of HER2 spanning the coding sequence of the first 191 amino acids. The first exon encodes the signal peptide of the pro-HER2 polypeptide. The promoter region of HER2 has been identified upstream to exon 1 by S1 nuclease mapping, using HER2 RNA and by a functional assay using a construct which contains the promoter region linked to the chloramphenicol acetyl transferase (CAT) gene. Two start points at nuc. -178 and -276 were identified utilizing $S_1$ protection analysis in the human placenta tissue, A-431 cells and in a breast carcinoma cell line. However, only one start point at nuc-178 appears in two solid tumors of mammary carcinoma while mammary carcinoma cell line MCF7 uses another two distinct upstream start points at nuc. -318 and -343. The various start point may play a role in the expression of these transcripts in the tumor and cell lines. The common start point at nuc. -178 active in the rest of the cell lines which were studied contains typical TATAA and CAAT boxes which are localized 21 and 70 bp, respectively, upstream to the mRNA initiation site. The sequence of the HER2 promoter shows no homology to that of the EGF receptor (HER1) and the GC boxes which are typical to the promoter of the EGF receptor gene are absent from the HER2 promoter. A typical repeating motif in HER2 promoter is the pentanucleotide GGAGG which appears 8 times.