GROWTH FACTORS, TUMOR PROMOTERS AND CANCER GENES

Nancy H. Colburn, Harold L. Moses and
Eric J. Stanbridge, Organizers
April 6 — April 13, 1986

Plenary Sessions

Poster Sessions

Cell Cycle and Differentiation — I

L1 ONCOGENES AND CELL CYCLE GENES, Renato Baserga and Bruno Calabretta, Department of Pathology, Temple University Medical School, Philadelphia, PA 19140

By differential screening of cDNA libraries, we have identified three sequences that are preferentially expressed when G_0 cells are stimulated to proliferate (1). The cDNA's corresponding to these sequences, designated as 2F1, 4F1 and 2A9, have now been isolated from a human Okayama-Berg library. All 3 sequences can be induced by different mitogenic stimuli, in different cell types, from different species (hamster, human, mouse and rat). However, 2A9 is not detectable in human lymphocytes stimulated by phytohemagglutinin, although it is expressed in fibroblasts and in monocytic cell lines.

All 3 sequences, (including 2A9) are highly expressed in human acute and chronic myeloid leukemias (2). Their level of expression has been compared to the levels of expression of genes known to be strictly related to cell cycle progression, like histone H3. In addition, these sequences, like c-myc and c-fos, are inducible by growth factors even in the presence of cycloheximide (at concentrations completely inhibiting protein synthesis) thus indicating that they are primary responders.

A number of functional and structural homologies with oncogenes will be discussed in support of our hypothesis that cellular oncogenes are only a subset of a larger group of genes that control the proliferation of animal cells.

1. Hirschhorn, R.R. et al. (1984) Proc. Natl. Acad. Sci. 81, 6004.
2. Calabretta, B. et al. (1985) Proc. Natl. Acad. Sci. 82, 4463

L2 GROWTH AND DIFFERENTIATION PROGRAMS IN NORMAL AND NEOPLASTIC LUNG CELLS, Curtis C. Harris, George H. Yoakum, John F. Lechner, Paul A. Amstad and Brenda I. Gerwin, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892

Pathways of growth and differentiation have been studied in normal human bronchial epithelial cells cultured in serum-free medium. Diminished responsiveness to inducers of terminal squamous differentiation (e.g., transforming growth factor type beta) and/or autocrine production of growth factors (e.g., gastrin-releasing factor) may provide lung carcinoma cells with a selective clonal expansion advantage. Human bronchial epithelial cells have also been employed to investigate the role of specific oncogenes in carcinogenesis and tumor progression. Using the protoplast fusion method for high frequency gene transfection, the v-Ha-ras oncogene initiates a cascade of events in the normal human bronchial cells leading to their apparent immortality, aneuploidy, and tumorigenicity with metastasis in athymic nude mice. Studies with other oncogenes associated with lung cancer are also ongoing. These results suggest that oncogenes may play an important role in controlling the balance between growth and terminal differentiation programs of human lung cells during carcinogenesis.

L3 PROLIFERATIVE CONTROL IN NORMAL AND NEOPLASTIC CELLS. Arthur B. Pardee, Henry C. Yang and Donald L. Coppock, Dana-Farber Cancer Institute and Dept. Pharmacology, Harvard Medical School, Boston, MA 02115

The onset of DNA synthesis is a process that closely regulates proliferation of mammalian cells. At the start of S phase DNA synthesis commences, but also other events related to DNA synthesis occur, including appearance of histones and replication enzymes. A single molecular signal generated in G1 phase by the action of growth factors may well simultaneously initiate these related processes (1). This intracellular signal is deranged in tumor cells, thus permitting their proliferation under conditions that do not allow proliferation of non-tumor cells. Therefore oncogenes such as myc and ras must function in this signalling process (2). Our studies are aimed at learning about these signals at both cellular and molecular levels. In summary, cells are fully activated about 2 hr before the onset of S phase, as shown by the dispensibility of growth factors (IGF-1) after this time. A requirement for rapid transcription is simultaneously dispensed with. Escape from translational requirements is more complex. A modest inhibition of protein synthesis (50-70%) specifically prevents progression to the "restriction point" (R) where the signal has been activated. The requirement for rapid protein synthesis is accounted for by a protein(s) that is specifically formed in G1, has a short halflife (2.5 hr) and must accumulate to a critical amount within the cell at R to activate entry into S 2 hr later. Tumor cells do not require such rapid protein synthesis to activate late G1 events necessary for S. In our model, stabilization or overproduction of this labile protein(s) in transformed cells results in the loss of regulated activation. Events which are controlled by this regulatory mechanism include DNA synthesis, synthesis of histones, new enzymes and arrangement of these enzymes into a multifunctional "replitase" complex that carries out DNA synthesis in the nucleus. At the molecular level, the principal difference we discern in the synthesis of proteins during G1 in normal and transformed cells is in the properties of a 68K protein (3).

1. Coppock, DL and AB Pardee (1985) Regulation of thymidine kinase activity in the cell cycle by a labile protein. J. Cell. Physiol. 124:269-274.

2. Campisi J, HE Gray, AB Pardee, M Dean and GE Sonenshein (1984) Cell-cycle control of c-myc but not ras expression is lost following chemical transformation. Cell 36:241-247.

3. Croy RG and AB Pardee (1983) Enhanced synthesis and stabilization of Mr 68,000 protein in transformed BALB/c-3T3 cells: Candidate for restriction point control of cell growth. Proc. Natl. Acad. Sci. USA 80:4699-4703.

Cell Cycle and Differentiation — II

L4 REGULATION OF DIFFERENTIATION BY CELL CYCLE-DEPENDENT MECHANISMS IN NORMAL HUMAN EPIDERMAL PROKERATINOCYTES AND MURINE MESENCHYMAL STEM CELLS, B. J. Hoerl, J. J. Wille Jr., M. R. Pittelkow, W. J. Hanson and R. E. Scott, Section of Experimental Pathology, Mayo Clinic/Foundation, Rochester, MN 55905

Mesenchymal stem cells of the 3T3 T type have been employed to establish that the control of adipocyte differentiation is regulated at a distinct complex cell cycle state designated $G_D/G_{D'}/TD$. It has been established that these cells must first growth arrest at the G_D state before differentiation can occur and that such cells then have the option to either reinitiate proliferation or to differentiate. If differentiation is induced, the cells first non-terminally differentiate into adipocytes at the $G_{D'}$ state while maintaining their potential to proliferate. Cells at the $G_{D'}$ state can also then be induced to undergo the terminal event in differentiation wherein they irreversibly lose proliferative potential. Detailed studies using this model system have demonstrated that specific human plasma proteins and other physiological products regulate each step in the differentiation process. Furthermore, these data prove that the integrated control of cellular differentiation and proliferation can be specifically regulated by cell cycle-dependent mechanisms. To evaluate whether normal human epidermal prokeratinocytes (HPK) express comparable or related mechanisms to regulate their differentiation, a tissue culture system was developed wherein HPK can be grown in defined medium. Protocols were then developed to arrest the proliferation of HPK via cell cycle-dependent processes. Thereafter, experiments were performed to evaluate whether keratinocyte differentiation is also regulated at a distinct type of cell cycle state. The results show that two types of growth arrest states can be identified in HPK. The first is a reversible growth arrest state in the G_1 phase of the cell cycle that is induced by culture in: 1) isoleucine-deficient medium or medium containing 2) human lymphokines, 3) β-TGF or 4) ethionine. The second type of growth arrest state is irreversible and is induced in G_1/G_2 by culture in: 1) a high calcium concentration in growth factor-deficient medium, medium containing 2) serum, 3) TPA or related agents, 4) razoxane or by 5) senescence or 6) culture of HPK in suspension. With these cell populations it was established that keratinocyte differentiation only occurs following growth arrest at the irreversible type cell cycle-dependent state. This was demonstrated by use of a variety of immunological, histochemical and morphological differentiation assays. These results therefore strongly suggest that in both murine mesenchymal stem cells and normal human epidermal prokeratinocytes, the control of cellular differentiation and proliferation is integrally regulated and that cell cycle-dependent events serve a significant role in this regulatory process.

L5 ABERRANT REGULATION OF DIFFERENTIATION IN EPIDERMAL CARCINOGENESIS. S. H. Yuspa, National Cancer Institute, Bethesda, MD.

The induction of mouse skin carcinomas by initiation-promotion protocols involves three operationally distinct stages. Initiation and promotion result in papillomas which can be converted to carcinomas by a promoter-independent event. Initiation appears to be a genetic change which results in an altered basal cell response to differentiation signals allowing the altered cells to proliferate away from the basement membrane. In cell culture, epidermal basal cells proliferate in medium with Ca^{2+} concentrations <0.1 mM, and are induced to terminally differentiate in medium with higher Ca^{2+}. Ca^{2+} induced differentiation is mediated via stimulation of phosphatidylinositol lipid metabolism and subsequent activation of protein kinase C. Cells isolated from initiated skin, from carcinogen treated basal cell cultures and from mouse papillomas continue to proliferate in medium with >0.1 mM Ca^{2+}. Phosphatidylinositol metabolism is not stimulated by Ca^{2+} in these "initiated cells". Phorbol esters also induce terminal differentiation in normal keratinocytes presumably by activating protein kinase C directly. "Initiated cells" are resistant to phorbol ester induced terminal differentiation and have a selective growth advantage during tumor promotion. Activated transforming genes, recognized by transfection into several test systems, have not been identified for "initiated cells". However, an activated ras gene may result in an "initiated cell" which requires a tumor promoter for mitogenic stimulation. In contrast, the introduction of an activated ras^{Ha} gene into "initiated cells" reproducibly accomplishes the malignant conversion step. This suggests that the ras locus may be a target for genetic damage during malignant conversion in vivo.

Gene Regulation by Growth Factors and Interferons (Joint)

L6 INTERNALIZATION OF FUNCTIONAL EPIDERMAL GROWTH FACTOR RECEPTOR/KINASE VESICLES AND THE ISOLATION OF A CALCIUM-BINDING 35-kDa SUBSTRATE, Stanley Cohen, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

We have previously reported the isolation of a 35-kDa protein from A-431 cells that, in the presence of Ca^{2+}, is an excellent in vitro substrate for the epidermal growth factor (EGF) receptor/kinase present in membrane preparations (1). We have also demonstrated that the phosphorylation of the 35-kDa protein is markedly enhanced in intact, ^{32}P-labeled A-431 cells following exposure of the cells to EGF. The 35-kDa protein immunoprecipitated from cells treated with EGF is phosphorylated to a 20-120-fold greater extent than comparable preparations from control cells. Both phosphotyrosine and phosphoserine residues are detected in the protein after treatment of the cells with EGF. EGF-dependent phosphorylation of the 35-kDa protein is barely detected unless the intact cells are exposed to EGF for periods greater than 5 min (2).

In parallel experiments we have isolated and partially purified an endosome fraction from A-431 cells. Exposure of intact A-431 cells to EGF leads to an accumulation of both the ligand and receptor/kinase in this endosome fraction. The accumulation is time- and temperature-dependent and is blocked by inhibitors of energy production. The EGF receptor in internalized vesicles is capable of autophosphorylation and, in the presence of Ca^{2+}, of phosphorylation of the previously isolated 35-kDa protein (3). The demonstration of an EGF-induced increase in kinase activity of an internalized vesicle fraction leads credence to the hypothesis that EGF-induced endocytosis of the receptor is of physiological significance in the response of cells to this ligand. In addition, these results are consistent with the hypothesis that the phosphorylation of the 35-kDa protein is associated with internalization of the EGF receptor/kinase complex.

Finally, (De and Cohen, unpublished data) we demonstrate that an antigenically similar 35-kDa protein is present in a number of normal mammalian tissues, and is especially high in lung and placenta. We have isolated this protein from pig lung and find that it not only is antigenically similar to the A-431 human protein, but that it also is a calcium-dependent substrate for the EGF receptor/tyrosine kinase. The protein was purified to homogeneity by gel filtration and ion-exchange chromatography. We have concluded that this 35-kDa protein is a calcium-binding protein by two criteria: calcium alters both its isoelectric point and chromatographic behavior on ion-exchange columns. In addition, calcium is required for the association of the protein with membrane vesicles. The function of this protein is not known.

(1) Fava, R. A. and Cohen, S. (1984) J. Biol. Chem. 259, 2635.
(2) Sawyer, S. J. and Cohen, S. (1985) J. Biol. Chem. 260, 12351.
(3) Cohen, S. and Fava, R. A. (1985) J. Biol. Chem. 260, 8233.

L7 FOS, A MULTIFACETED ONCOGENE: ASSOCIATIONS WITH TRANSFORMATION, PROLIFERATION AND DIFFERENTIATION, Tom Curran[1], Lidia Sambucetti[1], Robert Franza[2] and James I. Morgan[1]. [1]Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110 and [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

The v-fos oncogene is responsible for the induction of osteogenic sarcomas by the FBJ and FBR murine retroviruses. Virus-induced tumors often contain a variety of differentiated osteoid and chondroid cell types. Thus, the oncogenic phenotype associated with v-fos is a consequence of aberrant differentiation and proliferation. The cellular homologue, c-fos, is expressed in extraembryonal tissues and in differentiated macrophages. However, treatment of a variety of cell types with polypeptide growth factors, and other agents, results in an immediate but transient induction of the c-fos gene. Depending on the system studied, c-fos expression has been associated with either differentiation or proliferation. Because c-fos expression is closely linked to receptor-ligand interactions, it is thought to serve a role in coupling the short-term events, such as ion flux and protein phosphorylation, which occur on growth factor stimulation, to long-term alterations in gene expression. We have been studying the pheochromocytoma cell line, PC12, to determine the biochemical and biophysical processes which are involved in the transcriptional activation of c-fos. In these cells, the appearance of the c-fos protein closely parallels the dramatic increase in mRNA levels. The fos gene product is a 55 kilodalton acidic nuclear protein which undergoes extensive post-translational modification. As a result of these modifications, the fos product appears as a heterogeneous array of spots on 2-D gels. It exists in cells as a high molecular weight complex with a basic cellular protein of 39 kilodaltons. This complex, which exists only in the nucleus, displays some DNA-binding characteristics. The data suggest that c-fos may be involved in receptor-genome coupling by effects on gene transcription.

Gene Regulation by Tumor Promoters and Growth Factors

L8 GENES THAT COOPERATE WITH TUMOR PROMOTERS IN TRANSFORMATION, N.H. Colburn, M.L. Lerman, G.A. Hegamyer, K-T. Yao, A. Sakai, W.K. Dowjat, T. Shimada, Cell Biology Section, LVC, National Cancer Institute, Frederick, MD 21701-1013

Two novel genes have been cloned (1) that confer on resistant JB6 mouse epidermal cells sensitivity to promotion of neoplastic transformation by phorbol esters and other tumor promoters. These genetic sequences, termed pro-1 and pro-2 are structurally unrelated to any known oncogenes, though their biological activity can be mimicked by certain oncogenes. In addition to transferring susceptibility to promotion of transformation in JB6 preneoplastic mouse cells, pro-1 transfection into basal cell nevus genetically cancer prone human cells led to substantial life span extension of these cells. Hence, pro genes are apparently capable of cooperating with different genes to produce different endpoints. TPA exposure of JB6 promotion sensitive (P^+) cells yields a transient six to 8-fold stimulation in amount of cytoplasmic RNA hybridizing with a pro-1 probe, contrasted with a two-fold stimulation in promotion resistant (P^-) cells. Stimulation was maximal after 1 to 4 hours of TPA exposure. Characterization of pro-1 protein and RNA gene products is underway. It is postulated that pro gene expression switches on a separate transforming gene (2) that becomes constitutively expressed. Human tumor and normal human DNA have homologs of pro-1 and pro-2 as shown by hybridization with mouse pro gene probes. Human nasopharyngeal carcinomal cell DNA, but not normal human DNA transfers promotion sensitivity when transfected into mouse P^- cells. Screening a genomic library of these carcinoma cells with a mouse pro-1 probe has yielded biologically active clones of human pro-1 showing high molar specific activity. This suggests a role for pro genes in the etiology of human nasopharyngeal carcinoma.

References:

1) Lerman, M.I., Hegamyer, G.A., and Colburn, N.H.: Cloning and characterization of putative genes that specify sensitivity to induction of neoplastic transformation by tumor promoters. International Journal of Cancer, February, 1986.
2) Colburn, N.H., Lerman, M.I., Hegamyer, G.A., and Gindhart, T.D.: A transforming activity not detectable by DNA transfection to NIH 3T3 cells is detected by JB6 mouse epidermal cells. Molecular and Cellular Biology, 5: 890-893, 1985.

L9 MITOGEN NONRESPONSIVE 3T3 VARIANT CELL LINES. Harvey Herschman Dept. of Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024

We have developed a selection technique to isolate mitogen-specific nonproliferative variants from 3T3 cells, and used the procedure to isolate cells that are nonresponsive to either epidermal growth factor (EGF) or tetradecanoyl-phorbol-acetate (TPA). Somatic cell genetic studies on the dominance and recessiveness of these phenotypes will be described. The EGF nonproliferative variants are unable to bind EGF; they are functionally receptorless. Serologic and nucleic acid hybridization analyses for EGF-receptor-related products have been completed, and will be presented. The TPA nonproliferative variants demonstrate phospholipid/calcium-dependent TPA binding and normal levels of protein kinase C. Phosphorylation of protein kinase C substrates and other cellular responses in the TPA nonproliferative variants will be compared to those of the parental 3T3 cells.

L10 GENE ACTIVATION DURING MULTI-STAGE CARCINOGENESIS IN THE MOUSE SKIN, Peter Krieg, Karl Melber, G. Tim Bowden[1] and Gerhard Fürstenberger[2], Institutes for Virus Research and [2]Biochemistry, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG, [1]Univ. of Arizona, Tucson, Ariz., USA.

We have studied the alterations of the activity of cellular genes in different stages of the multi-step carcinogenesis in the mouse skin of NMRI mice. cDNA clone libraries were constructed using poly A^+ RNA isolated from mouse carcinomas that were induced by initiation with DMBA followed by multiple treatments with the tumor promoter TPA. Screening of 5000 cDNA clones by differential hybridization with radiolabeled cDNAs made from normal mouse epidermis and from carcinoma mRNAs as probes resulted in the isolation of cDNA clones displaying different hybridization signals (1). When used as probes in hybridization experiments these cDNA clones, designated pmal-1 to pmal-6, showed strong signals with carcinoma RNA but either a weak or no signal with RNA from normal epidermis. Studies on the expression pattern in vivo revealed transcriptional activation of mal-1, -2, and -3 but not of mal-4 related sequences already in the benign papilloma stage of multistep carcinogenesis. After a single application of TPA mal-related sequences were activated in the epidermis as soon as 2 h after TPA treatment. Transcription increased within 24 h to the same level found in the papilloma/carcinoma and decreased within 72 h. To study the expression pattern of mal-related sequences during the differentiation of epidermal cells, undifferentiated basal cells of the epidermis were separated from the differentiated suprabasal keratinocytes by percoll density gradients. mal-1 related sequences showed a strong transcriptional activity in the differentiated suprabasal keratinocytes and only a weak one in the undifferentiated basal cells. Transcriptional activity of mal-2 related sequences, however, was not detect in either cell type. In contrast, after in vitro cultivation of the basal cells a strong transcriptional activity of mal-2 related sequences was observed. These observations suggest that mal-1 expression can be used as a marker for in vivo differentiation, whereas mal-2 expression can serve as a marker for in vitro expression.

1) K. Melber, P. Krieg, G. Fürstenberger, and F. Marks, Carcinogenesis, in press.

Oncogene Products

L11 THE c-fms PROTO-ONCOGENE AND CSF-1 RECEPTOR. Charles J. Sherr, Martine F. Roussel, and Carl W. Rettenmier. Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38101.

The v-fms oncogene of the McDonough strain of feline sarcoma virus encodes an integral transmembrane glycoprotein of 140 kd (gp140v-fms) that is expressed at the cell surface and is active as a tyrosine kinase. Monoclonal antibodies directed to epitopes in the extracellular aminoterminal domain of gp140v-fms precipitate a 165 kd product of the feline c-fms proto-oncogene (1) that is restricted in its expression to cells of the mononuclear phagocyte lineage (2). Antisera to a bacterially produced v-fms-coded polypeptide react with a murine c-fms gene product that is related, and possibly identical, to the receptor for the mouse macrophage colony stimulating factor, CSF-1 (2). The c-fms-coded glycoprotein specifically binds CSF-1, and in vitro phosphorylation of the receptor on tyrosine is enhanced in the presence of the ligand. By contrast, gp140v-fms specifically binds CSF-1, but its activity as a kinase appears to be constitutive (3).

Cells transformed by v-fms show elevated specific activities of a guanine nucleotide-dependent, phosphatidylinositol-4,5-diphosphate-specific phospholipase C (4), pointing to enhanced production of inositol-1,4,5-triphosphate and diacylglycerol second messengers. However, gp140v-fms does not physiologically function as a phosphatidylinositol kinase. Thus, the increased rate of phosphatidylinositol turnover in transformed cells could reflect coupling of the v-fms gene product (together with a G protein) to phospholipase C.

The product of the human c-fms proto-oncogene is a 150 kd glycoprotein expressed in peripheral blood monocytes. A biochemically and immunologically indistinguishable product is also detected in human choriocarcinoma cell lines. These malignant trophoblasts specifically bind human urinary CSF-1 (Rettenmier, C.W. et al., submitted) suggesting that, in addition to serving as a lineage-specific growth factor in hematopoiesis, CSF-1 may play a role in normal trophoblast development.

References:

1. Rettenmier, C.W., Chen, J.H., Roussel, M.F. and Sherr, C.J. Science 228:320-322, 1985.
2. Sherr, C.J., Rettenmier, C.W., Sacca, R., Roussel, M.F., Look, A.T. and Stanley, E.R. Cell 41:665-676, 1985.
3. Sacca, R., Stanley, E.R., Sherr, C.J. and Rettenmier, C.W. These Proceedings.
4. Jackowski, S., Rettenmier, C.W., Sherr, C.J. and Rock, C.O. These Proceedings and J. Biol. Chem., in press.

Gene Cooperation

L12 ONCOGENE AND CHEMICALLY INDUCED NEOPLASTIC PROGRESSION: ROLE OF TUMOR SUPPRESSION

J. Carl Barrett, Minoru Koi, Mitsuo Oshimura, Lois Annab, O. Sugawara, N. Ozawa and Tona Gilmer, Natl. Inst. Environ. Hlth. Sci., Res. Tri. Prk., N.C. 27709

We have examined the ability of cloned viral oncogenes, alone or in combination, to induce neoplastic transformation of Syrian hamster embryo cells. We observed that following DNA transfection, the v-Ha-ras oncogene induced morphological transformation transiently but the cells senesced and did not form tumors. The v-myc oncogene alone also failed to induce neoplastic transformation of the cells. However, transfection of the cells with v-Ha-ras plus v-myc DNAs or with polyoma DNA and injection into nude mice resulted in progressively growing tumors as early as 3-4 weeks after injection (1). These results are consistent with our studies of chemical carcinogen-induced neoplastic transformation, which indicated that multiple steps, and hence multiple changes in genes, are necessary for neoplastic development of these cells (2). The number of steps necessary to convert a normal cell into a malignant cell is unknown. We analyzed the karyotypes of tumors formed after transfection of Syrian hamster embryo cells with either v-Ha-ras plus v-myc DNAs or polyoma DNA (3). Cells from tumors induced by polyoma DNA were diploid, whereas cells from tumors induced by v-Ha-ras plus v-myc oncogenes had a nonrandom chromosome change, loss of chromosome 15. Thus, an additional change, loss of chromosome 15, is required or advantageous for tumorigenicity induced by v-Ha-ras plus v-myc oncogenes. Other observations also support a three step model for neoplastic transformation. Carcinogen-induced, immortal cell lines are neoplastically transformed by v-Ha-ras oncogene alone, but the susceptibilities of cell lines vary. Tumorigenicity and anchorage-independent growth are recessive traits in hybrids between tumorigenic cells and normal Syrian hamster embryo cells. Some, but not all, immortal cells can suppress tumorigenicity. This ability decreases with passaging of immortal cell lines and subclones are heterogeneous in their ability to suppress tumorigenicity. Also, the susceptibility of immortal cell lines to neoplastic transformation by DNA transfection with viral oncogenes or tumor DNA is inversely correlated with suppresive ability in cell-cell hybrids. Taken together these observations indicate that neoplastic transformation of Syrian hamster embryo cells involves at least three steps: 1) induction of immortality; 2) activation of a transforming gene or oncogene; 3) loss or inactivation of a suppressor or growth arrest function. The loss of the cell's ability to suppress tumorigenicity appears to be a key step in the process of neoplastic progression.

(1) D.G. Thomassen, T.G. Gilmer, L.A. Annab and J.C. Barrett. Cancer Res., 45:726-732,1985.
(2) J.C. Barrett and P.O.P. Ts'o. Proc. Nat'l. Acad. Sci., USA, 75:3761-3765, 1978.
(3) M. Oshimura, T.M. Gilmer and J.C. Barrett. Nature, 316:636-639, 1985.

L13 CONTROL OF C-MYC GENE EXPRESSION, Neil M. Wilkie, Jas. Lang and Bruce Whitelaw, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland.

Alterations in the myc gene are frequently associated with experimentally induced and naturally occurring cancer. Alterations associated with naturally occurring cancer include chromosomal translocation and mutations affecting the amino sequence in Burkitt's lymphoma (1) amplification in tumour cell lines (2,3) and both insertional mutagenesis and transduction by Feline Leukaemia Virus (FeLV,4). Although the role of changes in amino acid sequence have not yet been resolved, analysis of the FeLV system strongly suggests that these are not essential but that alterations in the control of myc gene expression are important. (J. Neil, personal communication). In established cell lines in vitro, myc gene expression appears to by under the constant control of signal transduction systems associated with some growth factors (5,6).

We have investigated some aspects of myc gene regulation in transient expression assays. Basal promoter activity was found immediately 5' to the two cap sites. Further upstream, at least two other regulatory regions have been identified, one of which appears to be a target for negative regulation. This was confirmed by transfer of the sequence upstream to other promoters, and by competition assays. The data suggest that the human sequence is a target for protein(s) found in both human and murine cells. This region contains the DNase hypersensitive site II_2 (7) a conserved sequence shared by the murine and human genes, and a small region of potential Z DNA. In collaboration with the laboratory of R. Tijan in Berkeley, the protected sites associated with DNA binding proteins are now being investigated. It will be of considerable interest to determine how such regulatory proteins interact with the signal transduction systems.

(1) Rabbits TH, Hamlyn PH, Baer R (1983) Nature 306, 760-765.
(2) Schwab M, Ramsay G, Alitalo K, Varmus HE, Bishop JM, Martinsson T, Levan G, Levan A (1985) Nature 315, 345-347.
(3) Schwab M, Alitalo K, Klempnauer KH, Varmus HE, Bishop JM, Gilbert F, Brodeur G, Goldstein M, Trent J (1985) Nature 305, 245-248.
(4) Neil JC, Hughes D, McFarlane R, Wilkie NM, Onions DE, Lees G, Jarrett O (1984) Nature 308, 814-820.
(5) Kelly K, Cochran BH, Stiles CD, Leder P (1983) Cell 35, 603-610.
(6) Armelin HA, Armelin MCS, Kelly K, Stewart T, Leder P, Cochran BH, Stiles CD (1984) Nature 310, 655-660.
(7) Siebenlist U, Henninghausen L, Battey J, Leder P (1984) Cell 37, 381-391.

Suppression of Tumorigenicity

L14 RECESSIVE MUTATIONS PREDISPOSING TO HUMAN CANCER, Webster K. Cavenee, Department of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0524.

Predispositions to retinoblastoma and Wilms' tumor can be inherited as autosomal dominant traits. We compared DNA sequence polymorphisms in constitutional and tumor tissues from several children in order to determine the chromosomal mechanisms whereby initial predisposing germline mutations were expressed. These somatic mechanisms include mitotic nondisjunction and somatic recombination. In the bilateral form of retinoblastoma, inheritance of a germinal mutation at the RB1 locus at 13q14 predisposes each retinal cell to a subsequent second somatic event which results in the formation of the retinoblastoma tumor. Spontaneous unilateral tumors appear to arise following two sequential somatic events. Survivors of bilateral disease are at substantially increased risk for development of second primary cancers, particularly osteosarcoma, a bone cancer. Conversely, survivors of unilateral retinoblastoma exhibit the same likelihood of development of osteosarcoma as the general population. We determined restriction fragment length alleles at loci on chromosome 13 in DNA from constitutional tissues of bilaterally affected retinoblastoma patients and from their osteosarcoma tumors. Loss of constitutional heterozygosity occurred specifically for chromosome 13 and appeared to involve the same chromosomal region as that previously identified in retinoblastoma tumors. Similarly, specific loss of constitutional chromosomal 13 heterozygosity occurred in sporadic osteosarcomas suggesting that pleiotropic expression of recessive mutant alleles on chromosome 13 are involved in the genesis of both types of bone tumors as well as retinoblastoma. The significant clinical association of bilateral, but not unilateral, retinoblastoma with osteosarcoma indicates that both diseases are due to the unmasking of recessive mutants alleles at the RB1 locus by loss of the wild type chromosomal homologue. These findings provide a theoretical approach toward defining the number and location of genes whose recessive mutant forms predispose to cancer and suggest that the clinical occurrence of mixed cancer families may be due to the differential expression of a single recessive mutation. In pursuing this line of reasoning, the high frequency association between congenital malformations and embryonal tumors (hepatoblastoma, rhabdomyosarcoma and Wilms' tumor) in the autosomal dominant Beckwith-Wiedemann Syndrome became apparent. We determined constitutional and tumor genotypes at various autosomal loci in samples from several children with each tumor type. In each instance, loci on chromosome 11 showed clear monoclonal loss of constitutional heterozygosity in the majority of tumor samples. Loci on other chromosomes always showed maintenance of the constitutional genotype in the tumor samples. These results suggest that the high frequency association between these three tumor types may be due to recessive mutations of the WT1 locus on chromosome 11p13. They suggest a limited number of recessive oncogenes in the human genome, each with a broad but selective tissue specificity. Finally, they suggest a rational approach to the mapping of these loci based on clinical tumor-tumor and tumor-congenital anomaly associations.

L15 CHARACTERIZATION OF HUMAN GROWTH-INHIBITORY SEQUENCES BY DNA-MEDIATED GENE TRANSFER, Bruce Howard, Raji Padmanabhan, Tazuko Howard, Michael Fordis, and Mary McCormick, Laboratory of Molecular Biology, National Institutes of Health, Bethesda, MD 20892

Mammalian cell growth may be regulated by a balance between genes that act in stimulatory and inhibitory modes. We report a gene transfer assay designed to detect gene sequences that are inhibitory for the growth of HeLa cells. The essential steps in this assay are as follows: i) $CaPO_4$-mediated transfection of HeLa S3 cells with a mixture of genomic DNA and a selectable marker plasmid, pRSVneo; ii) selection against cells that continue to replicate when transferred into spinner culture by treatment with BrdUrd/Hoechst 33258 and fluorescent light; iii) monolayer culture of surviving cells in the presence of the antibiotic G-418 to eliminate nontransfected cells. Growth inhibitory activity is scored by an increase in the number G-418 resistant colonies (a function of the number of cells that fail to replicate during the BrdUrd/Hoechst 33258 negative selection step) relative to the number of G-418 colonies obtained following control transfection with an equivalent E. coli DNA/pRSVneo mixture. Low levels of growth inhibitory activity were found in genomic DNAs harvested from replicating HeLa cells, confluent NIH/3T3 mouse fibroblasts, confluent rat embryo fibroblasts, and confluent MRC-5 human embryo fibroblasts. Relatively high levels of growth inhibitory activity (5-20 fold greater than in HeLa DNA) were found in genomic DNAs from confluent WI38 human embryo fibroblasts and quiescent (serum-starved) MRC-5 human embryo fibroblasts. The requirement for serum starvation to detect growth inhibitory activity in MRC-5 cells suggests that expression of these sequences may be subject, under some circumstances, to an epigenetic control mechanism. Cosmid libraries constructed from E. coli, HeLa, and WI38 DNAs exhibited qualitatively similar growth inhibitory activities to the genomic DNAs from which they were derived; moreover, subfractionation of the WI38 cosmid library (sib selection) was consistent with the idea that sequences with the potential for growth inhibitory activity may be more abundantly represented in the mammalian genome than previously suspected.

L16 PHENOTYPIC REVERSION OF ras TRANSFORMED CELLS, C.J. Marshall, H.F.Paterson, R.Brown A.Hall, B.Reeves, J.L. Bos* and M.E. Furth†, Institute of Cancer Research, Chester Beatty Laboratories, London SW3 6JB, U.K., *Sylvius Laboratories, State University of Leiden, The Netherlands, †Memorial Sloan Kettering Cancer Centre, New York.

To investigate the contribution of mutant ras genes to the transformed phenotype of human tumor cells we have isolated flat revertants of HT1080 fibrosarcoma cells and EJ bladder carcinoma cells. HT1080 harbours a mutant N-ras gene (GLN61→LYS) and EJ a mutant c-Ha-ras-1 (GLY12→VAL). Revertants of both HT1080 and EJ are permanent cell lines that are unable to grow in anchorage independent conditions and are non-tumorigenic. Transfection experiments on to NIH3T3 cells show that all revertants retain the mutant ras gene, thus the mechanism of reversion does not involve complete loss of the ras transforming gene. Revertants can be re-transformed by transfection with their own cloned transforming gene and thus the mechanism of reversion is not resistance to the ras transforming protein. The sensitivity of the revertants to retransformation by ras oncogenes coupled with the retention of the ras oncogene by the revertants suggests that the mechanism of reversion may be due to a dosage effect This model has been supported by observations of the revertants of HT1080. By a combination of cytogenetic techniques and oligonucleotide probing we have shown that the revertants are tetraploid derivatives of HT1080 which contain only 3 instead of 4 copies of N-ras carrying chromosome 1. The underrepresented chromosome carries the mutant transforming allele, thus the revertants contain two copies of wild type N-ras but only one of the transforming allele. When rare re-revertants, which have regained tumorigenicity are examined they are found to contain 2 copies of the mutant transforming gene. To examine how these changes in dosage affect protein levels we have immunoprecipitated p21 and examined the precipitates on high resolution-2D gels. The level of the p21 ras transforming proteins are lower in the revertants than in the parental HT1080 cells and rise again in the revertants. We therefore conclude that the dosage of the transforming gene finely controls the expression of the transforming phenotype and that only small alterations in the level of the transforming protein are sufficient to render the cells non-tumorigenic.

L17 GENETIC STUDIES OF TUMOR SUPPRESSION, Ruth Sager, Ruth Craig and Wendy O'Brien, Division of Cancer Genetics, Dana-Farber Cancer Institute, Boston, MA 02115.

Cancer starts as a disease of individual cells, initiated by genetic abnormalities, either inherited or induced by DNA damage. The consequences at the cellular level include defective control of proliferation, inhibition of differentiation, and concurrently the continuing accumulation of chromosomal aberrations. These aberrations run the gamut from point mutations to much more frequent complex rearrangements. Associated with these rearrangements are genes involved in carcinogenesis. Rearrangements may activate genes that promote oncogenesis, the oncogenes, or inactivate genes that suppress it. This latter class of genes, associated with deletions, act as dominant suppressors of tumor formation, and are called anti-oncogenes or tumor suppressor genes. Two lines of experimentation in this laboratory have focussed our interest on tumor suppressor genes.

1. Suppression of tumor-forming ability in cell hybrids. We have observed suppression of tumor forming ability in hybrids from fusions of normal human or Chinese hamster (CHEF) cells with H-ras transfected tumorigenic cells. As reported (1) suppressed hybrids contain H-ras transfected DNA and express elevated p21. Thus suppression occurs post-translationally in this system. Experiments designed to induce suppression by transfecting a plasmid containing the normal H-ras allele with a 5' LTR into H-ras-transformed CHEF cells were unsuccessful. Studies using DNA transfer of subtracted cDNAs from normal cells to suppress the H-ras transfected cells are in progress.

2. Resistance of normal human cells to transformation. Many laboratories have reported their failure to induce tumorigenicity in human cells with chemicals, radiation, or viruses (2). We have previously described the inability of either SV40 DNA or the human mutant c-H-ras gene to induce tumor forming ability in diploid foreskin fibroblasts; and proposed that the resistance of human cells resulted from the evolution of strong tumor suppressor genes (3). We now report circumstances under which stable induction of tumor forming ability has been achieved with these cells, and discuss these results in relation to suppression.

(1) Craig, R. and R. Sager (1985) Suppression of tumorigenicity in hybrids of normal and oncogene-transformed CHEF cells. Proc. Natl. Acad. Sci. 82: 2062-2066.
(2) Antrup, H. and C. C. Harris, eds. (1983) Human Carcinogenesis. Academic Press, New York.
(3) Sager, R. (1984) Resistance of human cells to oncogenic transformation. Cancer Cell 2: 487-493.

L18 TUMOR-SUPPRESSOR GENES AND THE CONTROL OF NEOPLASTIC BEHAVIOR OF HUMAN CELLS, Eric J. Stanbridge, Andrew Geiser, Paul Saxon, Eri S. Srivatsan, and Joyce Wilkinson. Department of Microbiology and Molecular Genetics, University of California, Irvine, Irvine, CA 92717

Somatic cell hybrid studies have clearly established that stable suppression of the tumorigenic phenotype is found in hybrid cells derived from the fusion of human malignant cells with normal human cells. Specific chromosomes have been implicated in the control of tumorigenic expression. We now show that the transfer of single specific human chromosomes into human cancer cells may result in suppression of tumorigenicity. When cancer cells with activated oncogenes (e.g., EJ and HT1080) are used as recipients, suppression of tumorigenicity is accomplished, even though the product of the activated oncogene continues to be expressed. Strategies to identify the tumor-suppressor gene(s) will be discussed.

Inhibitors of Growth and Transformation (Joint)

L19 EXPRESSION OF THE GENES FOR THE TRANSFORMING GROWTH FACTORS-α AND -β, Rik Derynck, Ann Van Tilburg, Lucy Rhee, Ellson Chen,

Timothy S. Bringman, Marjorie E. Winkler, Jordan U. Gutterman* and David V. Goeddel, Genentech, Inc., South San Francisco, CA 94080, *M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

The two transforming growth factors, TGF-α and -β, are structurally unrelated polypeptides which bind to different receptors. cDNA analysis has revealed the structure of both TGFs and their precursors. Human TGF-α is a 50 amino acid peptide which is structurally related to EGF, recognizes the EGF receptor and is proteolytically derived from a 160 residue long precursor. Human TGF-β is a dimer of two identical 112 amino acid polypeptides which are synthesized as the C-terminal segment of a 391 amino acid precursor. Comparison of the human and murine TGF-β precursor sequences reveals that the mature TGF-β is extremely conserved. Also, the N-terminal third of the precursor exhibits very high sequence conservation, suggesting an important biological function for this polypeptide. The gene for human TGF-α is more than 70 kbp long and contains six exons, while the gene for the human TGF-β precursor contains five exons.

Human TGF-α synthesized in E. coli was used to raise polyclonal and monoclonal antibodies. These antibodies were used to develop a fast and sensitive TGF-α specific ELISA. This assay was used to evaluate expression by a variety of tumor cell lines. The expression of the TGF-α gene in various tumor cell lines and surgically removed tumors was also evaluated by Northern analysis of RNA from these cell sources. These analyses revealed that the TGF-α gene is expressed in several classes of solid tumors and not by hematopoietic tumors.

L20 TRANSFORMING GROWTH FACTOR-BETA (TGF-beta): REGULATOR OF CELLULAR PROLIFERATION, CELLULAR DIFFERENTIATION, AND CELLULAR FUNCTION, Anita B. Roberts, Mario A. Anzano, Sonia B. Jakowlew, John B. Kehrl, Lalage M. Wakefield, and Michael B. Sporn, National Cancer Institute, Bethesda, MD 20892

TGF-beta is a homodimeric disulfide-crosslinked peptide of Mr 25,000 which has been found in all tissues and cell lines examined (1) including cells of hematopoietic origin; the most concentrated source of TGF-beta is platelets. High affinity receptors for TGF-beta (Kd 10-40 pM) are found on all cell types examined. The actions of TGF-beta on cells are diverse. Although TGF-beta stimulates the anchorage-independent growth of

certain non-neoplastic fibroblasts (such as NRK and AKR-2B cells), it also acts to inhibit the growth of many other non-neoplastic and neoplastic cells. The ability of TGF-beta to inhibit the anchorage-independent growth of myc-transfected Fischer rat 3T3 cells depends on the other growth factors acting together with TGF-beta; TGF-beta inhibits EGF-dependent growth of these cells in soft agar, but stimulates their growth in the presence of PDGF. In monolayer culture, TGF-beta usually blocks the mitogenic effects of either EGF or PDGF on fibroblasts. In bronchial epithelial cells, TGF-beta inhibits growth and promotes differentiation of the cells. While its effects on cellular proliferation are often inhibitory, TGF-beta stimulates many cell functions such as glucose and amino acid transport, synthesis of prostaglandins, synthesis of collagen and proteoglycans, and in certain instances synthesis of EGF receptors. This suggests that TGF-beta might function as a trophic factor, stimulating growth of cells in the absence of cellular proliferation.

TGF-beta synthesis is regulated, at least in part, at the level of mRNA. Both viral transformation of murine fibroblasts and mitogen-activation of human lymphocytes result in increased secretion of TGF-beta, and in each case the level of TGF-beta mRNA is also increased. TGF-beta mRNA is optimally induced 2-4 hrs after activation of lymphocytes, yet maximum secretion of TGF-beta is delayed for approximately 72 hrs. Implications of these findings for lymphocyte function are currently being investigated.

1. for review: Roberts, A. B. and Sporn, M. B., Cancer Surveys, in press.

Signal Transduction in Neoplastic Transformation — I

L21 REGULATION OF PROTEIN KINASES AND CELLULAR PROLIFERATION BY TRANSFORMING PROTEINS, R.L. Erikson, J. Blenis, A. Bedard, and J. Martin-Perez, Department of Cellular and Developmental Biology, Harvard University, Cambridge, MA 02138

Normal cells cease division under conditions where malignant cells continue to divide. We study this feature of malignant transformation in our laboratory with the aim of describing the early biochemical events that may lead to cell division. A biochemical marker for quiescent cells deprived of serum growth factors is the unphosphorylated 40S ribosomal subunit protein S6. Following expression of a transforming protein such as $pp60^{v\text{-}src}$, or the addition of serum or tumor promoting phorbol ester, S6 is rapidly phosphorylated on serine residues. Thus, the mechanism of regulation of the enzymes involved offers the possibility of identifying additional tyrosine-specific protein kinase substrates. A chromatographically similar S6 protein kinase is stimulated several fold in each of these cell populations. Moreover, the stimulated S6 kinase is distinct from cAMP-dependent protein kinase and protein kinase C. We believe the S6 protein kinase is activated by covalent modification because it occurs in the presence of protein synthesis inhibitors.

In cells treated with phorbol ester, S6 protein activity transiently increases and declines to the initial level by 4 hours. In these cells the S6 protein kinase activity cannot be restimulated with an additional phorbol ester treatment apparently because of down regulation of the phorbol ester receptor, protein kinase C. The S6 protein kinase can be activated in these cells by addition of serum or the expression of $pp60^{v\text{-}src}$. These data indicate that it is likely there are at least two mechanisms through which S6 kinase activity can be regulated, one apparently utilizes protein kinase C whereas the other(s) does not. Preliminary evidence suggests that after expression of $pp60^{v\text{-}src}$ the phosphatase(s) responsible for the dephosphorylation of S6 is less active therefore contributing to the overall level of S6 phosphorylation in the transformed cell.

We have obtained cDNA clones of mRNAs expressed in transformed cells. Some of these mRNAs appear to be regulated by the expression of $pp60^{v\text{-}src}$ and thus may contribute to transformed phenotype. Characterization of certain cDNA clones will be discussed.

In addition to altered enzyme activity and gene expression, we have also observed greatly reduced levels of some proteins in cells after expression of $pp60^{v\text{-}src}$ or the addition of growth factors to quiescent cells. An example is a 20 kD protein secreted by quiescent confluent cells. Antibody has been prepared and immunoprecipitation analyses show that synthesis of the 20 kD protein is greatly reduced upon expression of $pp60^{v\text{-}src}$. Therefore these studies and reports by others suggest transformation or cellular proliferation may be accompanied by both increased or decreased gene expression.

L22 **ACTIONS OF TYROSINE PROTEIN KINASES ON STEROID HORMONE RECEPTOR PROPERTIES,** C. Fred Fox, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

Progesterone receptor purified from chicken oviduct is an effective substrate for phosphorylation by purified human EGF receptor kinase (1). Both the 80 and 105 kDa subunits are phosphorylated at identical sites as revealed by mapping of phosphopeptides derived by trypsin action, and stoichiometry of greater than unity has been achieved. The K_M for the reaction is 100 nM, and the V_{max} for progesterone receptor phosphorylation is of the same magnitude of that for synthetic peptide substrates, in the range of 1 μmol/min/mg protein. The progesterone receptor also has been characterized with PDGF and insulin receptor kinases, and is a substrate for the latter, but not the former (2). Comparisons of the actions of EGF and insulin receptor kinases on progesterone receptors indicate several differences: a) The EGF receptor phosphorylates both progesterone receptor subunits at equal rates and K_M values, whereas the 80 kDa progesterone receptor subunit is phosphorylated at a higher rate and with a higher K_M by the insulin receptor. b) The insulin and EGF receptors have specificities for different domains on progesterone receptor subunits.

Human mammary carcinoma cell lines display receptors for both progesterone and ligands activating tyrosine protein kinases. A number of such lines have been tested for the effects of such ligands on progesterone binding activity. Progesterone binding to these cells, or to particulate and supernatant fractions derived from them, revealed the presence off high and low affinity progesterone binding sites. These were detected in whole cell binding studies; and in studies with the subcellular fractions, the lower affinity progesterone receptors were present exclusively in the particulate fraction, and the higher affinity receptors, in the supernatant fraction (3). Treatment of these cells with EGF prior to or after progesterone binding resulted in a substantial reduction in their progesterone binding capacity, and this was isolated to the lower affinity, particulate fraction binding sites (4). Insulin addition had a similar effect, and the dose response curve indicated that insulin may have acted through association with somatomedin receptors. A progesterone analog has been shown to have a cytostatic action on human breast carcinomaa T47D cells (5); preliminary studies indicate that this action is attenuated by EGF. Supported by USPHS Grant No. AM25826 and ACS Grant No. BC-473.

1. Ghosh-Dastidar, P., Coty, W.A., Griest, R.E., Woo, D.D.L. and Fox, C.F. (1984) Proc. Natl. Acad. Sci. USA 81: 1654.
2. Woo, D.D.L., Fay, S.P., Griest, R.E., Coty, W.A., Goldfine, I.D. and Fox, C.F. (1986) J. Biol. Chem. 261: 460.
3. Rao, K.V.S., Sarup, J.C., Coty, W.A. and Fox, C.F. (1986) J. Cell. Biochem., Suppl. 10C, Abs. 306.
4. Sarup, J.C. and Fox, C.F., (1986) J. Cell. Biochem., Suppl. 10C, Abs. 310.
5. Horwitz, S.K. and Freidenberg, G. (1985) Cancer Res. 45: 167.

L23 THE ROLE OF PROTEIN KINASE C IN CELL SURFACE SIGNAL TRANSDUCTION.
Ushio Kikkawa and Yasutomi Nishizuka, Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan.

Information from certain extracellular signals, including a group of peptide hormones, some neurotransmitters and many other biologically active substances flows from the cell surface into the cell interior through two routes, Ca^{2+} mobilization and protein kinase C activation. At an early phase of cellular responses, inositol-1,4,5-trisphosphate mobilizes Ca^{2+}, whereas diacylglycerol (DG) activates protein kinase C. These two signal mediators are generated from the receptor-mediated hydrolysis of phosphatidylinositol-4,5-bisphosphate. By using Ca^{2+}-ionophore and permeable DG or tumor-promoting phorbol ester (as a substitute for active DG), it has been shown that two routes are both essential and often act synergistically to induce many of cellular responses such as exocytosis, smooth muscle contraction and metabolic activation. Similarly to these short-term responses, both protein kinase C activation and Ca^{2+} mobilization appear to be essential for long-term responses as well. However, additional receptor occupation by growth factor is necessary to induce full activation of cell proliferation, and the signal pathway through protein kinase C appears to be separate from and synergistic to that via growth factors. Immunohistochemical studies with monoclonal antibodies raised against protein kinase C suggest that this enzyme seems to be absent or in very low concentration in the nucleus; probably another step in signal translation is needed for the ultimate activation of nuclear events.

Several functional proteins in many tissues have been reported to serve as substrates of protein kinase C. The phosphorylation of some of these proteins such as membrane receptors (EGF receptor, insulin receptor, etc.), and Ca^{2+}-transport ATPase is apparently related to down regulation or negative feedback control of activation of cellular functions. It is possible that protein kinase C has dual actions in the positive as well as the negative phase of regulation depending on the function of each target substrate protein. However, crucial information of the physiological target proteins is still limited in most tissues. In this talk, results will be summarized of further studies on the mode of activation, intracellular localization and possible role of this unique protein kinase system.
(Supported by the Ministry of Education, Science and Culture, Japan)

L24 ONCOGENIC SUBVERSION OF EGF RECEPTOR FUNCTION - THE SEARCH FOR A MECHANISM, M.D.Waterfield, J. Haley, S. Clark, J. Downward, N. Whittle, L. Owen, M. Berger, C. Greenfield, W. Gullick, P. Parker and A. Ullrich*. Imperial Cancer Research Fund, London WC2A 3PX, *Genentech, Inc., San Francisco CA 94080.

The expression of an EGF receptor truncated at both amino and carboxyl termini by the erb-B oncogene is central to transformation and tumour production by Avian Erythroblastosis virus (AEV). Several different approaches to understanding the basis of subversion of signal transduction by the receptor will be described. The EGF stimulated tyrosine specific protein kinase remains the sole intrinsic receptor function. The activated kinase in intact cells autophosphorylates residues 1173 (site P1) of the receptor and in vitro (or in membrane preparations) will incorporate 2 additional moles of phosphate into sites P2 and P3 (residues 1148 and 1068). Studies with antisera raised to synthetic peptides indentical to the sequence of the autophosphorylation sites show that the autophosphorylation activity can be blocked by antibodies while the kinase continues to be fully active towards synthetic substrates. Detailed enzyme kinetic studies show that solublised receptor kinase in membranes having 0.2 - 2.8 moles/mole of phosphate has an unchanged kinase activity. The region of the receptor surrounding the ATP binding site has been defined by sequence analysis of FSBA labelled receptor and specific antibodies raised to a synthetic peptide associated with the region. The antibodies selectively inhibit the EGF receptor kinase without inhibiting other kinases (src, abl, C, A kinases). Using these antibodies the autophosphorylation site is shown to be in a distinct domain of MW 20,000 cleavable from the purified receptor by purified calpain.

The interaction of purified C kinase with ligand activatable purified receptor has been studied and the molecular mechanism of transmodulation of the receptor by phorbol esters and PDGF studied.

Since subversion by AEV of receptor function involves truncation, a series of deletion and site specific mutated receptor cDNAs have been generated allowing the expression of modified receptors. Structure-function studies made by microinjection of mRNA into firbroblasts and oocytes together with studies carried out in reticulocytes will be described. The use of various vectors to express mutant receptors in different cell types will also be discussed.

In conclusion the search for defective EGF receptor expression in human tumours of glial and squamous origin will be discussed.

Signal Transduction in Neoplastic Transformation — II

L25 SIGNALS AND SEQUENCES INVOLVED IN THE UV AND TPA DEPENDENT INDUCTION OF GENES, Peter Herrlich, Hans Jobst Rahmsdorf, Peter Angel, Christine Lücke-Huhle, Carsten Jonat, Anne-Marie Eades, Michael Karin[1)], Andrew Cato and Helmut Ponta, Kernforschungszentrum Karlsruhe, Institut für Genetik, Postfach 3640, D-7500 Karlsruhe 1, West Germany, and [1)]Dept. of Microbiology, University of Southern California, Los Angeles, California, USA

The end products of carcinogen action: the tumor cells carry various characteristic genetic changes including point mutations (1) and translocations (2). There is a considerable time lag between the primary carcinogen interaction and the appearance of the tumor cell and the presumed steps towards neoplastic transformation occur with unexpectedly high frequency and tissue specificity. These yet unexplained features suggest endogenous enzymatic processes for which genes may be induced by the carcinogen.

We have examined immediate genetic changes after treating cultured human cells with carcinogens or cocarcinogens. Several agents e.g. ultraviolet irradiation (UV), mitomycin C or Tetradecanoylphorbol-12-acetate(TPA) induce the appearance of the same set of new gene products (3). We are investigating their regulation and function using c-DNA and genomic cloning techniques and searching for phenotypic consequences of their expression.

With respect to their regulation, the set of genes is not homogeneous. The larger part of the genes is induced by DNA damage since in cells from patients with Xeroderma pigmentosum, about 20-30 fold less UV dose is required than in normal human cells. One of the genes (c-fos),however, responds to UV in the same dose dependent manner in both types of cells suggesting that DNA damage is not required.

The molecules linking the primary site of carcinogen or cocarcinogen action with the responding genes are yet largely unknown. Inhibition of proteinkinase C prevent the induction of c-fos by UV and by TPA. Using mutations in the 5' flanking regions of responsive genes, we have defined sequences which need to be present for regulation. The proteins working on these sequences have, however, not yet been found.

The current state of knowledge on regulation and function of the genes will be discussed using some of the following examples:

a) the human cellular fos gene, b) the MHC associated invariant chain as an example of a differentiation marker, c) the human metallothionein IIa gene, d) the major UV induced secreted protein XHF1, e) the UV induced secreted factor EPIF (3) which replaces the requirement for UV in the induction of genes, f) the mouse mammary tumor provirus, g) a gene amplification triggering function

(1) H. Zarbl et al., Nature 315, 382-385 (1985)
(2) J. Cairns, Nature 289, 353-357 (1981)
(3) M. Schorpp et al., Cell 37, 861-868 (1984), P. Herrlich et al., Adv. Enzyme Regulation, in press, P. Angel et al., Cancer Cells 3, 315-319 (1985).

L26 PROTEIN PHOSPHORYLATION IN VIRAL TRANSFORMATION AND GROWTH CONTROL
Tony Hunter, Kathleen L. Gould, Jonathan A. Cooper, Chris J. M. Saris and James R. Woodgett, The Salk Institute, P. O. Box 85800, San Diego, California 92138

Many growth factor receptors and viral transforming proteins are protein-tyrosine kinases (PTK). To determine whether tyrosine phosphorylation plays a role in growth control and viral transformation, we have identified several substrates for both types of PTK and asked whether phosphorylation of these proteins alters their function in a meaningful way. The substrates for the viral PTKs include vinculin, p81, p50, p36, enolase, LDH, and phosphoglycerate mutase. Vinculin, p36 and p81 form part of a submembraneous cortical skeleton in cultured cells, but to date there is no evidence that tyrosine phosphorylation of these proteins is involved in the transformed phenotype. p36 has been shown to be a Ca^{2+}/phospholipid binding protein. To learn more about its structure and function we have isolated and sequenced p36 cDNA clones.

We have previously found that protein kinase C (PKC) phosphorylates the EGF receptor at Thr 654 ten residues from the cytoplasmic face of the plasma membrane. This modification decreases the EGF-stimulated receptor PTK activity and diminishes the binding affinity for EGF and may be part of a feedback mechanism for regulating EGF receptor function by agents which increase phosphatidylinositol turnover and hence diacylglycerol (DAG) production. In an ongoing search for PTKs which might be regulated by PKC we have found that PKC phosphorylates $pp60^{c\text{-}src}$ at Ser 12. Phosphorylation of Ser 12 in $pp60^{c\text{-}src}$ is stimulated by treatment of intact cells with TPA or a synthetic DAG, as well as by addition of PDGF and bombesin, treatments which increase DAG levels. $pp60^{c\text{-}src}$ is associated with membranes via its N-terminal myristyl group, which means that Ser 12 lies in a position almost exactly homologous to that of Thr 654 in the EGF receptor. In contrast to the functional effects of Thr 654 phosphorylation on EGF receptor function, we have detected no consistent alteration in the enzymatic activity of $pp60^{c\text{-}src}$ consequent upon phosphorylation of Ser 12 . We are currently determining whether some other property of $pp60^{c\text{-}src}$, such as its distribution in the cell, is affected.

$pp60^{v\text{-}src}$ and $pp60^{c\text{-}src}$ display a considerable difference in PTK activity in vitro and this appears in part to be due to the phosphorylation of $pp60^{c\text{-}src}$ on a tyrosine residue. We have identified this residue as Tyr 527, which is in a sequence at the C-terminus of $pp60^{c\text{-}src}$ that is lacking in $pp60^{v\text{-}src}$. This type of negative regulation may also exist for other PTKs.

We have used polyclonal affinity-purified antibodies directed against rat brain PKC to study its phosphorylation, subcellular location and distribution in cell lines under various conditions. An 80 kDa ^{35}S-methionine-labeled protein, which comigrates with pure PKC and has an identical partial proteolysis map, can be immunoprecipitated from a wide variety of cells in amounts proportional to the number of PdBu binding sites they possess. Treatment of cells for 24 hr with TPA causes almost complete loss of the 80 kDa protein, in keeping with the known down regulation of PKC activity under these conditions.

Growth and Differentiation

L27 ABROGATION OF INTERLEUKIN-3 DEPENDENT GROWTH OF A MURINE MYELOID LEUKEMIC CELL LINE BY *src* AND *abl*,
Steven M. Anderson[1], Olga Agranovsky[2] and Allen Oliff[2], Merck Sharp & Dohme, West Point, PA and Memorial-Sloan Kettering Cancer Center, New York, NY.

Interleukin-3 (IL-3) is required to stimulate the *in vitro* proliferation of murine myeloid, macrophage and erythroid precursor cells. In addition, some leukemic cells also require IL-3 to maintain growth *in vitro*. An IL-3 dependent leukemic cell line was established from a mouse infected with Friend murine leukemia virus. This line does not form transplantable leukemias *in vivo* but is immortalized for growth *in vitro* when grown in the presence of IL-3. We have investigated the ability of various oncogenes to allow these cells to become IL-3 independent. *src* and *abl* both eliminated the IL-3 requirement for cell proliferation; although, Ki-*ras*, Ha-*ras*, *fos* and *mos* did not. Concomitant with the loss of growth-factor dependence, *src*- and *abl*-infected cells lose their ability to differentiate in response to hexamethylene bisacetamide or DMSO. We conclude at least two genetic events are required to convert murine hematopoietic precursors to tumorigenic cell lines. The first event permits immortal growth in the presence of specific growth factors, and the second abrogates the growth factor dependence.

L28 APPARENT AUTOCRINE GROWTH STIMULATION OF K562 ERYTHROLEUKEMIA CELLS BY HUMAN ERYTHROID-POTENTIATING ACTIVITY (EPA). Belinda R. Avalos, Masao Tomonaga, David W. Golde and Judith C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

EPA is a recently characterized hematopoietic growth factor which stimulates the growth of erythroid precursors. This 28,000 dalton glycoprotein has been purified to homogeneity from medium conditioned by the HLTV-II-infected Mo T-lymphoblast cell line. cDNA clones encoding EPA have been isolated and protein expressed in mammalian cells; purified biosynthetic (recombinant) EPA has all of the biological activities attributed to the purified natural protein. EPA stimulates the growth of primitive (BFU-E) and more mature (CFU-E) erythroid progenitors from human and mouse. Additionally, EPA enhances colony formation by the K562 human erythroleukemia cell line. High affinity receptors for EPA have been identified on K562 cells in equilibrium binding experiments with ^{125}I-EPA, consistent with their responsiveness in biologic studies. Using a sensitive radioimmunoassay and bioassay for EPA, medium conditioned by K562 cells has been found to contain high levels of biologically active EPA. Interestingly, EPA mRNA and protein from K562 cells are of slightly higher molecular weight than that produced by other cell lines. Colony formation by K562 cells in semi-solid medium is dependent upon the numbers of cells plated, suggesting production of autostimulatory factors by K562 cells. These studies together suggest that EPA acts as an autocrine growth factor for K562 cells. The abnormal size of the mRNA and protein may reflect aberrant expression of the EPA gene in these responsive cells.

L29 MICROTUBULE INVOLVEMENT IN INITIATION OF CELLULAR DNA SYNTHESIS BY GROWTH FACTORS, TUMOR PROMOTERS AND ONCOGENIC DNA VIRUSES. Rebecca L. Ball, T. Albrecht, W. C. Thompson and D. H. Carney, University of Texas Medical Branch, Galveston, TX 77550.

A number of studies have indicated that microtubule (MT) depolymerization is a signal for initiation of cell proliferation. For example, colchicine initiates DNA synthesis in arrested cells and acts synergistically with serum or growth factors to initiate DNA synthesis during the first 8 hours of the prereplicative lag period. In addition, taxol, a MT-stabilizing drug, blocks initiation of DNA synthesis by tumor promoters, growth factors and oncogenic DNA viruses. These viruses, like growth factors, initiate a single round of cell DNA synthesis in arrested cells. The taxol concentrations that half-maximally and maximally inhibit growth factor or virus-initiated DNA synthesis are similar and taxol blocks either type of mitogenic signal only during the first 8 to 12 hours of the prereplicative lag phase. To determine the effect of mitogenic agents on the tubulin-MT equilibrium in confluent cells, we have developed a radioactive antibody binding assay for MTs. Briefly, a monoclonal antibody to tubulin was radiolabelled in situ and binding of this antibody to extracted, fixed cytoskeletons was measured. Preliminary experiments showed an increase in polymerized tubulin in mouse embryo cells by 1 hour after addition of α-thrombin, EGF or phorbol myristate acetate, but not after addition of DIP-thrombin, a thrombin derivative that has lost the ability to stimulate DNA synthesis. The increased MT polymerization persists for at least 8 hours. We are further investigating the effect of oncogenic DNA viruses on MTs and if MT depolymerization occurs prior to 1 hour as part of the initial mitogenic signal. (AM-25807, CA-00805, GM-33505, AI42557 and R812086.)

L30 Lineage-specific transformation following differentiation of pluripontial murine stem cell containing a human oncogene.

J.C. Bell*, K. Jardine, M.W. McBurney and J.C. Foulkes.* Department of Medicine, University of Ottawa, Ontario, Canada K1H 8M5, and *National Institute for Medical Research, Mill Hill, London NW7 1AA, England.

We have transfected the human EJ bladder carcinoma oncogene (Ha-ras-^{EJ}1) into the pluripotential embryonal carcinoma cell, P19. The transgenic P19 (ras+) cells express the mRNA and p21^{EJ} protein derived from the oncogene. The P19 (ras+) cells differentiate in response to retinoic acid or DMSO, as efficiently as the parental P19 cells and the same spectrum of differentiated cells develops. Thus, it seems unlikely that the Ha-ras-1 proto-oncogene product plays a role in initiation of differentiation or in the choice of differentiated cell lineage. Following retinoic acid-induced differentiation of P19 (ras+) cells, post-mitotic neurons developed normally but the fibroblasts which formed appeared to be fully transformed (i.e. immortal and anchorage independent). Thus, transformation appeared to be lineage-specific with all fibroblasts and only fibroblasts developing the transformed phenotype. No detectable alteration in karyotype or genomic organisation of the Ha-ras-^{EJ}1 gene accompanied the development of these transformed fibroblasts, although some clones did have elevated levels of p21^{EJ} protein. The oncogene does not transform if transfected into fibroblasts derived from P19 cells, suggesting that transformation only occurs if the oncogene is present and expressed during early stages of the fibroblast developmental lineage. We are currently investigating the effect oncogenic tyrosine kinases have on the differentiation of EC cells.

L31 TISSUE PLASMINOGEN ACTIVATOR INTERACTS WITH THROMBIN RECEPTORS AND STIMULATES DNA SYNTHESIS IN MOUSE EMBRYO FIBROBLASTS. John S. Bergmann and Darrell H. Carney, Division of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550.

We recently demonstrated that thrombin mitogenesis involves two types of signals, one generated by receptor occupancy, the other by enzymic activity. To define the portion of the thrombin molecule responsible for receptor binding, we have synthesized a number of peptides based on the amino acid sequence of human prothrombin and determined their ability to bind thrombin receptors and activate mitogenic signals. One of these peptides which binds to thrombin receptors (J. Cell Biol. 101: 11a, 1985) is highly homologous to a portion of tissue plasminogen activator (TPA). Therefore, we tested TPA for its ability to interact with thrombin receptors and to initiate cell proliferation. We now report that TPA competes with ^{125}I-thrombin for receptor binding. Preincubation with TPA is not as effective in inhibiting ^{125}I-thrombin as TPA co-binding suggesting that the TPA effect is not simply related to proteolysis. Proteolytically active TPA is mitogenic for these cells at the same concentrations as those required to inhibit ^{125}I-thrombin binding. Since TPA is produced and secreted by a number of malignant cell types, these results may suggest that TPA elicits "autocrine" regulation of cell proliferation or facilitation of tissue invasiveness through interaction with thrombin receptors. (Supported by NIH Grants AM-25807 and CA-00805.)

L32 **NORMAL AND NEOPLASTIC DIFFERENTIATION: PROGRAMMING GENES, GENE DOSAGE AND SHUTTLE VECTORS,** R. Bertolotti and G. Lutfalla, Molecular Genetics, Gif sur Yvette, France.

Transfection of mammalian cells with total cellular DNA provides an opportunity to assay for genes that program cell differentiation (1). However, such a straightforward approach should not prove instrumental if gene amplifying processes were not available to overcome gene dosage problems that are essential to cell differentiation. Therefore, in order to generate dominant phenotypic alterations of recipient cells, we have devised a strategy that involves autonomously replicating vectors to transfer genomic libraries into target cells. Such vectors propagate as multicopy episomes and should offer the unique opportunity to efficiently amplify, from a pool of recombinant molecules, the very regulatory gene that would confer an appropriate selectable phenotype upon transfected cells.

Using either mouse mitochondrial DNA or polyoma clone 41 sequences, we have constructed first generation plasmids comprising the XGPRT gene as a selectable marker. In vivo new recombinant molecules were then isolated for their ability to shuttle from hepatoma cells to E. coli. Complementary investigations showed that our polyoma derivative behaves as a true classical shuttle vector while our mitochondrial DNA constructs are in fact integrating vectors. A new shuttling mechanism was thus evidenced and shown to be driven by homologous recombination (2). Adult, neonatal and foetal strains of well-differentiated hepatoma cells are currently being transfected with polyoma cosmid libraries and the screening for the aforementioned programming genes is performed with selective growth media specific for hepatic differentiation (1). A similar approach has been initiated for liver oncogenes.
1)& 2). Lutfalla,G., Blanc,H. and Bertolotti,R. (1985) Somat. Cell Mol. Genet.11: 223.

L33 Regulation of Chromosome and Growth Cycles in Chinese Hamster Ovary Cells After Removal of Growth Factors. William M. Bonner and Roy S. Wu, National Cancer Institute-National Institutes of Health, Bethesda, MD 20892.

We have previously shown that when protein synthesis is partially inhibited in proliferating CHO cells with cycloheximide, there is an immediate inhibition of histone and DNA synthesis (Wu and Bonner, Mol. Cell. Biol. 5: 2959-2966). However, after about an hour there is a recovery in the rate of histone protein and DNA synthesis which may approach control levels; the rate of nonhistone protein synthesis remains depressed. This process which we called chromosome cycle compensation may help explain the relative constancy of chromosome cycle progression when cells grow at quite different rates. A major part of the mechanism of chromosome cycle compensation is an elevation in the level of histone mRNA.

When proliferating CHO cells are placed in media with low or no serum, the same process of chromosome cycle compensation seems to occur. Cells complete the chromosome cycle in media without serum as quickly as in media with serum even though overall protein synthesis is decreased by approximately 50% in the former. Histone mRNA levels are found to increase approximately twofold.

However, once the chromosome cycle is completed, CHO cells in low serum have a much lower probability of initiating a new chromosome cycle than do those grown in cycloheximide, suggesting that the processes of chromosome cycle initiation and progression are regulated differently with respect to the rate of protein synthesis.

L34 EFFECT OF GRANULOCYTE-MONOCYTE COLONY STIMULATING FACTOR (GM-CSF) ON SYNCHRONIZED HL-60 CELLS. J.K. Brennan, C.N. Aboud, D. Fischer, P. Keng and C. Erickson-Miller. University of Rochester School of Medicine, Rochester, NY 14642. We found that exposure of HL-60 cells to dimethyl sulfoxide (DMSO) suppressed spontaneous proliferation and restored the requirement for exogenous GM CSF. Analysis of DNA content in these experiments indicated that the DMSO effect was correlated with an increase in the proportion of cells in G_1 to greater than 50% suggesting a CSF requirement during G_1. To investigate this question further, HL-60 cells were separated into G_1, S, and G_2M populations by centrifugal elutriation. The synchronized populations were exposed continuously to CSF, pulsed with CSF, or cultured without CSF, and their movements through the cell cycle compared by flow cytometry of mithramycin-stained cells over 36 hours. A two-hour exposure to CSF did not alter movement into, or from, any phase of the cell cycle. Early G_1 cohorts exposed continuously to CSF, moved more rapidly into S-phase by 10 hours. In contrast, cells synchronized in S-phase or G_2M did not differ from controls until 18 hours when increased S-phase proportions were observed. These late effects were the result of CSF stimulation of cells that had re-entered G_1 following synchronization. Thus, CSF appears to act predominently, if not exclusively on the G_1 phase of the HL-60 cell cycle. Current studies are underway to quantitate the effects of GM CSF on phase transit times using cells pulse-labelled with BrdU and identified with FITC-anti-BrdU monoclonal antibody.

L35 INTERFERON - γ MODULATES GROWTH OF A431 CELLS AND EXPRESSION OF EGF RECEPTORS, *Esther H. Chang, *Roberta Black, *Zhi-Qiang Zou, *Taras Masnyk, and +Joe B. Harford, *Dept. of Pathology, USUHS and +Cell Biology and Metabolism Branch, NICHD, Bethesda, MD 20814.

At 200U/ml, IFN-γ induced an elongated cell morphology in A431, a human epidermoid carcinoma cell line, and markedly inhibited cell growth within 3 days. No survivors were detected at 6 days of IFN-γ treatment. Analogous treatment with IFN-α or IFN-β did not result in this massive killing of A431 cells. The specific killing of A431 cells has been seen with several preparations of IFN-γ including highly purified recombinant IFN-γ .

A431 cells express levels of the EGF receptor (EGFR) that have been reported to be as much as 30-fold elevated over those seen in untransformed cells. The EGFR is the cellular homologue of the v-erb-B oncogene. Using Northern analyses with v-erb-B as a probe, the 10kb mRNA encoding the EGFR was found to be 3- to 7-fold elevated after 1 day of IFN-γ treatment. Treatment with IFN-α or IFN-β decreased receptor mRNA levels. Expression of the Ha-ras gene was also elevated 4- to 5-fold by treatment with IFN-γ but not by IFN-β treatment. The 1.6kb and 1.8kb mRNA species corresponding to 2'5'A synthetase were elevated 8-fold by IFN-γ treatment, 2-fold and 4-fold by treatment with IFN-α and IFN-β respectively. Metabolic labeling of A431 cells with [^{35}S]methionine and immunoprecipitation of the EGFR indicated that the receptor protein as well as its mRNA was elevated by IFN-γ treatment.

In several other epidermoid carcinoma cell lines, high expression of the genes for EGFR and Ha-ras have been observed.

L36 THE RELATIONSHIP BETWEEN PROTEIN SYNTHESIS, ADENYLATE CYCLASE ACTIVITY, AND β-ADRENERGIC RECEPTORS IN THE DIFFERENTIATION OF SALIVARY EXOCRINE CELLS, Leslie S. Cutler, University of Connecticut Health Center, Farmington, CT 06032

We have examined the sequence of events which occur during the functional differentiation of a model exocrine system (the acinar cells of the rat submandibular salivary gland) in which secretion is mediated by cyclic AMP. These studies revealed that exocrine cells initiate cell specific secretory protein production and develop the ability to synthesize and package secretory proteins (development of apical-basal polarity, accumulation of rough endoplasmic reticulum, and maturation of the Golgi apparatus) prior to attaining the ability to release the packaged proteins in response to hormonal stimuli. Membrane associated adenylate cyclase (AC) activity (the cyclic AMP generating system) develops in parallel with the protein synthetic components of the system. However, the AC system is not responsive to hormonal (β-adrenergic) stimulation until several days after the cells have attained enzymatic and structural maturity. Based on the direct measurement of receptors and evaluation of the guanyl nucleotide regulatory and catalytic subunits, this failure of AC to respond is due to the absence of β-adrenergic receptors at the cell surface. Appearance of β-adrenergic receptors at the cell surface is temporally correlated with the appearance of catecholamine containing nerve processes in the tissue. In vivo studies revealed that the appearance of β-adrenergic receptors is a specifically programmed developmental step which is associated with the degree of maturation attained by the cells and is not dependent on the presence of catecholamine containing nerve processes for expression.

This work supported by NIH grant DE-05632.

L37 Molecular genetic analysis of insulin receptor structure/function. Leland Ellis(1), Eric Clauser(1), David Morgan(2), Marc Edery(1), Richard Roth(2) and William J. Rutter(1). Hormone Research Institute, UCSF(1) and Dept. of Pharmacology, Stanford(2).

In order to explore the mechanism of transmembrane signalling, in the context of cellular responses to extracellular stimuli, we are studying the insulin receptor (IR), a transmembrane glycoprotein. As a first step, we have cloned and sequenced the human placental IR cDNA (Cell 40, 747-758, 1985). To begin to elucidate the role of deduced primary structural domains of the IR (esp. the tyrosine phosphokinase, TPK, domain) in the generation of the insulin response, we have established a system to express the human IR (hIR) protein in a heterologous cell. To this end, we have placed the hIR cDNA under the transcriptional control of the SV40 early promotor and, by the use of cotransfection and selection with the neomycin analog G418, established stably transformed CHO cell lines. The hIR is expressed in such cells, and processed into mature α- and β-subunits: we can distinguish the hIR from the endogenous rodent IR by the use of a monoclonal antibody specific for the hIR. Furthermore, the hIR is also functional: it exhibits insulin-stimulated autophosphorylation of the β-subunit, and mediates insulin-activated glucose uptake. We are currently using this system to begin to dissect primary structural features of the IR protein by making mutations in the cDNA, expressing such constructs in CHO cells, and analyzing the effects of these changes on IR function. Results derived from cell lines which express hIRs with alterations of the TPK domain (truncations and site-directed mutations of tyrosine residues) will be presented. Supported by grants from the NIH to WJR and RR.

L38 R.J.Ford, L.Yoshimura, I.Ramirez and C.G. Sahasrabuddhe, U.T.M.D.Anderson Hospital Houston, Texas 77030. Role of Growth Factors in Neoplastic B cell Proliferation.

Control of neoplastic human B cell proliferation is a subject of considerable importance both biologically as well as clinically. B cell tumors in general, retain lineage fidelity, reflected in normal appearing cell surface phenotypes and often closely resemble their normal B cell counterparts both functionally as well as morphologically. We have been interested to ascertain whether the same molecules are involved in the control of neoplastic B cell growth as those governing the activation and proliferation of normal human B cells. Our studies have shown that a variety of neoplastic human B cells including various non-Hodgkin's lymphomas (NHL) and chronic B cell leukemias (Hairy cell leukemia) (HCL) and chronic lymphocytic leukemia (CLL), can proliferate in vitro in response to low molecular weight (12-14KD) human B cell growth factor (BCGF), derived from normal human T cells. Growth factor-dependent tumor cell growth can be maintained in vitro for prolonged time periods without loss of original cell surface phenotype. We have also shown that at least some NHL and HCL contain a high molecular weight BCGF in the cytoplasm, which can be secreted into the culture supernatants. The high MW BCGF (60KD) that stimulates cell growth in both normal long term growth factor-dependent human B cell lines as well as the autologous tumor cells. This suggests that the tumor cell-derived BCGF is similar to the molecule(s) secreted by normal human T cells. In addition BCGF also stimulates the expression of the c-Myc protooncogene in neoplastic B cells in a manner similar to that of normal human B cells, again suggesting analogous mechanism for the control of cell proliferation in normal and neoplastic human B cells. Supported by NCI grants CA 31479 and CA 16672

L39 EFFECT OF ESTROGEN ON RAT LIVER PROLIFERATIVE AND 2-5 A SYNTHETASE ACTIVITY. Paul A. Galand, Martine Smekens and Jacques E. Dumont, Institute for Interdisciplinary Research and Lab. of Cytology and Exptl. Oncology. Free Univ. of Brussels.

In the course of studies aimed at testing the hypothesis that 2-5 A synthetase activity might exert negative control on normal cell growth and multiplication, we show here that a treatment of ovariectomized rats with a single dose of estradiol 17 β (100 μg/100 g b.w.) induced a transient increase in the ^{3}H thymidine labelling index (L.I.) in the liver after 24 hours and markedly decreased the 2-5 A synthetase activity. A time course study revealed that 2-5 A synthetase activity started to decrease after 3 hours, to reach a minimal value (10% of the control level) after 12 hours, then slowly increased to come back to control level at 48 hours.

These results, together with similar data on regenerating liver (Smekens et al. Europ. J. Biochem. 130, 269-273, 1983) suggest that low 2-5 A synthetase activity might be permissive for acquisition of proliferative "competence" by G_0 cells.

L40 Effects of interleukin 3 on cell metabolism.
J.M. GARLAND, A. KINNAIRD (K. ELLIOT. Depts. Immunology & Biochemistry, Manchester Medical School.

The murine growth factor interleukin 3 (IL3) is unusual in that its presence is required continually by IL3 dependent cell lines. In its absence, cells die usually within 24 hours. Withdrawal of IL3 is accompanied sequentially by: reduction in production of lactic acid, reduction in incorporation of labelled thymidine, reduction in protein synthesis and loss in membrane integrity. There is preferential arrest of cells in G2/M. To further understand this sequence of events, we have examined cell metabolism with a view to understanding the very early (1 hr) reduction in lactic acid production. We have found glucose transport remains intact but there appears to be a defect in glucose phosphorylation, the lactate: pyrovate levels are altered, but ATP levels are not changed. Protein synthesis appears unchanged even when lactate is reduced to 50% of control values, but eventually (4 hrs) both glucose transport and protein synthesis/amino acid uptake are reduced. Intracellular calcium levels are unaffected by IL3 withdrawal as determined by Quinn-2, neither does re-addition of IL3 create a calcium transient. We have been unable to show any affect of stimulators of protein kinase C on dependent cells, although both trifluoroperazine and compound "W7" inhibit IL3, suggesting a role for calcium/calmodulin. Our data will be presented in detail.

This work was supported by the Cancer Research Campaign.

L41 Relationship between differentiation and respiration in factor-induced differentiation of marrow progenitor cells.
J.M. GARLAND, A. KINNAIRD & F. KATZ. Depts. Immunology, Manchester Medical School and Institute of Child Health, London.

Many tumour cells appear to have enhanced glycolysis which, it is considered, gives them a metabolic selective advantage (Warburg's hypothesis). Recently, leukaemia cells have been shown to express phenotypes corresponding to progenitor cell status, and some may be induced to differentiate when malignant potential is lost. We have studied the effects of respiratory inhibitors on mouse marrow cells, and growth and differentiation of marrow colony-forming cells (G/M-CFC) stimulated by G/M-CSF. Whereas the proliferation and fresh marrow cells (which are 99% terminally differentiating cells) is completely inhibited by inhibitors of oxidative phosphorylation, (Cyanide or Antimycin A) G/M-CSF dependent colonies grew in the presence of both; however, differentiation to mature granulocytes was inhibited or completely arrested. Our experiments show that progenitor cells, unlike terminally-differentiating cells, have enhanced anaerobic metabolism, and we hypothesise that differentiation is linked to the establishment of oxidative phosphorylation. Similar results have been found with human marrow progenitor cells. Our findings thus offer an explanation for Warburg's hypothesis, progenitor cell status of leukaemic cells and the loss of malignancy if differentiation can be induced.

This work was supported by the Cancer Research Campaign.

L42 EXPERIMENTAL TUMOR PROMOTERS OR TRANSFORMING GROWTH FACTOR-β INDUCE SQUAMOUS DIFFERENTIATION OF CULTURED NORMAL HUMAN BUCCAL EPITHELIAL CELLS.
Roland C. Grafström, Kristina M. Sundqvist, Kari Ormstad and Kristina Arvidson, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Normal human buccal epithelial cells were serially grown at serum-free conditions using slightly modified methods and media established for human epidermal and bronchial epithelial cell cultures. At clonal density in optimized chemically defined MCDB 153 medium, subcultured buccal epithelial cells grow on fibronectin/collagen-coated dishes at 3-8% colony forming efficiency and divide at a mean rate of 1 population doubling per day. For example, epidermal growth factor optimize the clonal growth rate at a concentration of 0.3 ng/ml. Transforming growth factor-β between 0.1-1.0 pM cause terminal squamous differentiation as indicated by an increased surface area and decreased clonal growth rate. Similar results are obtained with 2% blood-derived serum. Phorbol-12-myristate-13-acetate at 1 nM, or cigarette smoke condensate fractions with tumor promoting activity in the mouse skin carcinogenesis model, also induce differentiation of buccal epithelial cells. Extracellular Ca^{2+} between 0.1 to 0.3 mM gives optimal growth whereas 1 mM induce squamous differentiation. The results show that normal human epithelial cells from buccal mucosa can now be cultured and their growth and differentiation regulated by physiological endogenous as well as exogenous agents. Buccal epithelial cells are similar to other normal human epithelial cell types in their response to several different growth or differentiation stimuli including their ability to undergo differentiation upon exposure to experimental animal tumor promoters.

L43 IDENTIFICATION OF PROMOTER ELEMENTS NECESSARY FOR TRANSCRIPTIONAL REGULATION OF A HUMAN HISTONE GENE IN VIVO, Sarah Hanly and Nathaniel Heintz, Rockefeller University, New York, NY 10021.

The replication dependent hisone genes have been shown to be cell cycle regulated at both transcriptional and post-transcriptional levels. Transcriptional induction of histone gene expression in S phase has been reproduced in vitro using S phase extracts (Heintz and Roeder). Furthermore our analysis of deletion mutants in the promoter of an H4 gene has identified elements upstream from the TATA box which are necessary for S phase specific transcription (Hanly et al). Recently, we have identified at least two and possibly three proteins which bind to different upstream elements of this gene (Dailey, Hanly and Heintz). We are currently addressing the question of how these promoter elements function in vivo, in particular, to discern which distal element confers cell cycle regulation on this gene. Stable cell lines containing H4 promoter mutant genes have been constructed and initial results indicate that the steady state levels of histone message in cell lines containing H4 genes which lack the upstream promoter elements fluctuate normally during S phase. This result is not surprising considering the powerful post-transcriptional regulatory mechanisms which operate to control histone mRNA concentration. Therefore we have begun an analysis of the rate of transcription of H4 mRNA from the mutant genes and have demonstrated that the wild type gene is normally regulated. Our present efforts are directed toward analysis of the mutant genes and identification of the upstream sequence elements responsible for transcription regulation.

L44 REGULATED EXPRESSION OF LOW MOLECULAR WEIGHT KERATINOCYTE ANTIGENS DURING DIFFERENTIATION AND CARCINOGENESIS, P. Hawley-Nelson, D. R. Roop, S. H. Yuspa, N.C.I., Bethesda, MD 20892

Mouse epidermal cytosol contains 3 antigens of MW 10-12 kd which react with a polyclonal antiserum raised against a parvalbumin of the panniculus carnosus but which are distinct from that antigen. In keratinocyte cultures these antigens are synthesized by basal cells maintained in culture medium with 0.05 mM Ca^{2+}. Synthesis of all 3 antigens is coordinately depressed if the basal cells are induced to terminally differentiate by raising the Ca^{2+} in the culture medium to 1.2 mM. The tumor promoter TPA also induces differentiation in epidermal basal cells, and this agent decreases the synthesis of all 3 antigens transiently. While the regulation of these antigens appears to be coordinate in normal cells, their expression in malignant keratinocytes and regulation in response to Ca^{2+} or TPA are variable and distinct for different malignant lines. In studies utilizing pulse labeling with $^{32}PO_4$, one antigen of 11 kd is phosphorylated. All 3 antigens are translated in vitro in a reticulocyte lysate system with newborn mouse epidermis mRNA or mRNA from keratinocytes cultured in low Ca^{2+} medium. Thus it is unlikely that any antigen represents processed or degraded proteins. A cDNA clone has been identified in a λgt 11 expression library of mouse epidermal mRNA which, on hybrid selection, rescues mRNA which translates into all 3 antigens in a reticulocyte assay. These results suggest a role for these closely related antigens in keratinocyte proliferation, differentiation and transformation. Further results on sequence of the cDNA and transcriptional control of the messages will be discussed.

L45 THE POLYPURINE.POLYPYRIMIDINE SUPERFAMILY OF REPETITIVE SEQUENCES: AN APPROACH TO STUDY THEIR ROLE IN NORMAL GROWTH AND DIFFERENTIATION AS WELL AS IN MALIGNANCY, Barbara Hoffmann-Liebermann and Dan Liebermann, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA, 19104.

We have cloned sequences homologous to the IVR-OD of the TU transposons (Nature, 306, 342,1983) from a human genomic library.. Studies with these clones led to the discovery of the polypurine.polypyrimidine superfamily of repetitive sequences that have been shown to be intrinsic to the eukaryotic genome, to have the potential to alter DNA conformation in chromatin, and to be assymetrically transcribed in a tissue specific and developmentally regulated manner. Taken together, these findings suggest an important biological role for this superfamily of repetitive sequences. Currently we are using λgt10 libraries constructed using mRNA from both normal and leukemic white blood cells of the myeloid lineage representing all stages of myeloid cell differentiation, as well as genomic libraries, to identify and isolate clones of developmentally regulated genes which also contain sequences homologous to sequences of the polypurine.polypyrimidine superfamily. We intend to use these clones to study the role of polypurine.polypyrimidine elements in the regulation of gene expression during normal growth and differentiation and in leukemia.

L46 EPIGENETIC CONTROL OF TUMOR CELL PHENOTYPE, Raymond J. Ivatt, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

Tumor cell heterogeneity is a major complication for effective cancer therapy. This heterogeneity can arise as a consequence of long-term, genetic alterations and can also arise as a result of short-term effects. We have been studying the latter, specifically, the short-term heterogeneity of tumor cell adhesiveness during the cell cycle and in response to cellular contacts. Our studies with the murine teratocarcinoma system have identified a carbohydrate-mediated cellular adhesive system. The system is characteristic of the stem cells and is lost on differentiation. The expression of the carbohydrate moiety is conserved during the cell cycle and is modulated by cellular contacts. In contrast, the expression of the carbohydrate receptor shows a periodic variation during the cell cycle and very little alteration in response to cellular contact. As the carbohydrate is the limiting component, the net result is that cells maintain their cohesiveness during periods of continued proliferation but have decreased adhesiveness as the number of cellular contacts increase. This may provide a homeostatic mechanism for regulating the strength of adhesive contacts. Understanding how intrinsic and extrinsic factors regulate the strength of cellular adhesiveness is an important part of understanding the metastatic phenotype.

Supported by Basil O'Connor Starter Research Grant, March of Dimes 5-365, and Grants CD-153 and BC-426 from The American Cancer Society.

L47 CELL CYCLE AND INFECTION STATUS OF THE CFU-S COMPARTMENT IN FV LEUKEMIC MICE, Candace S. Johnson and Philip Furmanski, AMC Cancer Research Center, Denver, CO 80214

Animals infected with FV-P or FV-A develop a lethal erythroleukemia. A variant strain, RFV, induces an initially identical disease except that it spontaneously regresses in 50% of infected mice. Regression is dependent on normal macrophage function; in progressor leukemic mice, macrophage precursors (CFU-C) are productively infected with virus and produce infected, defective, mature macrophages. To determine whether the pluripotent stem cell (CFU-S) in progressor mice is infected with virus and thus is precluded from repopulation of the CFU-C compartment, we tested bone marrow and spleen CFU-S from leukemic mice for susceptibility to cytotoxicity by monospecific anti-viral-gp70 antiserum. CFU-S in progressively leukemic (FV-A, FV-P and RFV progressor) mice, became infected at 21 days post virus. Infection of CFU-S correlated with increased proportions of replicating (S-phase) CFU-S in these populations. Splenic CFU-S from RFV regressors (leukemic mice that will regress) were not productively infected with virus or in active cell cycle regardless of time post virus inoculation. Bone marrow CFU-S in RFV regressors, as well as RFV, FV-P and FV-A progressors, remained uninfected and were not induced to increased cell cycling. The difference in splenic CFU-S infection and cycle in progressor and regressor mice may be related to disease reversal, but the results also demonstrate that infection of the CFU-S compartment is not an obligatory event in the pathogenesis of erythroleukemia as animals may become fully leukemic without evidence of infection of their stem cells. Supported by a gift to the AMC Cancer Research Center from David W. Garlett.

L48 LONG-TERM DOWN-REGULATION OF EPIDERMAL GROWTH FACTOR RECEPTORS BY NERVE GROWTH FACTOR IN PC12 CELLS. Philip Lazarovici and Gordon Guroff, Section on Growth Factors, NICHD, NIH, Bethesda, Md. 20892.

Cells of the rat pheochromocytoma clone PC12 possess receptors for both nerve growth factor (NGF) and epidermal growth factor (EGF), thus enabling the study of the interaction of these receptors in the regulation of proliferation and differentiation. Treatment of the cells with NGF induces an 80% decrease in the specific binding of EGF starting at 24 hrs and complete within 4 days. Scatchard analyses of this decrease indicates a reduction in receptor number. Using affinity labeling of PC12 cell membranes by crosslinking of receptor-bound ^{125}I-EGF, we found 60-90% decrease in the labeling of 170 and 150 kD receptor bands in cells treated with NGF for 4 days. Analysis of ^{35}S-labelled EGF receptors isolated by wheat germ lectin-Sepharose chromatography from detergent extracts of PC12 membranes showed a 60-90% decrease in the 170 kD band in NGF-differentiated cells. No differences could be detected in rates of internalization or degradation of ^{125}I-EGF between proliferative and NGF-differentiated PC12 cells. The data indicate that heterodown regulation of EGF receptors by NGF in PC12 cells is mediated by some alteration on EGF receptor synthesis. It can be suggested that this heterodown regulation is part of the mechanism by which differentiated cells become desensitized to mitogens.

L49 DOWN REGULATION OF C-MYC EXPRESSION BY INTERFERON AND BY DIFFERENTIATION AGENTS, Bernard Lebleu, Nadir Mechti, Jean-Marie Blanchard, Tamim Salehzada, Marc Piechaczyk and Philippe Jeanteur, University of Montpellier II, 34060 Montpellier cedex, France.

IFN treatment of Daudi lymphoblastoid cells leads to a preferential degradation of c-myc mRNAs while leaving unchanged the transcriptional activity of c-myc gene, as revealed by run-off experiments in isolated nuclei (Dani et al., 82 (1985) 4896-4900). A screening of several human lymphoblastoid cell lines differing in the structure of c-myc gene has revealed a possibly interesting correlation between the presence of exon 1, the down regulation of c-myc by IFN and the growth inhibitory activity of IFN, which is presently under closer investigation. Experiments aiming at establishing to which extent c-myc protein level itself is affected and the role of the 2-5A dependent RNase in the accelerated degradation of c-myc mRNA will be described.
Such a mechanism of down regulation of c-myc expression rapidly takes place as well in Friend erythroleukemia cells induced to terminal differentiation with Me_2SO. This and previous observations of our group (Dani et al PNAS 81 (1984) 7046-7050) concerning the unusually low stability of c-myc mRNA in most cell lines point to the major role played by post-transcriptional steps in the regulation of c-myc expression.

L50 PROLIFERATIVE RESPONSE AND ONCOGENES EXPRESSION INDUCED BY EGF IN EL2 RAT FIBROBLASTS, E. Liboi, U. Testa, E. Pelosi, C. Peschle and G. B. Rossi. Istituto Superiore di Sanità, Roma. In this study we have investigated the response to growth factor (EGF) of a new rat cell line, EL2, recently isolated from rat embryo fibroblasts, which is highly susceptible to a number of different transforming genes (Liboi et al. Mol. Cell. Biol. 4: 2925-2928, 1984) It is generally conceded that in 3T3 cells entry in S phase of the cell cycle is mediated by two sequential events, which are controlled by different serum components: (i) a competence state induced by platelet-derived growth factor (PDGF) and (ii) progression into the cycle, mediated by EGF or other factor(s) present in platelet poor plasma. Our results afford clear evidence that EGF alone leads EL2 cells to proliferate in serum-free conditions, at a rate corresponding to 50-60% of that observed in the presence of 10% calf serum. The addition of anti-PDGF antibodies to the culture medium does not inhibit the EGF-induced growth of EL2 cells. In contrast, PDGF alone is unable to sustain the proliferation of EL2 cells. However PDGF, added with EGF, slightly potentiates the EGF-induced proliferation of EL2 cells. At the molecular level, we show that in resting EL2 cells exposed to EGF, the transcription of both c-myc and c-fos is markedly induced. Altogether, these observations suggest that: (i) in contrast with the model of cell growth mentioned above, EL2 fibroblasts require presence of a single growth factor (EGF) for induction of DNA synthesis and (ii) the expression of myc and fos protooncogenes may represent an obligatory step in the pathway of the commitment to cell proliferation. From the present study it results that EL2 cells represent a unique model to investigate the events responsible for the control of cell growth by EGF at cellular and molecular level.

L51 MOLECULAR GENETICS OF NORMAL GROWTH AND DIFFERENTIATION AND THE BREAKDOWNS WHICH LEAD TO MALIGNANCY, Dan Liebermann and Barbara Hoffman-Liebermann, Department of Biochemistry and Biophysics, University of Pennsylvania School of medicine, Philadelphia, PA, 19104.
We have constructed λgt10 cDNA libraries with mRNA from both normal and leukemic murine white blood cells of the myeloid lineage representing all stages of myeloid cell differentiation. We are using these libraries in conjunction with normal and leukemic genomic libraries to identify and isolate both cDNA and genomic clones of genes that encode for:
1. mRNAs turned on or off at various times during normal myeloid growth and differentiation
2. Early regulated mRNAs that may encode for growth and differentiation regulatory functions
3. mRNAs that exhibit a cell lineage specific pattern of expression, i.e. expressed specifically in myeloid cells or, furthermore, in either granulocytes or macrophages
4. mRNAs that are abnormally expressed in myeloid leukemic cells, including genes that are developmentally regulated during normal myeloid growth and differentiation.
We intend to use these cDNA and genomic clones to study the molecular genetics of normal growth and differentiation and the breakdowns which may lead to leukemia.

L52 Modification and Reorganization of Phosphotyrosine-Containing Proteins in Cells Treated with Growth Factors, P.A. Maher, E.B. Pasquale and S.J. Singer, University of California at San Diego, La Jolla, CA.
In an attempt to understand the molecular basis for the changes in cellular morphology brought about by treatment of cells with growth factors we have utilized a polyclonal antibody to phosphotyrosine (P-Tyr)(PNAS 82:6576) along with a battery of antibodies to cytoskeletal proteins to analyse cytoskeletal changes both immunocytochemically and biochemically.In normal fibroblasts P-Tyr is localized to the focal contacts along with the proteins vinculin and talin.Immunoblotting of cell extracts showed only one major P-Tyr-containing protein.Following treatment of quiescent fibroblasts with PDGF a rapid change in the quantity and distribution of P-Tyr-containing proteins was observed.This was followed shortly by a dramatic change in the cytoskeletal organization.Microfilament bundles were disrupted and vinculin and talin disappeared from the focal contacts.The major tyrosine-phosphorylated protein was the PDGF receptor although immunoblotting revealed several other as yet unidentified P-Tyr-containing proteins not seen in normal cell extracts.The amount of P-Tyr in the PDGF receptor began to decrease by 60 min after PDGF addition, concomitant with a return of the normal cytoskeletal architecture.These changes in cytoskeletal organization and in the quantity and distribution of P-Tyr-containing proteins that were observed following treatment of cells with PDGF were very similar to those seen when fibroblasts infected with a temperature-sensitive mutant of Rous sarcoma virus were shifted from the nonpermissive to the permissive temperature suggesting a similar mechanism may be operating in both cases.

L53 LEAD-INDUCED ACTIVATION OF PROTEIN KINASE C IN RAT BRAIN MICROVESSELS, J. Markovac and G.W. Goldstein, University of Michigan Medical Center, Ann Arbor, MI 48109
Microvessels isolated from rat brain provides a model system to study the toxic effects of lead on the nervous system. Since proliferating brain capillary endothelial cells appear especially vulnerable to lead, we investigated the effect of lead upon the activation of protein kinase C (PKC) in immature brain microvessels. In the other biologic systems, lead may act as a calcium agonist or antagonist. Microvessels were isolated from six day old rat brain by density gradient centrifugation and glass bead filtration. The resulting suspension of highly purified microvessels was homogenized and assayed for PKC activity using exogenous lysine-rich histone as a substrate. The activity of PKC in the brain microvascular cytosol was 14.3 ± 1.8 pmol/mg/min in the presence of 10 uM calcium; 25.2 ± 2.3 in the presence of 10 uM lead; and 57.2 ± 2.0 in the presence of 10 uM calcium and 10 uM lead. Half maximal activation of PKC by lead in the presence of 10 uM calcium occured at 0.1 uM lead. We find that lead is more potent than calcium and may have a synergistic action with calcium in the activation of PKC in cerebral microvessels. Alteration of this regulatory pathway may underlie some of the toxic effects of lead upon immature brain microvessels.

L54 TYPE β TRANSFORMING GROWTH FACTOR INDUCTION OF SQUAMOUS DIFFERENTIATION OF NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS IS ANTAGONIZED BY EPINEPHRINE AND CHOLERA TOXIN, Tohru Masui, Lalage M. Wakefield*, John F. Lechner, Moira A. LaVeck, Brenda I. Gerwin, Michael B. Sporn*, and Curtis C. Harris, Lab. of Human Carcinogenesis and *Lab. of Chemoprevention, Division of Cancer Etiology, NCI, Bethesda, MD 20892

Type β transforming growth factor (TGF-β) irreversibly inhibited growth and induced terminal squamous differentiation in cultured normal human bronchial epithelial (NHBE) cells. In contrast, TGF-β did not inhibit DNA synthesis or induce differentiation in human lung carcinoma cell lines, even though all the normal and carcinoma cells examined possess comparable numbers of high affinity TGF-β receptors. Therefore, aberrations of differentiation induction in lung carcinomas may occur at pathways distal to the ligand-receptor interactions. Epinephrine and cholera toxin antagonized the differentiation-inducing effect of TGF-β in NHBE cells. Though epinephrine and cholera toxin stimulated growth and enhanced cyclic AMP levels in the cells, TGF-β inhibited growth in epinephrine- or cholera toxin-free medium and prevented growth enhancement by epinephrine or cholera toxin without lowering the level of cyclic AMP in the cells. Therefore, the cyclic AMP level does not appear to mediate the effect of TGF-β.

L55 SELECTION OF HEMATOPOIETIC PRECURSOR CELLS AND CLONING OF MULTIPOTENTIAL B-LYMPHOCYTE PRECURSORS. J.P. McKearn, J. McCubrey and B. Fagg, E.I. du Pont de Nemours & Co., and Sandoz Ltd., Glenolden, PA 19036 and Basel Switzerland.
A simple one-step isolation technique significantly enriched mouse fetal liver cells that respond to interleukin 3 (IL-3), a multilineage hematopoietic growth factor. The fetal liver cell subpopulation isolated with monoclonal antibody (clone AA4) contained 50- to 100-fold higher frequencies of multipotential (CFU-mix) or restricted (CFU-G/M and BFU-E) erythroid/myeloid precursors as well as precursors that differentiate to become mature B lymphocytes. The B lymphocyte precursors could be cloned in single-cell cultures when IL-3-containing supernatants were present. Growth of these clones was supported by purified IL-3 but not purified IL-2. Stable growth has been maintained for greater than 6 months in the presence of IL-3. Such clones express on their cell surface low amounts of class I major histocompatibility complex antigens and high amounts of AA4, GF1, and leukocyte common glycoprotein (LGP) 200 antigens. They lack detectable IgM, kappa light chain, Thy 1, Lyt-1, Lyt-2, class II MHC or J11d antigens. Genes encoding Ig heavy chains or light chains were found to be in embryonic configuration. However, these clones could be induced to generate mature IgM-secreting B cells in vitro. These responses required approximately 10 days to reach maximal levels and each clone produced B cells committed to several different variable region specificities.

L56 The Influence of the Extracellular Matrix on the Response of Human Skin Fibroblasts to Platelet Derived Growth Factor in vitro. John McPherson and Roberta Rhudy, Collagen Corporation, Palo Alto, Ca. 94303.
The proliferation of low passage, adult human skin fibroblasts was evaluated on plastic and various fibrillar collagen matrices using [3-H] thymidine incorporation as a means to quantitate mitotic activity. The proliferation rate of these cells was initially determined on type I fibrillar collagen (35 mg/ml) and compared to the rate observed on plastic when the cells were cultured in 10% fetal bovine serum. Adult human skin fibroblasts exhibited a 30-40% decrease in proliferation rate when cultured on fibrillar collagen as compared to plastic. It was noted that normal murine mammary gland epithelial cells exhibited less than a 10% decrease in proliferation rate under the same conditions. The proliferation of the human skin fibroblasts in the presence of 0.5% fetal bovine serum was 5-10 fold greater on the fibrillar collagen than it was on plastic. The addition of platelet derived growth factor (PDGF) to cells cultured under low serum conditions on plastic surfaces resulted in greater than a 10 fold increase in proliferation rate, whereas the addition of PDGF to cells grown on fibrillar collagen resulted in approximately a two fold increase in proliferation rate. Fibrillar collagen preparations that were air dried, to form a collagen film, supported fibroblast proliferation in a similar fashion to that observed for cells grown on plastic as opposed to those cultured on fibrillar collagen suspensions. The results of these experiments suggest that the extracellular matrix can significantly influence the response of cells to growth factors.

L57 Down regulation of c-myc expression by interferon and by differentiation agents, Bernard Lebleu, Nadir Mechti, Jean-Marie Blanchard, Tamim Salehzada, Marc Piechaczyk and Philippe Jeanteur, University of Montpellier II, 34060 Montpellier cedex, France.

IFN treatment of Daudi lymphoblastoid cells leads to a preferential degradation of c-myc mRNAs while leaving unchanged the transcriptional activity of c-myc gene, as revealed by run-off experiments in isolated nuclei (Dani et al., 82 (1985) 4896-4900). A screening of several human lymphoblastoid cell lines differing in the structure of c-myc gene has revealed a possibly interesting correlation between the presence of exon 1, the down regulation of c-myc by IFN and the growth inhibitory activity of IFN, which is presently under closer investigation. Experiments aiming at establishing to which extent c-myc protein level itself is affected and the role of the 2-5A dependent RNase in the accelerated degradation of c-myc mRNA will be described.
Such a mechanism of down regulation of c-myc expression rapidly takes place as well in Friend erythroleukemia cells induced to terminal differentiation with Me_2SO. This and previous observations of our group (Dani et al PNAS 81 (1984) 7046-7050) concerning the unusually low stability of c-myc mRNA in most cell lines point to the major role played by post-transcriptional steps in the regulation of c-myc expression.

L58 RECIPROCAL EXPRESSION OF c-myc AND MUSCLE CREATINE KINASE mRNAs DURING MYOGENESIS, Eric N. Olson*, Gwendolyn Spizz* and Michael D. Schneider+. UTHSC and M. D. Anderson Hospital and Tumor Institute* and Baylor College of Medicine+, Houston, TX.

The nonfusing mouse muscle cell line, BC_3H1, previously has been shown to express high levels of mRNA encoding the muscle isozyme of creatine kinase (MCK) after withdrawal from the cell cycle in mitogen-depleted media (Olson et al., J. Biol. Chem. **258**, 3784-3790). In this study, we have examined possible involvement of the proto-oncogene, c-myc, as a regulator of BC_3H1 cell differentiation. Proliferating, undifferentiated cells contained relatively high levels of c-myc mRNA and undetectable levels of MCK mRNA. Withdrawal from the cell cycle was accompanied by down-regulation of c-myc mRNA and subsequent induction of MCK mRNA. Differentiated cells challenged by mitogens showed reappearance of c-myc mRNA followed by disappearance of MCK mRNA. Rates of c-myc transcription changed in parallel with levels of c-myc mRNA. Up-regulation of c-myc mRNA did not require protein synthesis, whereas down-regulation of MCK mRNA did; this suggests that synthesis of one or more early gene products is necessary to repress differentiation. This reciprocal relationship between c-myc and MCK mRNA expression suggests possible involvement of c-myc as a negative regulator of differentiation. To test the functional role of c-myc in myogenesis, a series of modified BC_3H1 cell lines was constructed by DNA-mediated gene transfer of pSVc-myc-1 and pSV2neo, conferring neomycin resistance as a dominant co-selectable marker. Myc-cotransfected BC_3H1 cells exhibited enhanced cloning efficiency and morphologic alteration (formation of dense foci) not seen in colonies resulting from neo alone. Functional consequences of transfection with c-myc for differentiation of BC_3H1 cells will be discussed. (Supported by grants from the American Heart Assoc., March of Dimes, and N.I.H.).

L59 CHORIOCARCINOMA CELLS EXPRESS AN ALTERED mRNA FOR PLACENTAL ALKALINE PHOSPHATASE, C. Ovitt, A. Strauss, D. Alpers, J. Chou and I. Boime, Washington University School of Medicine, St. Louis, MO 63110 and NIH, Bethesda, MD 20205.

The human alkaline phosphatases (AP) are found in a variety of tissues and in many malignant tumors. Although their biological function has not been established, AP may be involved in the dephosphorylation of phosphotyrosine containing proteins. Because AP are membrane glycoproteins, they may also be involved in cell surface alterations of malignant cells. Human placental AP (PLAP) is both a membrane-bound and secreted protein which is normally under developmental regulation in the placenta. Trophoblast derived tumor lines, choriocarcinomas, synthesize a PLAP enzyme which is immunologically related, but not identical to placental PLAP. We have compared the expression of PLAP in the placenta to that of three choriocarcinoma cell lines. Term placental RNA directs the synthesis of two PLAP translation products, which are encoded by separate mRNAs. Translation of choriocarcinoma RNA yields a single PLAP product, with a MW intermediate between the placental products. A cDNA clone for PLAP was isolated by antibody screening of a placental cDNA library in lambda gt11. Examination of PLAP mRNAs by blot analysis shows that the placental PLAP mRNA is 3.0 kb in size, but the choriocarcinoma PLAP mRNA is 2.6 kb. The latter is induced by sodium butyrate. Choriocarcinoma PLAP mRNA may be derived from a tumor-specific splicing of the normal PLAP transcript, or it may represent the expression of a unique PLAP-like gene in these cells. The correlation of the altered PLAP mRNA with choriocarcinoma cells may imply a role for the tumor specific PLAP-like protein in the generation of malignancy.

L60 IN VITRO RETROVIRAL TRANSFER OF RAS GENES TO SINGLE PLURIPOTENT HEMOPOIETIC PROGENITORS, Pamela N. Pharr, Makio Ogawa and W. David Hankins, Department of Medicine, Medical University SC, VA Medical Center, Charleston, SC and NCI-NIH, Bethesda, MD.

A new type of murine hemopoietic colony that consists of undifferentiated blast cells and has a high secondary replating efficiency has previously been identified in this laboratory. Such colonies are a unique source of primitive hemopoietic progenitors. Cells from these blast cell colonies were used to study the infection of pluripotent hemopoietic stem cells by Harvey sarcoma virus. Individual colonies were exposed to virus and replated. Secondary colonies were examined for lineage expression and for the expression of the viral oncogene, Ha ras. Seventeen percent of the secondary colonies examined were positive for the product of the ras gene. No ras expression was seen in the uninfected controls. Single cells isolated from blast cell colonies by micromanipulation were infected. A variety of types of positive colonies were seen including a macrophage (m)-neutrophil (n)-erythroid (E)-mast cell(mast)-megakaryocyte (M) colony, a m-E-mast-M colony, a m-n-mast colony, m-n-E colonies, m-n colonies and m colonies; thereby providing evidence for a direct effect of the virus on hemopoietic progenitors. The development of a culture system in which the target for virus infection is a single cell circumvents many problems of interpretation and should therefore facilitate the study of the effects of ras oncogenes or other genes on the growth and development of hemopoietic cells.

L61 REGULATION OF GENE EXPRESSION DURING B CELL ACTIVATION, Nancy E. Phillips and David C. Parker, University of Massachusetts Medical Center, Worcester, MA.

As a model for studying how complexes of IgG antibody and antigen downregulate humoral immune responses, we have used rabbit IgG anti-mouse Ig antibodies to study the effects on mouse B cell activation of crosslinking B cell Fcγ receptors to membrane Ig. Fab'$_2$ anti-Ig, but not IgG anti-Ig, will induce DNA synthesis and Ig secretion in the presence of the appropriate helper cell factors. Furthermore, the whole molecule will inhibit activation by the Fab'$_2$ molecule. This inhibition is not T cell or macrophage-mediated and occurs prior to entry into S phase [J. Immunol.130:620(83)]. We have therefore compared the two molecules in their ability to induce known early events in B cell activation such as blastogenesis, increased expression of class II molecules, and elevation of c-*myc* mRNA. IgG anti-Ig induces only a small increase in cell volume compared to the Fab'$_2$ anti-Ig. Both IgG and Fab'$_2$ anti-Ig, however, induce comparable levels of class II molecules at 1 or 10 μg/ml, but the IgG anti-Ig is less effective at lower doses. We have recently begun to examine steady state levels of c-*myc* mRNA which have been shown to increase in response to the B cell mitogen LPS [Kelly *et.al.*, Cell 35:603(83)]. We have found that a mitogenic dose (10 μg/ml) of Fab'$_2$ anti-Ig increases the expression of this proto-oncogene to the same extent as LPS. Experiments comparing 10 μg/ml Fab'$_2$ anti-Ig with IgG anti-Ig or submitogenic doses of Fab'$_2$ anti-Ig indicate that levels of c-*myc* mRNA expression correlate with the mitogenicity of the stimulus.

L62 Characterization of high specificity polyclonal antiphosphotyrosine antibodies. Sydonia I Rayter[1], John C. Bell[1], A. Raymond Frackleton Jr.[2], and J. Gordon Foulkes[1] 1. National Institute for Medical Research, Mill Hill, London NW7 1AA, England. 2 Roger Williams General Hospital, Brown University, Providence, RI 02908, U.S.A.

Nearly half of all known oncogenes, as well as the receptors for several growth factors encode protein-tyrosine kinase activities. Physiologically important substrates for these enzymes, however have yet to be identified for any of the enzymes. To isolate phosphotyrosine containing proteins, which may be present at very low concentrations, one of us (A.R.F.) had previously developed a monoclonal antiphosphotyrosine antibody using azobenzyl phosphonate as the hapten. (Mol. Cell Biol. 3, 1343-1352). Although these antibodies have proved very useful (JBC 260, 8070-8077; JBC 259, 7909-7915), they have a number of limitations: they are low affinity; they are unable to act in either an immunoprecipitation assay or a western blot; they cross react with adenine nucleotides as well as the phosphohistidine residue of ATP citrate lyase . We have now raised a polyclonal antiphosphotyrosine antibody (using phosphotyrosine as hapten) which appears to have overcome all of the above problems. In addition, these antibodies show a marked difference in immunofluorescent staining of normal versus transformed cells. We are now using these antibodies to determine the subcellular localization and identity of the major phosphotyrosine containing proteins in both transformed cells, as well as the developing mouse and xenop us embryos. The results of these experiments will be reported.

L63 A kinetic study was carried out to assess the stability of the intracellular messenger(s) generated by insulin in quiescent cells for the stimulation of DNA synthesis. Using murine lens epithelial cells and Swiss 3T3 cells in culture, it was found that insulin stimulated DNA synthesis after a lag of 16 hours. If, however, 6 hours after the addition of insulin to the cells, the insulin containing media was totally removed followed by the addition of fresh media (even if insulin was returned to the mediun within ten minutes) a 16 hour lag still remained after insulin readdition before DNA synthesis started. In another set of experiments the insulin was removed by diluting it's concentration approximately 60 thousand fold. In this case it was found that insulin had to be at the diluted concentration for approximately 30 minutes before the lag time necessary for the start of DNA synthesis, after the readdition of insulin, was a full 16 hours. The half time for loss of signal was 2 minutes for total washout and 12 minutes for the dilution experiment.

These results indicate that the intracellular messenger(s) for DNA synthesis produced by the binding of insulin to its cellular receptor are extremely transitory in nature. The signal disappears at the same rate that insulin dissociates from the receptor. Thus, insulin must be constantly binding to the membrane receptor inorder to keep the key messenger(s) at a high enough level for the cell to progress on to S phase. Addition of vanadate to the cell is found to stabilize the messenger such that there is no loss of signal when insulin is removed. These data are consistant with tyrosine-phosphorylated insulin receptor being the second messenger.

L64 PRODUCTION OF MOUSE-MONOCLONAL ANTIBODIES DIRECTED AGAINST ACIDIC ISOFERRITIN, Feickert, H.J., Dorner-Huntsberry, M., Broxmeyer, H. and Riehm, H., Medizinische Hochschule Hannover, Zentrum Kinderheilkunde, 3000 Hannover 61, Federal Republik of Germany.

Isoferritin and related substances seem to play a major role in the regulation of normal hematopoesis. Acidic isoferritin (AIF) derived from tumor cells is an extremly strong inhibitor of the normal hematopoesis in vitro. A role of AIF in the development of anemia is suspected in neuroblastoma (and other) patients with progressing tumors and high serum ferritin levels. To enable the investigation of this problem the production of monoclonal antibodies (mAbs) was undertaken

AIF produced by the HL-60 cell line was biochemically purified and separated into heavy and light chains, used for immunizations of C57BL6 x F1 female mice and monoclonal antibodies produced according standard procedures.

Two clones could be identified binding to either light or heavy chain of AIF which were subsequently subcloned.

In vitro studies demonstrated that the blocking effect of AIF on colony formation of hematopoetic stem cells (CFUc) could be reversed by mAb aAIF-21 directed against the heavy chain of AIF, but not by the mAb aAIF-19 directed against the light chain. This indicates that mAb aAIF-21 recognizes the relevant epitope responsible for suppression of normal hematopoesis. This also supports earlier findings that the heavy chain of AIF is responsible for its biologic effects.

L65 Differentiation and Transformation in Human Neuroblastoma - Association with $pp60^{c-src}$ Activation and N-myc Expression. N. Rosen.*, J.B. Bolen†, J.L. Biedler††, C.J. Thiele**, and M.A. Israel**. *Medicine Branch, †Laboratory of Tumor Virus Biology, **Pediatric Oncology Branch, National Cancer Institute, Bethesda, MD and ††Sloan-Kettering Cancer Institute, Rye, NY.

Two oncogenes are commonly activated in human neuroblastoma cell lines; N-myc is amplified and overexpressed and $pp60^{c-src}$ protein kinase activity is elevated. We have isolated neuroblastoma cell cultures which contain neuroblastic and epithelial cell types. Clones of both types amplify and overexpress N-myc and share common cytogenetic markers. Neuroblastic clones have elevated $pp60^{c-src}$ kinase activity and N-myc mRNA; they are tumorigenic and grow in an anchorage independent fashion. When they are terminally differentiated to a neuronal phenotype, N-myc expression decreases and $pp60^{c-src}$ activity is further increased. Epithelial clones overexpress N-myc, have less than 5% the $pp60^{c-src}$ activity of neuroblasts, and are neither tumorigenic nor anchorage independent. $pp60^{c-src}$ is therefore associated with both the transformed and neural phenotypes of these cells.

L66 gp160, A CELL SURFACE GLYCOPROTEIN INVOLVED IN THE RESISTANCE OF TUMOR CELLS TO IMMUNE DESTRUCTION. Rosenstein, Y., Sánchez, I., Pedraza, G. and Michalak, C. Departamento de Inmunología, Instituto de Investigaciones Biomédicas, UNAM, Apartado Postal 70228, México 04510, D.F.

LPC-1 murine myeloma cells possess certain alterations of cell surface antigenicity. When the tumor is grown in ascites form, cells show a periodic resistance to lysis by cytotoxic T lymphocytes (CTL): if harvested 4 days after transplantation ("early" LPC-1), cells are lysed by CTL. However as the tumor develops, cells become progressively less reactive with CTL and when harvested on day 12 ("late" LPC-1) they are completely resistant to lysis by CTL. This correlates with the production and accumulation of a trypsin-sensitive glycoprotein of approximately 160 Kd (gp160) on the tumor cell surface. gp160 consists of a single acidic peptide chain, rich in sialic acid residues (10% of total M.W.). Liposomes with gp160 and H-2 antigens fail to induce a secondary CTL anti H-2 response in vitro, suggesting that gp160 masks MHC products thereby providing LPC-1 cells with a mechanism to escape immune response. gp160 was identified by immunofluorescence in bone marrow cells and plasmacytes of BALB/c, BALB.K, and C57BL/6 but was absent on corresponding BALB.B cells (the only strain making anti-gp160 Abs). gp160 is possibly an oncofetal antigen, the accumulation of which on LPC-1 cell surface protects tumor cells from being destroyed by CTL by masking H-2 antigens and by preventing the cells being recognised as foreign by the organism.

L67 ALTERNATIVE INDUCTION OF MEGAKARYOCYTIC, GRANULOCYTIC, AND ERYTHROID ANTIGENS ON MULTIPOTENT HUMAN LEUKEMIC CELL LINE K652. P.T. Rowley, J.F. Leary, B.M. Ohlsson-Wilhelm, R. Giuliano, S. LaBella, and B. Farley. University of Rochester, Rochester, NY.

K562 cells have been reported to display a variety of nonerythroid properties. Using 46 lineage-specific monoclonal antibodies, we systematically analyzed which antigens are present spontaneously and which are inducible with a variety of agents. Induction protocols, chosen on the basis of changes in morphology with Wright's stain or by reaction with granulocyte- or monocyte-specific stains, were phorbol dibutyrate (PDB) 4×10^{-8} M, daunorubicin (DNR) 1.9×10^{-6}M, or thymidine-hypoxanthine (THX) each 10^{-4} M. After culture with inducer, cells were incubated with a specific monoclonal antibody or with an irrelevant antibody of the same Ig subclass and then with FITC-labelled $F(ab)_2$ fragment of goat anti-mouse Ig and analyzed on an EPICS V multiparameter flow cytometer, excluding dead cells. Of megakaryocytic-specific antibodies, untreated cells bound 0/15, PDB induced 12/15, DNR induced 1/3, THX induced 2/3. Of granulocytic-specific antibodies, untreated cells bound 9/18. PDB decreased 6/10 and did not change 4/10. DNR increased 3/18, decreased 1/18, and did not change 14/18. Of monocytic-specific antibodies, untreated cells bound 1/7 and none were inducible. Of erythroid-specific antibodies, untreated cells bound 3/3, PDB decreased 2/3, and DNR increased 3/3. Thus (1) antigens of a common lineage tend to be expressed en bloc, most present or most absent under a given condition. (2) Inducers have some lineage-specificity, e.g. PDB induces only megakaryocytic antigens. (3) A given inducer may influence antigens of different lineages in opposite directions, e.g. PDB, not only induced megakaryocytic antigens (absent in untreated cells), but also decreased granulocyte and erythroid antigens (present in untreated cells). Thus, the K562 cell, despite its malignant origin, retains the capacity for expression of alternative programs of differentiation, a characteristic of the normal multipotent hematopoietic stem cell.

L68 EXPRESSION OF DIFFERENTIATED PROPERTIES IN NORMAL AND TRANSFORMED HUMAN MAMMARY EPITHELIAL CELLS, Martha Stampfer, Gordon Parry, and Jack Bartley, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Control of growth and differentiation in human epithelial cells is not yet clearly understood. Malignant transformation likely involves aberrations in these control pathways. We have used the human mammary epithelial cell (HMEC) system developed in our laboratory to examine growth potential and expression of differentiated properties in response to varying culture conditions. HMEC have been grown in two different media: MM, which contains fetal calf serum and conditioned media; and MCDB170, which is serum-free but contains bovine pituitary extract. HMEC grown in MM display 10-15pd; in MCDB170, 40-65pd are possible. However, only a small fraction of the original cell population expresses this long-term growth capacity, and some specimens require a cAMP stimulator. Expression of differentiated properties, e.g., glycogen and lactate synthesis, human mammary milk fat globule antigens, synthesis of casein and other milk-related proteins, is much greater in cells grown in MM compared to MCDB170. However, cells grown for >20pd in MCDB170 can display these more differentiated phenotypes after transfer to MM. Exposure of HMEC to the chemical carcinogen, benzo(a)pyrene, can extend the reproductive lifetime in culture, and two immortal, continuous cell lines have emerged form these extended life cells (184A1 and 184B5). Additionally, we have obtained one immortal cell line from one non-BaP treated cell culture. Expression of differentiated properties in 184A1 and 184B5 has been compared to normal parental cells, with 184A1 generally expressing a less and 184B5 a more differentiated phenotype.

L69 INVESTIGATION OF PHORBOL ESTER BINDING IN RAT KIDNEY CORTEX MEMBRANES
Susan Jaken, George Andrews and James Stevens, Center for Drugs and Biologics, FDA, NIH, Bethesda, MD 20892

The proximal tubule of kidney is a polarized epithelium which sorts signals in a bipolar manner. There is little information on the distribution of protein kinase C (PKC) in proximal tubule. We prepared brush border membranes (BBM) from rat kidney cortex bymagnesium precipitation (Malathi et al. BBA 554,259,1979) and measured phorbol dibutyrate (PDBu) binding. The BBM marker, gamma-glutamyl transpeptidase (gGT) was purified 10 fold, and PDBu binding 6 fold compared to the whole homogenate (homogenate, Bt=0.55 pmol/mg; BBM, Bt=2.9 pmol/mg). The receptor was solubilized with 1 mM EGTA and 0.2% Triton X-100 (64%, Bt=2.7 pmol/mg), or 1.5% octyl glucoside (65%, Bt=3.2 pmol/mg), and with sonication in 1 mM EGTA alone (35%, Bt=4.9 pmol/mg), suggesting loose membrane association.

Barrett et al. (BBRC 128,494,1985) recently reported enrichment of PKC activity in rabbit kidney cortex BBM. Though basolateral membranes (BSLM) were not prepared, distribution of kinase activity and N,K-ATPase suggested that activity was not present in BSLM. We prepared BSLM and BBM from rat kidney cortex by sedimentation in Percoll gradients. PDBu binding purified with both gGT (BBM) and N,K-ATPase (BSLM); BBM, Bt=1.8 pmol/mg; BSLM, Bt=1.9 pmol/mg; homogenate, Bt=0.3 pmol/mg. The data suggest that the phorbol ester receptor is enriched in both BSLM and BBM. The possibility that the difference in the results for binding and PKC activity are due to different requirement for PKC activity in membrane fractions is under investigation.

L70 Growth regulation of cultured GC cells by L-triiodothyronine.
Martin I. Surks, Charles R. DeFesi, Michael J. Miller, and Lawrence E. Shapiro. Montefiore Medical Center and The Albert Einstein College of Medicine, Bronx, N.Y. 10467.

Incubation with T3 results in a dose-dependent increase in the growth rate of cultured GC cells, a GH-producing pituitary cell lines. The T3-induced increase in growth rate which is not caused by secreted GH results mainly from a shortening of the G1 period from 79.4±4.3 h (-T3) to 10.0±0.9 h (0.3 nM T3) in asynchronous cells and from >40-50 h to 13.4±2.1 h (n=7) in cells synchronized at the beginning of G1 by mitotic selection. This action of T3 was limited to the first 4-6 h of the G1 period and was 1/2-maximal at physiological T3 (0.17 nM) which is 1/2-maximal for occupancy of the nuclear T3 receptor and other T3 induced responses in this cell line. We have recently tested the effect of conditioned medium from GC cells (0.2 nM T3) on -T3 cells after addition of anti T3-antiserum. The antiserum effectively prevented T3 from entering the cultured cells. Conditioned medium stimulated cell growth as much as T3 itself! Despite significant growth stimulation ($p < 0.001$), GH production remained depressed. These findings suggest that T3 induction of GC cell growth is mediated by the nuclear T3 receptor and is associated with the production/secretion of a growth factor which, in turn, stimulates cell growth.

L71 ALTERATIONS IN GENE EXPRESSION DURING RETINOIC ACID(RA) INDUCED DIFFERENTIATION OF HUMAN NEUROBLASTOMA(NB), Carol J. Thiele, Catherine McKeon, Lorraine Cazanave+, Neal Rosen* and Mark A. Israel. Pediatric and Medicine* Branches, NCI/NIH and Children's Hospital Washington D.C.+

NB is an embryonal tumor of the peripheral nervous system which seems to arise from changes affecting cellular maturation. Since RA treatment of NB cell lines results in cell growth arrest and morphological differentiation, we have utilized this system and two approaches to study genes associated with cessation of cell growth, differentiation and development of the neuronal phenotype. 1) Since it is known that proto-oncogenes are differentially expressed during development and may be important in control of cell growth, we examined mRNA levels of several proto-oncogenes in NB pre and post RA treatment. We reported that N-myc is markedly decreased in NB after 2d in RA and this decrease occurs prior to differentiation and isn't associated with growth arrest. In contrast c-ets expression increases 3-fold at 2d while levels of the transforming gene N-ras are unaltered even after 14d in RA. 2) We have constructed cDNA libraries to NB and RA-NB and utilized differential cDNA screening to identify clones differentially expressed. We have identified several clones which are expressed at low levels in NB and whose mRNA levels increase 2-20 fold in RA. Like the neurofilament gene whose expression is markedly increased after 2d in RA, these clones may be associated with committment to a neuronal celltype or cell growth arrest. We are evaluating panels of such genes to study the molecular mechanisms by which their expression is regulated during differentiation.

L72 THE LEVELS OF C-MYC AND C-FOS mRNAS IN CULTURED RAT THYROID CELLS ARE REGULATED BY THYROTROPIN, IGF-I, AND DIBUTYRYL CYCLIC AMP, Donatella Tramontano, Alan C. Moses, William W. Chin, and Sidney H. Ingbar, Harvard Medical School, Boston, MA. 02135.

Growth factor-induced cell proliferation is associated with increased levels of proto-oncogene mRNAs, thus suggesting that products of proto-oncogenes such as c-myc and c-fos may play a key role in regulating cell proliferation. FRTL5 cells provide an interesting model for studying cell proliferation since they respond both to the thyroid specific growth factor (thyrotropin, TSH) and to growth factors such as IGF-I that stimulate a wide variety of cells in culture. TSH and IGF-I both stimulate FRTL5 cell growth, although they differ in their relative ability to stimulate ^{3}H-thymidine incorporation into DNA and cell proliferation. Thus, while 10^{-9} M TSH or IGF-I increase ^{3}H-thymidine incorporation 10-fold, TSH stimulates cell proliferation 3 times more than IGF-I. In FRTL5 cells, 10^{-9} M TSH produces a rapid but transient increase (10-fold) in the levels of c-myc (60 min) and c-fos (30 min) mRNAs. 10^{-9} M IGF-I stimulated c-myc mRNA only 2-fold. These increases in c-myc mRNA correlated better with cell proliferation than with ^{3}H-thymidine incorporation into DNA. While the mechanisms by which TSH and IGF-I exert their mitogenic effects are not clear, cAMP mediates most of the biological effects of TSH but not those of IGF-I. In FRTL5 cells, 10^{-3} M dibutyryl cAMP, a cAMP analogue, mimics TSH by increasing c-myc and c-fos mRNA levels and suggests that this TSH effect also is mediated by cAMP. In epithelial cells such as FRTL5, as well as in mesenchymal cells, increased c-myc and c-fos mRNAs are early markers of cell proliferation.

L73 Differential sensitivity of two malignant mesothelioma cell lines and two benign mesothelial cell lines for Hydrocortisone and Epidermal Growth Factor

M.A. Versnel, H. Hoogsteden, M.J. Bouts, M. Delahaye, Th. van der Kwast and A. Hagemeijer
Dept. of Cell Biology, Immunology and Genetics, Dept. of Pulmonary Disease and Dept. of Clinical Pathology, Erasmus University and Academic Hospital Dijkzigt, Rotterdam, The Netherlands

Two malignant mesothelioma cell lines (Mero-14 and Mero-25) were isolated from two patients. Also two non-malignant mesothelial cell lines (M1.1 and M1.2) were established: M1.2 in medium supplemented with both Epidermal Growth Factor (EGF; 10 ng/ml) and Hydrocortisone (HC; 0.4 μg/ml), and M1.1 in medium without supplementation. Mero-14 and Mero-25 were found to have several chromosomal aberrations in contrast to M1.1 and M1.2.
The proliferation of normal mesothelial cells is generally promoted by the simultaneous supplementation of EGF and HC. The proliferation of the malignant mesothelioma cell lines Mero-14 and Mero-25, however, was inhibited under these conditions. This appeared to be due to the HC, since EGF supplementation alone did promote the growth of these two malignant mesothelioma cell lines. We investigated the effect of HC and EGF on these malignant and benign mesothelial cell lines in more detail. The number of EGF receptors and several cytological criteria were determined. No major differences in the number of EGF receptors were observed when Mero-14 and Mero-25 were grown under different conditions. However, several cytological parameters did change. The latter was also observed in the benign mesothelial cell lines.

Supported by the Dutch Cancer Foundation (Koningin Wilhelmina Fonds).

L74 EARLY GENE ACTIVATION IN MONOCYTIC DIFFERENTIATION OF A VARIANT HL-60 CELL LINE, Marie-Cecile Wetzel, Jacqueline Zemmour and Yvon Cayre, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

A cloned variant cell line, 1F10, was derived from the human promyelocytic leukemia cell line HL-60. 1F10 cells are resistant to the adhesion and growth inhibition effects normally observed when original HL-60 cells are treated with either phorbol 12-myristrate 13-acetate (PMA) or 1,25 $(OH)_2$ vitamin D_3 (vit D_3), two inducers of monocytic differentiation. However, 1F10 cells can be induced to become monocyte/macrophage-like cells and to withdraw from the cell cycle when these two inducers are applied concomitantly. Similarly to HL-60 cells, development of this differentiation process in 1F10 cells is accompanied by a drastic decrease in the level of c-myc mRNA. Phenotypic and cell cycle characteristics of variant cells maintained with one inducer (either PMA or vit D_3) reveal that they are partially differentiated, and - since no decrease in the level of c-myc mRNA was detected - may represent early differentiation steps. Complementation experiments involving sequential use of the two inducers, revealed that early events, induced by vit D_3 and persisting after complete removal of this inducer, render 1F10 cells competent to further differentiate and withdraw from the cell cycle by treatment with PMA alone. Such an effect was not obtained when cells were pretreated with PMA, and treated with vit D_3 as a second inducer. In order to clone genes specifically activated by vit D_3, a cDNA library was constructed with cDNA from vit D_3-treated cells substracted with mRNA from PMA-treated cells. We present here the results of our initial screening.

L75 TRANSIENT ACCUMULATION OF c-myc PROTO-ONCOGENE mRNA DURING DIFFERENTIATION OF LENS EPITHELIAL CELLS INTO LENS FIBERS. P. Zelenka, P. Nath, and L. Pallansch, NEI, NIH, Bethesda, MD

Differentiation of lens epithelial cells to form lens fibers is accompanied by withdrawal of dividing cells from the cell cycle. In vivo studies of Zwaan and Kenyan have shown that withdrawal occurs as cycling cells complete mitosis and enter the next G_1 phase. Since expression of c-myc proto-oncogene has been associated with cell division in a variety of cell types, we examined the levels of c-myc mRNA in differentiating explants of embryonic chicken lens epithelia during the time when cells are withdrawing from the division cycle. Differentiation was initiated by adding vitreous humor to the culture medium and RNA was extracted at subsequent times for Northern blot analysis. Levels of 2.4 kb c-myc mRNA were elevated about 5-fold within 30 min of exposure to vitreous humor, and remained elevated for at least 5 hr before dropping to very low levels after 24 hr or longer. In contrast, δ-crystallin mRNA levels remained constant for the first 5 hr and were elevated at 24hr, in agreement with earlier studies. Thus, a transient elevation of c-myc mRNA precedes the loss of c-myc mRNA and the withdrawal of cells from the cell cycle.

Gene Regulation, Growth Factors and Tumor Promoters

L76 HUMAN T-CELL GROWTH FACTOR (INTERLEUKIN 2) AND GAMMA INTERFERON GENES: EXPRESSION IN HUMAN T-LYMPHOTROPIC VIRUS TYPE III AND TYPE I INFECTED CELLS, Suresh K. Arya and Robert C. Gallo, Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Acquired immune deficiency syndrome (AIDS) is characterized by severe depletion of $OKT4^+$ T-lymphocytes and leukemia is associated with abnormal proliferation of maturation-arrested lymphocytes. Human T-lymphotropic virus type III (HTLV-III) or lymphoadenopathy virus (LAV) and type I (HTLV-I) are etiologically linked to AIDS and adult T-cell leukemia/lymphoma, respectively. T-cell growth factor (TCGF) (interleukin 2) is required for the growth of activated T-cells which play an important role in immune regulation. Gamma interferon (γIFN) is also implicated in immune modulation. It was possible that T-cell depletion in AIDS could be due to an impairment of TCGF synthesis and that adult T-cell leukemia could be due to unregulated production of TCGF. We find that the transcription of the TCGF gene and production of TCGF is not impaired in cultured HTLV-III-infected cells. It is readily induced by mitogen phytohemagglutinin (PHA) and tumor promoter tetradecanoyl phorbol acetate (TPA). Paradoxically, the TCGF gene in HTLV-I-infected cells is transcriptionally inactive and uninducible by PHA and TPA. The reverse is the case for the γIFN gene -- it is actively transcribed in HTLV-I-infected cells but not in the HTLV-III-infected cells. It thus appears that both in HTLV-III and HTLV-I infection, growth control and immune regulatory mechanisms may bypass a modulatory role of TCGF and possibly of γ-IFN as well.

L77 Phorbol ester induced degradation of immunoprecipitable protein kinase C, Roymarie Ballester and Ora M. Rosen, Memorial Sloan-Kettering Institute for Cancer Research, New York, NY 10021

The effect of phorbol 12-myristate 13-acetate (PMA) on protein kinase C was studied by metabolically labeling GH_3 cells with [^{35}S]methionine and using a polyclonal antibody raised against rat brain protein kinase C to immunoprecipitate the enzyme. PMA accelerates the loss of immunologically reactive protein kinase C from the cells in a time- and dose-dependent manner. The half-life of the enzyme in cells treated with 400 nM PMA was 2 h whereas in control cells 60-70% of the enzyme was still detectable after 24 h. PMA also induced the translocation of [^{35}S]Met-labeled protein kinase C from the cytosol to the membranes in a concentration-dependent manner. When cells were exposed to 400 nM PMA most of the labeled protein kinase C became membrane-associated and was rapidly degraded. In cells treated with 20 nM PMA, dissappearance of [^{35}S]Met-labeled protein kinase C from the cytosolic fraction occured in two phases, a rapid decrease characteristic of the membrane-associated enzyme, followed by a slower loss similar to that seen in control cells. The rate of degradation of membrane associated protein kinase C was the same at both concentrations of PMA.The results indicate that turnover of protein kinase C is enhanced by membrane association. The effect of subcellular localization on the phosphorylation state of protein kinase C will be discussed.

L78 Establishment of Myeloid Cell Lines from the Recombinant Inbred BXH-2 Mouse Strain. Hendrick G. Bedigian, The Jackson Laboratory, Bar Harbor, ME 04609.

The BXH-2 recombinant inbred mouse strain has been identified as a highly leukemic line with 89% incidence of myeloid leukemia by one year of age. A B-ecotropic murine leukemia virus is associated with the disease. The B-ecotropic virus induces myeloid leukemias when inoculated into other strains. We have established long term cell lines from naturally occurring and induced leukemias from BXH mice. The majority of cell lines established are of the myeloid lineage; they are not dependent on the addition of interleukin-3 (IL-3) for growth and are highly leukemogenic. Each of the cell lines were positive for nonspecific esterase and chloroacidesterase but were negative for myeloid peroxidase. These cells were also negative for several T- and B-cell surface antigens as determined by cytoflurometry and immunofluorescence. The cell lines are presently being analyzed for their ability to differentiate in the presence of phorbol esters and various growth factors in addition to any changes in viral and/or oncogene expression.

L79 Differential Gene Expression During The Progression of Benign to Malignant Epidermal Tumors. G.T. Bowden, L.M. Matrisian, R. Breathnach, P. Krieg and G. Fürstenberger, Univ. of Arizona, Tucson Ariz., USA (G.T.B.); Institut de Chimie Biologique, Strasbourg France, (L.M.M., R.B.); Deutsches Krebsforschungszentrum, Heidelberg, Germany (P.K., G.F.). In the multistage process of epidermal carcinogenesis, the formation of malignant squamous cell carcinomas is proceeded by the induction of benign papillomas. A study was undertaken to identify genes that are upregulated in their expression during this step from a benign to malignant tumor. We have detected in carcinomas the enhanced expression of a sequence termed TR1. This TR1 sequence was isolated and sequenced as a cloned cDNA corresponding to an mRNA present in significantly higher levels in rat cells treated with epidermal growth factor and transformed by polyoma virus and oncogenes (EMBO J. 4, 1435, 1985). We have detected by Northern analysis the expression of a TR1 sequence (1.9 kb transcript) in carcinomas but not in papillomas or normal adult epidermis. Screening λgt10 cDNA libraries made from papilloma and carcinoma poly A^+ RNA for TR1 positive clones confirmed the greatly enhanced level of expression of TR1 related sequences in the carcinoma. The tumor promoter 12-0-tetradecanoylphorbol-13-acetate was found to enhance the level of expression of TR1 in normal adult epidermis 4 hr. after application. The mechanism of activation and the functional role of TR1 is being studied using a cloned mouse TR1 cDNA sequence.

L80 SYNTHESIS OF INSULIN-LIKE GROWTH FACTOR-II FOLLOWING T-LYMPHOCYTE ACTIVATION, T.J. Brown, M.N. Lioubin, G.J. Todaro and B.H. Ginsberg, Oncogen, Seattle, WA and Veterans Administration Medical Center/University of Iowa, Iowa City, IA USA

Lectin activated human T-lymphocytes have been reported to lose insulin-like growth factor-II (IGF-II) receptors preceeding the onset of DNA synthesis when cultured in a chemically defined medium (CDM) (1). This receptor decay indicated the potential for in situ synthesis of IGF-II as a consequence of activation. Radioimmunoassay (RIA) specific for IGF-II detected 8.4 + 0.7 ng/ml in acidified CDM. No immunoreactive IGF-I was detected by IGF-I RIA. Following acid gel filtration of CDM three major M_r regions of IGF-like activity were demonstrated by crossreaction in insulin radioreceptor assays. Purification of the 5-10 KDal activity by rp-HPLC yielded a chromatographically distinct fraction with the ability to compete on an equimolar basis with IGF-II for [^{125}I]-iodo IGF-II binding to activated T-lymphocytes. This fraction was found to stimulate glucose oxidation in fatty tissue and to substitute for serum during thymidine labeling of T-lymphocyte proliferation consistent with the known metabolic and growth regulatory action of IGF-II. IGF-II stimulation was recently reported to be a prerequisite for cellular transformation by beta-transforming growth factor (2). These data indicate that human T-lymphocytes represent an inducible site for the expression of IGF-II in the adult. Immune IGF-II may function normally in the clonal expansion of lymphocyte subpopulations as well as abnormally in the process of leukemogenesis.
(1) J. Receptor Res. 5(4):297, 1985; (2) J. Biol. Chem. 260(8):4551, 1985.

L81 INDUCTION OF ADENOVIRUS GENE EXPRESSION BY PHORBOL ESTERS IN TRANSFORMED HUMAN CELLS, Timothy Carter, Calvin James, Brian Green, Emia Chan and Willard Lou, St. John's University, Jamaica, NY 11439

Tumor promoters accelerate the onset of transcription from type 5 human adenovirus (HAd5) early genes in infected HeLa cells, at least in part by altering the specificity of host RNA polymerase. We now report that cytoplasmic RNA from both E1A and E1B genes in several transformed human cell lines was elevated up to 10-fold within 90 min of exposure to 100 ng/ml TPA, with half-maximal stimulation occurring at 5-7 ng/ml. As in lytic infection, the effect was not inhibited by cycloheximide and was elicited by only those phorbol esters with tumor promoting activity. Nuclear runoff transcription showed transient stimulation of E1A, E1B, B-actin and c-myc RNA synthesis during the first 60 minutes. Cytoplasmic E1A RNA remained elevated for several hours in the presence of TPA, whereas E1B RNA returned rapidly to basal levels; cycloheximide caused accumulation of even higher levels of viral RNA from both regions in response to TPA, and E1B RNA persisted under these conditions. These results are consistent with a rapid, transient stimulation of E1A (and E1B) transcription by TPA in transformed human cells that express basal levels of these gene products, and implicate a cellular factor in regulation of viral mRNA stability. The response of the integrated Ad5 E1A and E1B genes thus resembles that reported by others of B-actin and c-myc, respectively, and suggests that DNA sequence elements of the virus E1 region determine responsiveness to TPA independently of overall genetic context.

L82 THE CHARACTERISTICS OF THE INSULIN RECEPTOR AND ITS EFFECT ON THE EXPRESSION OF HBsAg ON HUMAN HEPATOMA CELLS, Chen-Kung Chou, Veterans General Hospital,Taipei,Taiwan,Republic of China

Specific insulin binding on cultured human hepatoma cells Hep3B was examined. The Kd of the insulin receptors on Hep3B cells is 0.5nM and each cell has about 50,000 receptors. The tyrosine protein kinase activity associated with those human hepatic insulin receptors was demonstrated. Only one phosphorylated protein with apparent molecular weight 95K dalton was specifically immunoprecipitated by insulin receptor antiserum B-9. We also examined the growth promoting activity of insulin towards serum starved Hep3B cells. The result showed that insulin not only stimulates cell proliferation of Hep3B cells, but also suppress the hepatitis B surface antigen(HBsAg). At the same time, the production of -fetoprotein by Hep3B cells was not affected. Suppresion of HBsAg production by insulin was correlated with the decrease of HBsAg mRNA in Hep3B cells. The concentration of insulin required to show half maximum suppression of HBsAg is about 1nM which closes to the Kd of the insulin receptors on Hep3B cells. Therefore, Hep3B cells provide an excellent model to study the control mechnism of cell growth and HBsAg expression on human hepatoma.

L83 STUDIES ON A cDNA CLONE OF THE mRNA ENCODING MURINE MAJOR EXCRETED PROTEIN, David T. Denhardt, Richard T. Hamilton, Craig L.J. Parfett, Dylan R. Edwards, Paul Waterhouse, Rebecca St. Pierre, and Marit Nilsen-Hamilton, Cancer Research Laboratory, University of Western Ontario, London, Canada N6A 5B7

Using an antiserum raised against murine major excreted protein (MEP, M.M. Gottesman, PNAS 75, 2767, 1978) to probe an expression library in λgt11, we have isolated recombinant phage expressing an MEP epitope. This clone was used to isolate additional MEP cDNA clones, some of which are full length, or almost so. The DNA sequence of the cDNA has been determined and will be presented; the inferred protein product possesses substantial regions of homology with several thiol proteases (papain, actinindin, cathepsin H) leading us to propose that the protein is a thiol-dependent protease. A number of different cell lines secrete detectable amounts of protein. MEP secretion is enhanced by stimulation with growth factors, and we have confirmed that at least part of this control is at the level of mRNA production. However, transcription of the gene does not appear to vary during a normal cell cycle in exponentially growing Ehrlich ascites cells.

(Supported by the NCI of Canada and MRC of Canada.)

L84 A HUMAN PRO GENE HOMOLOG TRANSFERS PROMOTION SENSITIVITY, W. K. Dowjat, A. Sakai, K-T. Yao, M. I. Lerman and N. H. Colburn, National Cancer Institute, Frederick MD

Recently two genes designated pro-1 and pro-2, that determine sensitivity to promotion of neoplastic transformation have been cloned ( ). These genes cloned from promotion sensitive (P^+) mouse cells transfer sensitivity to induction of anchorage independent transformation by tumor promoters when transfected into insensitive (P^-) cells. Initial observations suggested that structurally similar genes appeared to be present in normal and tumor cells of various species, including human. It was further observed that DNA isolated from human nasopharyngeal carcinoma lines (CNE_1 and CNE_2) showed P^+ activity when transfected into mouse P^- cells. The question addressed by these studies was whether this P^+ activity of CNE cell DNA could be attributed to homologs of mouse pro-1 or pro-2. A Charon 4A genomic library of CNE_2 DNA was constructed. Screening with mouse pro-1 and pro-2 probes, yielded three pro-2 and six pro-1 homologs. The transfection assay for promotion sensitivity (P^+ activity) showed that some of the pro-1 clones possess P^+ activity of similar magnitude to that observed for mouse pro-genes. In contrast, none of three pro-2 homologs from the CNE_2 library was active in this assay. The P^+-biologic activity of the pro-1 homolog was located within a 6 kb XbaI fragment. The restriction maps of the pro-1 clones revealed the existence of restriction site polymorphism. Pro-1 homologs occurred in the CNE_2 human library with a frequency of 10-50 copies per genome. These findings suggest possible activation by structural alterations and or gene amplification. Hence the promotion sensitivity activity of human CNE DNA can be attributed, at least in part to a biologically active homolog of mouse pro-1.

L85 GENETIC CONTROL OF POST-INITIATION EVENTS IN MURINE HEPATOCARCINOGENESIS
N. R. Drinkwater, J. Ginsler, M. Hanigan, and C. Kemp, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, WI 53706

We have studied the divergent susceptibilities of C3H/HeJ and C57BL/6J mice to hepatocarcinogenesis by analysis of the number of induced liver tumors per animal as a quantitative genetic trait. Treatment of newborn male mice with diethylnitrosamine (DEN) (0.1 mol/g body weight) resulted in averages of 34 and 0.3 tumors per animal at 32 wk of age for C3H and C57BL/6 mice, respectively. Quantitation of liver tumor induction in parental, F1 hybrid, and F2 mice, and in backcrosses between F1 and parental mice indicated that approximately 90% of the difference in susceptibility between the two parental strains resulted from an allelic difference at a single locus. The parental strains did not differ in the levels of ethylated bases in hepatic DNA isolated after treatment of newborn male mice with 14-C-DEN. Studies of the development of glucose-6-phosphatase-deficient hepatic foci in parental or F1 hybrid mice treated with N-ethyl-N-nitrosourea (ENU) indicated that the major difference between the strains was in the growth rate of the putative preneoplastic lesions. Analysis of the development of preneoplastic lesions and liver tumors in female mice and in male mice treated with hexabromobiphenyl indicated that the action of the C3H susceptibility allele was largely independent hormonal status and exogenous tumor promotion. Current studies are directed toward mapping the location of the susceptibility gene and determining its cellular site of action.

L86 EGF and retinoic acid stimulate EGF receptor synthesis, H. Shelton Earp, Lester W. Lee, Victoria W. Raymond, Joyce Blaisdell, Kathy Austin, and Joe W. Grisham.

Until recently experiments defining total cellular receptor number and rates of receptor synthesis have relied upon labeled-ligand binding studies. Assessment of receptor synthesis after stimulation of a cell with ligand e.g., EGF has been particularly difficult. We have used antiserum directed against the rat hepatic EGF receptor to follow the synthesis and fate of ^{35}S-methionine labeled receptor. Immunoprecipitation followed by gel electrophoresis fluorography, and gel slice counting has been used in a line of hepatic epithelial cells (WB) which respond to EGF. Most intriguing was the response to EGF. As expected, receptors prelabeled for 18h were degraded upon addition of EGF, however when ^{35}S-methionine and EGF were added concurrently it was clear that receptor synthesis increased by at least 3 fold. Increased receptor synthesis was stimulated by as little as 3ng/ml EGF and was demonstrated within 2h of EGF binding. Prolonged treatment with high doses of EGF reduced surface receptor number and attenuated but did not abolish EGF-enhanced receptor synthesis. Northern analysis using probes to both the cytoplasmic and extracellular coding domains revealed that EGF increased receptor mRNA levels by 3-5 fold. (probes generously provided by Axel Ullrich). Retinoic acid (R.A.) responsive liver cells derived from WB cells by carcinogen treatment exhibit R.A. induced increases in EGF binding. R.A. produced dose and time dependent increases in ^{35}S-labeled EGF receptor protein synthesis. (3-5 fold) EGF receptor synthesis was clearly increased within 4h of R.A. treatment. Studies are determining whether the effect of R.A. involves increases p170 mRNA.

L87 CYTOSKELETAL AND EXTRACELLULAR MATRIX GENE EXPRESSION IN EMBRYONIC CHICKEN CHONDROCYTES TREATED WITH PHORBOL MYTISTATE ACETATE, Mitchell Finer, Helga Boedtker and Paul Doty, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge , MA 02138 and Louis Gerstenfeld, Department of Orthopedic Research, The Childrens Hospital, Harvard Medical School, Boston, MA 02215

The temporal pattern of cytoskeletal and extracellular matrix gene expression during the switch from growth of embryonic chicken chondrocytes in suspension to growth of cells in monolayer culture has been examined. Primary chondrocytes grow in suspension and produce a cartilage specific extracellular matrix. These cells proceed synchronously through a defined program upon growth in the presence of the potent tumor promotor, phorbol myristate acetate (PMA). Attachment and induction of β-actin is followed by induction of fibronectin and lastly, type I procollagen. Following attachment, cartilage specific macromolecules are no longer synthesized. Control of the levels of these gene products is primarily transcriptional, however there is also translational control and control at the level of mRNA processing. Partial reversion of chondrocytes to the differentiated phenotype is possible providing type I collagen has not been synthesized. These revertant cells remain attached and have a polygonal morphology. This suggests that there is a pathway which chondrocytes move through during the establishment of the fibroblastic phenotype, which contains uncommitted states and states committed to the establishment of the fibroblastic phenotype. Therefore, this is an ideal system to study coordinate control of genes responsible for adhesion and a simple model system to study commitment to a differentiated phenotype.

L88 PROTEIN RESPONSES TO SERUM FACTORS AND DNA-DAMAGING AGENTS SCORED BY QUANTITATIVE 2D GEL ANALYSIS. James I. Garrels, Michael E. Lambert, & B. Robert Franza, Jr., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 11724

Proteins responding to growth stimulatory factors, DNA damaging agents, and activated oncogenes are being systematically studied in REF52 cells and its transformed derivatives using the QUEST system of quantitative two-dimensional gel analysis. By accurate 2D gel quantitation and automated pattern matching, databases are being built which can be used to assess the levels of synthesis of more than 1000 proteins in each radiolabeled cell preparation.

Proteins induced by adding fresh serum to serum-deprived cells have been compared in normal REF52 cells, and in REF52 cells transformed by SV40 or adenovirus. Of the serum-induced proteins in normal cells, only half respond to serum in minimally altered SV40-transformants, only 35% in anchorage-independent SV40-transformants, only 30% in tumorigenic SV40-transformants. Most strikingly, in adenovirus-transformed REF52 cells, almost all serum-stimulated inductions of protein synthesis are abolished.

PCNA and a co-regulated nuclear protein of 85KD, proteins which respond to serum-stimulation in REF52 cells and are highly sensitive to rate of proliferation in normal cells, are unresponsive to serum in all transformants studied.

Exposure of REF52 cells to chemical carcinogens results in growth arrest and induction of a cellular response to DNA damage. Using the 2D gel database, protein changes involved in these two effects can be distinguished. Proteins specifically induced by activated chemical carcinogens will be described.

L89 DIFFERENTIAL REGULATION OF β- AND γ-CYTOSKELETAL ACTIN GENE TRANSCRIPTION BY EPIDERMAL GROWTH FACTOR. Michael J. Getz, Paula K. Elder and Lucy J. Schmidt, Department of Cell Biology, Mayo Clinic/Foundation, Rochester, MN 55905

The addition of epidermal growth factor (EGF) and somatomedin C to quiescent AKR-2B mouse embryo cells in culture results in an increased accumulation of specific β-actin mRNA within 20 minutes. This is preceded by a rapid but transient stimulation of β-actin gene transcription as measured by a nuclear run-off transcription assay. In contrast to β-actin, γ-actin gene transcription and mRNA accumulation appear completely refractory to EGF-stimulation during this early time period. Treatment of cells with cycloheximide, an inhibitor of protein synthesis, causes a time-dependent increase in both β and γ transcription and increases the sensitivity of both genes to subsequent stimulation by EGF. This effect is specific for the cytoskeletal actin genes as evidenced by a lack of a similar effect on two sarcomeric actin genes or on an α-tubulin gene. These data indicate that the specificity of isoactin expression following EGF binding is largely determined by the differential control of gene transcription. Furthermore, the data suggest the involvement of a labile protein in modulating the strength of EGF-dependent, positive transcription signals. (supported by NIH grants GM25510, CA33643, and the Mayo Foundation)

L90 Ornithine Decarboxylase Expression in TPA-Treated Mouse Skin and Epidermal Tumors. Susan Gilmour and Thomas G. O'Brien, The Wistar Institute, Philadelphia, PA.

One of the earliest events after treatment of mouse skin with the tumor promoter TPA is the induction of ornithine decarboxylase (ODC) activity. Epidermal tumors exhibit constitutively high levels of ODC activity compared to basal epidermal levels. We used an immunocytochemical method to localize epidermal cells containing induced levels of ODC during two-stage tumorigenesis. CD-1 mice chronically treated with TPA exhibited heterogeneous epidermal staining for ODC predominantly in the suprabasal cells lining the hair follicles. The induction of ODC-specific staining after TPA treatment was transient, and was inhibited by retinoic acid and cycloheximide. However, treating mice several times with hyperplastic agents such as mezerein, ethylphenylpropiolate, or catharidin resulted in epidermal hyperplasia but no ODC-specific staining in cells surrounding hair follicles. Cellular heterogeneity in ODC-specific staining was also seen in epidermal tumors but, unlike normal tissue, it was constitutively expressed as evidenced by intensely staining tumor cells appearing more than one week after the last TPA treatment. Northern blot analysis of poly(A^+) RNA isolated from TPA-treated mouse skin or from papillomas revealed one species of ODC mRNA of 2.1 kb, while mouse carcinoma contained additional ODC mRNA species of sizes 2.8 and 1.6 kb. These data indicate that both mouse epidermal cells as well as tumor tissue display heterogeneity in ODC expression, and that neoplastic progression to a malignant state results in differential regulation of ODC. Supported by grant ES-01664 from NIH, DHHS.

L91 BLOOD PLASMA FROM TUMOUR BEARING ANIMALS : DECREASED BINDING OF EGF AND DISTURBED CAPACITY TO INHIBIT THE INTERACTION OF EGF WITH ITS RECEPTORS, G.V.Glinsky, A.B.Ivanova and V.B.Vinnitsky, Institute for Oncology Problems Acad.Sci.Ukr.SSR, Kiev, USSR

Ultrafiltration of blood plasma with ^{125}I-EGF revealed a 1.7-fold decrease in the binding of EGF in blood plasma (BP) from animals with tumours. Disturbed binding of EGF in BP from tumour bearers was also found for the systems with competing binding : BP-EGF-BSA and BP-EGF- rat liver microsomes (RLM). Radioreceptor analysis showed that the capacity of BP from tumour bearers to inhibit the binding of EGF to its receptor is 34 times decreased. Specific binding of EGF to RLM is increased more than 100-fold in the presence of BP from tumour bearers as compared with the control BP. Contrary to EGF, the binding of somatostatin (SRIF) in BP from tumour bearers is increased. Competing binding assay in the system BP-SRIF- mouse brain microsomes revealed specific binding of SRIF in BP from tumour bearers only. The changes in peptide-protein binding in BP from tumour bearing animals were found to be polyamine-dependent. The binding of EGF and SRIF in BP is one of the mechanisms controlling their bioactivity. As the binding of α-TGF to the EGF-binding proteins is altered, the mechanism may be ineffective with respect to α-TGF. In malignant growth disturbances in this regulatory mechanism form molecular microenvironment favouring the proliferation of tumour cells. In confirmation to this idea, normal blood plasma in vitro was shown to block completely the mitogenic effect of EGF on target cells, and blood plasma from tumour bearers fails to exert inhibitory activity.

L92 CORRELATION BETWEEN mRNA-cDNA, HFGPF RECEPTORS PROTEIN PHOSPHORYLATED IN RESPNSE TO HFGPF AND CELLULAR TUMORIGENICITY. Anwar A. Hakim. Loyola University Medical Center. Maywood. Illinois 60153.

Earier studies led to the isolation and characterization of a human fibroblast growth-promoting factor- HFGPF (Hakim, Experientia 34: 1515-1519, 1978). Although the binding of HFGPF to specific receptors on the cell surface has been well documented, the events bridging the initial interaction of HFGPF with target cells and the induction of unique gene products remain obscure. Possible processes effecting the transfer of mitogenic information from the plasma membrane to the nucleus include changes in intracellular calcium concentration and phosphorylation of cytoplasmic and membrane associated proteins. Human skin fibroblasts (HSF), embryonic lung fibroblasts WI-38, malignant amelanotic (HMMC-SR) and malignant melanotic (HMCC-ShA HMMC-WJP) differ in their tumorigenic ability in Nude mice and in their response to HFGPF. The present studies contribute to the understanding of some of the processes responsible for this variation. HSF, WI-38, HMMC-ShA, HMMC-WJP and HMMC-SR were cultured in absence (control) and in media supplemented with HFGPF. mRNA were isolated from these cells and complementary cDNA to each of these mRNAs were prepared. Only mRNA and cDNA preparations from cultured cell in prence of HFGPF coded for a protein kinase which specifically phosphorylated tyrosine. The results indicate a linkage between the protein-kinase mediated tyrosine specific phosphorylation and tumorigenesis : 1. HFGPF-receptors isolated from senescent HFGPF-insensitive cells, in contrast to those obtained from young HFGPF-responsive cells, did not exhibit tyrosine-specific auto-phosphorylating activity.2. A close sequence homology of the v-erb B oncogene product and the transmembrane, kinase-associated region of the HFGPF-binding domain.

L93 CLUSTERING OF CHROMOSOME BREAKPOINTS 5' TO THE C-MYC LOCUS IN BURKITT LYMPHOMAS HAVING THE t(8;14) TRANSLOCATION, F.G. Haluska, Y. Tsujimoto, S. Finver, and C.M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia, Pa. 19104

Burkitt lymphoma is a B-cell neoplasm characteristically displaying one of three chromosome translocations, each of which results in the constitutive expression of c-myc. One approach to understanding this loss of transcriptional control has been to explicate the mechanism of translocation. We now demonstrate a cluster of breakpoints at least 13 kb 5' to the c-myc gene. We previously obtained a cell line carrying both t(8;14) and t(14;18) translocations from a young male with acute lymphoblastic leukemia, and from this cell line cloned the t(8;14) breakpoint. From the breakpoint clone, we subcloned a probe, p380j9SS, which hybridized to Southern blots from somatic cell hybrids containing human chromosome 8, but not to those containing 14. Using this probe, we have demonstrated DNA rearrangements by genomic Southern blots in two Burkitt lymphoma cell lines, P3HR-1 and Daudi. These lines were obtained from different African Burkitt lymphoma patients, contain t(8;14) translocations, and are EBNA positive; in both, c-myc is translocated to the 14q+ chromosome. By somatic cell genetic techniques, chromosome 14 in P3HR-1 has been shown to break in the J_H segment, whereas in Daudi the break is in the V_H segment. We prepared a partial Sau 3A digest of P3HR-1 DNA, and constructed a genomic library in the phage EMBL 3A. Two clones were isolated which hybridized to both a J_H probe and p380j9SS. We conclude that in the 380 ALL, the BL P3HR-1, and probably in the BL Daudi, the t(8;14) translocation involves breakage of the same segment of DNA. We speculate that the same mechanism is responsible for these translocations, and that this mechanism is operative early in B-cell ontogeny. F.G.H. is supported by the Medical Scientist Training Program, NIH 5-T32-GM 07170.

L94 C-fos Protooncogene Expression is Necessary for Normal Growth of Mouse 3T3 Cells. JT Holt,TV Gopal,AW Nienhuis.CHB,NHLBI, Bethesda,MD. Inhibition of expression of proto-oncogenes in intact cells may provide information about the roles of these genes in vivo. We have produced "antisense" RNA complementary to c-fos mRNA in mouse 3T3 cells by gene transfer techniques. Transcriptional units were constructed consisting of an inducible MMTV promoter, a 196 bp fragment from the 5' untranslated region and exon I of the human c-fos gene in the "sense" or "antisense" orientation, and splice and polyadenylation signal from the human beta globin gene. The theoretical Tm of the human-mouse RNA-RNA hybrid at physiologic salt concentration is 75°, consistent with the greater than 80% overall homology between human and mouse c-fos genes. A neomycin resistance gene was included in the constructs to allow isolation of stable transformants. There was a 10 fold decrease in the number of stable transformants isolated when the "antisense" plasmid was transfected in the presence of steroid (but no decrease with the control sense plasmid). There was a 10 to 50 fold induction of the expected mRNA by steroid in all clones studied. The growth rate of three "antisense" transformants was reduced 50-80% by steroid. Untransformed 3T3 and "sense" transformants are not significantly inhibited by steroid. Preliminary studies suggest that the antisense transformants show a blunted DNA synthetic response to PDGF in the presense of steroid. These results suggest that "antisense" RNA may be useful in elucidating proto-oncogene function in normal cells, and provides evidence that the c-fos gene product has a required role in normal cell division.

L95 ISOLATION AND NUCLEOTIDE SEQUENCE ANALYSIS OF cDNA CLONES FROM RAT BRAIN USING OLIGONUCLEOTIDE PROBES TO PROTEIN KINASE C AND PROTEIN KINASE A

Gerard M. Housey, Catherine A. O'Brian, Mark D. Johnson, Paul T. Kirschmeier, Jeffrey S. Roth and I.B. Weinstein, Cancer Center/Institute of Cancer Research, Columbia University, New York, NY 10032

We have synthesized two 53 bp oligonucleotide probes corresponding to amino acid sequences which are present in rat brain protein kinase C (PKC) and bovine heart protein kinase A (PKA). The amino acid sequence of rat brain PKC was partially determined by endo lys C cleavage of purified enzyme followed by gas phase sequence analysis of several HPLC-purified cleavage peptides. The sequence of bovine heart PKA (catalytic subunit) has already been determined . The two amino acid sequences are identical at 14 out of 19 positions. Initial screening of 2.5 x 10^5 clones from a rat brain cDNA library yielded several positive clones with the PKC probe but only 2 positive clones with the PKA probe. Northern blot analysis with PKA-related clone A1 shows a 5 kb transcript in rat brain, heart, and liver. The relative levels of expression of this transcript parallel the PKA enzymatic activities in these tissues. Partial nucleotide sequence analysis of clone A1 shows an open reading frame with approximately 90% homology to the bovine heart amino acid sequence. All of the PKC-related clones appear to be identical based on restriction mapping and Southern blotting. Northern blot analysis with one of these clones (P4) shows a 7 kb transcript with the highest levels of expression in brain. Partial nucleotide sequence analysis of clone P4 shows an open reading frame with all of the conserved amino acid regions expected for a protein kinase. This work was supported by NCI Grant CA-26056.

L96 ANALYSIS OF ANTI-SENSE EGF RECEPTOR mRNA IN INHIBITING EGF RECEPTOR HYPERPRODUCTION, J. Hunts[1], G. Merlino[2], I. Pastan[2] & N. Shimizu[1,3], [1]Dept. Mol. & Cell. Biol., Univ. Arizona, Tucson, Az, [2]Lab. Mol. Biol., NCI, Bethesda, MD, [3]Dept. Mol. Biol., Keio Univ. Sch. Med., Tokyo.

We are actively analyzing a potential involvement of the EGF receptor hyperproduction in the development of squamous cell carcinomas. The present approach we are using to study this problem is the construction of vectors which will express anti-sense EGF receptor mRNA for the inhibition of receptor expression. The main plasmid vector used was derived from psV2-neo, which is readily selectable. Three unique restriction sites, ClaI, HindIII and BamH1 are also present for insert versatility. DNA transfection is used to introduce these vectors into recipient cells. We are presently testing three EGF receptor cDNA fragments, a 5', a middle, and a 3' region for differences in efficiency as anti-mRNAs. EGF receptor inhibition can be assayed by EGF binding. Results will be presented regarding the efficiences of these vectors in reducing the EGF receptor levels in hyperproducing cell lines. The use of these vectors should give both a greater insight into the possible role of the EGF receptor in cellular transformation and a better understanding of the basic biological functions of the EGF receptor in normal cells.

L97 Transforming Growth Factor ß Promotes the Production of the Extracellular Matrix. Ronald A. Ignotz and Joan Massague, University of Massachusetts Medical Center, Worcester, MA 01605

Transforming growth factorß (TGFß) affects the morphology, proliferation and differentiation of many cell types. Because the extracellular matrix can also affect these activities, we have examined whether the extracellular matrix is a biochemical target for TGFß. We observe that TGFß increases several fold the expression of the major extracellular matrix proteins, fibronectin and collagen in both the matrix and the medium. This effect is seen in both primary cultures and established cell lines from chick, human, and rodent origins, including normal and transformed cells. The effect of TGFß on fibronectin in chick embryo fibroblasts is selective, rapid in onset and persistant, half maximal at about 100pM TGFß, and not mimicked by other growth factors tested. TGFß increases the intracellular as well as the extracellular levels of labeled fibronectin and the effect is sensitive to Actinomycin D. Cells treated with TGF also show an increased ability to incorporate fibronectin into the matrix. In a functional assay, TGFß promotes the growth of NRK cells in soft agar. Exogenous fibronectin can substitute for TGFß in the induction of colony formation by NRK cells in soft agar. TGFß-induced colony formation can be inhibited by the hexapeptide Gly-Arg-Gly-Asp-Ser-Pro in the soft agar. This peptide corresponds to the site in the fibronectin molecule that binds to specific cell surface receptors. These results suggest a model for TGFß action based on the control of the extracellular matrix in target cells.

L98 EPIDERMAL GROWTH FACTOR RECEPTORS IN THE RAT GASTRO-INTESTINAL TRACT EPITHELIUM AND IN THE COLONIC TUMORS INDUCED BY 1,2-DIMETHYLHYDRAZINE Yu.D.Ivashchenko,I.T.Gut,L.V.Garmanchuk,Institute for oncology problems Acad.Sci.Ukr.SSR,Kiev USSR

Using Scatchard analysis of 125I-EGF binding we have shown that the plasma membranes of gastric and small intestinal epithelial cells contain approximately 20 times less EGF-receptors(EGF-R)(25-60fmol/mg;Kd=1,4-4,0nM) than liver cells:1200fmol/mg,Kd=40nM.

Investigation of the phosphorylation activity of EGF-R-kinase was performed in vitro on plasma membranes from intestine,intestinal tumors,liver, A-431 cells.The main phosphorylated at tyrosine protein in the tumors was p34-39 but the intensivity ofEGF-R autophosphorylation and its total protein-tyrosine kinase activity was reduced(compare with small-intestinal and liver membranes).Considering that EGF-R was phosphorylated in tumors not at tyrosine(as was shown by treating gels with 1M KOH for 2h at 55°C), quite possible that this phenomen depends on activation of C-kinase.On contrary,in the tumors of the colon induced by DMH increased binding of 125I-EGF has been observed compared to normal colonic enterocytes and increased number of EGF-R was clearly demonstrated.Phosphorylation of p34-36 in the intestinal membranes was much more intense than phosphorylation of EGF-R.

L99 ISOLATION OF A DNA SEQUENCE WHOSE TRANSCRIPTION IS INDUCED BY TPA, Mark D. Johnson, Gerard M. Housey, Paul Kirschmeier, I. Bernard Weinstein, Cancer Center/ Institute of Cancer Research, Columbia University, New York, N. Y. 10032

A cDNA library was constructed from C3H 10T1/2 mouse embryo fibroblast poly A^+ RNA, collected 4 hrs after treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). Screening of the library with cDNA probes synthesized with poly A^+ RNA from control and TPA-treated cells identified sequences whose expression was either induced or inhibited by TPA treatment. Northern blot analyses revealed that one of the induced clones hybridized to a single 1 kb transcript which demonstrated a 10-fold induction in poly A^+ RNA from TPA-treated cells, relative to control cells. This transcript was also identified in Northern blots from a carcinogen-transformed C3H 10T1/2 line and from mouse skin carcinomas induced by DMBA and TPA treatment. The TPA-induced sequence was not, however, detected in RNA from control mouse liver or spleen, or from papillomas induced by DMBA alone or DMBA and TPA treatment. These results suggest that a DNA sequence induced in TPA-treated C3H 10T1/2 cells is constitutively expressed in carcinogen-transformed C3H 10T1/2 cells and also in mouse skin carcinomas. Supported by NCI Fellowship CA07870-01 and NCI Grant CA 26056.

L100 PURIFIED PROTEIN KINASE C BINDS TO DNA. A. Christie King and Soon Ok Moon, Department of Biochemistry, University of Illinois, Chicago, Illinois, 60612.

Transcription of two cellular oncogenes, c-fos and c-myc, is stimulated after quiescent cells are treated with mitogens or phorbol ester tumor promoters. One enzyme which might be involved in transduction of this signal from the cell surface to the nucleus is Protein Kinase C, an enzyme activated either by diacylglycerol generated from hydrolysis of phosphoinositides or by phorbol esters which substitute for this natural activator. The mechanism for transcriptional regulation of other cellular genes by steroid hormones involves direct binding of receptors to specific DNA sequences. Thus, we have proposed that C-kinase may regulate expression of c-fos and c-myc genes in an analogous fashion. Using a nitrocellulose filter adsorption assay, highly purified C-kinase binds to DNA, showing a preference for single stranded DNA. The binding is saturable with respect to C-kinase concentration and the complex formed is stable to salt concentrations of 200 to 300 mM. A 100-fold excess of unlabeled DNA reduces binding to a labeled, nick translated plasmid by 85 to 95%. A salt-inhibited, ATP-dependent topoisomerase activity is also measured in this C-kinase end preparation. Studies are underway to learn whether binding of C-kinase to cloned fragments of the myc and fos genes might have a higher affinity for the enzyme relative to nonspecific sequences contained in nonregulated genes. Ours is the first evidence that C-kinase binds directly to DNA, although earlier studies provided evidence for the existence of a small population of nuclear binding sites for phorbol esters. These studies may provide invaluable insight into the mode of regulation of primary gene transcripts by growth factors and tumor promoters.

L101 INFLUENCE OF THE TUMOUR PROMOTER TPA ON DRUG RESISTANCE

A.R. Kinsella and M. Fox, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, England

The effect of the tumour promoter TPA on the frequency of mouse and hamster cells resistant to methotrexate (MTX), N-phosphonacetyl-L-aspartate (PALA) and cadmium has been examined. TPA was shown to enhance manifold the recovery of mouse cell clones resistant to all three drugs. Detailed examination of the recovery of MTX-resistant 3T6 mouse cell clones has shown TPA to enhance recovery in both single-step and multi-step selection protocols. However, quantitation of the levels of the dihydrofolate reductase gene product in these clones by dot blot hybridisation and FACS analysis showed that TPA-induced enhancement of MTX-resistant colony recovery was not due to gene amplification in this system.

L102 OVERPRODUCTION OF A mRNA ENCODING A GLUTATHIONE-S-TRANSFERASE RELATED PROTEIN IN CHEMICALLY INDUCED RAT HEPATOCELLULAR CARCINOMAS, Brian J. Knoll, Maryann Longley and Stewart Sell, Dept. of Pathology and Laboratory Medicine, Univ. of Texas Medical School, Houston, Tx. 77025

We have conducted a screen for mRNA sequences which are more abundant in rat hepatocellular carcinomas than in normal liver. The screen was done by differential colony hybridization of a cDNA library constructed from the mRNA of a primary tumor induced by diethylnitrosamine (DEN). We isolated one cDNA which hybridizes to a 900 nucleotide mRNA present in abundance in two primary DEN induced hepatocellular carcinomas, three Morris hepatomas and one AAF induced tumor in its first passage. This transcript is not detectable in normal adult or fetal (17 day) liver. The abundance of the transcript in these tumors, determined by hybridizing back to the cDNA library, is about five parts per thousand. The cDNA was completely sequenced, and then translated to amino acids by computer. The protein encoded by the cDNA is related to the family of glutathione-S-transferases (GSTs) found in normal rat liver. These normal rat liver GSTs are not usually elevated in rat hepatomas; in contrast, the GST-related mRNA is elevated at least 100 fold above normal liver. The protein encoded by our cDNA may be the placental isoenzyme of GST, recently shown by Sato and his colleagues to be consistently elevated in hepatomas and in neoplastic nodules (Proc. Nat. Acad. Sci. USA 82:3964-3968). We are now exploring the mechanism by which the expression of the GST-related mRNA in enhanced in hepatomas and preneoplastic cells.

L103 In Vivo and in Vitro Expression Pattern of Tumor-specific Overexpressed Genes.

P. Krieg, G. T. Bowden*, K. Melber and G. Fürstenberger, German Cancer Research Center, Heidelberg, FRG and *Univ. of Arizona, Tucson, Ariz., USA

Previously we described the isolation of sequences (mal-1 to mal-6) that were activated during multi-stage carcinogenesis in the skin of NMRI mice (K. Melber et al., Carcinogenesis, in press). We studied the expression of these mal-related sequences during differentiation of epidermal cells in the mouse epidermis and in in vitro cultivated basal cells and keratinocytes. It was shown that mal-1 expression is specific for differentiated keratinocytes, whereas mal-3 and mal-4 expression is specific for undifferentiated basal cells. Expression of mal-2, however, could not be detected in either cell type. In contrast, after in vitro cultivation of the undifferentiated basal cells a high level of expression of all the mal-related sequences was observed. A similar expression pattern was shown in several cell lines of transformed keratinocytes. In vivo a transcriptional activation of mal-related sequences was observed after treatment of the mouse skin with the tumor promoter 12-0-tetradecanoyl-phorbol-13-acetate. Studies on the kinetics of this activation as well as studies on the expression pattern in individual cells by in situ hybridization should help to clarify a possible role of mal-related sequences in the molecular process of the multistep carcinogenesis of the mouse skin.

L104 EXPRESSION OF A TRANSFECTED HUMAN TRANSFORMING GROWTH FACTOR-α cDNA IN MAMMALIAN CELLS, Patricia B. Lindquist, Arnon Rosenthal, Tim S. Bringman, David V. Goeddel and Rik Derynck, Genentech, Inc., South San Francisco, CA 94080.

Two structurally unrelated polypeptides have been given the name of transforming growth factors (TGFs). TGF-α is a 50 amino acid peptide which is derived from a 160 amino acid precursor and binds to the same receptor as EGF. TGF-β is a dimer of two identical subunits of 112 amino acids derived from a larger precursor of 391 amino acids, which binds to a unique receptor. The sequence coding for the 160 amino acid human TGF-α precursor has been ligated to the 3' flanking region of the Hepatitis B virus surface antigen gene and incorporated into expression plasmids under the control of the SV40 early promoter or the Rous sarcoma virus LTR. To permit selection of transfected cells, genes for dihydrofolate reductase and neomycin resistance under the control of the SV40 early promoter, are also present on the plasmids. Clones of transfected cells were assayed for the expression of TGF-α in a specific ELISA which uses antibodies raised against bacterially synthesized human TGF-α. Chinese hamster ovary cells transfected with a TGF-α expression vector and amplified by exposure to increasing concentrations of methotrexate were found to synthesize increasing amounts of TGF-α. The results of similar transfection experiments using other cell lines will be discussed.

L105 Isolation and characterization of genes activated during the progression of chemically induced mouse skin tumors.

K. Melber, P. Krieg, G. Sauer, G. Fürstenberger and F. Marks, German Cancer Research Center, Heidelberg, FRG.

We have studied alterations in the expression of cellular genes in different stages of the multistep carcinogenesis process in the mouse skin. A cDNA library was constructed using poly A^+ RNA from carcinomas induced by 7,12-dimethylbenz[a]anthracene followed by multiple treatments with 12-0-tetradecanoyl-phorbol-13-acetate (TPA). Screening of 5,000 clones by hybridization with both a carcinoma-specific ^{32}P-cDNA and a ^{32}P-probe made from normal epidermal RNA resulted in identification of 35 cDNA clones displaying stronger hybridization signals with carcinoma-specific cDNA. Eight out of these clones designated pmal-1 to pmal-8 could be verified in Northern blots as transcriptionally activated in carcinomas compared to normal epidermis. Three of them (pmal-2, -7 and -8) turned out to be identical. pmal-1, -2 and -3 were overexpressed at the earlier benign papilloma stage. In vitro studies using mouse keratinocytes demonstrated that pmal-1 and -2 could be stimulated with both serum and TPA. pmal-1 was also activated in several virus transformed fibroblasts but not in the normal parental cells. Presently we are sequencing the mal clones in order to determine if these sequences have homology with known genes.

L106 INTERLEUKIN 1 ENHANCEMENT OF LYMPHOKINE-ACTIVATED KILLER ACTIVITY. Glenn A. Miller, Joachim Buck, Ulrich Hammerling, Roland Mertelsmann and Karl Welte. Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Lymphokine-Activated Killer cell(LAK) activity against a NK cell insensitive, human melanoma cell line (Mel-30) was seen in normal peripheral blood mononuclear cells (PBMC) and Leu-11b enriched cells incubated for 3-7 days with Interleukin 2 (IL2). The Leu-11b antibody, recognizing predominantly large granular lymphocytes (LGL) and NK cells, was used in an immunorosetting cell separation procedure to enrich for a population of cells having high LAK activity when compared to PBMC or Leu-11(-) cells. Adherent cell enriched fractions of PBMC yielded a supernatant capable of enhancing the LAK activity of, IL2 stimulated, Leu-11b enriched cells. As IL1 has been shown to be a constituent of adherent cell supernatants, ultrapure monocyte-derived human Interleukin 1 (IL1) and IL1-like activity purified to apparent homogeneity from Epstein-Barr Virus transformed B-cell line supernatants were added to IL2 containing cultures of Leu-11b enriched lymphocytes. Cytotoxic (LAK) activity against the Mel-30 cell line was measured using a 4 hour ^{51}Cr release assay at days 3,5 and 7 of culture. Results of these LAK assays demonstrated enhancement of LAK activity an average of 2.5 fold in the presence of IL1 or IL1-like activity. Experiments are currently underway to elucidate the mechanism of this activity and further isolate the responding cell population.

L107 TRANSCRIPTION OF INT-2, A PUTATIVE ONCOGENE IMPLICATED IN MOUSE MAMMARY TUMORIGENESIS, Robert Moore, Mark Dixon, Gordon Peters and Clive Dickson, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, U.K.

In the mouse the activation of one or both of two genes (int-1 and int-2) has been implicated in most cases of virally induced mammary tumorigenesis. The int-2 locus is a 30kb region on mouse chromosome 7 which, in mammary tumors, is often found to be interrupted by a mouse mammary tumor virus proviral integration. Proviral integration in this region activates transcription of the int-2 gene. In most cases the provirus is oriented such that viral transcription is directed away from the activated gene and it is believed that activation is due to enhancement. In most tumors the major transcript is a 3.2kb polyadenylated RNA, there are however a number of minor transcripts, both larger and smaller, which are also present. In the few cases in which the provirus is inserted in the promoter insertion mode the pattern of RNA transcription is even more complex and the level of expression is higher. In order to determine how the different transcripts are derived and how they are related to each other we have made cDNA libraries from a number of different tumors and have isolated a large number of int-2 cDNA clones. Analysis of these clones, taken in conjunction with S1 protection and primer extension experiments is starting to reveal the structure of this putative oncogene.

L108 REGULATION OF FERRITIN HEAVY CHAIN AND C-MYC GENE EXPRESSION IN CELL LINES DERIVED FROM HUMAN LYMPHOID MALIGNANCIES. V. Kermani-Arab, Ph.D., W. Salser, Ph.D., C.C Chou, M.S., R.A. Gatti, M.D., F. Naeim, M.D. UCLA Departments of Pathology and Biology, Los Angeles, CA.

Both ferritin and c-myc oncogene expression are known to be important in the growth and differentiation of hematopoietic cells. In this study the effects of 1,25-dihydroxy Vitamin D_3 (Vit D_3), phorbol ester (TPA), and histamine on expression of these genes were investigated by probing northern blotts (Poly A^+ cytoplasmic RNA) with ^{32}P-labeled cDNA ferritin heavy subunit (HL-217) and c-myc probes. The cell lines tested were derived from hairy cell leukemia (W17), Burkitt's lymphoma (Raji), adult T-cell leukemia (Jurkat) and normal peripheral blood B lymphocytes (62). The HL-217 and c-myc probes hybridized with 1,100-and 3,100-nucleotide transcripts respectively and were expressed at much higher levels in T-cell leukemia than in Burkitt's lymphoma and hairy cell leukemia cell lines. Vit D3 markedly reduced c-myc and ferritin mRNA expression in the T-cell leukemia line while TPA and histamine treatments gave increased c-myc and ferritin mRNA levels in a cell line derived from hairy cell leukemia. Inducing agents may exert a selective role on expression of c-myc and ferritin heavy chain which in turn may play a role in control of tumorigenesis of certain variants of lymphoid malignancies.

L109 REGULATION OF EXPRESSION OF HUMAN GM-CSF IN ACTIVATED T LYMPHOCYTES. S.D. Nimer, J. Chan, J.C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Our laboratory has purified a 22,000 MW human granulocyte-macrophage colony-stimulating factor (GM-CSF) from medium conditioned by the HTLV-II-infected Mo T-lymphoblast cell line. GM-CSF stimulates proliferation of hematopoietic progenitor cells and primes neutrophils for enhanced responsiveness to physiologic stimuli. Using a GM-CSF cDNA probe, we have detected GM-CSF mRNA in activated but not resting T lymphocytes and in HTLV-infected T-lymphoblast cell lines. Low level constitutive production of GM-CSF by HTLV-infected T-cell lines can be increased (as much as tenfold) by stimulation with phorbol diester (TPA) and either phytohemagglutinin (PHA) or the OKT3 monoclonal antibody.

To define the regulatory region required for this increased expression of GM-CSF in activated T cells, we prepared recombinant constructions linking a 650 bp fragment of GM-CSF genomic DNA that contains the promoter and sequences 5' to the first exon to the marker gene CAT. HTLV-infected T-cell lines were transiently transfected with this construct and CAT activity assayed in both stimulated and unstimulated cells. Increased CAT activity (tenfold) could be seen with stimulation by PHA and TPA or OKT3 and TPA; no CAT activity was detectable using the construct in the reverse orientation. Thus, regulatory sequences involved in enhanced GM-CSF expression in activated T cells are present in this 650 bp region. We are currently testing constructions containing various deletions within this 650 bp region to further define the location of these regulatory sequences.

L110 Decreased pp60$^{c\text{-}\underline{src}}$ tyrosine kinase specific activity in avian erythroblastosis virus-infected quail cells. Sarah J. Parsons, Deborah J. McCarley and J. Thomas Parsons. University of Virginia, Charlottesville, VA 22908.

The genome of the avian erythroblastosis virus (AEV) contains two oncogenes, erb-A and erb-B. erb-B encodes a truncated form of the receptor. To study the possible consequences of transformation by AEV on the proto-oncogene product, pp60$^{c\text{-}\underline{src}}$, a continuous cell line from infected quail embryo fibroblasts was derived. Clones of this line were virus non-producers and exhibited a typical transformed phenotype, including morphological alterations, density-independent growth, increased glycolysis and tumor development in nude mice. A single, unique band of ca. 68-70,000 molecular weight was immunoprecipitated from extracts of AEV quail cells with anti-ASV gag sera. This peptide is thought to be the gag-erb-A fusion protein encoded by AEV.

To investigate pp60$^{c\text{-}\underline{src}}$ activity and structure, immune complexes of pp60$^{c\text{-}\underline{src}}$ were prepared from extracts of both AEV infected and uninfected quail cells with specific monoclonal antibodies. These complexes were analyzed for auto- and transphosphorylation activities utilizing denatured rabbit enolase as substrate. The amount of c-src protein in the complexes was estimated by determining the amount of ^{35}S-methionine incorporation into the 60 kD band. It was found that the specific kinase activity of pp60$^{c\text{-}\underline{src}}$ in AEV quail cells was reduced to 3-10% of that found in uninfected quail cells. In addition, the level of ^{32}P-orthophosphate incorporation into pp60$^{c\text{-}\underline{src}}$ was reduced in the transformed cells. Similar results were found with pp60$^{c\text{-}\underline{src}}$ precipitated from AEV chick cells. Analyses of enzyme kinetics and structural modifications of pp60$^{c\text{-}\underline{src}}$ are being investigated.

L111 MITOGENIC AND ANTIMITOGENIC GROWTH REGULATORS SECRETED BY Ad2- AND SV40 TRANSFORMED HAMSTER CELLS. C. Patch, K. Akagi, K. Murai, and A. Levine. NICHD, NIH, Bethesda, Maryland. Syrian hamster embryo (SHE) cells transformed by Ad2 or SV40 differ in several phenotypic properties. The small round Ad2-transformed SHE cells accumulate little fibronectin and actin; they are sensitive to in vitro lysis by activated macrophages and natural killer cells; they induce tumors in immunoincompetent hamsters but rarely in adult animals. In contrast, the fibroblastic SV40-transformed SHE cells accumulate large amounts of fibronectin and actin; they are relatively resistant to lysis by nonspecific immune effector cells, and they readily induce tumors in immunocompetent syngeneic and allogeneic hamsters. Many investigators have suggested that transforming growth factors (TGF) govern several properties such as morphology, autocrine growth, and anchorage independent growth. We have recently investigated TGFs secreted by two lines of transformed SHE cells, one line transformed by Ad2 and one by SV40. Media conditioned by these two lines as well as by untransformed SHE cells was fractionated by HPLC. The TGFs from both transformed lines were virtually identical with respect to competition with EGF; both were different from competition by factors secreted by SHE cells. The HPLC fractions from both cell lines exhibited similar patterns in their ability to induce DNA synthesis in quiescent SHE and NRK cells. When tested on serum stimulated cells, a powerful anti-mitogen was revealed in the TGFs secreted by SV40-transformed cells but not in the TGFs of the other cells. Current studies will determine whether the amount of this inhibitory factor is typical of TGFs secreted by other cell lines independently transformed by these two viruses, and whether it might influence the phenotypic differences between these two types of transformed cells.

L112 POST-TRANSCRIPTIONAL REGULATION OF c-MYC ONCOGENE IN NORMAL AND TUMOR CELLS
Marc Piechaczyk, Jean-Marie Blanchard, Christian Dani, Anne Bonnieu, Nadir Mechti, Bernard Lebleu and Philippe Jeanteur. UA CNRS 541, University of Sciences of Montpellier, 34060 Montpellier cedex, France.

c-myc RNA is known to be extremely unstable (Dani et al. (1984) PNAS 81 7046). We have shown in various experimental models affecting cellular division that c-myc gene expression is regulated at the post-transcriptional level and when possible, that variations in steady state level of c-myc RNA is accounted for by a variation of its half-life (Dani et al. (1985) PNAS 82 4896; Blanchard et al. Nature (1985) 317 443).

Recently, we have reported that the very high levels of abnormal c-myc RNA found in murine and human B tumors where c-myc gene structure is altered are partially accounted for by a significant stabilization of the cytoplasmic transcripts (Piechaczyk et al. (1985) Cell 42 589; Eick et al. (1985) EMBO J. in press). Furthermore, we have shown that rearranged c-myc genes could be transcribed at very different rates as compared to their normal counterparts.

L113 **E. PRINCIPAUD, E. JEUNET, J. ALBERT, J.J. LAWRENCE**
DRF/Biologie Moléculaire du Cycle Cellulaire, CENG, 85 X, 38041 Grenoble, France
A flow cytofluorimetry study on the expression of cellular oncogenes along the cell cycle.

We have stained several cell line in vivo by Hoerchst 33342 (REF, FR3T3 and their transformants by SV40 (type N and type A)) and sorted pure fractions of cells in the various phase of the cell cycle in a flow cytofluorimeter. This technical approach has allowed us to study the expression of several cellular oncogenes along the cell cycle with minimal disturbance of the cell cycle. Nuclear and cytoplasmic RNAs have been isolated from the sorted fractions and analysed by dot blot hybridization. Our results show that c-myc and c-fos are expressed all along the cell cycle as previously found by an other technical approach. On the contrary c-myb is transcribed only in the G2 phase of the cell cycle in FR3T3 cells, while it is transcribed in the S phase only in type A transformant. In both cases the mRNA was found only in the nuclear fraction, suggesting a post transcriptional control of the mRNA accumulation.

When antibodies are available a direct estimation of the accumulation of the oncogene product as a function of the cell cycle is possible after double stainig of the cells and analysis in the flow cytofluorimeter. Preliminary results will be presented.

L114 GENES AND GENE PRODUCTS IDENTIFIED AS PART OF THE MAMMALIAN GENETIC STRESS RESPONSE,
Hans Jobst Rahmsdorf, Peter Angel, Marina Schorpp, Christine Lücke-Huhle and Peter Herrlich. KFK, IGT, P.O.Box 3640, D 7500 Karlsruhe 1, F.R.G.
Several carcinogenic and cocarcinogenic agents induce new gene products in mammalian cells including primary human fibroblasts. We characterized them on the protein level and by cDNA cloning techniques and begin to understand the function of some of the induced proteins. These include: (1) the MHC-associated invariant chain as an example of a differentiation marker, which is expressed at high level in resting splenic B-cells, but not in Pre-B-cells. It can be induced in these cells by carcinogenic treatment. Genomic clones of the murine invariant gene have been transfected into human fibroblasts, and stable transfectants are used to define promoter and regulatory segments. (2) The fibroblast specific secreted protein XHF_1. This protein is expressed in very low levels in SV40 transformed and in starved fibroblasts as compared to primary, growing fibroblasts and can be induced about 10 fold in all these cells by tumor promoters and carcinogens. (3) The metallothionein IIa gene. (4) The oncogene c-fos. (5) An extracellular protein synthesis inducing factor (EPIF): UV-irradiated primary human fibroblasts secrete a protein factor into the culture medium, which when given to non-treated fibroblasts, induces in these cells the cellular stress response. The factor has similarity to interleukin 1. (6) A gene amplification triggering function: Several carcinogens, including α-irradiation induce the amplification of integrated SV40 sequences and of neighboring genes in a Chinese hamster cell line. We showed by somatic cell fusion involving non-irradiated and irradiated partners, that the inducing factor is formed in the irradiated cell and act on the non-treated nucleus.

L115 SECRETION OF PDGF-LIKE MOLECULES BY HUMAN ERYTHROLEUKEMIC CELL LINES: DIFFERENTIATION-SPECIFIC EXPRESSION AND PARACRINE SECRETION TO ENHANCE CLONAL GROWTH, E.W. Raines, T. Papayannopoulou, S.J. Collins, B. Nakamoto, and R. Ross, University of Washington, Seattle, WA 98195

Examination of 10 nonlymphoid human hematopoietic cell lines demonstrated that the cell lines of erythroleukemic origin (HEL, K562(S), K562(C-16), OCIM1, OCIM2) secrete the highest levels of molecules which are antigenically and functionally similar to purified PDGF. This secretion of PDGF-like molecules by the erythroleukemic cells is enhanced by treatment with TPA, which stops cell proliferation and induces differentiation, but is not affected by treatment with inactive phorbol or thrombin. TPA-induced secretion appears to be due to de novo synthesis based on kinetics of secretion and inhibition of secretion by cycloheximide and actinomycin. Consistent with this is the finding that treatment with TPA also increases expression of the sis gene (the gene for the B chain of PDGF) by Northern analysis. Induction of PDGF-like molecules in the erythroleukemic cells is accompanied by coordinate enhancement of megakaryocyte/platelet-specific markers. However, unlike the platelet and megakaryocyte, which store PDGF intracellularly, the cell lines secrete more than 90% of the PDGF produced. Neither the erythroleukemic cells nor their normal counterparts appear to be directly responsive to PDGF. However, clonal growth of the erythroleukemic cells is enhanced in the presence of fibroblasts which are stimulated to grow by the secreted PDGF-like molecules. Whether secretion of PDGF-like molecules by erythroleukemic cells represents differentiation-specific expression or a competitive advantage by enhancing clonal growth in the presence of fibroblasts, these studies demonstrate that erythroleukemic cell lines are a paracrine source of PDGF-like molecules.

L116 DELAYED EXPRESSION OF THE C-FOS AND C-FMS PROTO-ONCOGENES DURING INTERFERON GAMMA INDUCED HUMAN MONOCYTIC DIFFERENTIATION, Eric P. Sariban, Thomas M. Mitchell, James D. Griffin and Donald W. Kufe, Dana-Farber Cancer Institute, Boston, MA 02115.

Transcripts of the c-fms proto-oncogene, which encode for the CSF-1 receptor, were induced in HL-60 promyelocytes by recombinant interferon gamma (rIFN-γ). A decrease in c-myc expression followed by an increase in c-fos RNA preceded the appearance of c-fms transcripts which were detected after 7 days of rIFN-γ treatment. Expression of the Class II major histocompatibility antigen, HLA-DR was the earliest marker of differentiation detected within 12 h of IFN-γ treatment, while a decline in c-myc transcripts was detectable only by 72 h. Furthermore, expression of the cell surface monocyte differentiation antigens Mø2 and MY4 were early events occurring prior to the detection of c-fos and c-fms RNA. In the human myeloid U937 cell line, c-myc transcripts also decreased after treatment with rIFN-γ. However, there was no detectable increase in expression of c-fos, c-fms, HLA-DR or monocyte differentiation antigens. In contrast, TPA treatment of these U937 cells resulted in the induction of c-fos and c-fms transcripts, as well as the monocytic phenotype. Thus, neither the rapid decline in c-myc transcripts nor the early expression of the c-fos and c-fms proto-oncogenes are necessary events in the initiation of rIFN-γ-induced differentiation along the monocytic pathway. However, expression of the mature monocytic phenotype appears to be associated with c-fos and c-fms expression.

L117 cDNA CLONING OF PROTEIN-TYROSINE KINASE SUBSTRATE p36. Chris Saris[1], John R. Glenney Jr.[1], Brian E. Tack[2] & Tony Hunter[1]. [1]The Salk Institute, San Diego, CA 92138; [2]Scripps Clinic and Research Foundation, La Jolla, CA 92037.

We have constructed a λgt_{11} expression library using murine fibroblast RNA which had been enriched for p36 mRNA by size selection. Immunoscreening of this library yielded a clone which encodes the C-terminal half of the p36 protein. The identity of this clone was established by peptide mapping of the *in vitro* translation product of hybrid-selected RNA and of the bacterially synthesized βgalactosidase fusion protein. In addition, the deduced amino acid sequence shows extensive homology with a partial protein sequence of p36 isolated from bovine intestine. Southern analysis of genomic DNA indicates that there may be one or more p36-related genes. Full-length p36 clones will be used to study the effects of over-expression of the wild-type protein and of site-directed mutations in critical regions of the molecule. The altered expression, including repression by anti-sense RNA, may gain insight in the as yet unknown function of p36.

L118 AXC/SSh RAT PROSTATE CANCER CELL PROLIFERATION IN VITRO IS ANDROGEN MODULATED, Sydney A. Shain and Rachel I. Huot, Department of Cellular and Molecular Biology, Southwest Foundation for Biomedical Research, San Antonio, TX 78284

We characterized clonal AXC/SSh rat prostate cancer cell lines obtained by limit dilution of heterogenous parental cultures maintained on medium containing 10% fetal bovine serum (FBS), FBS plus 10^{-7} M 5α-dihydrotestosterone, or FBS plus 10^{-7} M testosterone. Isolated cell lines are identified, respectively, as C, D, or T derived cells. All cell lines morphologically are epithelial and contain phenotypic markers characteristic of prostatic epithelium. Total androgen receptor content of C, D, and T derived cells respectively was 131 ± 61 (mean ± SD), 43 ± 32, and 274 ± 96 fmol/100 μg DNA. Proliferation of C or D clonal cells propagated on steroid depleted medium was insensitive to changes in medium testosterone concentration through the range 10^{-6} to 10^{-9} M. T5 cell proliferation rate was decreased, whereas T1 cell proliferation rate was increased by concentration dependent changes in medium testosterone content. Elaboration of proteins secreted into culture medium was cell line specific and either was, T1 and T5 cells, or was not, C and D derived cells, modulated by testosterone. Effects of testosterone were reversed by the antiandrogen RU 23908. Our data document establishment of clonal rat prostate cancer cell lines, showing multiple androgen specific effects on proliferation, which should permit detailed analyses of relationships between androgen responsive gene function and prostate cell proliferation.

L119 STIMULATION OF PROLIFERATION AND GENE EXPRESSION IN AKR-2B CELLS INVOLVES THE ACTIVATION OF PROTEIN KINASE-C (PK-C). G. D. Shipley[1], R. T. Abraham[2], and H. L. Moses[3] Dept. of Cell Biol. & Anat., Oregon Health Sciences Univ., Portland, OR[1]; Dept. of Immun., Mayo Clinic, Rochester, MN[2]; Dept. of Cell Biol., Vanderbilt Univ., Nashville, TN[3].

To determine whether PK-C activation by polypeptide growth factors may be involved in the stimulation of gene expression and DNA synthesis in mouse AKR-2B cells, studies were initiated utilizing growth factors, phorbol esters, and a diacylglycerol (DAG), dioctanoyl glycerol (DiC_8). AKR-2B cells can be maintained in serum-free medium and stimulated to proliferate by the addition of purified polypeptide growth factors (Shipley et al., P.N.A.S. 82:4147). Epidermal growth factor, EGF, and heparin-binding growth factor type-β, HBGFβ, stimulate DNA synthesis in these cells and their activity is synergistic with insulin-like growth factor type I (IGF-I), which has essentially no effect when added alone. EGF and HBGFβ, but not IGF-I, stimulate the accumulation of two proliferation-related mRNAs (JE and VL30) within 4 hrs post stimulation while the expression of other genes is unaffected. Phorbol esters, TPA and 4β-PDD, that are known to bind and activate PK-C also stimulate DNA synthesis in AKR-2B cells. The activity of these phorbol esters is synergistic with IGF-I. However, the PK-C inactive phorbol (4α-PDD) has no effect on DNA synthesis in these cells either when added alone or in the presence of IGF-I. Both TPA and 4β-PDD, but not 4α-PDD, stimulate the accumulation of mRNA coding for JE and VL30 within 4 hrs post stimulation. DiC_8 also stimulates the expression of these genes and (to a lesser extent) the entry of the cells into S phase. These results suggest that EGF and HBGFβ, but not IGF-I, stimulate the release of DAG from membranes, and that subsequent DAG activation of PK-C plays an important role in the pathway of biochemical events (including the expression of specific mRNAs) leading to the initiation of DNA synthesis in AKR-2B cells.

L120 REGULATION OF C-MYC GENE EXPRESSION DURING ACTIVATION OF NORMAL HUMAN B CELLS Erlend Smeland, Tore Godal, Klaus Beiske, Susan Pfeifer Ohlsson and Rolf Ohlsson. Lab. for Immunology, Dept. of Pathology, NHIK, The Norwegian Radium Hospital, N-0310 OSLO 3, Norway

The myc proto-oncogene appears to play an important role during the induction pathway from quiescence to competence for cell proliferation. We have studied the c-myc gene expression in resting human peripheral blood B lymphocytes stimulated to proliferate by antibodies to surface immunoglobulin (anti-μ) and B cell growth factor (BCGF). Initial activation from G0 to G1 could be triggered by polyclonal anti-μ alone, without subsequent S phase entry unless BCGF was added. The G0 to G1 transition was accompanied by a specific 10-50 fold transient increase in c-myc mRNA levels (1). Nuclear run-off experiments showed that this increase was, at least partly, due to transcriptional activation of the c-myc gene. The monoclonal anti-μ, AF6, and the monoclonal antibody 1F5 (against the Bp35 or CD20 antigen) were both capable of inducing a similar increase in c-myc transcription and -expression. However, these antibodies only weakly synergized with BCGF for DNA synthesis. This implies that other inducible function(s) must be present to potentiate the myc specific function, in order for human B cells to acquire competence to respond to BCGF. Our findings differ from other examples, notably fibroblasts, where G0 is operationally defined by serum starvation. In these cases the increased myc mRNA levels during G0 to G1 transition appears to result from stabilization of c-myc mRNA, rather than by increased rate of transcription (2). These data will also be discussed in relation to c-myc protein levels during B cell activation.

References: 1. Smeland, E. et al. (1985) PNAS 82:6255
2. Blanchard, J.M. et al. (1985) Nature 317, 443

L121 ISOLATION OF A TUMOUR PROMOTER INDUCIBLE cDNA CLONE FROM A JB6 MOUSE EPIDERMAL CELL LINE, James H. Smith and David T. Denhardt, Cancer Res. Lab., University of Western Ontario, London, N6A 5B7, Canada. A cDNA library was prepared from the tumour-promotable JB6 mouse epidermal cell line, C122, which had been treated with 12-O-tetradecanoyl-phorbol-13-acetate (TPA), for 10 days. We screened the library for clones corresponding to mRNAs that change in abundance as a result of TPA treatment. A clone, p2ar, which represents a 1.6-kb mRNA and is inducible with TPA, has been isolated. This mRNA species exhibits a biphasic induction. A rapid increase, peaking at 6-9 hrs, which then returns to non-induced levels within 24 hrs, is initially seen. Continued exposure to TPA induces a second increase in 2ar mRNA, detectable at approximately 48 hrs and plateauing at 4-5 days. Aplysiatoxin, teleocidin and phorbol 12,13-dibutyrate induce 2ar mRNA to similar levels as TPA in both phases of induction. Phorbol 13-monoacetate, a non-promoting, biologically less active phorbol has no inducing activity in either phase. Epidermal growth factor and platelet-derived growth factor have little effect initially, but induce 2ar levels 2-3 fold higher than TPA in the second phase. Induction of 2ar in the second phase can be effectively inhibited by all-trans retinoic acid, an inhibitor of tumour promotion and transformation. However, the 6-9 hr induction is essentially refractory to this treatment. (Supported by the NCI(C) and MRC of Canada).

L122 SYNTHESIS OF THE mRNA FOR THE HEMATOPOIETIC GROWTH FACTOR GM-CSF BY MOUSE PERITONEAL MACROPHAGES, Bernard Thorens, Jean-Jacques Mermod*and Pierre Vassalli, Dept Pathology, University of Geneva, CH 1211 Geneva 4, and *Biogen S.A., 1227 Carouge.

GM-CSF is a growth factor promoting the proliferation and differentiation of hematopoietic progenitors along the granulocytic macrophage pathway. While its effect has been well studied, the conditions of its synthesis and release by potential hematopoietic regulatory cells are less clear (except for its production by T lymphocyte clones). The present experiments show the conditions of induction of GM-CSF mRNA synthesis by mouse peritoneal macrophages: adherence to plastic is both inductive by itself and required for superinduction by other stimuli, such as exposure to bacterial lipopolysaccharide. The accumulation of GM-CSF mRNA in stimulated cells is preceded by a transient accumulation of c-fos mRNA, and is rather brief, with a peak amount about 3 hours after stimulation. Simultaneous exposure of the macrophages to other agents active on macrophages, such as γ IFN, modulate GM-CSF mRNA accumulation. Synthesis of GM-CSF mRNA by T lymphocytes is not induced by the same stimuli, and shows slower kinetics.

L123 ONCOGENE EXPRESSION IN HUMAN HEPATOMA CELLS. Ling-Pai Ting, King-Song Cheng, Hsiao-Kuey Chang, Chungming Chang, Tsong-Seng Su, Shou-Hwa Han, Fung-Ku Peng and Cheng-Kung Chou, Graduate Institute of Microbiology and Immunology, National Yang-Ming Medical College, and Department of Medical Research, Veterans General Hospital, Shih-Pai, Taipei, Taiwan, Republic of China

Expression of several known oncogenes such as c-myc, c-fos and c-Ha-ras has been demonstrated under cell cycle control in normal cells. We have particularly studied the expression of c-myc, c-Ha-ras, c-Ki-ras and c-mos at different cell cycle stages in human hepatoma cell line HA22T/VGH to elucidate their relationship with the growth control. In the exponentially growing hepatoma cells, c-myc mRNA level is intermediate compared to the high level of c-Ki-ras, but is undetectable in the Go phase of cell cycle. As other cells already studied, c-myc mRNA level increases at the early stage of a proliferative response and drops down in the S phase of hepatoma cells. The mRNA level of c-Ki-ras is much higher than that of c-Ha-ras in exponentially growing hepatoma cells. However, in contrast to c-Ha-ras which mRNA level stays quite constant throughout Go and G_1 phases and slightly increases in the S phase, the c-Ki-ras mRNA level is greatly reduced in the Go phase, coming back in the G_1 phase and stays constant in the S phase. The c-mos expression is particularly interesting to study in these hepatoma cells. Results show that its mRNA level is intermediate in exponentially growing hepatoma cells, undetectable in the Go phase, slightly and maximally increased in the early G_1 and S phase respectively. 2F1 gene expression has also been studied.

L124 INDUCTION OF ODC mRNA BY DIFFERENT TUMOR PROMOTERS IN RAT LIVER, Coen F.van Kreijl, L.v.d.Zande, A.Bisschop and B.Wieringa, National Institute of Public Health and Environmental Hygiene, Lab.Carcinogenesis and Mutagenesis, 3720 BA Bilthoven, The Netherlands.

Ornithine Decarboxylase (ODC) is a cytosolic decarboxylase, which catalyses the first and rate limiting step in polyamine biosynthesis. Increased ODC-activity can be observed after a wide variety of proliferative stimuli, and the enzyme is therefore believed to play a crucial role in the onset of cellular growth.

A rat cDNA bank was constructed from testosteron induced rat kidney poly A^+ RNA. One positive clone (pE10) was isolated upon screening of this bank with a mouse ODC cDNA clone (p OD32; obtained from P.Coffino). This rat clone was characterized by restriction enzyme analysis, and found to be nearly full length (2.4 kb). Comparison with mouse revealed a striking similarity in the 3' halves of both cDNA's, and preliminary sequence analysis showed the mouse-rat homology in that region to be 96%. Significant differences were observed in the 5' halves, especially in the 5' leader sequence which has been postulated to be involved in translational control.

Northern blot analysis demonstrated a single length mRNA species of ~2.4 kb in both testosteron stimulated kidney, normal liver and in liver- and bladder tumors of the rat. The effect of different promoters on ODC mRNA induction in rat liver in vivo will be presented and discussed.

L125 STIMULATION OF GROWTH HORMONE SECRETION IN RAT PITUITARY CELLS BY GHRF AND TUMOR PROMOTING PHORBOL ESTERS: A COMPARATIVE STUDY, G. Velicelebi and S. Patthi, SIBIA, 505 Coast Blvd. So., La Jolla, CA. 92037

Growth hormone (GH) release by primary cultures of rat anterior pituitary cells can be stimulated in a dose-dependent manner by growth hormone releasing factor (GHRF) and tumor promoting phorbol ester, phorbol 12-myristate 13-acetate (PMA). We investigated the relationship between these two mechanisms of stimulation. The EC50 values for stimulation of GH secretion by GHRF and PMA were 20 pM and 1 nM, respectively, while the kinetics of the two responses were comparable. Maximal stimulation observed with GHRF treatment was 2-3 times greater than that caused by PMA. A non-tumor promoting phorbol ester, 4-α-phorbol 12,13-didecanoate, was ineffective in stimulating GH release. Somatostatin treatment caused a ten-fold increase in the EC50 for GHRF response, but did not alter the PMA dose-response curve. Furthermore, pretreatment with GHRF resulted in decreased responsiveness to GHRF but did not affect PMA-stimulated GH secretion. Likewise, pretreatment with PMA led to rapid decrease in responsiveness to PMA without affecting the stimulation of GH secretion in response to GHRF. These results suggest that the secretion of GH by rat pituitary cells in vitro can be regulated by two distinct mechanisms, one mediated by GHRF and involving cAMP-dependent kinase and other mediated by phorbol esters and involving protein kinase C.

L126 **INDUCTION OF BIOENERGETIC HOUSEKEEPING mRNAs BY VIRAL TRANSFORMATION. Keith A. Webster, Stanford University, California 94306**

The relative transcriptional rates and total cellular levels of eight specific mRNAs were measured in confluent cultures of human and rodent cells transformed with fragments of Adenovirus or SV40 DNA. The mRNAs were assayed by hybridization to specific cDNA probes isolated from a rat muscle cDNA library, these included five glycolytic enzyme cDNAs (Lactate Dehydrogenase, Pyruvate Kinase, Triosephosphate isomerase, Aldolase and Glyceraldehyde-3-phosphate Dehydrogenase), Cytochrome c which is a component of the mitochondrial respiratory chain, Actin and Collagen. The cellular levels of the bioenergetic related, housekeeping mRNAs were increased by varying amounts up to six fold, wheras the levels of Actin and Collagen were both decreased in transformed cells relative to control (non transformed) cells. Transcriptional rate changes, as determined by isolated nuclei run off transcription assays from stationary cultures, were at least partially responsible for these changes in cellular mRNA levels.

L127 B2 repeats containing transcripts in transformed and normal proliferating cells

Karl-Heinz Westphal and Peter W. J Rigby

A class of transcripts containing B 2 repetitive elements was originally observed in a number of transformed and embryonic cells. The pattern in embryonic cells is very complex. However the transcripts appearing in the transformed cells show some similarities to the RNAs in mitogen treated normal cells.
We will present the effects of cell physiological parameters like cell contact and proliferation in both transformed and normal cells on the B2 elements containing RNAs. The influence of the treatment of normal cells with mitogens and tumor promotors on these RNA species will be discussed. We also present data showing the effects of interferon on those transcripts.
The role of this special type of transcripts in the establishment and maintenance of the transformed phenotyp will be discussed.

L128 MITOGENIC REGULATION OF POLYAMINE BIOSYNTHESIS BY INCREASES IN mRNA LEVEL AND EFFICIENCY OF TRANSLATION, 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 S-adenosylmethionine decarboxylase (SDC) and ornithine decarboxylase (ODC) 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. Comparisions of mRNA levels and changes in either synthetic rate or activity of these proteins revealed 2-fold and 5-fold discrepancies for SDC and ODC, respectively. ODC and SDC mRNAs shift into larger polysomes after ConA addition, demonstrating an increase in efficiency of translation. This change in translational efficiency is not a general effect of stimulation by ConA on all message species, since the size of polysomes containing actin or c-myc mRNAs was identical in resting and activated cells. In conclusion, modulation of the key regulatory proteins of polyamine biosynthesis, SDC and ODC, during lymphocyte mitogenesis, is controlled at two levels: 1.) regulation of mRNA increases mediated by a pathway involving activation of protein kinase C and 2.) control of mRNA translational efficiency mediated through changes in initiation.

L129 MOLECULAR CLONING OF GENE SEQUENCE EXPRESSED IN THE INITIATION STAGE OF THE RAT HEPATOCARCINOGENESIS, Kazushige Yokoyama,The institute of Physical and Chemical Research, Wako,Saitama,Japan 351.

Chemically induced hepatocarcinogenesis has been described as a multistep process in which the emergence of hepatocellular carcinoma is proceeded by focal and hyperplastic lesion in the liver. We have forcused on the initial commitment stage of the chemical carcinogenesis to identify the rare gene sequences transcribed specifically in the initiated liver by the chemical carcinogen, DEN(diethylnitrosamine) administration. A modified cloning method designed to produce differentialcDNA libraries permits the isolation of sequences that are present in RNA populations of any initiation stage,but are not present or are much less abundant in normal stage of rat hepatocarcinogenesis.One of 7 independent clones is studied in detail(pIn-3).The level of this specific transcript(2.3 kb) increased from 10 to 30 fold at 36 h following DEN injection.This increasing level is continued more than 4 monthes and then decreased gradually.In vitro transcription run-on assay demonstrated that the increment of this transcript is due to the transcriptional activation. We have observed no elevation of the transcripts on phenobarbital induced promotion and by other promoters. These results strongly suggest that In-3 sequence is expresed at the initiation process of the hepatocarcinogenesis.The similar results were obtained by other carcinogens(AAF, DAB,3-M-DAB,AAT etc) to show the incresing of this transcript.The series of the hepatoma cell strains from rat,mouse and human were screened by In-3 cDNA probe. These studies imply that the In-3 sequence is not only specific common indicator at the initiation but also a useful diagnostic marker of the hepatocarcinogenesis. Only the initiated liver expressed this sequence by the tissue specific manner. The genomic blot also showed it is composed of a multigene family. The nucleotide sequence and the expression studies will be also presented.

L130 TUMOR PROMOTERS AND PHOSPHOLIPASE C INCREASE THE SYNTHESIS OF Mr 31K IN MUSCLE CELLS IN CULTURE. Bianca M. Zani, Sergio Adamo, Clara Nervi, & Mario Molinaro. Inst. Histology & gen. Embryology, Univ."La Sapienza" Rome, Italy.

Phospholipase C (PLC) treatment, in differentiated myogenic cells, mimics the effect of tumor promoters increasing the synthesis of a particulate fraction-associated polypeptide of Mr 31K, that we already observed in these cells after exposure to TPA (Cancer Res. 43, 3748-3753, 1983). The synthesis of this polypeptide is evident after 2hr of TPA, PLC, mezerein and teleocidin treatment reaching the maximum level at 6hr. After longer incubation with the drugs (10-24hr) the synthesis of 31K decreases while inhibition of the synthesis of contractile protein (myosin heavy chain) takes place. The Mr 31K is easily extracted from the particulate fraction by non ionic detergent while it is not extracted by 0.1 N NaOH or by high ionic strength buffer, behaving as an integral membrane protein. The possibility that the expression of 31K may reflect the activation of protein kinase C is based on the following results: treatment with phospholipase C induces rapid and constant breakdown of phosphatidylinositol, resulting in the production of diacylglycerol, the physiological activator of protein kinase C, as detected by the level of inositol-1-phosphate after 10-20 min of treatment: phospholipase A, which does not induce diacylglycerol production, fails to stimulate the synthesis of Mr 31K: phosphorilated polypeptides were also observed after brief treatment (30 min) with TPA or PLC.

L131 PDGF, PLATELETS, LEUKEMIA AND ADENOCARCINOMA CELLS SHARE AN EPITOPE IDENTIFIED BY THE MONOCLONAL ANTIBODY DU-ALL-1, Nancy J. Zeleznik, Michael A. Hollingsworth, and Richard S. Metzgar, Duke University Medical Center, Durham, NC 27710

The DU-ALL-1 monoclonal antibody (Mab), which was raised against leukemic cells from an ALL patient, recognizes a molecule of 24,000 daltons (24kd) present on human ALL cells and ALL cell lines. DU-ALL-1 also recognizes an epitope on platelets and a variety of epithelial cells. By western blotting DU-ALL-1 binds to a 20,000 dalton molecule (20kd) from a pancreatic carcinoma cell line as well as to both 20kd and 24kd molecules on human platelets. We have now shown that the DU-ALL-1 Mab is able to precipitate purified human platelet derived growth factor (PDGF). This suggests that PDGF and the DU-ALL-1 20kd and 24kd molecules share a common antigenic determinant. Although the DU-ALL-1 20kd and 24kd molecules share an epitope with PDGF, these molecules are different by several criteria. The DU-ALL-1 molecule consists of a single polypeptide chain of 20kd or 24kd by immunoprecipitation from radiolabeled cell lysates under reducing and nonreducing conditions. In contrast PDGF is a 30kd dimer under nonreducing conditions and is two polypeptides (18kd and 16kd) under reducing conditions. The Mab-affinity purified DU-ALL-1 20kd molecule directly stimulates phosphorylation of phosvitin or casein substrates at serine residues whereas PDGF requires interaction with its receptor in order to phosphorylate tyrosine residues. We are currently studying the growth promoting activity of antibody affinity purified DU-ALL-1 antigen and amino acid sequence homologies between this antigen and PDGF.

Oncogene Products/Gene Interaction

L132 CHROMOSOMAL ABNORMALITIES AND DOSE-EFFECTS OF AMPLIFIED ONCOGENES IN TUMOR CELLS
Kari Alitalo, Lea Sistonen, Robert Winqvist and Olli Jänne
Department of Virology and Recombinant DNA laboratory, University of Helsinki, 00290 Helsinki, FINLAND.

Regulatory or structural alterations of cellular oncogenes have been implicated in the causation of various cancers. Amplification of cellular oncogenes can augment their expression by increasing the amount of DNA template available for the production of mRNA. It appears that amplification of certain oncogenes is a common correlate of the progression of some tumors and also occurs as a rare sporadic event affecting various oncogenes in different types of cancer. Amplified copies of oncogenes may or may not be associated with chromosomal abnormalities signifying DNA amplification: double minute chromosomes (dmin) and homogeneously staining chromosomal regions (HSR:s). Amplified oncogenes, whether sporadic or tumour type-specific, are expressed at elevated levels, in some cases in cells where their diploid forms are normally silent. Increased dosage of an amplified oncogene may contribute to the multistep progression of at least some cancers.

We have examined the expression of the transformed phenotype in a series of clonal lines of dihydrofolate reductase (dhfr)-deficient CHO cells transfected with the human c-Ha-$ras^{Val\ 12}$ oncogene and mouse dhfr-gene. Examination of various transformation parameters in the transformed cell clones indicated that cells expressing low levels of mutant ras-protein had lost some of their extracellular fibronectin network, and were altered in their cytoskeleton, whereas cells having amplified c-Ha-ras gene and expressing abundant $p21^{c\text{-}Ha\text{-}ras}$ had lost both their actin and fibronectin networks and secreted increased amounts of plasminogen activator activity. Cells with amplified c-Ha-$ras^{Val\ 12}$ grew better in low serum, formed large colonies in soft agar and showed enhanced activity of ornithine decarboxylase, the rate-controlling enzyme in polyamine biosynthesis. Amplification of the c-Ha-$ras^{Val\ 12}$ oncogene was also associated with enhanced RNA levels for the transforming growth factor-alpha. These results point to the importance of the dosage level of mutant oncogenes in transformed cells.

Sequences of the short arm of chromosome 2 containing the N-myc oncogene at 2p23-p24 are often involved in DNA amplification in neuroblastomas. We have searched for other genes in this chromosomal region. We have found that the human ornithine decarboxylase (ODC) sequences map to chromosome 2, region 2p23-pter, and are expressed as a mRNA species of 2.2 kb in several tumor cell lines. However, the ODC sequences are not coamplified with the N-myc oncogene. Our further experimental studies should clarify, whether N-myc is included in the amplicon resulting from a selection pressure for overexpression of ODC in neuroblastoma cells.

1. ALITALO, K. and SCHWAB, M.: AMPLIFICATION OF CELLULAR ONCOGENES IN CANCER CELLS. ADV. CANCER RES. vol 47, in press
2. Alitalo, K., Saksela, K., Winqvist, R., Keski-Oja, J., Alitalo, R., Ilvonen, M., Knuutila, S. and de la Chapelle, A. Acute myelogeneous leukemia with c-myc amplification and double minute chromosomes. The Lancet, in press, 1985.
3. Winqvist, R., Mäkelä, T., Seppänen, P., Jänne, O., Alhonen-Hongisto, L., Jänne, J., Grzeschik, K.-H., and Alitalo, K. Human ODC sequences map within chromosomal region 2p23-pter but are not amplified concomitant with N-myc. submitted for publication
4. Pohjanpelto, P., Holttä, E., Jänne, O., Knuutila, S. and Alitalo, K.: Amplification of ODC gene in response to polyamine starvation in chinese hamster ovary cells. J. Biol. Chem. 260, 8523-8537, 1985

L133 THE SECOND CELL-DERIVED INSERT OF THE KIRSTEN AND HARVEY SARCOMA VIRUSES REPRESENTS ANOXIC STRESS RESPONSE GENES. Garth R. Anderson, Becky K. Farkas, Daniel L. Stoler and Jack R. Fabian, Roswell Park Memorial Institute, Buffalo, NY 14263.

The ras oncogene of the Kirsten and Harvey sarcoma viruses represents a cellular gene closely related to the G-protein regulatory pathway. This system allows cells to respond to a variety of external signals. Each of these two sarcoma viruses also contain a second 3 kb cell-derived insert, related to sequences in the VL30 family. This second insert has now been found homologous to functional genes expressed by normal cells as a response to a specific stress, anoxia.

Anoxic stress induces uninfected rat fibroblasts to express approximately 10,000 copies per cell of a Kirsten related transcript; in contrast uninduced cells express approximately 50 copies per cell of this RNA. The induced transcript of 5.3 kb is polyadenylated and polysome associated. Anoxia causes no parallel increase in ras expression.

The anoxic stress response is associated with production of two polypeptides of 56,000 and 34,000 daltons. The 34,000 dalton polypeptide efficiently binds nucleic acids, binds a p21 which appears related to p21 ras gene products, and has lactate dehydrogenase activity. The function of the 56,000 dalton polypeptide is unclear.

Human cancer patients express elevated levels of the 34,000 dalton polypeptide in their sera and urine. This polypeptide, known as LDH_K, is under development as a cancer marker.

L134 EXPRESSION OF P53 ONCOGENE PROTEIN IN FRESH HUMAN LEUKEMIA CELLS. Michael Andreeff, James Squires, and Jan Bressler, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021

The nuclear protein p53 is found at elevated levels in tumor cells of different tissue types and from different species. P53 has been implicated both in the regulation of the cell cycle, and in the induction of a transformed phenotype when co-transfected with an activated c-Ha-ras gene into primary rat embryonic fibroblasts. The presence of p53 in fresh leukemic cells from three patients with acute lymphocytic leukemia (ALL) and one patient with biphenotypic leukemia (AML/AUL) was shown by immunoprecipitation using an anti-murine p53 monoclonal antibody (PAb-122) that has been found to cross-react with the human antigen. An irrelevant murine monoclonal antibody, Daudi cells which are known to express the protein, and HL-60 leukemic promyelocytes from which the p53 gene has been deleted were used as positive and negative controls in these experiments. A simultaneous flow cytometric assay of immunofluorescence and total DNA content performed on aliquots of the same patient samples showed high levels of p53 and p21ras in G_1 cells. P21ras was measured using a monoclonal antibody (Y13-259, courtesy of Dr. Mark Furth, Sloan-Kettering Institute) that binds to the products of the human c-Ha-ras, c-Ki-ras, and N-ras genes. The sequential expression of p53, p21ras, and c-myc in leukemic cells is currently being examined by multi-parameter flow cytometry using two-color immunofluorescence (FITC and biotin-avidin phycoerythrin) and Hoechst 33342 for DNA to investigate the progression from kinetic quiescence into the cell cycle.

L135 ACTIVATION OF THE NEU ONCOGENE, Cornelia I. Bargmann, Mien-Chie Hung, and Robert A. Weinberg, Whitehead Institute and Department of Biology, M.I.T., Cambridge, MA, 02142

The neu oncogene is specifically activated in neuro- and glioblastomas that arise after transplacental mutagenesis of rats with ethylnitrosourea. Transfection of DNA from such tumors onto NIH 3T3 cells results in foci which contain the neu gene and its product, the 185,000 kilodalton transmembrane protein p185. Neu is related to but distinct from the erbB gene, which encodes the EGF receptor. We have isolated biologically active genomic and cDNA clones of normal and transforming alleles of neu. The DNA sequence of such clones encodesa putative protein product of 1260 amino acids. This product is homologous to the EGF receptor, with 50% amino acid identity in the tyrosine kinase domain. Approximately 600 residues of the neu product are extracellular, including 50 cysteines which are absolutely conserved in position between neu and the EGF receptor. These data strongly suggest that neu encodes the receptor to an as yet unidentified growth factor.

By exchanging restriction fragments of the normal and transforming versions of the neu cDNAs, we have identified a region which confers transforming ability on the active allele. DNA sequencing of this region reveals that neu appears to be activated by a point mutation in the coding region. This contrasts with the known activations of the EGF receptor, which depend on massive truncation of the gene. We believe that the activating mutation of neu will shed light on the mechanism by which receptors transduce signals through the membrane.

L136 ALTERED EXPRESSION AND STRUCTURE OF ONCOGENIC SEQUENCES IN HUMAN BRAIN TUMORS,
N. Blin, R. Müller-Brechlin, E. Meese, J. Maurer & K.D. Zang,
Institute of Human Genetics, University of Saarland, D-665 Homburg, F.R.G.

In several tumors of human origin well defined chromosomal alterations have been demonstrated. Next to viral integration into the genome specific translocations have been implied as a means to activate oncogene expression. On the other hand, it has been suggested that losses of genomic sequences (suppressor genes) will result in oncogene activation and finally lead to tumorgenesis (Antioncogene hypothesis: COMINGS, 1973;KNUDSON, 1983). The best example studied so far is the homozygous loss of a region assigned to chromosome 13 (13q14) in retinoblastoma. To investigate whether the observed absence of both chromosomes 13 in a glioblastoma cell line (HeRo) and of one chromosome 22 in meningiomas is related to an antioncogene mechanism we have first studied the activity pattern of oncogenes in HeRo. Lack of chromosomes 13 and overexpression of abl, erbB, cmyc and Hras is concordant with this hypothesis. We then analyzed the DNA organization in the remaining chromosome 22 of meningiomas to check for a possible, minute deletion postulated by the hypothesis. After establishing the restriction pattern of the c-sis sequence in a random population, tumor samples and corresponding normal material were compared. Data suggesting a change in Pst I pattern are being tested using additional tumor material. Application of further probes from a chromosome 22-specific library will extend this search. At the same time, we are studying the oncogene expression pattern of meningiomas to collect further evidence for the antioncogene hypothesis so far examined in retinoblastomas and Wilms' tumor only.

L137 The role of the Ha-ras oncogene in malignant transformation of normal and two established cell lines of human keratinocytes

P. Boukamp[+], E. J. Stanbridge[++], P. A. Cerutti[+++], and N. E. Fusenig[+]
[+]DKFZ Heidelberg, FRG; [++]UCI Irvine, CA; [+++]ISREC Epalinge, CH.

The activated ras-oncogene family has been identified in tumors of various tissue types including carcinomas and may be an important intermediate in the process of malignant transformation. In order to explore this we transfected normal human foreskin and adult keratinocytes as well as a nontumorigenic keratinocyte line immortalized by SV-40 DNA (HaSV) with a plasmid containing the EJ Ha-ras oncogene. While the normal cells senesced before further characterization could be performed, a clone of the HaSV cells (HaSV-EJ) formed tumors when injected into athymic mice. Transfection with a plasmid containing only the neo gene did not lead to tumorigenicity.
To examine whether immortal growth per se complements the action of Ha-ras, we transfected a cell line which has spontaneously arisen from a long-term primary keratinocyte culture (HaCaT). Of 21 clones examined in more detail, five displayed the parental Ha-ras pattern upon Southern blot analysis. However, nine clones contained a single additional integrant and in seven clones two or more extra bands were identified. At the level of expression, a significant increase in Ha-ras RNA could only be observed in one clone which had multiple integrants of the Ha-ras gene. The tumorigenic potential of this as well as other clones will be discussed.

L138 Differences between c-fms and v-fms Gene Products at the C-Terminal end may account for the v-fms Transforming Potential

P.J. Browning, H.F. Bunn, T.V. Gopal, A. Kline, M. Shuman, A.W. Nienhuis
(CHB, NHLBI) Bethesda, Md.

The c-fms gene encodes for a protein closely related or identical to the receptor for M-CSF (CSF-1), a hematopoietic growth factor. The c-fms gene is homologous to v-fms, a transforming gene found in the McDonough strain of feline sarcoma virus (FeSV). We have isolated a 1.6 Kb human c-fms cDNA clone that encodes for the C-terminal half of the protein. Comparison of the human c-fms and v-fms DNA sequences reveal 94% homology to 33 nt from the v-fms termination codon. The c-fms reading frame continues 120 nt beyond the end of the c-fms and v-fms homology. Thus the v-fms encoded protein is truncated at the C-terminal end compared to the c-fms gene product. Either the entire tyrosine kinase encoding domain or the 3' 160 nt of the v-fms gene in the FeSV proviral genome were replaced with the corresponding segments of the human c-fms cDNA. The FeSV genome containing v-fms gave 51 foci/ug when transfected into 3T3 cells whereas neither of the hybrid v-fms/c-fms genes gave transformed foci. Thus differences at the C-terminal end of the c-fms and v-fms encoded proteins appear to account for the transforming potential of the v-fms gene product.

L139 ANALYSIS OF GROWTH AND DIFFERENTIATION USING RETROVIRUS VECTORS FOR VIRAL ONCOGENES. V. Cherington, M. Brown, B. Morgan, and T. M. Roberts, Dana-Farber Cancer Institute, Boston, MA. 02115

We have constructed recombinant murine retroviruses which code for individual polyoma virus T antigens, large T (PyLT), middle T (PyMT), and small T (PyST), and for SV40 large T (SVLT). With these vectors we can express individual T antigens in a wide variety of cell types, including mouse fibroblasts and rat pituitary cells to study T antigen effects on growth and gene expression normally regulated by growth factors and hormones, and mouse preadipocytes to study T antigen effects on differentiation. PyST retrovirus infection increases the saturation density of NIH3T3 cells. PyMT retroviruses are sufficient to transform NIH3T3 cells to become tumorigenic and to form foci in monolayer cultures. PyMT, but not PyLT, retrovirus infection eliminated the growth requirement for EGF in a defined medium and permitted growth in platelet poor plasma, indicating PDGF independence as well. Insulin and transferrin requirements are retained by PyMT transformed cells. Prolactin synthesis in clonal rat pituitary cells (GH4C1) is regulated by EGF. PyMT expression in these cells increases basal prolactin synthesis indicating that the signals generated by PyMT to eliminate the EGF growth requirement in fibroblasts may also be utilized to modulate prolactin synthesis in rat pituitary cells. PyLT and SVLT individually retard normal differentiation in mouse pre-adipocytes (3T3-F442A). The possibility that this effect is a result of T antigen modulation of adipocyte specific gene expression is currently being examined in collaboration with Bruce Spiegelman (DFCI). The ability of these viral oncogenes to alter cellular growth and differentiation will allow analysis of cellular regulatory pathways at a genetic and biochemical level. (Supported in part by NIH Grants CA30002 and CA15751.)

L140 ROLE OF raf ONCOGENES IN CARCINOGENESIS, J. L. Cleveland, S. Storm, M. Huleihel and U. R. Rapp, NIH, NCI/FCRF, Frederick, MD 21701-1013

The identification of the v-raf oncogene from the defective retrovirus 3611-MSV has allowed the isolation of a new family of proto-oncogenes. The active homolog of v-raf, c-raf-1, is located at position p25 on chromosome 3 in man, a site that is frequently altered in a variety of neoplasia, including small cell lung carcimoma and heriditary renal carcinoma. Moreover, transforming versions of c-raf-1 have been identified in human stomach cancer and glioblastoma. Expression of c-raf-1 in these neoplasias is currently under investigation. Genes related to c-raf-1 map on human chromosomes Xp12, 7p13, and 4 near the entromere. The gene mapping on the X chromosome, δ-raf, is closely related to c-raf-1 with 70% nucleic acid and 76% amino acid homology, and behaves as a oncogene when inserted into a retrovirus. To study the role of raf in carcinogenesis in vivo, we have developed an animal model where 90% of newborn mice treated transplacentally with ethylnitrosourea and promoted at birth with butylated hydroxytoluene develop lung tumors and lymphomas within 5 to 14 weeks. High c-raf-1 expression in tumors and established cell lines has been determined by Northern blot and immunoflorescence analyses. Southern blot analysis of DNA from these tumors for rearrangements of c-raf-1 is currently in progress. Moreover, we have now defined conditions using growth modulators and hormones for the differential inhibition of raf-transformed, but not untransformed control cell lines in vitro. These regimens are now being tested in our animal model system, in conjunction with vaccination experiments using purified raf protein, to examine their potential inhibitory actions on the development of these tumors.

L141 PRODUCTION OF Ras-ENCODING RETROVIRAL VECTORS AND GENERATION OF FULLY-PROCESSED c-Ha-Ras GENES, Michael J. Corbley, Larry A. Feig, Van Cherington, Channing J. Der, Geoffrey M. Cooper and Thomas M. Roberts, Dana-Farber Cancer Institute, Boston, MA 02115

In order to study the biochemistry of transformation by the ras oncogenes, we have generated c-Ha-Ras cDNAs and have produced ras-encoding retrovirus stocks by a unique method. We inserted the coding regions of wild-type and codon-61 activated genomic c-Ha-Ras into a derivative of the G418-resistant retroviral shuttle vector pZipNeoSV(X)1, transfected the recombinant plasmid into psi-2 packaging cells to make viral stocks and used these stocks to infect NIH3T3 cells. After selection with G418 for infected NIH cells, circularized plasmids bearing one LTR, Neo and the corresponding wild-type or activated ras cDNAs were rescued as described by Cepko et al. (Cell 37:1053, 1984). As an alternative to excising the cDNAs and re-inserting them into fresh vectors to make ras cDNA virus stocks, we fused the G418-resistant NIH colonies back to psi-2 packaging cells and collected transient supernatants which containedvirus in titers of $2x10^3$ CFU/ml. Stable NIH/psi-2 producing lines were generated by co-selection for the G418 and Ecogpt markers; these lines made virus with titers as high as 10^5 CFU/ml. Furthermore, in contrast to the original infections with virus encoding the activated genomic ras, in which most of the colonies were flat, greater than 95% of these ras cDNA colonies were clearly transformed.

We are currently using the processed ras genes to over-produce the ras proteins in bacteria for biochemical studies and the retroviruses to produce transformation-defective mutants in ras.

L142 SEQUENCE ANALYSIS OF THE RAS PROTEIN FAMILY. Marc De Braekeleer[1], Paul N. Draper[2] and Chyi Chyang Lin[1]. [1]University of Alberta, Edmonton, Alberta, Canada [2]University of Calgary, Calgary, Alberta, Canada.
To date, 19 protein sequences of the ras family have been reported. This study was undertaken to determine any specific conserved aminoacid (AA) regions and the degree of evolutionary conservation within this family. Using the human H-ras protein (p21) as a reference, the protein alignment program of Queen and Korn was run against the other sequences. Five regions of p21 were found to be highly conserved (with few conservative changes): AA7-17 (including Gly-12), AA32-41, AA53-63 (including Ala-59), AA114-120 (including Asn-116) and AA185-186 (including Cys-186). The region AA89-98 was shown to be less conserved although most substitutions were conservative. Many non-conservative substitutions were found in region AA125-180. Specifically, the region AA125-166 was divergent between the species considered (Drosophila, Yeast, Dictyostelium, Man); however, within Man (H-, K- and N-ras) and Drosophila (ras1 and ras2), this same sequence was highly conserved. The region AA167-180 showed extensive aminoacid changes between species as well as within species (Man and Drosophila). The results suggest that the 5 regions that are highly conserved represent common functions (such as GTP binding, GTPase activity, etc.). Regions of divergence may represent different interspecies functions but related intraspecies functions.

L143 AN ONCOGENIC Ki-RAS PROTEIN AND SERUM GROWTH FACTORS DIFFERENTLY PROMOTE G_2 TRANSIT OF tsK-NRK CELLS, Jon P. Durkin, and James F. Whitfield, Division of Biological Sciences, National Research Council of Canada, Bldg. M-54, Montreal Road Campus, Ottawa, Canada K1A OR6

NRK cells infected with a ts Kirsten sarcoma virus (ts371-KSV) are transformed at 36°C, but are untransformed at 41°C which inactivates the abnormally thermolabile oncogenic $p21^{Ki}$ product of the viral v-ras^{Ki} gene. At 41°C, tsK-NRK cells were arrested in G_0/G_1 when incubated in serum-free medium, but could then be stimulated to transit G_1, replicate DNA and divide (1) by adding serum at 41°C or (2) by dropping the temperature to a p21-activating 36°C without adding serum. When quiescent cells at 41°C were stimulated to transit G_1 in serum-free medium by activating p21 at 36°C and were then shifted back to the p21-inactivating 41°C in mid S-phase, they continued replicating DNA but could not transit G_2. Reactivating p21 in the G_2-arrested cells, by once again lowering the temperature to 36°C, stimulated a rapid entry into mitosis. Thus, besides directly stimulating G_1 transit, p21 directly promotes G_2 transit. By contrast, adding serum (to 10%) at 41°C did not stimulate these G_2-arrested cells to enter mitosis. Since serum stimulated quiescent G_0 cells to replicate DNA and enter mitosis, but, unlike p21, did not directly affect G_2 transit, serum growth factors must trigger events during the G_1 phase that ultimately determine G_2 transit. Therefore, $p21^{Ki}$ and serum growth factors differently promote cell proliferation.

L144 CHARACTERIZATION OF THE TRANSCRIPTIONAL AND TRANSLATIONAL PRODUCTS OF dbl, A NEWLY ISOLATED HUMAN TRANSFORMING GENE, A. Eva, S. Srivastava, S. Tronick, G. Vecchio, D. Ron, S. Aaronson, National Cancer Institute, Bethesda, MD 20892

A human B cell lymphoma was shown to contain a transforming gene apparently unrelated to the ras gene family and 17 other known onc genes. This transforming gene, named dbl, was cloned in biologically active form as a 45-kb human DNA fragment in a cosmid vector. Specific fragments have been isolated from the cloned DNA and used as probes to identify dbl mRNA as well to determine the gene structure. Up to now at least three exons have been identified and a 2.9-kb mRNA was detected in transfectant cell lines. A third-cycle transfectant cell line was injected in NFS mice and sera from tumor-bearing animals were examined for reactivity with any proteins which were induced specifically by the dbl transforming gene. We found that a polypeptide was specifically precipitated from all dbl transfectant clones, while it was not detectable in other transformed cells or in untransformed NIH/3T3. Analysis with the same antisera of a spontaneous mutant of dbl indicated that this polypeptide is the protein encoded by the transforming dbl gene. Finally, a cDNA library was constructed from a third-cycle transfectant and screened with specific probes isolated from cloned dbl. Results of the characterization of several cDNA clones will be discussed.

L145 OVER-EXPRESSION OF c-sis ONCOGENE IN DIPLOID HUMAN FIBROBLASTS YIELDS AN ANCHORAGE-INDEPENDENT PHENOTYPE, William E. Fahl, Craig W. Stevens, Jeffrey A. Burgess and T. Herbert Manoharan, McArdle Laboratory, Univ. of Wis., Madison, Wis. 53706

In several human mesenchymal tumors, anchorage-independent growth as well as over-expression of the c-sis gene coding for PDGF have been observed. In our experiments, we co-transfected an SV40 -promoted c-sis cDNA and pSV2neo into diploid human fibroblasts and characterized phenotypic abberrations in the G418-resistant cell populations. Large diameter (1.7-2.2 cm) colonies showing dense fibroblast cords in sis (pSM-1 plasmid) plus neo (psv2neo) dishes were isolated, expanded and compared to expanded control colonies (0.3-0.6 cm) from neo alone dishes. A 3.6 kb sis transcript was measureable in mRNA isolated from each of the large diameter colonies; no sis-hybridizing transcript was detected in neo alone control colonies. The ability of each clonal cell population to grow in soft agar (i.e. form colonies >75 um diameter) was also determined. Our results indicate that the efficiency with which human fibroblasts grew in agar was directly relatable, in a dose-dependent fashion, to the level of sis transcripts generated from the transfected SV40 promoter-c-sis cDNA chimeric gene.

Supported by NIH grant number CA-42024.

L146 ISOLATION AND CHARACTERIZATION OF CLONES ENCODING BASIC AND ACIDIC FIBROBLAST GROWTH FACTORS, John C. Fiddes, Ayalew Mergia, Jacqueline Wang, Annette Turolo, Jeff Friedman, Kathryn A. Jarvis, Denis Gospodarowicz[+] and Judith A. Abraham. California Biotechnology, Inc., 2450 Bayshore Frontage Road, Mountain View, CA 94043 and [+]Cancer Research Institute, University of California, San Francisco, CA 94143.

Growth factors that are both mitogenic for cultured capillary endothelial cells and angiogenic in vivo have been purified from several sources. The recent discovery that these growth factors all show a high affinity for heparin and are either cationic or anionic, has raised the possibility that there are only two major endothelial cell mitogens, basic and acidic fibroblast growth factor (FGF). Basic FGF is probably identical to beta-heparin binding growth factor (beta-HGF), macrophage derived growth factor (MDGF), tumor angiogenesis factor (TAF) and cationic hypothalamus derived growth factor (cHDGF) while acidic FGF is probably identical to endothelial cell growth factor (ECGF), alpha-heparin binding growth factor (alpha-HGF) and anionic hypothalamus derived growth factor (aHDGF). Despite the pI differences these two growth factors have a similar range of biological activities.

We have isolated and characterized cDNA and genomic clones for both of these growth factors. Nucleotide sequence analysis shows that basic and acidic FGF are homologous and have introns located at equivalent positions. Genomic Southern blotting experiments indicate that both growth factors are encoded by single copy genes. Even at reduced levels of stringency they do not cross hybridize with each other or with any other sequences, thus strengthening the conclusion that this growth factor family has only two members.

The physiological functions of basic and acidic FGF are unclear but endothelial cell mitogens have been implicated in processes such as tissue repair, placental development and the growth of capillary beds around solid tumors. This possible role as tumor angiogenesis factors is supported by our isolation of basic and acidic FGF clones from a cDNA library generated from a human breast carcinoma.

L147 EXPRESSION OF c-Ha-ras ONCOGENE AND CORRELATION WITH TUMORIGENICITY IN HUMAN HYBRID CELLS, Andrew G. Geiser and Eric J. Stanbridge, University of California, Irvine, CA 92717.

The involvement of the activated c-Ha-ras oncogene in the tumorigenicity of certain human cells was studied in a hybrid cell system. Normal human fibroblasts were fused with the EJ bladder carcinoma cell line expressing the activated (mutated at codon 12) c-Ha-ras oncogene. All of the resultant hybrids were found to be suppressed for tumorigenicity but continued to express the EJ c-Ha-ras oncogene at nearly the same level as the parental EJ cell line. Tumorigenic segregants of the hybrids arose in culture but maintained the same c-Ha-ras DNA pattern and expression level as that of the suppressed hybrids. In an attempt to increase expression of activated c-Ha-ras in the suppressed hybrids, resulting in nontumorigenic transfectants (continued suppression). Expression of c-Ha-ras, however, was only increased about twofold. In order to increase expression even further, the EJ c-Ha-ras oncogene was introduced into an inducible expression vector utilizing the glucocorticoid responsive MMTV LTR. Nontumorigenic hybrid cells transfected with the inducible c-Ha-ras plasmid construct will be discussed in terms of oncogene expression and tumorigenicity.

L148 IDENTIFICATION AND DIFFERENTIAL TISSUE EXPRESSION OF TWO SETS OF CELLULAR POLYPEPTIDES RELATED TO THE v-ets ONCOGENE OF AVIAN LEUKEMIA VIRUS E26.

J.Ghysdael, A.Gegonne, P.Pognonec, D.Dernis, D.Leprince and D.Stehelin. INSERM UNITE 186 -INSTITUT PASTEUR - LILLE - FRANCE

E26 is a replication defective retrovirus that induces erythroblastosis and myeloblastosis in chickens. The genome of E26 contains two distinct cell-derived oncogenes v-myb and v-ets expressed together with a partial viral gag gene as a 135 kd protein (P135). A portion of v-ets has been expressed in bacteria and the ets-encoded polypeptide used to raise antisera that specifically precipitate the E26-encoded P135. These antisera also identify two distinct sets of polypeptides in several avian tissues and cell-lines. The first set includes a major protein of 54 kd (P54) and a minor one of 56 kd (P56). Both of these proteins share most of their (^{35}S) methionine-containing tryptic peptides with the v-ets-encoded domain of P135. Both of these proteins are expressed at high levels in the blast fraction of thymic and bursal lymphocytes. The second set includes two proteins of 64 kd and 62 kd highly related to each other which share a limited domain of homology with P54 and P135. The 64/62 kd proteins are expressed at high levels in both quiescent and dividing macrophages and their synthesis is specifically and rapidly induced upon differentiation of AMV-transformed myeloblasts into macrophages after TPA treatment, raising the possibility that the expression of these polypeptides might be of importance to macrophage differentiation.

L149 STRUCTURE-FUNCTION OF Ha-ras p21. Jackson B. Gibbs, Irving S. Sigal, Jill D'Alonzo, Bohdan Wolanski, Phillip Hew, Susan Socher and Edward M. Scolnick, Virus & Cell Biology Research, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486.

A series of mutations have been introduced into normal Ha-ras and oncogenic Ha[Val 12 Thr 59] by oligonucleotide-directed mutagenesis. The proteins encoded by the various ras genes were expressed in E. coli and purified in order to study their intrinsic biochemistry, immunological properties and biological activity upon microinjection into NIH 3T3 cells. This analysis has identified residues 16 and 119 within the guanine nucleotide site of p21 and has indicated that Lys 16 interacts with the phosphate groups of GDP and that Asp 119 interacts with the purine 2-amino group. These points of interaction between p21 and GDP are homologous to those determined in the structure of procaryotic EF-Tu * GDP. Sequential single residue mutations have begun to identify sites that alter p21 biology without changing guanine nucleotide binding and sites that are important for monoclonal antibody recognition. Proteins that are unable to morphologically transform NIH 3T3 cells are also unable to complement normal yeast RAS function.

L150 REQUIREMENTS FOR TRANSFORMATION BY THE v-rel ONCOGENE OF RETICULOENDOTHELIOSIS VIRUS STRAIN T. T.D. Gilmore, B.S. Sylla, and H.M. Temin. McArdle Laboratory for Cancer Research, Univ. of Wisconsin, Madison, Wisconsin 53706.

Reticuloendotheliosis virus strain T (REV-T) is an avian retrovirus that transforms chicken spleen cells. However, REV-T does not transform chicken embryo fibroblasts (CEF). We have been studying p59$^{v\text{-}rel}$, the product of the v-rel oncogene of REV-T, in order to understand the basis for this cell-type specific transformation by REV-T. p59$^{v\text{-}rel}$ is present at the same levels and is phosphorylated at identical serine residues in REV-T infected spleen cells and CEF. However, immunofluorescence reveals that p59$^{v\text{-}rel}$ is a nuclear protein in CEF, but is primarily a cytoplasmic protein in REV-T transformed spleen cells. Thus, there is a correlation between subcellular localization of p59v-rel and its ability to induce transformation. We are currently examining the distribution of p59$^{v\text{-}rel}$ in other types of cells infected with REV-T, and we are constructing viruses encoding hybrid v-rel proteins with sequences that localize p59v-rel to different subcellular compartments.

To define the changes necessary for the generation of the transforming property of v-rel, we have constructed a number of recombinant viruses containing DNA sequences from v-rel and the turkey proto-oncogene c-rel. Analysis of these recombinant viruses indicates that sequences at the 5' end of v-rel are essential for transformation of spleen cells. Sequences at the 3' end of c-rel can be substituted for 3' v-rel sequences without affecting the transforming activity of rel proteins.

L151 REQUIREMENTS FOR TRANSFORMATION BY THE MYB ONCOGENE, Thomas J. Gonda+, Julie de Blaquiere+ and Gregory Johnson*. Ludwig Institute for Cancer Research+ and Walter and Eliza Hall Institute of Medical Research*, P.O. Royal Melbourne Hospital, Victoria 3050, Australia.

Comparison of the nucleotide sequence of murine c-myb cDNA clones (Gonda et al., EMBO J. 4, 2003-2008 (1985)) with the sequences of v-myb oncogenes and analysis of tumour cells bearing c-myb genes which have been disrupted by retroviral insertion both suggest that truncation of the c-myb protein may be required to generate a transforming gene product. In particular the products of the v-myb genes of the avian leukemia viruses AMV and E26 are truncated at both the amino and carboxy termini with respect to the c-myb protein. Therefore, we have constructed versions of the murine c-myb cDNA clones which should encode proteins with truncated amino and/or carboxy termini as well as the normal protein. These constructs, in addition to the v-myb gene, have been inserted into retrovirus vectors and are being used to infect murine hemopoietic cells, in order to assess their transforming potential. The results of these experiments will be presented.

L152 STRUCTURAL ANALYSIS OF THE MYC PROTEINS, Stephen R. Hann and Robert N. Eisenman Fred Hutchinson Cancer Research Center, 1124 Columbia St., Seattle, WA 98104

In all species examined thus far we have shown that two major proteins and at least one minor protein are specifically immunoprecipitated with myc antisera. The synthesis of these proteins appear to be regulated independently during cellular proliferation of normal and transformed fibroblasts and B cells. In addition, we have found that there are dramatic changes in their ratios in cells which have alterations at the myc locus, such as in human Burkitt's lymphomas and avian bursal lymphomas. All of these proteins were found to have extremely short half-lives, except for a minor species which appeared much more stable in fibroblasts. Also, all of them have similar isoelectric points. Using a variety of specific proteases and chemical reagents we have examined these different forms for structural differences. All of them appeared to be phosphorylated on their C-terminal domain and there were no apparent differences in their phosphorylation. The difference between the proteins was found to be in their N-terminal domain. Structural differences between the c-myc and v-myc proteins will also be discussed.

L153 TRANSFECTION OF EJ^{Ha} INTO KERATINOCYTES DERIVED FROM CARCINOGEN-INDUCED MOUSE PAPILLOMAS CAUSES MALIGNANT PROGRESSION, J.R. Harper, D.R. Roop, and S.H. Yuspa, National Cancer Institute, Bethesda, MD 20892.
The development of malignant tumors in carcinogen-treated mouse skin appears to involve several genetic changes. The initiation step involves alterations in the normal pattern of epidermal differentiation, resulting in benign tumors following exposure to tumor promoters. Subsequent genetic changes appear to be required for malignant conversion of papillomas to epidermal carcinomas. We derived several cell lines from chemically-induced mouse skin papillomas that have many properties expected of initiated cells. Even though these cells retain some differentiated functions, they exhibit an altered differentiation program, i.e., the ability to proliferate under culture conditions favoring terminal differentiation. When DNA from 3 separate cell lines was tested in the NIH-3T3 transfection assay, active transforming activity was not detected. However, when the EJ ras^{Ha} gene was introduced into papilloma cells by DNA transfection, transfectants showed an enhanced capacity to proliferate under differentiating culture conditions and formed rapidly growing, anaplastic carcinomas in nude mice. Our findings suggest that in papilloma cells, a genetic change distinct from ras^{Ha} activation may produce an altered differentiation program associated with the initiation step, and this genetic alteration may act in a cooperating fashion with an activated ras gene to result in malignant transformation.

L154 **GLUCOSE DEPRIVATION, VIRAL TRANSFORMATION, AND HEXOSE TRANSPORTER POLYPEPTIDES;** Howard C.Haspel, Eugene W. Wilk, Morris J. Birnbaum, and Ora M. Rosen. Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021. Using antisera to the 55 kD human erythrocyte hexose transporter (GT) we probed the effects of glucose (Glc) deprivation ("starvation") and viral transformation on GT polypeptide (pp) content and structure. Glc deprivation of 3T3-C2 murine fibroblasts elicited a reversible (upon refeeding 12 h) time (12-72 h) and Glc concentration (0-4 g/l) dependent increase (30-40 fold by 48 h) in total GT pp content. This increase includes a 5-10 fold increase in the 55 kD form of the GT and the appearance of a 42 kD pp. The appearance of the 42 kD pp correlates with lower threshold Glc concentations. The near identity of the Mr of the 42 kD pp with the pp observed after tunicamycin treatment and the inability of the 42 kD pp to adsorb to agarose bound wheat germ agglutinin (in contrast to the 55 kD GT pp) suggest that this pp is an aberrently glycosylated form of the GT. Hexose phosphate formation and interaction of sugars with the GT were both implicated in the accumulation of total GT pp. Appearance of the 42 kD pp, but not accumulation of the 55 kD pp, was dependent on protein synthesis. When oligosaccharide biosynthesis proceeded normally the 42 kD pp was not observed. Using rat and mouse fibroblasts infected with transforming RNA tumor viruses we observed a transformation dependent >10 fold increase in GT pp. The levels of translatable GT pp-RNA observed with starvation or transformation were similar to those of control cells. The results suggest that the accumulation of GT pp elicited by starvation or transformation involves post-transcriptional mechanisms. The relationship between the Glc deprivation- and transformation-induced increase in GT pp is currently being examined. Supported by grants from NIH and ACS.

L155 **TUMOR PROMOTERS AND A SERUM FACTOR ENHANCE EXPRESSION OF THE TRANSFORMED PHENOTYPE IN RAT 6 FIBROBLASTS TRANSFECTED WITH AN ACTIVATED ONCOGENE.** **W.-L.Wendy Hsiao, Cecilia A. Lopez and I.Bernard Weinstein. Cancer Center/Institute of Cancer Research, Columbia University, New York, N.Y. 10032.**

Rat 6 embryo fibroblasts were transfected with the activated human c-Ha-ras oncogene T24 and grown in the absence or presence of the tumor promoters 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or teleocidin. The presence of TPA or teleocidin led to a 6 to 14 fold increase in the number of transformed foci obtained. Time course studies indicated that maximum enhancement could still be obtained when the addition of TPA was delayed until 7 days after transfection, and some enhancement was seen even when addition of TPA was delayed until 16 days. Parallel transfection studies with the drug resistance markers gpt or neo, neo/c-Ha-ras co-transfection experiments and Southern blot analyses indicate that the enhancement seen with the c-Ha-ras oncogene in rat 6 cells is not simply due to a mitogenic effect of TPA nor an effect on the process of DNA transfection. We have also found that a factor present in fetal calf serum (FCS) is even more potent that TPA in producing this type of enhancement of cell transformation. This factor has been isolated by HPLC following Bio-gel P10 gel filtration. Other known growth factors including EGF, TGF-α, TGF-β, insulin and PDGF produced no effect on T24 induced transformation in this system. Thus, tumor promoters and a serum factor may act synergistically with activated oncogenes during multistage carcinogenesis. Supported by NCI Grant CA-26056.

L156 COOPERATIVITY BETWEEN v-erbA AND v-src-TYPE ONCOGENES IN ERYTHROID CELL TRANSFORMATION. Patricia Kahn, Lars Frykberg, Hartmut Beug, Irene J. Stanley, Björn Vennström and Thomas Graf. European Molecular Biology Laboratory, 6900 Heidelberg, West Germany.

Avian erythroblastosis virus (AEV-ES4) causes erythroleukemias and sarcomas in infected chickens and transforms bone marrow-derived erythroblasts as well as fibroblasts in culture. This virus carries two independent oncogenes. V-erbB encodes the complete transforming capacity and is a truncated and mutated derivative of the EGF receptor gene. V-erbA by itself has no detectable oncogenic capacity in chickens but enhances the transformed phenotype of v-erbB-transformed erythroblasts in vitro by blocking their capacity to differentiate spontaneously and by reducing the stringency of their growth requirements. We have investigated further the functions of the v-erbA gene and find that: 1) v-erbA cooperates in a similar manner with other oncogenes which transform avian erythroid cells, including v-src, v-fps and v-Ha-ras. 2) A retroviral construct encoding both v-src and v-erbA induces acute erythroblastosis as well as sarcomas, indicating that v-erbA alters the pathogenic spectrum of v-src. 3) Superinfection of v-erbB- or v-src-transformed erythroblasts with a v-erbA-encoding retroviral construct enables these cells to proliferate under a much wider range of pH and ionic strength, suggesting that this gene might affect some aspect of ion metabolism or transport.

L157 EXON-INTRON MAPPING OF THE EGF RECEPTOR GENE, Derek Kinchington, Nigel Whittle, John Haley and Michael Waterfield.

Genomic fragments of the EGF receptor gene isolated from several different cell lines and cDNA fragments isolated from an A431 library were used to construct an exon-intron map of the EGF receptor gene by heteroduplex analysis. These data agree well with restriction and hybridisation data. The EGF receptor gene is approximately 110 kb in length and contains 24 coding regions (3.9 kb).

L158 EVIDENCE FOR "HIT-AND-RUN" TUMORIGENESIS IN CHINESE HAMSTER EMBRYO FIBROBLAST CELLS, Ching C. Lau, Inder K. Gadi, and Ruth Sager, Dana-Farber Cancer Institute, Boston, MA 02115

We have previously described the recovery of tumorigenic foci from Chinese hamster embryo fibroblast (CHEF/18) cells following transfection with plasmids pSV2gpt, pSVneo, and J132, a recombinant of Harvey murine sarcoma virus LTR and the normal human c-Ha-ras1 in pBR322 (PNAS 82, 2843). Unlike foci from transfection with plasmids containing known oncogenes, these foci and their tumor derivatives do not retain any plasmid DNA. Several criteria were used to distinguish these plasmid-induced foci from spontaneously transformed cells. We interpret these results as evidence of "hit-and-run" tumorigenesis.

Associated with the hit-and-run process is the presence of chromosomal aberrations in the focal and tumor-derived cells. Most notable is trisomy for chromosome 3q or rearrangement involving 3q which is characteristic also of tumorigenic CHEF cells transformed by other methods. In addition, translocation and amplifications involving chromosomes 7 and 9 were also consistently observed. The analyses of these genomic rearrangements in hit-and-run tumorigenesis will be presented.
This research was supported by NIH grants CA24828 and CA09361

L159 THYROID HORMONE EFFECTS THE EXPRESSION OF NEOPLASTIC TRANSFORMATION INDUCED BY DNA-TRANSFECTION. Susan W.C. Leuthauser & Duane L. Guernsey, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242

The involvement of thyroid hormone in the initiation of x-ray and chemical induced neoplastic transformation has been reported previously. When C3H 10T 1/2 mouse embryo cells were grown in thyroid hormone-depleted media, they become resistant to transformation. To examine the role of thyroid hormone in the expression of the transforming event, we transfected DNA from transformed cells (by x-rays & MCA) to normal C3H 10T 1/2 fibroblasts adapted to hypo- or euthyroid hormone (10^{-9} M T_3) conditions. Control experiments determined that the uptake and integration of exogenous DNA was not affected. However, a T_3 dependency was observed in the expression of the transformed phenotype. A reduced number of transformed foci were observed under hypothyroid conditions. K-ras has previously been reported to be activated in MCA-transformed 10T 1/2 cells (Parada and Weinberg, 1983, Molec. Cell. Biol. 3:2298-2301). The expression of this activated oncogene was unaffected by the T_3 status of the culture media. Thus we suggest that thyroid hormone may play a role in modulating the expression of the transformed phenotype by regulating endogenous genes/proteins.

L160 STRUCTURE AND EXPRESSION OF R-*RAS*, A MAMMALIAN GENE RELATED TO THE *RAS* PROTO-ONC GENES, David G. Lowe, Eric Delwart, Daniel J. Capon and David V. Goeddel, Genentech, Inc., South San Francisco, CA 94080

Low stringency hybridization of a v-Ha-*ras* probe to a human genomic DNA library identified a *ras* related gene, R-*ras*. The predicted 218 amino acid sequence of R-*ras* shares 47% similarity to c-Ha-*ras*-1 p21. The homology of R-*ras* and p21 extends over several highly conserved domains, including regions predicted to comprise the p21 guanine nucleotide binding site, and the C-terminal sequence required for p21 membrane attachment. The R-*ras* gene is split into at least 6 exons spanning 5 kilobases of DNA. There is no similarity in the positions of intervening sequences in the R-*ras* gene compared to the H-, K-, or N-*ras* genes when their coding sequences are aligned by amino acid homology. cDNA clones of mouse R-*ras* mRNA were isolated with a human R-*ras* cDNA probe. The human and mouse R-*ras* cDNAs show 88% DNA sequence similarity, and 94% predicted amino acid homology. Experiments on the expression of the human R-*ras* gene transfected into mammalian cells, and in E. coli, will be described.

L161 A LYMPHOCYTE-SPECIFIC PROTEIN-TYROSINE KINASE GENE IS REARRANGED AND OVEREXPRESSED IN THE MURINE T CELL LYMPHOMA LSTRA, Jamey D. Marth, Richard Peet, Edwin G. Krebs, and Roger M. Perlmutter, Howard Hughes Medical Institute SL-15, Department of Pharmacology, Department of Medicine, University of Washington, Seattle, Washington 98195.

Protein-tyrosine kinases are implicated in control of normal and neoplastic cell growth. We have used molecular cloning strategies to isolate and characterize cDNAs encoding the major protein-tyrosine kinase present in the Moloney Murine Leukemia Virus-transformed T cell lymphoma LSTRA. Sequence analysis predicts a translation product of 509 amino acids within which all protein-tyrosine kinase sequence motifs are found. Comparisons with other members of the protein-tyrosine kinase family show closest homology to avian src and yes gene products. In normal murine tissues, RNA transcripts from this gene, designated lsk^T, accumulate only in tissues of lymphoid origin with highest levels appearing in the thymus. Furthermore, expression of lsk^T is confined to lymphocytes and especially to T lymphocytes, implying that this protein may aid in the transduction of proliferative or differentiative signals unique to these cells. In LSTRA cells, the lsk^T gene is rearranged and overexpressed suggesting that alterations in the structure or expression of this protein-tyrosine kinase gene may in some cases mediate neoplastic transformation.

L162 Regulation of cell division and protein phosphorylation by serum and RSV transformation product in CEF cells. Jorge Martin-Perez and Raymond L. Erikson, Harvard University.Cambridge, MA 02138

In searching for specific effects induce by the oncogen product, the protein $pp60^{v\text{-}src}$, we have compared a number of cellular events when quiescent cultures of CEF cells are stimulated by serum or transformed by a temperature sensitive mutant of the RSV, NY 68. Addition of serum or a shift to permisive temperature to quiescent cultures of CEF and NY68 infected cells(CEF.NY68)induce cells to leave the G_0 phase ,to enter into the cell cycle and ultemately to divide.Analisis of [^{3}H]-Thymidine uptake and cell number have shown that in both cell types the G_1 phase is about 9hr,the S phse is 2-3hr and cell division takes place at about 15hr.One of the early events found upon cellular stimulation is the multiole phosphorylation of the 40S ribosomal protein S6. Both cell types, the serum-stimulated CEF and the CEF.NY68 transformed cells showed a similar time course of S6 phosphorylation,clearly observed at 15min.and reaching the maximal extent of phosphorylation after 2hr of cellular stimulation. This is follow by a 2 fold increase on protein synthesis observed at 2hr and maintained for at least 24hr.Because cellular transformation by RSV causes a drastic rearrangement of the intracellular infrastructure,we have looked for a possible specific distribution of phosphorylated ribosomes in serum-stimulated CEF and CEF.NY68 cells.We have found that after 30min. in both cell types the most highly phosphorylated ribosomes are associated with membranes and cytoskeleton fractions when compared with free ribosomes in the cytosplasm.As shown by others, S6 is a cell cycle dependent event in serum-stimulated cells, whereas this protein is always highly phosphorylatedin RSV-transformed cells. We have therefore looked for the tunrover of phosphatein S6 in the 2 cell types and we have found that in the presence of serum 40% of the phosphate label turns over in 3hr,whethe phosphate appears to be metabolicaly stable in RSV-transformed cells, suggesting that $pp60^{v\text{-}src}$ specifically inhibits a serine phosphatase activity.

L163 onc D, A NEW HUMAN TRANSFORMING GENE IS A HYBRID BETWEEN A TROPOMYOSIN GENE AND A NOVEL TYROSINE KINASE LOCUS. D. Martin-Zanca, S.H. Hughes, & M. Barbacid, Frederick Cancer Research Facility, Basic Research Program, P.O. Box B, Frederick, MD 21701.

onc D is a new transforming gene isolated from a human colon carcinoma by transfection of DNA into NIH/3T3 cells. A cDNA library, consisting of 10^6 clones was derived from third cycle NIH/3T3 transformants. An onc D cDNA that is capable of transforming NIH/3T3 cells was isolated from this library. It has an insert 2.3 kbp long, approximately the same size as the onc D mRNA (2.5 kb), and contains a 1923 base open reading frame. The 5' portion of the onc D message contains the first seven coding exons (221 amino acids) of a non-muscle tropomyosin gene. The central portion contains sequences encoding 25 hydrophobic amino acids, flanked by by six basic amino acids, a characteristic feature of a transmembrane domain. The 3' portion contains sequences encoding a tyrosine kinase domain which has extensive homology with retroviral tyrosine kinase oncogenes and with the EGF and insulin receptors, but is different from all known tyrosine kinases. These features suggest that onc D is a hybrid gene generated by a rearrangement involving a non-muscle tropomyosin gene and a tyrosine kinase gene, possibly one coding for a new growth factor receptor. Analysis of DNA from the original colon carcinoma and from normal tissue from the same patient revealed that the rearrangement took place specifically in the tumor tissue, indicating a direct involvement of onc D in the oncogenic process. Research sponsored by the National Cancer Institute, DHHS, under contract No. NO1CO-23909 with Litton Bionetics, Inc.

L164 ANALYSIS OF C-K-RAS IN LUNG CANCER REVEALS NO A-C, A-G POINT MUTATION AT POINT 61. Antonio Milici, Mark Blick and Jordan U. Gutterman, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Molecular characterization of the C-ras oncogene family indicates that they have acquired transforming ability through a single point mutation which alters the nucleotide sequence of either first or second exon of the gene and creates in some instances a new restriction enzyme cleavage site and a fragment length polymorphism (RFLP) detected by Southern blot analysis. To determine whether or not two possible point mutations, CAA-CGA and CAA-CGA, might be present at position 61 of the c-K-ras oncogene, we digested 30 fresh lung tumors using two restriction enzymes, AvaII and TaqI each of which recognizes the respective specific mutations. We detected no RFLPs in any of these fresh tumors which suggest that the CAA-CCA and CAA-CGA point mutations at position 61 of the c-K-ras oncogene are rarely present in fresh uncultured lung carcinoma. We have also extended our search for restriction enzymes, possibly useful in detecting somatic alterations in the ras family, to position 13 and 59 which are two additional codons where point mutations can confer transforming ability to c-ras. We have identified 2 restriction enzymes at position 13 and 59 of c-H-ras, 2 at position 13 and 59 of c-K-ras, and 1 at position 13 and 59 of N-ras. The use of these restriction enzymes will allow us to expand the analysis of specific point mutations that may occur in the c-ras oncogene family and will assist in understanding and better characterize the role of such mutagenic events in the development of human cancer.

L165 TRANSFORMATION OF C3H/10T1/2 FIBROBLASTS: DEPENDENCE UPON PLATELET-DERIVED GROWTH FACTOR (PDGF). Lawrence J. Mordan, Cancer Research Center of Hawaii, 96813.

Promotion of tumor formation in initiated mouse skin by TPA or wounding expose initiated cells to plasma and platelet polypeptide growth factors including somatomedin, platelet-derived growth factor (PDGF), type B transforming growth factor (TGF-B), and an epidermal growth factor (EGF)-like polypeptide. Transformation of C3H/10T1/2 fibroblasts by chemical carcinogens has been shown to depend upon the serum concentration of the culture medium. To examine the role of serum growth factors in the preneoplastic progression of initiated cells, C3H/10T1/2 fibroblasts initiated with methylcholanthrene were cultured in 10% plasma-derived serum (PDS) supplemented with an aqueous lysate of platelets. In the absence of platelet lysate (PL) initiated fibroblasts failed to transform. Reconstitution of the culture medium with 5% FBS or with enough PL in PDS to induce a mitogenic response equivalent to 5% FBS, restored the ability of initiated cells to transform, suggesting that the conversion of an initiated cell to a transformed cell is dependent upon one or more platelet factors. A serum-free medium containing defined amounts of PDGF, EGF, TGF-B and multiplication stimulating activity was developed which replicates the growth promoting ability of FBS. Transformation of initiated C3H/10T1/2 fibroblasts was dependent upon the PDGF concentration. Further experiments are being conducted to determine the roles in transformation of EGF and TGF-B. These results are interpreted as indicating that neoplastic transformation of fibroblasts is dependent upon molecular events induced in initiated cells by platelet polypeptide growth factors, especially PDGF.

L166 ROLE OF LOSS OF CHROMOSOME 15 IN SYRIAN HAMSTER TUMORS INDUCED BY V-HA-RAS PLUS V-MYC ONCOGENES, Mitsuo Oshimura, M. Koi, N. Ozawa, O. Sugawara, P. Lamb, L. Annab, T. Gilmer, J.C. Barrett, Natl. Inst. Environ. Hlth. Sci., Res. Tri. Prk., NC

Recently, it has been shown that normal, Syrian hamster embryo (SHE) cells can be neoplastically transformed by transfection with two cooperating oncogenes, myc plus ras. However, the number of genetic alterations necessary to convert a normal cell into a malignant cell is unknown. We have examined the cells from the tumors which formed following v-Ha-ras plus v-myc cotransfection and found that they had a nonrandom chromosome change, monosomy of chromosome 15. The tumors were also monoclonal in origin. These results suggest that ras plus myc were necessary but not sufficient for neoplastic transformation of normal SHE cells and that an additional, specific change was required. In order to clarify the role of monosomy 15, molecular and cytogenetic studies of hybrid cells and normal SHE cells have been made with the following results: 1) The tumorigenicity of these ras/myc tumors cells was suppressed by fusion with normal cells; 2) RNA expression of ras/myc oncogenes was observed even in the suppressed hybrids; 3) When the hybrid cells were passaged, anchorage-independent variants appeared in the cultures which was accompanied by morphological changes and tumorigenicity; 4) A nonrandom loss of chromosome 15 was observed in the transformed segregants of the hybrid cells. These results suggest that the loss of chromosome 15 results in the loss of a cellular gene which effects a phenotypic change necessary for neoplastic development.

L167 A CONSERVED NON-ENZYMATIC DOMAIN IN CYTOPLASMIC PROTEIN-TYROSINE KINASES IS IMPORTANT FOR TRANSFORMING ACTIVITY. Ivan Sadowski and Tony Pawson. Division of Molecular and Developmental Biology, Mt. Sinai Hospital Research Institute, 600 University Av., Toronto, Ont. M5G 1X5, Canada.

Protein-tyrosine kinases can be divided into two classes, transmembrane receptors and cytoplasmic proteins. Approximately 80 amino acids amino-terminal to the ATP-binding site of the conserved tyrosine kinase catalytic domain receptors possess a hydrophobic membrane-spanning region. At the analogous position in cytoplasmic tyrosine kinases is a higly conserved 40-residue sequence, which is found in the products of the src, fps, abl, yes and fgr genes. Dipeptide insertions in this region of Fujinami sarcoma virus P130gag-fps destroy both transforming ability and kinase activity. However expression of the kinase domain C-terminal to this secondary region of homology in bacteria yields enzymatically active fps polypeptides. We suggest that this non-enzymatic domain diagnostic of cytoplasmic tyrosine kinases folds with and regulates the catalytic domain. Peptide insertions apparently interfere with folding of the kinase-active region. The importance of this N-terminal region is indicated by its exact retention in viral transforming proteins in which a heterologous coding sequence is fused to a cytoplasmic tyrosine kinase domain (i.e. gag-abl, actin-fgr).

The use of high-level bacterial epxression to analyze structure-function relationships in the fps tyrosine kinase will be presented.

L168 CLONING OF A FULL LENGTH cDNA OF AVIAN pp60c−src AND THE ANALYSIS OF ITS ASSOCIATED PROTEIN AND PHOSPHATIDYLINOSITOL KINASE ACTIVITIES.

Helen Piwnica−Worms, David R. Kaplan, Malcolm Whitman and Thomas M. Roberts, Dana Farber Cancer Institute, Boston, MA 02115.

The cellular proto−oncogene src encodes pp60c−src, a tyrosine−specific protein kinase of 60K. The low level of pp60c−src in normal cells and the lack of an available cDNA has hindered a detailed analysis of its biochemical activities. To study the role of pp60c−src in normal cellular growth and differentiation and its involvement in polyoma virus transformation, we have constructed a recombinant murine retrovirus which efficiently transduces avian pp60c−src into murine cells. Several NIH−3T3 lines overproducing avian pp60c−src were isolated after infection with recombinant retroviral stocks. All lines appeared morphologically normal and none formed foci even at levels of pp60c−src 15 fold above normal endogenous levels. All lines were tested for their in vitro kinase activities, this included autophosphorylation of pp60c−src as well as phosphorylation of exogenous substrates (enolase and phosphatidyl inositol). These analyses were carried out in the presence and absence of polyoma virus middle t antigen (MTAg). We find polyoma virus MTAg is able to activate the kinase activity of avian pp60c−src in vitro. Finally, we have isolated the first cDNA of pp60c−src by the rescue fusion protocol. This cDNA has been cloned into an expression vector which directs the synthesis of enzymatically active pp60c−src in bacteria. We are currently co−expressing pp60c−src and polyoma virus MTAg in bacteria to study their interactions in greater detail.

L169 TRANSFORMATION OF RAT FIBROBLASTS BY Ela-myc CHIMERAS, Robert Ralston and J. Michael Bishop, G.W. Hooper Research Foundation and Department of Microbiology and Immunology, University of California, San Francisco, CA 94143

The myc oncogene and Adenovirus Ela region are functionally similar in their ability to "immortalize" primary cells and to cooperate with activated ras genes to cause neoplastic transformation. The proteins encoded by myc and Ela also display some structural similarity and weak sequence homology. This observation raised the possibility that the two proteins might function via similar mechanisms. Alternatively, this structural similarity might reflect requirements for processing and/or nuclear transport. We have investigated the structural relationship between the myc and Ela proteins by constructing chimeras. Portions of the two genes that had been shown to be biologically inactive by themselves using mutational or deletion analysis were fused to produce intronless chimeric genes. The chimeras were composed of portions of the first exon of Ad 5 Ela and the third exon of chicken or human c-myc. The chimeras were then examined for function in two contexts: cooperation with ras, and as recombinant retroviruses.

The Ela-myc chimeras were biologically active in both assays. When introduced into primary rat embryo fibroblasts together with ras, they produced large foci at a slightly lower efficiency than either myc or 12S or 13S Ela (gifts of N. Jones). Cells from these foci grew efficiently in soft agar and exhibited a very round, refractile morpholcgy similar to cells transformed by myc plus ras. In contrast, cells transformed by Ela plus ras were fibroblastic (12S) or epithelial/cubiodal (13S). Molecular analysis of the cells transformed by the chimeras showed transcripts that hybridized to both myc and Ela probes, and novel proteins that were precipitated by antisera or monoclonal antibodies to myc or Ela proteins. Chimeric proteins were also demonstrated by expression in COS-7

cells using replicating vectors. A Moloney MLV vector carrying one of the chimeras was capable of transforming Rat-1 cells to growth in soft agar. The colonies produced were similar to those observed with transformation by an MLV vector containing the v-myc oncogene (gift of A. Bruskin). However, the MLV chimera was less efficient in focus formation than MLV v-myc.

These results suggest that a functional protein can be reconstituted from portions of myc and Ela proteins that are inactive by themselves. Analysis of the properties of these chimeras may help to identify functional domains of the parental proteins.

L170 IDENTIFICATION OF THE N-myc GENE PRODUCT: Gary Ramsay and J. Michael Bishop, Department of Microbiology and G.W. Hooper Foundation, University of California, San Francisco, California 94143.

The N-myc gene was discovered by its shared nucleotide sequence homology with the previously characterized proto-oncogene c-myc. In order to further explore the resemblance between N-myc and c-myc, we have identified and characterized the N-myc gene product.

We immunized rabbits with a synthetic oligopeptide corresponding to a region near the amino-terminus of the N-myc protein. We obtained antisera that specifically precipitates a protein doublet of 65 kd (p65) and 67 kd (p67) from cell lines expressing high levels of N-myc RNA. In studies on cells transfected with a genomic clone of the N-myc gene, the antisera detects elevated levels of p65/p67 providing additional evidence that this doublet represents the gene product(s) of N-myc.

Comparison of the biochemical properties of the N-myc and c-myc gene products has revealed remarkable similarities. Both proteins have extremely fast turnover rates ($t_{\frac{1}{2}}$ = 30-40 minutes), are located predominantly in the nucleus, bind to DNA, and show the same discrepancy between the molecular weights predicted from deduced amino acid sequences and the apparent molecular weights as judged by electrophoretic mobility. These findings further establish functional relationships between these two genes.

L171 EXPRESSION OF THE p53 ONCOGENE IN HUMAN TRANSFORMED CELL LINES, Varda Rotter, Nic Nicholas Harris, David Wolf, Meron Prokocimer*, Naomi Goldfinger, Einat Brill and Orit Shohat, The Department of Cell Biology, The Weizmann Institute of Science, Rehovot, and *Beilinson Hospital, Petah-Tikva, Israel.

Human transformed cells have been found to express more than one species of the p53 proteins; which appear to be encoded by a single gene. To investigate whether differential expression of the single p53 gene was the result of transcriptional or post-translational control, a lambda gt 10 cDNA library was prepared from SV80 transformed fibroblasts and a number of full-length p53 cDNA clones were isolated. Construction of the clones in the pSP65 RNA transcription vector and employment of the SP6 bacteriophage RNA polymerase facilitated the generation of p53 transcripts, which were in turn translated in a cell-free system. This rapid transcription-translation assay provided the means for isolating cDNA clones that code for the individual p53 species expressed in vivo. One representative clone, p53-H-1, encodes a p53 protein physically indistinguishable from the smaller species synthesized by SV80 cells, whereas another clone, p53-H-19, codes for the larger p53 species expressed by these cells. While both of the in vitro synthesized proteins are immunoprecipitated efficiently with anti-p53 monoclonal antibodies, they exhibit variable partial peptide maps. Detailed restriction map analysis of these representative cDNA clones showed complete homology, except for variation in the 5' region. The possibility that the respective cDNA clones reflect the existence of different mRNA species as a consequence of differential initiation or alternative splicing is currently being studied.

L172 SPECIFIC BINDING OF THE MONONUCLEAR PHAGOCYTE COLONY STIMULATING FACTOR,CSF-1, TO THE PRODUCT OF THE v-fms ONCOGENE, Rosalba Sacca, E.Richard Stanley, Albert Einstein College of Medicine, Bronx, NY 10461 & Carl W. Rettenmier, Charles J. Sherr, St. Jude Children's Research Hospital, Memphis,Tenn. 38105

Cells transformed by the McDonough strain of feline sarcoma virus(SM-FeSV) express a v-fms-coded glycoprotein whose expression at the cell surface correlates with the transformed phenotype. The mouse mononuclear phagocyte growth factor, CSF-1, specifically binds to SM-FeSV transformed cells at high affinity sites indistinguishable from those detected on normal feline macrophages. A monoclonal antibody to a v-fms-coded epitope competed for CSF-1 binding to SM-FeSV-transformed cells, and chemical crosslinking demonstrated that murine CSF-1 bound to the v-fms gene product at the cell surface. Tyrosine phosphorylation of the v-fms gene products in membranes was observed in the absence of CSF-1 and was not enhanced by addition of the purified murine growth factor. Although SM-FeSV-transformed fibroblasts lines were found to secrete CSF-1 , effects of exogenous murine CSF-1 on the growth of transformed cells were not demonstrated. The fact that the v-fms-coded glycoprotein has a functional ligand binding domain for CSF-1 supports the hypothesis that the c-fms proto-oncogene product is related, and possibly identical, to the CSF-1 receptor, and suggests that the v-fms-associated kinase functions in the absence of an exogenous growth factor.

L173 EVIDENCE FOR THE EXPRESSION OF A MYC-PROTOONCOGENE HOMOLOG IN THE NATURALLY SYNCHRONOUS MITOTIC CYCLE OF PHYSARUM

Helmut W. Sauer, Gregory L. Shipley, Mark E. Christensen, Werner Nader, John Diller and Jung Choi, Texas A&M University, College Station, TX 77843

Evidence for a myc gene related function in Physarum includes cross-reactivity of the 1.5 kb v-myc DNA probe from MC29 virus with genomic Southern blots and a 4.5 kb fragment of PpM1 isolated from our genomic EMBL3 library; and with Northern blots of total RNA and poly (A) RNA. Furthermore, an anti-myc polyclonal antibody (obtained by K. Alitalo, Helsinki) specifically reacts with a 70 K nuclear protein from Physarum following Western-blotting and visualization with HRP-conjugated second antibody.

The level of myc-related RNA remains rather constant during the mitotic cycle. However, the steady state level of the nuclear 70 K protein fluctuates dramatically during the mitotic cycle from its absence in metaphase to a maximal level in late G_2-phase. We speculate that the myc-related protein enters a newborn nucleus, somehow represses the differentiation program of Physarum and activates late genes that are essential to initiate mitosis after reaching a threshold level.

L174 N-TERMINAL AND C-TERMINAL DELETION MUTANTS OF v-SIS DEFINE THE MINIMAL TRANSFORMING REGION AND DEMONSTRATE THAT DIMERIZATION IS REQUIRED FOR TRANSFORMATION, Monica K. Sauer, Mark Hannink and Daniel J. Donoghue, UCSD, La Jolla, CA 92093.

V-sis encodes the B-chain of platelet derived growth factor, PDGF. However, it also encodes additional amino acids at its N-terminus and C-terminus, which are not represented in the sequence data of PDGF. We have constructed a series of N-terminal and C-terminal deletion mutants in v-sis to define the minimum region required for transformation. C-terminal deletion mutants encoding truncated proteins up to 57 residues shorter than the wild type $p28^{v-sis}$ are capable of transforming cells. Correspondingly, an N-terminal deletion mutant in which the v-sis signal sequence is linked to residue #127 is capable of transformation. The minimal transforming region of v-sis defined by these endpoints has 15 residues missing at the N-terminus and 6 residues fewer at the C-terminus compared to the B chain. With regards to the C-terminal deletion mutants, there are only 10 residues separating the closest transforming and nontransforming gene products including 2 cysteine residues, #208 and #210. One or both of these residues is also necessary for dimerization of the v-sis gene product, since all gene products missing those residues are unable to form dimers. Similarly, at the N-terminus there are only 2 residues separating the closest transforming and nontransforming gene products. One of these is cysteine #127. Site directed mutagenesis of the 10 cysteine residues is currently being used to examine their roles in dimerization and transformation. These results demonstrate a strong correlation between dimerization and transformation by v-sis, as well as defining a minimal transforming region of v-sis which is smaller than the PDGF-B chain.

L175 GAG-ONC TRANSFORMING PROTEINS CONTAINING TWO DIFFERENT FATTY ACIDS, Alan Schultz and Stephen Oroszlan, National Cancer Institute-Frederick Cancer Research Facility, Frederick, MD 21701

Fatty acid components of transforming proteins have been shown to be relevant to the oncogenic potential of at least two oncogenes. In $p21^{ras}$, palmitate incorporated in an alkali-sensitive linkage (Sefton et al, Cell 31:465,1982) is essential for transformation (Willumsen et al, Nature 310:583,1984) and in $p60^{src}$ alkali-stable N-terminal myristate (Schultz et al, Science 227:427,1985) is required (Krueger et al, Mol.Cell.Biol.4:454,1984; Kamps et al, PNAS 82:4625,1985). We have previously shown that the *gag-ras* fusion protein $p29^{gag-ras}$ of rat sarcoma virus is a myristylated protein. In SDS-PAGE we now find that $p29^{gag-ras}$ migrates as a doublet, both members of which label with ^{3}H-myristate. However only the lower (mature protein) band labels with ^{3}H-palmitate. Hydrolysis and fatty acid identification confirm these observations. By a similar analysis we show that another oncogene protein, $gP180^{gag-fms}$ which is an integral membrane protein (Rettenmier et al, Cell 40: 971,1985), also contains two fatty acids. In addition to the incorporation of ^{3}H-myristate observed for both $gP180^{gag-fms}$ and its $p60^{gag}$ fragment, the *fms* sequence incorporates ^{3}H-palmitate. Since the *gag* sequences are myristylated and not glycosylated, $gP180^{gag-fms}$ apparently does not become glycosylated via a *gag* leader sequence but instead must possess an internal signal sequence. This suggests that membrane localization specified by the myristate of the *gag* sequences is incompatible and that cleavage of the *gag* sequences from *fms* sequences of $gP180^{gag-fms}$ may be necessary for *fms* function.

L176 Cloning and sequencing of the S13-transforming gene - a new oncogene.
Smith, D.R., Shaw, L.J., Enrietto, P.J., Vogt, P.K., Hayman, M.J.

S13 is an avian retrovirus which is acutely oncogenic in chickens causing fibrosarcomas and erythroid proliferation in the bone marrow that results in severe and fatal anemia. The gene product responsible for the oncogenic effects is a hybrid 155,000 Dalton transmembrane glycoprotein. This protein is post-transcriptionally cleaved into two parts, one of which (gp85) contains only env sequences while the other (gp70) contains tyrosine protein kinase activity.

A portion of the S13 genome from an integrated provirus was cloned from a rat cell line using an env-specific probe to select for the transforming region. A 2.5 kb region including the transforming gene was then sequenced. The completed sequence includes env and LTR sequences that are related to other avian retroviruses, however the env gene is interrupted by a foreign sequence of about 1.1 kb near the end of the gp37 coding region (this is the only known example of an env-linked oncogene). A search of the available protein and nucleic acid databases revealed a region of the gene with limited homology to other known tyrosine kinase transforming proteins (also egf and insulin receptor). However no extended homology with any other known gene or protein was found.

L177 MULTISTEP TRANSFORMATION OF C3H 10T½ CELLS: THE ROLE OF myc. Vincenzo Sorrentino, Vladimir Drozdoff, Louis Zeitz and Erwin Fleissner. Sloan-Kettering Institute, New York, New York 10021.

C3H 10T½ cells are a cell line widely used in studies of carcinogenesis. Transfection of a viral k-ras gene into these cells does not generally result in completely transformed cells. Transfer of myc into cells already harboring ras consistently causes an increased ability to grow in semi-solid medium. The growth of such cells is sensitive to the number of cells plated in agarose; conditioned medium from monolayer cultures stimulates the growth of these cells, but not of C3H cells minimally transformed by ras alone. These properties, together with the increased sensitivity to purified growth factor preparations observed in C3H cells to which myc genes alone have been transferred, support a role for the myc gene in the pathway of growth factor stimulated cell growth.

The effect of an active myc gene on radiation- or carcinogen-induced transformation of C3H 10T½ cells has also been studied. Data indicate that in the presence of an activated myc gene, under Mo-MuLV LTR transcriptional control, transformation frequencies are increased by an order of magnitude. These and the above results will be discussed in relation to multistep theories of malignant transformation.

L178 DETECTION OF ONCOGENE MESSENGER RNAs BY IN SITU HYBRIDIZATION, Gary Stoner, Yian Wang, Ming You, G. Colin Budd, Medical College of Ohio, Toledo, OH 43699

In situ hybridization techniques were used to identify RNA transcripts of viral oncogene (src gene) in a Rous sarcoma virus transformed rat cerebral endothelial cell line and cellular oncogene (ras gene) in a human ras gene transformed murine fibroblast cell line. Both cell lines were cultured at 37°C in DMEM medium containing 10% fetal bovine serum. The cells were fixed in acetic acid/ethanol (1:3) and ^{32}P-labeled v-src and v-Ha-ras cDNA probes were hybridized for 48 h at 25°C. The hybridizations were evaluated semiquantitatively by counting silver grains in the autoradiographic emulsion over the cells. It was found that the majority of the transformed rat cerebral endothelial cells contained src gene mRNA, and nearly all of the transformed murine fibroblasts contained ras gene RNA transcripts. Both mRNAs were present in significant, but highly variable, amounts. The results of the present study indicate that in situ hybridization can be used to detect and quantitate both viral and cellular oncogene mRNAs in malignant tumor cells and tissues. Further application of this technique to oncogene studies is under investigation.

Supported by NCI Grant No. CA 28950

L179 THYROID HORMONE REGULATION OF THE LEVEL OF K-RAS PROTO-ONCOGENE RNA CORRELATES WITH ACTIVATION OF K-RAS BY METHYLCHOLANTHRENE (MCA). James E. Thomas and Duane L. Guernsey, Department of Physiology and Biophysics, University of Iowa, Iowa City, IA 52242

It has previously been shown that thyroid hormone regulates neoplastic transformation in vitro induced by x-rays, benzo(a)pyrene and N-methyl-nitro-N-nitroguanidine. In an attempt to elucidate the mechanism of thyroid hormone effect we are addressing the hypothesis: that thyroid hormone regulation of the expression of the proto-oncogene at the time of exposure to the carcinogen will determine the susceptibility of the gene to mutation, and subsequent activation. MCA transformation of C3H/10T 1/2 cells has been demonstrated to activate the k-ras oncogene (Parada & Weinberg, 1983, Molec. Cell. Biol. 3:2298-2301). We report here that thyroid hormone (T_3) modulates MCA transformation of C3H/10T 1/2 cells in a dose-dependent manner. Additionally, the T_3 dose-related transformation frequency correlates closely to the T_3 close-related levels of k-ras proto-oncogene RNA in the C3H/10T 1/2 cells. These data support the hypothesis that T_3 regulation of k-ras proto-oncogene expression at the time of exposure to MCA regulates the activation of the oncogene and subsequent cell transformation.

L180 LARGE SCALE PRODUCTION OF ACTIVE, RECOMBINANT HUMAN PLATELET DERIVED GROWTH FACTOR. Arlen Thomason, Margery Nicolson, Ming Hu, Julia Tseng and Renee Yew; Amgen, Thousand Oaks, CA.

We have used recombinant DNA techniques to express and purify human platelet-derived growth factor (PDGF) in quantities heretofore unattainable. The final product is >99% pure; no contaminants are detectable using sensitive methods. Amino acid sequence analysis confirmed that the protein is bona fide human PDGF. It reacts with antisera to PDGF purified from human platelets, as well as with antisera to synthetic peptides based on the known PDGF amino acid sequence. It competes with PDGF from platelets in a receptor binding assay. The mitogenic activity of the recombinant PDGF is equal to or greater than PDGF from platelets in the NIH3T3 cell bioassay. The active protein is a heat-stable 31,000 MW dimer; reduction yields an inactive 15,500 MW monomer. Availablility of large amounts of this important human growth factor will allow extensive studies of its biological properties and effects.

L181 ACQUISITION OF METHIONINE-DEPENDENCE ASSOCIATED WITH TRANSFORMATION BY THE ACTIVATED ONCOGENE c-Ha-Ras. Luc vanhamme and Claude Szpirer, Free University of Brussels, Departement de Biologie Moléculaire, 67,rue des Chevaux, 1640 Rhode-St-Genèse.Belgium

Methionine-dependence is a metabolic defect exhibited by some malignant human or animal established cell lines; this defect is defined as the inability of cells to grow in a culture medium where methionine has been replaced by its immediate metabolic precursor, homocysteine. We show here that acquisition of methionine-dependence can be associated with transformation by an activated cellular oncogene.
We transfected clone 9-3, a subclone of an epitheloid cell line established from normal rat liver with pSV2-Neo-EJ; clone 9-3 is a methionine-independent cell line; pSV2-Neo-EJ is a plasmid carrying the cellular human oncogene Ha-Ras activated in the cell line EJ and a bacterial gene conferring resistance to G418 (this plamsid was kindly given to us by Chris Marshall).
We isolated 10 G418-resistant clones; seven of them were demonstrated to be transformed and grew in soft agar while the three other ones were anchorage-dependent. The integrity of the transfected oncogene has been analyzed by Southern blot. Its expression has been confirmed by Northern blot analysis. We found that the seven transformed clones became methionine-dependent while the three normal clones remained methionine-independent (like the parental clone 9-3). In conclusion, our results show a perfect correlation between transformation by a activated oncogene and the acquisition of a methionine metabolism defect.

L182 Characterization of the c-myc protein, Rosemary Watt[1], Cecelia Green[1], Philip Schein[1] and Neil Sullivan[2], Smith Kline and French Laboratories, Philadelphia, PA 19101[1] and Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724[2]

To investigate the role of the c-myc protein in growth regulation and cell differentiation, the entire coding sequence of the human c-myc gene has been expressed in E.coli and the protein product of the gene has been purified. The gene product is a DNA binding protein which, after microinjection into the cytoplasm of mammalian cells, rapidly localizes to the nucleus. This localization is not prevented by treatment of the cells with inhibitors of transcription or translation or with blockers of electron transport. The protein appears to localize in discrete granules within the nucleus which are non-nucleolar. We have demonstrated that the c-myc protein synthesized in E.coli is functionally active by co-microinjection of the myc protein and the activated ras protein into primary rat embryo fibroblasts which resulted in transient focus formation. We are investigating the domains of the myc protein required for the nuclear localization of the protein and for its interaction with ras and we will determine whether these functions can be blocked by treatment with anti-myc antibodies.

L183 ANTI-RAS MONOCLONAL ANTIBODY INHIBITS GUANINE NUCLEOTIDE BINDING IN VITRO, Maureen O. Weeks and Andrea Fattorossi, Laboratory of Tumor Immunology and Biology, NCI, Bethesda, MD 20892 and Rep. Igiene ed Immunologia, CSRMA, Rome, Italy

Ras oncogenes and their translational products have been implicated in the multistep process of carcinogenesis by virtue in a variety of systems. Since the only known function of the ras gene product p21 is its ability to bind and hydrolyze guanine nucleotides it is particularly striking that point mutations affecting codon 12 have also been shown to abrogate p21 GTPase activity. Therefore, any agent capable of blocking the guanine nucleotide binding site on the p21 molecule might also effect its biochemical properties and therefore its biological activities. Mulcahay et al. (1985) showed that one anti-ras antibody Y-259, but not a second, Y-238 (Furth et al, 1982), was able to neutralize the transforming activity of a coninjected cellular ras protein. Therefore we have attempted to demonstrate that Y-259 recognizes a determinant at or in close proximity to the guanine nucleotide binding site of p21. In a modified ^{3}H-GDP binding assay using detergent disrupted HaNIH cells as a source of p21 it can be shown that GDP binding by extracts preabsorbed by Y-259 is 22% of that exhibited by unabsorbed extracts; neither a related anti-v-Ha-ras antibody Y-172 nor controls, rat IgG or antibody MOPC-21, interfere with the ability of p21 to bind GDP in these assays. Since low levels of GDP binding by p21 can be detected in the presence of Y-259 alone, this suggests that the nucleotide binding site on p21 and the epitope recognized by Y13-259 are not identical, but rather that the sites are situated such that binding of MAb Y13-259 makes successive binding of guanine nucleotides difficult.

Enhancers, Promoters and Transacting Elements
Use of Genetic Variants to Find Genes for Growth Regulation or Transformation
Genetics of Multistage Carcinogenesis in Mice and Humans

L184 The evidence and evaluation of epidermal growth factor receptors (EGF-R) and EGF like factors (EGF-F) as prognostic agents in ovarian carcinomas, Thomas Bauknecht 78 Freiburg, Hugstetterstr. 55, Uni-Frauenklinik, FRG.

EGF-R can be detected in spontaneously originated ovarian carcinomas. These malignant tumors contain also EGF-F. The purpose of this paper was to investigate the physico-chemical properties and prognostic values of EGF-R and EGF-F in advanced ovarian carcinomas. EGF binding and the content of EGF-F were measured by a radioreceptorassay. EGF-F was further analysed by isoelectric focusing. 35% of ovarian carcinomas (n=100) are EGF-R positive. The correlation of EGF-R status with clinical data showed no differences to tumor stage, histological and an inverse correlation to steroidhormonereceptor status. Significant differences were noticed in the responserate of tumors to chemotherapy with 52% responders in EGF-R positive and 14% responders in EGF-R negative group. The mean survival time of patients with EGF-R positive carcinomas was 27 months, in EGF-R negative group 17 months. In extracts of these tumors and normal ovaries EGF-F were detected. 18/42 tumors contained high factor levels of 6-17 EGF units per mg protein. In nonmalignant tissues factor levels did not exceed 6 ng EGF units. High factor levels were mainly found in EGF-R negative carcinomas. These patients had also a poor prognosis. The tissue extract originated EGF-F obviously consist of several components as demonstrated by isoelectric focusing. In malignant tissues an additional peak of EGF-F activity at pH 6.8 was detected. In contrast to native EGF the extracted EGF-F stimulate the growth of arrested 3T3 cells in culture. We hope to clarify by further experiments the mechanism of EGF-R and EGF-F interaction with the influence to clinical prognosis of ovarian carcinomas.

L185 FURTHER EVIDENCE THAT PROLACTIN MAY BE AN ENDOGENOUS TUMOR PROMOTER, Arthur R. Buckley, Charles W. Putnam and Diane H. Russell, University of Arizona, Department of Pharmacology, and Veterans Administration Medical Center, Tucson, AZ.

Prolactin (PRL), in many target tissues, rapidly induces ornithine decarboxylase (ODC) and plasminogen activator (PA), markers of cell-cycle progression elicited by mitogens, tumor promoters and other growth stimulatory substances. These observations led to the hypothesis that PRL may be a general tumor promoter. S/D rats received 102mg/kg diethylnitrosamine (DEN), followed by thrice weekly ovine-PRL (11mg/kg for 6 wks or 5.5mg/kg for 23 wks). Organ/body weight ratios, hepatic γ-glutamyltranspeptidase (GGTase) activity and development of GGTase-positive foci were assessed. Ovine-PRL for 6 wks with and without DEN increased liver to body weight ratio ($p<0.01$). Increased GGTase activity ($p<0.001$) and an increased number of GGTase-positive foci were demonstrated. Twenty-three wks of PRL further increased GGTase activity ($p<0.01$), and positive foci. In rats given PRL at 12h intervals for 48h, hepatic DNA synthesis was 182% ($p<0.05$) of controls. Hepatic cytochrome P-450 at 24h, also was induced ($p<0.001$). We conclude that the significant hepatic hypertrophy and hyperplasia in response to PRL indicate that it is a potent liver mitogen. PRL administration following initiation increases both GGTase and P-450. These data strongly suggest that PRL is a promoter of initiated hepatocytes. Since hyperprolactinemia is common in humans, we suggest that PRL may accelerate the timing and increase the incidence of certain tumors in man.

L186 ONCOGENIC TRANSFORMATION OF FIBROBLASTS BY DNA FROM WALKER CARCINOMA MITOCHONDRIA. Néstor González-Cadavid, Francisco Arvelo, Olga Antuña and José L. Pérez, Universidad Central de Venezuela, P.O. 21201, Caracas, Venezuela.

We have reported the oncogenic transformation of NIH3T3 fibroblasts by transfection with total DNA from ascites cells of the rat Walker carcinosarcoma. Now we found that DNA isolated from DNAse/RNAse treated mitochondria elicits a similar response in these cells and in non-immortalized rat embryo fibroblasts (REF cells). DNA from the top band in CsCl/EtBr gradient where no bottom band was detectable (mtDNA II*), as well as its Hind III fragments cloned in plasmid HSV 106 (pMT II*), induced oncogenic transformation more efficiently than total DNA. This was shown by foci in monolayer cultures, colonies in soft agar, TGF activity in the conditioned medium, chromosome polyploidy, and tumorigenicity in the chorioalantoic membrane of the chicken. The bottom DNA band containing closed circles (mtDNA I), isolated by a modified procedure, induced a reversible transformation followed by cell degeneration at 13 weeks. This DNA has Eco RI, Hind III, and Bam HI restriction patterns resembling those of rat liver mtDNA, without visible contaminants. No viral particles were seen by electron microscopy. However, sequences homologous to a retrovirus LTR probe were detected by Southern analysis. Total DNA and RNA from immortalized cells hybridized with this probe and with a feline c-myc oncogene, whereas REF cells gave weaker signals. The respective nuclear DNAs are being examined for the possible presence of integrated sequences,using both LTR and mtDNA I probes,to determine whether the transforming activity is located in the mitochondrial genome and transposes to the nucleus after transfection.

L187 DIFFERENTIAL ACTIONS OF N-METHYL,N'-NITROSOUREA AND PHORBOL MYRISTATE ON PANCREATIC TUMORIGENESIS IN YOUNG AND SENESCENT MICE, Jay Zimmerman, Karen Zelinsky-Papez and Timothy Carter, St. John's University, New York, New York 11439.

Our previous studies have reported that 20% of aged (22 mo), but no young (4-6 mo), C57Bl/6J mice developed pancreatic acinar cell carcinoma 5 months after exposure to a single i.p. dose of 37.5 ug/gm of N-methyl-N'-nitrosourea (NMU). We have sought to minimize the potential effects of age-related immunological differences on the tumor frequency by exposing the animals to NMU in vivo and removing the pancreases to tissue culture 6-8 weeks later. In addition, the tumor promotor 12-O-tetradecanoyl phorbol-13-acetate (TPA) was added to some of the cultures during outgrowth. Cell type and transformation were determined by a combination of morphologic and growth characteristics, isozyme patterns and lectin staining. No cells could be established in culture from young mice treated with NMU alone (0/19), whereas 21% of NMU-treated old mice (3/14) gave rise to transformed lines with acinar cell characteristics. TPA in culture allowed 27% of the pancreases from NMU-treated young mice (4/15) to give rise to transformed acinar lines, whereas the promoter did not increase the frequency of cell outgrowth from NMU-treated old mice (3/13). Eight cloned lines (4 young and 4 old) all expressed c-myc RNA by "Northern" blot analysis, but in differing amounts. Taken together, the results suggest that age-related factors may play a significant role in the response of an individual to both carcinogenesis and promotion at the cellular level.

L188 SUBVERSION OF STRUCTURE AND FUNCTION OF THE EGF RECEPTOR, Stella Clark, John Haley+, Lindsey Owen, Nigel Whittle, Clive Dickson, Michael Waterfield and Axel Ullrich. Protein Chemistry Laboratory, Imperial Cancer Research Fund, London, WC2A 3PX, UK and + Department of Molecular Biology, Genentech, South San Francisco, California 94080, USA.

The expression of a truncated EGF receptor by the Erb-B oncogene in some way mediates transformation by Avian Erythroblastosis Virus (AEV). To probe the structure, function and transforming potential of the receptor, a full length human receptor cDNA has been constructed from overlapping portions of cDNA clones, and this cDNA has been employed to generate a variety of constructs which can be used to express normal or mutant receptors in vitro or in transfected cells.

A number of different mutant receptors have been generated by deletion or site specific mutagenesis. The function of such normal or mutant receptors has been studied by several techniques. Receptor RNA generated by transcription for the Sp6 promoter and capped in vitro can be translated in reticulocyte lysates with microsomal membranes to give 170,000 MW glycosylated receptor. Through the microinjection of such RNAs the expression of receptors in oocytes, fibroblasts and NR6 (which lack EGF receptors) cells is being investigated. Studies employing various mutant cDNAs in retroviral vectors are being used to explore the effects of specific autophosphorylation site mutations on the transformation capacity of the receptor.

L189 SKIN TUMOR PROMOTION SUSCEPTIBILITY IN INBRED MOUSE STRAINS, John DiGiovanni, Kristine J. Chenicek and M. Winnann Ewing, The University of Texas, Science Park-Research Division, Smithville, TX. 78957

Tumor promotion appears to be a major determinant in susceptibility of mice to chemical carcinogenesis. Several inbred mouse strains (DBA/2, C57BL/6 and C3H have been compared with SENCAR mice for their sensitivity to skin tumor promotion. 12-O-tetradecanoylphorbol-13-acetate (TPA) did not promote skin papillomas in C57BL/6 mice. Conversely, DBA/2, C3H and SENCAR mice responded to TPA promotion. To examine the inheritance of susceptibility to skin tumor promotion, sensitivity to TPA promotion is being examined in B6D2F_1, D2B6F_1 and B6C3F_1 mice. Preliminary data in the B6D2F_1 hybrid suggests susceptibility is a dominant trait since these hybrids are highly sensitive to TPA promotion. The number of genes controlling susceptibility to skin tumor promotion in DBA/2 and C3H mice is being examined in backcross and F_2 mice. These experiments are currently in progress. Finally, a number of histological evaluations have been performed. SENCAR, DBA/2, C3H and B6D2F_1 mice responded with a slight to moderate hyperplasia after single applications of TPA, whereas with multiple applicatons (i.e., 4 or more) all 4 mouse lines responded with a potentiated hyperplasia. C57BL/6 mice on the other hand, responded with only a slight hyperplasia after both single and multiple applications of TPA. Examination of the other mouse lines is in progress. The results to date suggest that promotion susceptibility is directly correlated with sustained epidermal hyperplasia in response to phorbol esters. Supported by USPHS Grant CA 38871.

L190 DIFFERENT GROWTH STIMULATION BY TCPOBOP OF NDEA-INITIATED LIVER NODULES IN B6C3 AND B6C MICE. T.A. Dragani, G. Manenti and G. Della Porta - Istituto Nazionale Tumori, Milan, Italy.

Spontaneous incidence of hepatocellular tumors is high in (C57BL/6J x C3Hf)F1 (B6C3) mice and low in (C57BL/6J x BALB/c)F1 (B6C) mice. Both hybrids were treated with a single dose of diethylnitrosamine (NDEA) at 1 week of age followed by 1,4-bis 2-(3,5-dichloropyridyloxy) benzene (TCPOBOP), a phenobarbital-like promoter of liver carcinogenesis, or by vehicle, and sacrificed at 30 weeks of age. The frequency of hepatocellular nodules was similar in the two hybrids. However, the mean volume of nodules (MVN) was about 10 fold greater in B6C3 than B6C male mice (0.713 vs. 0.055 mm^3) receiving NDEA plus vehicle, whereas the treatment with TCPOBOP after NDEA resulted in a MVN of 3.303 mm^3 in B6C3 and 0.450 mm^3 in B6C males. No differences in MVN were seen in the females of both hybrids after NDEA and vehicle, whereas upon NDEA and TCPOBOP the MVN was 25-fold greater in B6C3 than in B6C females (1.355 mm^3 vs. 0.054 mm^3). TCPOBOP alone induced the same level of DNA synthesis and of aminopyrine-N-demethylase in the liver of both hybrids. Therefore, the different susceptibility to hepatocarcinogenesis in B6C3 and B6C mice may be related to a higher susceptibility of B6C3 than B6C initiated liver cells to growth stimulation.

L191 **RETROVIRAL ACTIVATION OF THE INTERLEUKIN-2 (IL-2) GENE IN A GIBBON APE T-CELL LINE** D.B. Durand, M. Kamoun, C.A. Norris, J.S. Greengard, J.A. Kant, and G.R. Crabtree. Departments of Pathology, University of Pennsylvania and Stanford University.

MLA is a Gibbon Ape Leukemia Virus (GaLV) infected T-cell line derived from the lymphosarcoma of a gibbon ape. The line constitutively expresses IL-2 and its receptor, implying an autocrine mechanism of malignant cell growth.

Southern analysis (S.J. Chen et al., PNAS, in press) defined two sites of rearrangement in the MLA IL-2 gene, one at the 5' and one at the 3' end. Sequencing of IL-2 cDNA clones revealed a GaLV insertion in the 3' IL-2 non-translated region which results in a lengthened IL-2 transcript terminating in the viral long terminal repeat (LTR) (Ibid). By Northern analysis the predominant MLA IL-2 mRNA is longer than that of normal gibbons.

To further define the extent of the 3' insertion and to determine the nature of the 5' rearrangment we obtained cosmid clones of the MLA IL-2 alleles. The clones define a normal allele, and an abnormal allele with 5' and 3' GaLV insertions. The 3' insertion within the IL-2 non-translated region is a provirus with both LTR's intact, but with a 3 kilobase (kb) internal deletion. The LTR's are in the same transcriptional orientation as the IL-2 gene. The 5' insertion is located approximately 1.4 kb 5' to the first exon of the IL-2 gene and is a complete LTR alone, with a transcriptional orientation opposite that of the IL-2 gene. Of note is the presence of a 94 bp direct repeat in the LTR's of the 3' (Ibid) and 5' insertions. Such direct repeats have been shown to have enhancer activity; hence, possible mechanisms of activation of the IL-2 gene include enhancement of transcription by the LTR's of the 5' and 3' insertions and/or stabilization of the lengthened mRNA transcripts resulting from the 3' insertion.

L192 ALTERED DIFFERENTIATION AND CHROMOSOMAL CONSTITUTION DURING EPIDERMAL CELL TRANSFORMATION IN VITRO, Norbert E. Fusenig, Rule Dzarlieva-Petrusevska and Dirk Breitkreutz, Div. Differentiation and Carcinogenesis in vitro, Inst. of Biochemistry, German Cancer Research Center, 6900 Heidelberg, F.R.G.

Sequential phenotypic and genotypic changes occurring at different stages of spontaneous cell transformation were studied in primary cultures of newborn mouse skin keratinocytes. After a phase of adaptation to continued growth in vitro, cells acquire immortality and grow in sequential subcultures at high and later clonal density. The cell lines exhibit malignant growth behaviour (invasion, tumorigenicity and formation of metastases) either at earliest subcultures (< 10) or at later passages (> 20). During adaptation cells accumulate structural and numerical chromosomal alterations. Transformed cell lines are aneuploid at earliest passages with characteristic under-(7,14) and overrepresentation (5,6) of chromosomes. Cell line specific and stable marker chromosomes (structural aberrations) are present in early subcultures including HSR and DM or are acquired later coincident with tumorigenicity. All cell lines exhibit distinct alterations in the expression and regulation of keratinization, but the extent of altered differentiation is not directly correlated to the degree of malignancy. It is assumed that early stages (adaptation and immortality) of transformation are caused by numerical chromosomal changes with the consequence of gene imbalance. By further genetic changes (visible as structural chromosomal alterations) critical alterations in growth and differentiation control are induced leading to malignant growth behaviour.

L193 DNA POLYMORPHISMS AND CHARACTERIZATION OF GENES ACTIVE DURING PROMYELOCYTE DIFFERENTIATION, Richard A. Gatti, Roy Shaked, Richard C. Davis, Chuan-Chu Chou and Winston Salser. UCLA, Departments of Pathology and Biology, Los Angeles, CA 90024.

We are characterizing genes regulated during cell differentiation and identifying associated DNA polymorphisms. A cDNA library was constructed from mRNA species which were up- or down-regulated during differentiation of HL60 (promyelocytic leukemia) cells to granulocytes (with DMSO and amiloride) or macrophages (with phorbol esters). The majority of regulated clones so far identified have been members of large gene families. These include genes for the heavy- and light-chains of ferritin, alpha-tubulin, and beta- and gamma-actin. All regulated clones were used to probe Southern blots containing genomic DNA from Caucasians, AmerIndians, Orientals, Blacks and HL60 cells. The DNAs were digested with one of four restriction enzymes: Eco RI, Bam HI, Msp I or Taq I. We identified eighteen polymorphisms. These include three polymorphisms with a ferritin heavy-chain probe (mHL217): 217/Bam/6 kb, 217/Taq/6 kb and 217/Msp/1.2 kb. A 1.7 kb polymorphic Taq I fragment was observed with a ferritin light-chain probe (mHL227). Two polymorphisms were found with an alpha-tubulin probe (mHL1301): 1301/Msp/2.2,2.0 kb and 1301/Taq/3.2,2.9 kb. Using a beta-actin probe (mHL1216), three polymorphisms have been noted: 1216/Bam/8,5,3.4 kb, 1216/Taq/1.0,0.5 kb and 1216/Eco/4.7 kb. These polymorphisms segregate as Mendelian genes. Further studies are underway with large families in the CEPH consortium to characterize gene frequencies and to identify the chromosomal locus of each polymorphic fragment.

L194 ONCOGENE ACTIVATION IN RODENT TUMORS INDUCED BY DIRECT-ACTING CARCINOGENS, Anne E. Hochwalt and Seymour J. Garte, New York University Medical Center, NY, NY 10016

Beta-propiolactone (BPL) and dimethylcarbamyl chloride (DMCC) are potent, well characterized carcinogens in several animal model systems. DNA from 1 of 2 mouse squamous cell skin carcinomas, 1 of 4 fibrosarcomas, and 3 of 4 rat nasal epithelial carcinomas induced by BPL were positive in the NIH3T3 transfection assay. A focus from a mouse carcinoma DNA was picked, and DNA from these cells was transfected into rat-2 fibroblasts in a secondary round of transfection. Foci were picked, the DNA was extracted and analyzed by Southern blot hybridization. A mouse derived exogenous Bam HI restriction fragment of H-ras was seen in addition to the endogenous rat sequences. A functional restriction map of the activated H-ras gene showed loss of transforming activity after Pst I digestion, but not after digestion with Eco RI, Bam HI or Hind III. Structural mapping of the gene has also been done. DNAs from 10 DMCC induced mouse squamous cell skin carcinomas, 5 fibrosarcomas, and 2 rat nasal epithelial carcinomas were negative in transfection. DNA from the 2 rat nasal epithelial carcinomas were tested in the nude mouse cotransfection assay. Neomycin resistant colonies were grown in the presence of geniticin (1.2 µg/ml) until colonies formed. Colonies were grown to mass culture, pooled, and injected into nude mice at 1 X 10^7 cells per mouse. A nude mouse tumor that arose from the injected cells was excised, and part was used to develop a tumor cell line. Southern blot analysis of the tumor DNA has not yet detected activation of a known oncogene. The animal model systems used here are valuable, because they can elucidate the relationship between species, tissue, and/or carcinogen specificity, and oncogene activation.

L195 CHANGES IN GENE EXPRESSION BY TEMPERATURE SHIFT IN TS-SRC TRANSFORMED RAT FIBROBLASTS. Detlev Jaehner and Tony Hunter, Salk Institute San Diego,CA,92138

The viral protein tyrosine kinase $pp60^{v\text{-}src}$ of the mutant avian sarcoma virus ts LA23 is active at 34°C but inactive at 39°C. Rat NRK cells infected with ts LA 23 virus,LA23 NRK, can be arrested in cell growth by serum starvation at 39°C. Addition of serum at 39°C or temperature shift to 34°C without medium change both induce the reinitiation of the cell cycle, resulting in DNA synthesis 8 to 12 hr later.To study this mitogenic activity of $pp60^{v\text{-}src}$ we are trying to identify genes that are rapidly induced by temperature shift of arrested LA23 NRK cells. We have constructed a c-DNA library enriched for those mRNAs. Clones of interest will be identified by comparative hybridization to RNA from both growth states and will be analyzed in further detail.

L196 DETECTION OF DOMINANT TRANSFORMING GENE(S) IN RADIATION INDUCED MOUSE SKIN TUMORS, Deborah Jaffe and G. Tim Bowden, University of Arizona Health Sciences Center, Tucson, AZ 85724

The objective of the current study is to investigate the role of oncogene activation in radiation-induced carcinogenesis. We have selected the two-stage mouse skin model for our studies because, 1) induction of tumors has been shown to occur with both chemical and physical agents, and 2) it is possible to isolate tissue at several defined biological stages of tumorigenesis, and this offers an opportunity to study progressive changes which occur during discrete stages of tumor development. The dorsal skin of female Sencar mice (5-7 wks) was irradiated with a 4 MeV linear accelerator using x-rays at a dose rate of 0.31 Gy/ min. Animals received a single dose of ionizing radiation ranging from 0.5 to 11.25 Gy followed two weeks later by twice-weekly applications of 8 nmoles of 12-O-tetradecanoyl phorbol-13-acetate (TPA). There was a significant increase in tumor incidence in animals that were irradiated and promoted over control groups. In order to examine the role of oncogenic activation, DNA was isolated from both benign and malignant tumors as well as from normal skin. The DNA was used in NIH/3T3 transfection assays to identify dominant transforming genes. Papillomas, squamous cell carcinomas, adenocarcinomas and basal cell carcinomas were all positive in the NIH/3T3 transfection assay. DNA isolated from normal and TPA-treated epidermis was negative. We are currently investigating the identity of the dominant transforming gene(s). Preliminary evidence suggests that the family of ras genes is not actively involved in the transformation event. Supported by NIH grant CA-40584.

L197 CHARACTERIZATION OF THE PROTO-ONCOGENE c-myb FROM DROSOPHILA MELANOGASTER: Alisa L. Katzen[1,2], Barry Drees[1], Thomas Kornberg[1], and J. Michael Bishop[2], [1]Department of Biochemistry and Biophysics, and [2]Hooper Foundation, University of California, San Francisco, California 94143.

Eukaryotic organisms contain a proto-oncogene c-myb that encodes a nuclear protein. Its functions in the cell and in the organism as a whole are not well understood. Studying this gene in Drosophila, however, provides us with a genetically manageable multicellular organism in which to approach these questions.

We have examined the genome of Drosophila melanogaster and identified the presence of a single myb-related gene. Sequence analysis of genomic clones revealed that the Drosophila c-myb gene shares a conserved domain with the chicken c-myb gene of at least 125 amino acids in which 73% are identical. This conserved domain lies within an open reading frame capable of encoding a protein of at least 55,000 daltons. In order to further define the structure and sequence of the Drosophila c-myb gene, cDNA clones have been isolated and analysis is in progress.

The Drosophila c-myb gene maps to chromosomal location 13F on the X-chromosome. Based upon this information, we have obtained D. melanogaster strains suitable for the generation of Drosophila myb mutants. The mutagenesis strategy will be discussed. The myb gene is transcribed throughout Drosophila development as a polyadenylated RNA species of ca. 3.2 kb. We are now undertaking experiments to determine whether the Drosophila c-myb gene is expressed in a tissue specific-manner.

L198 ONCOGENES IN HUMAN MAMMARY CARCINOMA. S. Kozma, K. Buser, R. Jaggi, B. Groner and N. Hynes. Ludwig Institute for Cancer Research, Inselspital, 3010 Bern, Switzerland.

We are characterizing genes which are involved in the malignant transformation of human mammary gland cells. The assay involves transfecting DNA isolated from human mammary tumors into NIH/3T3 cells and then testing the transfected cells for tumorigenicity in nude mice. The human mammary carcinoma DNAs are from three sources: a cell line (MDA-MB231), a breast carcinoma metastasis (HM347) and human breast carcinomas growing as xenografts in nude mice (obtained from H.H. Fiebig, Freiburg, BRD).

Two different tumors arose from NIH cells transfected with MDA-MB231 DNA. One tumor contains at least 50 copies of the human Ki-ras gene. The original cell line contains a single copy of the Ki-ras gene. The second tumor does not contain a human ras oncogene. This tumor DNA has been used in secondary and tertiary transfections and the resulting tumors show similar patterns of human repetitive DNA containing restriction enzyme fragments. These fragments are currently being cloned in a λ vector and are being tested for their homology with other known oncogenes.

The nude mouse tumors induced by NIH cells transfected with HM347 DNA contain a single copy of the human N-ras gene. We are currently analyzing this gene with specific probes to determine if the original HM347 DNA contains an activated N-ras gene.

Nude mouse tumors have also been induced by NIH cells transfected with different xenograft DNAs. These tumors appear not to contain members of the human ras gene family and they are currently being analyzed in a second round of transfection.

L199 CELL CYCLE REGULATORY ELEMENT OF A HAMSTER HISTONE GENE . Amy S. Lee, Alexander Artishevsky, Scott Wooden, and Steven Wells. Department of Biochemistry, Univ. of Southern California, School of Medicine, Los Angeles, CA 90033. Our laboratory is interested in the molecular control mechanisms by which cells regulate the temporal events during the cell cycle. Our approach to identify the elements of the mammalian genome which control the cell cycle expression of specific sets of genes utilizes the well-studied histone gene system. To test the hypothesis that sequences in or around cell cycle regulated histone gene may represent specific control elements necessary for the temporal regulation of transcription of this gene, we constructed hybrid genes in which the 5' sequence of a hamster H3 gene was fused to the bacterial neomycin-resistance gene (neo). Upon transfection into the hamster fibroblast cell line, K12, the hybrid genes exhibited cell cycle dependent regulation which was evidenced by the maximal accumulation of the neo transcripts during the S phase. In addition, cells arrested In G1, as a consequence of the K12 temperature sensitive mutation, produced significantly lower levels of the neo mRNA. Deletion analysis of this regulatory sequence is in progress to locate the the cell-cycle regulatory element.

L200 MUTATIONS IN THE CYTOPLASMIC KINASE DOMAIN OF THE EGF-RECEPTOR: EFFECTS ON INTERNALIZATION AND LATERAL MOBILITY OF THE RECEPTOR. Etta Livneh, Morris Benbenisty, Ron Prywes and Joseph Schlessinger. The Weizmann Institute of Science, Rehovot 76100, Israel.

The EGF receptor is a 170,000 dalton membrane glycoprotein, that is composed of three major functional domains; an extracellular EGF-binding domain, a hydrophobic transmembrane region and a cytoplasmic kinase domain. EGF binds to dispersed receptors which rapidly cluster and become internalized via coated pits regions, delivering the occupied receptors to lysosomes where both EGF and the receptor are degraded. We have addressed various questions concerning the mechanism of action of the EGF receptor and its dynamic properties by creating EGF-receptor mutants with increased deletions in the C-terminus cytoplasmic kinase domain, including mutants devoid of Thr654 - the kinase C major phosphorylation site. The role of C-terminus autophosphorylation sites, the kinase activity and Thr654 domains in regulating the internalization process of the EGF receptor in response to EGF binding in cultured stable CHO and NIH 3T3 cell lines is examined. In collaboration with A. Ullrich (Genentech Inc.) preliminary data indicates that mutating Thr654 does not affect the internalization process in these cell lines. Along a different line of experiments the intact EGF receptor gene and the various derived mutated constructs were transiently expressed in monkey cells and their lateral diffusion compared. Our results show that large deletions in the cytoplasmic domain of the EGF receptor do not affect its lateral mobility and even a mutant that has only 8 amino acids in the cytoplasmic domain still translates with the same lateral diffusion as the intact receptor.

L201 FIBROBLASTS FROM PATIENTS WITH INHERITED PREDISPOSITION TO RETINOBLASTOMA ARE SLIGHTLY MORE SENSITIVE THAN NORMAL CELLS TO THE CYTOTOXIC EFFECTS OF IONIZING RADIATION, BUT NOT TO ITS MUTAGENICITY. J. Justin McCormick, Yenyun Wang, William C. Parks, Jeffrey C. Wigle, Suzanne Kateley-Kohler, and Veronica M. Maher, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824. Retinoblastoma (RB) is a disease characterized by cancer of the retina developing in early childhood. Fibroblasts from bilateral RB patients, an inherited form of the disease, have been shown to be abnormally sensitive to the cytotoxic effects of ionizing radiation. We compared fibroblasts from 6 bilateral RB patients and 3 normal individuals for their sensitivity to the mutagenic effects of γ-radiation (^{60}Co), using resistance to 6-thioguanine (TG) as the genetic marker. There was no significant difference between the two types of cell lines. The slope of the least squares line representing the frequency of TG resistant cells induced in the RB populations as a function of dose was 23 per 10^6 cells per Gray with an intercept of 0.26 Gray; that for the normal cells was 24 per 10^6 cells per Gray with an intercept of 0.3 Gray. We also compared 8 bilateral RB cell lines and 9 age-matched normal cell lines for sensitivity to the cytotoxic effect of ^{60}Co, using survival of colony-forming ability. The cloning efficiency of the unirradiated RB cell lines ranged from 22% to 76% with an average of 52%; that of the normal cell lines from 21% to 89% with an average of 64%. The mean D_0 for the RB cell lines ranged from 0.99 to 1.69 Gray with an average of 1.44 Gray; that of the normal cell lines ranged from 1.42 to 2.24 Gray, with an average of 1.82 Gray. If these slight differences in sensitivity to the killing effect of ^{60}Co reflect a fundamental difference between RB and normal cells, the mechanism responsible does not apply to the mutagenic response of the cells. (Supported by NCI Grants CA32924, CA21253, CA21289, and DOE Contract EV04659.)

L202 CHARACTERIZATION OF AN F9 VARIANT WHICH UNDERGOES INCOMPLETE DIFFERENTIATION AND REMAINS TUMORIGENIC WHEN TREATED WITH RETINOIC ACID. Emma E. Moore, Leonor Y. Wenger, and Judith A. Pasternak, National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, 80206

We have isolated an F9 variant, 5C, which undergoes incomplete differentiation and remains tumorigenic when treated with trans-retinoic acid (RA). 5C cells have normal levels of the RA-specific cytoplasmic binding protein which is thought to mediate the initial step of differentiation. 5C cells treated with RA initiate differentiation in a normal fashion but fail to complete the process. Somatic cell hybrids prepared between F9 and 5C cells remain tumorigenic in RA and differentiate to a degree that is intermediate to the F9 and 5C cells. This observation indicates that 5C cells (over)produce some factor which interferes with the loss of tumorigenicity normally induced by RA, and that this same factor probably also prevents complete differentiation. Na butyrate suppresses the tumorigenic phenotype of both F9 and 5C cells but does not induce their differentiation. Although neither RA nor Na butyrate induce differentiation of 5C cells, the combination of these two agents is quite effective. In order to elucidate the lesion in 5C cells, we are currently comparing the expression and structure of various (proto-)oncogenes in F9 and 5C cells, grown in the absence and presence of the various inducing agents. Furthermore, we have undertaken the cloning of the abnormal gene in 5C cells which is responsible for their persistent tumorigenicity and incomplete differentiation. This work is supported by a NCI grant, CA39131.

L203 THE p53 CELLULAR ONCOGENE IN ERYTHROLEUKEMIA INDUCTION, M. Mowat*, V. Chow#, S. Benchimol+ and A. Bernstein#. *Manitoba Institute of Cell Biology, Winnipeg, Manitoba, Canada. #Mount Sinai Hospital Research Institute, Toronto, Ontario and +Ontario Cancer Institute, Toronto, Ontario.

The objective of these studies is to determine the role the p53 oncogene plays in the multistage erythroleukemia induced by Friend leukemia virus. We have recently described several erythroleukemia cell lines with altered p53 expression (Mowat et.al. (1985) Nature 314:633-636). Some of these cell lines do not express p53 whereas others express very high levels of a truncated form of the protein. Cell lines not expressing p53 show reduced tumor forming ability when compared to p53 expressing cell lines. Using a p53 cDNA probe, we have found alteration of the p53 gene in these cell lines. We have isolated cell lines from the same mouse that either express a normal p53 protein or do not express p53. To determine whether the p53 positive and p53 negative cell lines were the result of the same or different transformation events in vivo, we have utilized the integration sites of Friend murine leukemia virus (F-MuLV) as an unique clonal marker. Southern gel analysis indicates that the p53 positive and negative cell lines from the same mouse were not clonally related, suggesting that at least two independent transformation events had taken place in these mice. We have also molecularly cloned the normal p53 gene from an expressing cell line and a rearranged p53 gene from a non-expressing cell line. The rearranged p53 gene has undergone a 2Kb deletion resulting in loss of several p53 exons. Preliminary results show that F-MuLV was not the cause of this deletion. (Supported by the MRC, NCIC, LRF and MCTRF).

L204 **THE B CHAIN OF PDGF ALONE IS SUFFICIENT FOR MITOGENESIS**, M. Murray*, J. Kelly*, E. Raines+, R. Ross+, *ZymoGenetics, Inc., 2121 N. 35th Street, Seattle, WA 98103, +Dept. of Biochemistry, Univ. of Washington, Seattle, WA 98195

The B chain of PDGF is highly homologous to a portion of $p28^{sis}$, the transforming protein of simian sarcoma virus. It has been suggested that $p28^{sis}$ exerts its transforming potential by mimicking the growth promoting activity of PDGF and stimulating the cell in an autocrine manner. We have directly examined the mitogenic potential of $p28^{sis}$ and the B chain homologous region by expressing these heterologous sequences in the yeast Saccharomyces cerevisiae. In our constructions, these proteins are encoded by portions of the v-sis gene. Expression and secretion from the yeast cell is achieved by using a yeast promoter and the α-factor pheromone secretory leader. The $p28^{sis}$ and B chain proteins thus expressed and secreted are immunoreactive with anti-PDGF goat antisera and are mitogenic for cultured fibroblasts and 3T3 cells. Furthermore, they mediate this mitogenic activity by specific binding to the PDGF cell surface receptor. Preincubation of these products with the anti-PDGF antisera or chemical reduction eliminates all the receptor binding and mitogenic activities. Gel electrophoresis and cell binding analysis indicates that the mitogenic material secreted from yeast is primarily a disulphide bonded dimer. These experiments directly demonstrate that $p28^{sis}$ is a mitogen and that a polypeptide corresponding to the B chain alone is sufficient to account for the mitogenic activity attributed to PDGF. Using in vitro mutagenesis techniques, we have altered the B chain sequence in an attempt to secrete a biologically active molecule which does not form a dimer. The results obtained with these mutants will be presented.

L205 GENETIC ANALYSIS OF SMALL CELL LUNG CANCER, Susan L. Naylor, John Minna, and Alan Y. Sakaguchi, University of Texas Health Science Center, San Antonio, TX 78284 and NCI Naval Med. Oncology Branch, Bethesda, MD 20014

A deletion in the short arm of human chromosome 3 (p14-p23) associated with small cell lung cancer was examined using 6 polymorphic DNA probes, two in the region of the deletion. Of 9 paired normal and SCLC DNAs tested by filter hybridization, 8 were heterozygous for one or both 3p DNA markers in their normal DNA. Seven corresponding tumor DNAs displayed only one of the alleles present in their normal DNA, indicating that material from one chromosome 3 homolog had been lost. Analyzed with 4 other chromosome 3 polymorphic probes, some tumors had become homozygous at all alleles, whereas in others, only short arm markers had become homozygous. One exception showing heterozygosity of a 3p marker in a tumor sample may indicate either that the marker lies outside the deletion in this particular sample or that a deletion may not be found in all SCLC cases. SCLC cells produce the growth factors transferrin and gastrin releasing peptide, a bombesin-like molecule. To determine if TF and GRP are located on 3p and might be affected by deletion, we determined the chromosomal location of both genes. TF was localized to 3q1-qter and GRP to chromosome 18. Filter hybridization indicates that neither gene is grossly rearranged or amplified in SCLC. Our data suggest that reduction to homozygosity of chromosome 3 markers is a common finding in SCLC. Whether this allows expression of a recessive oncogene or loss of activity of a regulatory gene is not known. Additional probe in the 3p deletion are being isolated for fine structural analysis and for searching for sequences expressed from this region in SCLC.

L206 THE ROLE OF ALTERATIONS IN ION TRANSPORT IN SIGNAL TRANSDUCTION BY PHORBOL ESTERS, Thomas G. O'Brien, Ralph Prettyman and Mortimer M. Civan, The Wistar Institute, Philadelphia, PA 19104 and The Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

The post-receptor events responsible for phorbol ester-induced changes in gene expression, proliferation, and differentiation are largely unknown. To study this problem we have used two different experimental systems to ask whether changes in ionic fluxes trigger biologic effects in phorbol ester-treated cells and tissues. In one system, BALB/c 3T3 cells and a mutant cell line defective in $Na^+K^+Cl^-$ cotransport [a known target of phorbol esters] are compared for their responses to TPA including induction of the enzyme ornithine decarboxylase (ODC) and stimulation of cell proliferation. The results indicate that mutation of this ion transport system in these cells eliminates responsiveness to TPA. In the second system, the intact epidermis of the frog, TPA is known to rapidly stimulate the transepithelial transport of Na^+ (Civan et al., 1985, Am. J. Physiol. 248:C457). The longer term consequences of TPA treatment were then examined in frog epidermis in vivo. As in the mouse epidermis, TPA is a potent mitogen in this epithelium and also induces ODC. The results of experiments designed to prove or disprove a cause-and-effect relationship between ionic changes and longer term events such as ODC induction will be presented. It is our hypothesis that rapid changes in ion fluxes after phorbol ester treatment, whether they occur in cells in culture or epithelia in vivo, act as intracellular signals for some of the sequelae produced by tumor promoters, especially those involved at the level of gene expression. (Supported in part by research grants CA-36353, ES-01664 and AM-20632 from NIH, DHHS).

L207 C MYC EXPRESSION, A LATE EVENT IN RODENT LIVER TUMOURIGENESIS?

C.M. Pearson[1], S.A. Withe[2], P.J. Pinto[3], S. Sinha[4], G.E. Neal[5].
[1,2,3]Imperial Chemical Industries PLC, Alderley Park, Nr. Macclesfield, Cheshire, UK.
[4,5]MRC Unit, Carshalton, Surrey, UK.

There have been several reports of elevations in c-myc transcripts in regenerating liver, liver tumour cell lines, and liver tumours induced by carcinogens in rats. We can confirm previous reports concerning Morris hepatomas in culture, but were unable to detect c-myc expression at any time up to 24 hours following partial hepatectomy in rats. A series of liver cell lines were studied in an attempt to evaluate if elevations in c-myc expression were required for cell turnover, or were associated with the later events in liver tumourigenesis. No elevated expression was observed in immortalised cells isolated from animals exposed to Aflatoxin B. These cells were not transformed or tumourigenic, and were of epithelial morphology. In contrast, c-myc expression was detected in tumourigeneic cells isolated from Aflatoxin B induced tumours in the same animals. Interestingly, immortalised cells transformed by transfection with pSV_2neo plasmids containing Ha-ras also had elevated levels of c-myc, but transformants[2] with N-ras constructs did not. These data indicate that elevated c-myc expression may not be a pre-requisite for cell turnover but could be associated with transformation and the later events in tumourigenesis in rodent liver.

L208 SELECTION OF GLUCOCORTICOID RECEPTOR MUTANTS IN A RAT FIBROBLAST CELL LINE: A GENERAL SELECTION SCHEME FOR MUTANTS IN ENHANCER BINDING PROTEINS, Magnus Pfahl, La Jolla Cancer Research Foundation, La Jolla, CA 92037

The activated glucocorticoid receptor binds to specific DNA sequences in the vicinity of glucocorticoid-responsive genes. The interaction of the receptor with these specific sequences is necessary for hormone-responsive transcription of the relevant genes. It has recently been shown that the glucocorticoid-responsive DNA element (GRE) can act in both orientations 5' and 3' of genes. The GRE therefore belongs to the class of DNA elements termed enhancers, and its activator protein is the glucocorticoid receptor. Here we have used a hybrid promoter and a selector gene, the Herpes simplex virus (HSV) thymidine kinase (tk) gene to select glucocroticoid receptor mutants. The hybrid promoter consists of the GRE of mouse mammary tumor virus (MMTV) and a partial tk promoter. This construct was introduced into a tk^- derivative of Rat-1 cells (a rat embryonic fibroblast line) by DNA transfection. Tk^+ derivatives were selected in HAT medium containing 10^{-6} M dexamethasone (Dex). All tk^+ derivatives were dependent on the presence of the hormone for the tk^+ phenotype; conversely, they only grew in BudR medium in the absence of Dex. Using mutagenesis and a two-step selection procedure in which we selected for tk^- phenotypes in BudR medium, we were able to obtain clones which have altered glucocorticoid receptors. All receptor mutants found were of the nuclear transfer-defective type. The principles of the selection scheme described here can be used to obtain mutants in other steroid hormone receptors and tissue-specific enhancer-binding proteins.

L209 THE SEQUENTIAL ANALYSIS OF PROGRESSION OF MOUSE SKIN PAPILLOMAS TO CARCINOMAS STUDIED WITH MARKERS. A. Lakshma Reddy and Mark Caldwell, Dept. Medicine, University of Washington, Seattle, Wa. 98195.

To understand the mechanism of progression of skin papillomas to malignant carcinomas, we followed the growth of papillomas by serial photographs, coordinates, histopathological evaluation, and X-chromosome-linked phosphoglycerate kinase(PGK) cell markers. The skin papillomas were induced on the backs of BALB/c PGK mosaic mice by initiation with 200 ug of 7,12, dimethylbenz(a)anthracene(DMBA) and promotion with 10 ug of 12-0-tetradecanoyl phorbol--13-acetate(TPA) three times a week. The location, histopathology, and PGK phenotype of of tumors were examined three times; at the first biopsy after 15 weeks of promotion; at the second biopsy after 30 weeks of promotion; and finally at autopsy. In this experiment, we examined the progression of 209 and 184 clonal (PGK-A or PGK-B) papillomas from mice promoted for 15 weeks (group 1) and 30 weeks (group 2), respectively. In both groups, about 82% of papillomas regressed by the time of autopsy. Further examination of the PGK phenotype of clonal papillomas that remained until autopsy revealed that about 20-25% of them changed their phenotype. Hence, it is possible theoreitically that about 40-50% of the papillomas, induced by above initiation-promotion protocol, have not advanced sequentially although exhibited morphological progression. Therefore, it is concluded that morphological progression of a induced papillom to a carcinoma does not always mean the step-wise progression of the same clone. Other initiated epidermal cells may grow in some of these papillomas, and some times completely replacing the original papillom clone. Supported by NIH grant CA32716

L210 NUCLEOTIDE SEQUENCE ANALYSIS OF THE NORMAL AND ACTIVATED myb GENE. Dan Rosson and E. Premkumar Reddy, Department of Molecular Oncology, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

The myb oncogene codes for the transforming properties of the Avian myeloblastosis virus (AMV). This oncogene has also been implicated in two mouse lymphoid tumors as well as human myeloid and colon tumors. To investigate the mechanism of activation of this oncogene, we have undertaken molecular cloning and nucleotide sequence analysis of several c-myb cDNAs in order to compare the normal and activated forms of this gene.

To date the complete nucleotide sequence of the coding region of the normal myb messenger RNA from chicken has been obtained. Comparison of the deduced amino acid sequence of the normal protein with that of the oncogenic myb gene product from AMV reveals the viral protein has acquired substantial deletions of amino acids from both its N-terminal and C-terminal ends.

A similar situation is found in two mouse model systems, that of the plasmacytoid lymphosarcomas (PL tumors) and the myeloid tumors induced by the CAS-Br-MuLV. In the former, tumors are associated with integration of a helper virus within the 5' portion of the mouse myb gene truncating the 1st 129 amino acids in the resulting gene product. Similarly, in NSF-60 tumors, viral integration results in loss of the last 246 amino acids from the protein. These results implicate a qualitative mechanism of activation in which loss of amino acid residues from one or both ends of the protein are responsible for the acquisition of its oncogenic potential.

L211 CLONING OF AN ABNORMAL C-MYC GENE DERIVED FROM A HUMAN LEUKEMIC T CELL LINE, Giuseppe Saglio, Angelo Guerrasio, Anna Serra, Patrizia Spinelli, Cristina Giubellino, G. Carlo Avanzi,*Deborah Aghib and* Sergio Ottolenghi. University of Turin and * University of Milan, Italy.

The human leukemic T cell line Hut 78, derived from a Sézary syndrome patient, shows a rearrangement of the c-myc gene beginning within 500 bp 3' to c-myc exon 3. This abnormal c-myc also appears to be duplicated compared to the normal allele. Chromosome analysis reveals that trisomy is the only cytogenetic anomaly involving chromosome 8, suggesting that the duplicated chromosome is the one carrying the abnormal c-myc and ruling out a Burkitt's type translocation event. This was also excluded by Southern blotting analysis, which showed a germ line configuration of the heavy and light chain immunoglobulin genes. Similarly the involvement of the and chain T cell receptor genes was excluded. Compared to other human leukemic T cell lines, the Hut 78 cells express high amount of c-myc transcript, suggesting that the 3' c-myc anomaly may cause a deregulation of the expression of the gene. The transmission of this c-myc abnormality through multiple cell passages and its duplication implies a possible relationship either with with the leukemic phenotype in culture. In order to better define this c-myc alteration, we have recently cloned in the 788 arms a 13.8 kb genomic Hind III fragment derived from the Hut 78 DNA, containing the entire c-myc and a 4.5 kb of the 3' rearranged sequences. The abnormal portion is rich of human repetitive sequences.

L212 ACTIVATION OF MYC AND RAS ONCOGENES IN RADIATION-INDUCED RAT SKIN TUMORS, Mary J. Sawey, and Seymour J. Garte, New York University Medical Center, New York, NY 10016

Rat skin tumors of various histologic types induced by ionizing radiation were examined for activation of oncogenes. Six of twelve tumors tested were positive in the NIH 3T3 transfection assay and the rat K-ras oncogene was found to be activated in representative transformed foci. Similar experiments, using N- and H-ras probes revealed only the endogenous mouse fragments in transfectant DNA. Activation of the c-myc oncogene was observed in the original tumor DNA's. Southern analysis of the tumor DNAs revealed evidence for c-myc gene amplification and restriction enzyme polymorphisms in 10 of the 12 tumors. Northern blot analysis of poly A^+ RNA indicated enhanced c-myc gene expression in 3 of 5 tumors examined as compared to normal rat epidermis. When these same RNA samples were hybridized with H-ras or K-ras probes, no increase in expression of these genes over normal skin levels was seen for any tumor. These results indicate that certain radiation induced rat skin tumors contain an activated K-ras oncogene, as well as an amplified and over expressed c-myc oncogene. There appears to be a correlation between transforming activity of the DNA, myc gene activation, and the histologic type of the tumor. This is the first known example of concurrent activation of oncogenes from the ras and myc complementation groups in primary tumors in a well defined experimental model of carcinogenesis.

L213 DIVERGENT EFFECTS OF RECOMBINANT HUMAN TUMOR NECROSIS FACTOR-ALPHA ON CELL GROWTH *IN VITRO*, H.M. Shepard, B.B. Aggarwal, P.E. Hass, I.S. Figari, M.A. Palladino, Jr., and B.J. Sugarman.

Modulation of the *in vitro* growth of human and murine cell lines by recombinant human tumor necrosis factor-alpha (rTNF-α) and recombinant human interferon-gamma (rIFN-γ) was investigated. rTNF-α had cytostatic/cytolytic effects on only some tumor-cell lines. rTNF-α synergized with rIFN-γ to cause enhanced antiproliferative effects on a subset of the cell lines tested. In contrast to its effects on sensitive tumor cells, rTNF-α augmented the growth of normal diploid fibroblasts. Variations in the proliferative response induced by rTNF-α were apparently not due to differences in either the number of binding sites per cell nor their affinity for rTNF-α. These observations indicate that the effects of rTNF-α on cell growth are not limited to tumor cells, but rather that this protein may have a broad spectrum of activities *in vivo*. We have selected murine and human tumor cell lines which are resistant to the antiproliferative effects of rTNF-α. These mutants are now being characterized in order to understand the mechanism by which rTNF-α can induce divergent effects on cell growth.

L214 STRUCTURE AND EXPRESSION OF THE DROSOPHILA EGF RECEPTOR HOMOLOG, Ben-Zion Shilo, Eyal Schejter, Lillian Glazer and Daniel Segal*, Departments of Virology and Neurobiology*, The Weizmann Institute of Science, Rehovot 76100, Israel.

A single copy gene termed DER was isolated from a Drosophila genomic library, using the chicken v-erbB kinase domain as a probe. Characterization of DER genomic and cDNA clones revealed extensive homology to the human EGF receptor sequence, in both intracellular and extracellular domains. The DER extracellular domain is longer than the equivalent human domain as a result of a duplication of one of the two cysteine-rich domains. Comparison of the cysteine domains of the Drosophila and human receptors allows us to trace the evolution of this gene family.

Analysis of DER cDNA clones reveals an alternative splicing pattern which generates different coding possibilities for the N-terminal part of the extracellular domain. These alternatives may modify the affinity of the receptor to the hormones which trigger it. In situ hybridization of DER probes shows that during embryogenesis the transcripts are uniformly distributed. In the larval period the transcripts are localized to the nervous system and the imaginal discs which are the only diploid tissues undergoing normal cell division at that stage. In the adult, the transcripts are most prominent in the cortex of the brain and the gangelions. The distribution of DER transcripts suggests that the protein may have a crucial role not only in the control of cell proliferation, but also in tissues which have undergone differentiation. The different splicing alternatives have a similar tissue distribution.

L215 MULTI-STEP NATURE OF RADIATION-INDUCED CARCINOGENESIS IN REF CELLS. Elaine Sierra[1], Larry W. Oberley[1], and Duane L. Guernsey[2], [1]Radiation Research Laboratory, and [2]Department of Biophysics and Physiology, University of Iowa, Iowa City, Iowa 52242.

In an attempt to further characterize the development of X-ray induced neoplasia, cultures of primary rat embryo fibroblasts (REF) were irradiated with X-rays (3 Gy). A phase of rapid proliferation after irradiation was observed, followed by a decline (crisis) leading to senescence. After crisis, six clones from irradiated cells gave rise to continuous cell lines; the newly immortalized cells differed in morphology, growth patterns, and tumorigenicity. One of these cell lines consists of highly stable, serum dependent, non-tumor producing cells. Upon exposure to a second dose of X-rays, or transfection with T-24 E-J bladder carcinoma DNA (C-Ha-ras), the cells did not undergo transformation. These results suggest that X-ray induced immortalization may be due to an oncogene which is not complimentary to ras oncogene in its induction of full neoplastic transformation, or that more than two steps are required for X-rays to neoplastically transform normal REF cells. Supported by PHS grants CA36483-01 awarded by NCI and Training Grant T32-CA09125.

L216 Fis-1, A PUTATIVE ONCOGENE INVOLVED IN F-MuLV-INDUCED LYMPHOID AND MYELOID LEUKEMIAS, IS VERY CLOSELY LINKED TO Int-2, AN ONCOGENE INVOLVED IN MAMMARY CARCINOMA. Jonathan Silver and Charles Buckler, LMM, NIAID, NIH, Bethesda, MD.

Friend murine leukemia virus (F-MuLV) proviruses were found to be integrated in a 1.5 kb region on mouse chromosome 7 in 4 of 35 independant F-MuLV-induced tumors; 2 of these 4 tumors were lymphomas and 2 were myeloid leukemias (1). This common integration region for F-MuLV, designated Fis-1, is very closely linked to Int-2, an oncogene involved in mouse mammary tumor virus (MMTV)-induced breast carcinomas. No recombinants between Int-2 and Fis-1 were found in over 30 backcross mice and 51 recombinant inbred strains, indicating that these loci are within 1 centimorgan (about 1000 kb) of one another. However, molecular clones of 30 kb surrounding Fis-1 show no overlap with molecular clones of 30 kb surrounding Int-2, and F-MuLV insertion in Fis-1 does not induce Int-2 mRNA. F-MuLV insertion in the Fis-1 region is associated with transcription of cellular sequences downstream of the provirus. Fis-1 may represent a novel oncogene very closely linked to Int-2, or this region of mouse chromosome 7 may contain a hot spot for proviral integrations.

(1) J. Silver and C. Kozak, J Virol (in press)

L217 HORMONAL INFLUENCES ON THE PROMOTION AND PROGRESSION OF HEPATIC PRENEOPLASTIC AND NEOPLASTIC LESIONS DURING CHEMICAL CARCINOGENESIS, Tracy C. Sloop, Diane B. Campen, Geoffrey I. Sunahara, Karen G. Nelson and George W. Lucier, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Our studies utilize "multi-stage" rodent models to examine the effects of tumor promoting agents on the formation of preneoplastic and neoplastic lesions following diethylnitrosamine (DEN) initiated hepatocarcinogenesis. In the present study, the hepatic tumor promoters 17α-ethinylestradiol (EE_2) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were examined. Ovariectomized rats were administered a single dose of DEN (200 mg/kg, i.p.) followed by chronic exposure to the promoting agent. EE_2 (90 µg/kg/day) was implanted s.c. in silastic capsules and TCDD (0.1 µg/kg/day) was administered by gavage. Marked changes in hepatic biochemistry were observed following EE_2 promotion. A progression of liver lesions (preneoplastic foci, nodules, tumors) occurred within 40 weeks in DEN-EE_2 animals. Nuclear estrogen receptor occupancy increased 6-8 fold in all but control animals. Alterations in hepatic biochemistry were also observed following TCDD promotion. Hepatic aryl hydrocarbon hydroxylase activity and Ah receptor concentrations were significantly increased in TCDD treated rats. Although TCDD is reported to be a potent hepatic tumor promoter in intact female rats, no neoplastic lesions were observed in ovariectomized TCDD promoted rats. Nevertheless, an increase in preneoplastic δ-glutamyltranspeptidase positive lesions were observed in DEN-TCDD animals. Hormonal estrogens may play a critical role in the promotion and progression of hepatocarcinogenesis for a number of structurally-divergent promoting agents.

L218 THE SECOND CELL-DERIVED INSERT OF THE KIRSTEN AND HARVEY SARCOMA VIRUSES REPRESENTS ANOXIC STRESS RESPONSE GENES. Garth R. Anderson, Becky K. Farkas, Daniel L. Stoler and Jack R. Fabian, Roswell Park Memorial Institute, Buffalo, NY 14263.

The ras oncogene of the Kirsten and Harvey sarcoma viruses represents a cellular gene closely related to the G-protein regulatory pathway. This system allows cells to respond to a variety of external signals. Each of these two sarcoma viruses also contain a second 3 kb cell-derived insert, related to sequences in the VL30 family. This second insert has now been found homologous to functional genes expressed by normal cells as a response to a specific stress, anoxia.

Anoxic stress induces uninfected rat fibroblasts to express approximately 10,000 copies per cell of a Kirsten related transcript; in contrast uninduced cells express approximately 50 copies per cell of this RNA. The induced transcript of 5.3 kb is polyadenylated and polysome associated. Anoxia causes no parallel increase in ras expression.

The anoxic stress response is associated with production of two polypeptides of 56,000 and 34,000 daltons. The 34,000 dalton polypeptide efficiently binds nucleic acids, binds a p21 which appears related to p21 ras gene products, and has lactate dehydrogenase activity. The function of the 56,000 dalton polypeptide is unclear.

Human cancer patients express elevated levels of the 34,000 dalton polypeptide in their sera and urine. This polypeptide, known as LDH_K, is under development as a cancer marker.

L219 CHARACTERIZATION OF THE ACTIVATING LESIONS OF ONCOGENES IN CHEMICALLY-INDUCED RAT AND MOUSE LUNG TUMORS, Shari J. Stowers, Paul L. Glover, Steven H. Reynolds, Lawrence R. Boone, Robert R. Maronpot and Marshall W. Anderson, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

In this study, mice and rats were exposed to the chemical, tetranitromethane (TNM), by inhalation. High molecular weight DNA was isolated from tumors of these TNM-treated animals and was transfected onto NIH/3T3 mouse fibroblasts. Southern blot analysis showed that the transforming activity found in the rat transfectants was due to the transfer of an activated K-ras oncogene. Cloning and sequencing of the first exon of the activated K-ras oncogene showed that the activating lesion in two of the transfectants was a mutation in the 12th codon with a GGT → GAT change. Oligonucleotide hybridization of the TNM rat transfectants showed that 7/7 had this change. The rat TNM tumor DNA are also being examined for their activating lesions using this method. The mouse tumors are now being screened for their transforming activity. Oligonucleotide hybridization should show whether the mouse tumors have an activating lesion similar to the rat or not.

L220 TUMORIGENIC TRANSFORMATION OF HUMAN TERATOCARCINOMA CELLS BY ACTIVATED RAS ONCOGENES BUT NOT THE HOMOLOGOUS PROTO-ONCOGENES, Michael A. Tainsky, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Activated ras oncogenes are forms of the germ-line proto-oncogenes with specific point mutations which when transfected onto NIH-3T3 murine fibroblasts produce foci of morphologically altered cells. Approximately 15% of human tumors contain activated transfectable ras oncogenes. However, the role in human oncogenesis of these activated ras genes is unclear. It has yet to be determined whether these genes have a causal role in tumor induction or are mutated as a consequence of their presence in tumor cells which may simply have increased mutation rates. It has not been demonstrated that these genes are responsible for tumorigenic transformation in these systems. The present study addresses this issue using PA-1 human teratocarcinoma cells. This cell line was isolated by culturing the ascites fluid from a 12 year old female with an ovarian teratocarcinoma. PA-1 cells at early passages do not form tumors in athymic nude mice while late passage cells (>100) readily form tumors in athymic nude mice. In PA-1 cells, the presence of an activated N-ras oncogene was directly correlated with the ability of those cells to form tumors in athymic nude mice. Late passage cells contain a N-ras gene which is activated by a point mutation at the codon for amino acid 12 of the p21 ras protein. Evidence will be presented that the N-ras has a causal role in the tumorigenesis of these teratocarcinoma cells in nude mice and that the oncogene allows the tumorigenic cells to escape host immune defenses. This mechanism differs from the way cells are transformed by overexpression of proto-oncogenes.

L221 A HYBRID RNA TRANSCRIPT MAY BE RESPONSIBLE FOR met ONCOGENE ACTIVATION, G.F. Vande Woude[1], M. Park[2], M. Dean[1], D. Blair[2], [1]LBI-Basic Research Program and [2]Laboratory of Molecular Oncology, NCI-Frederick Cancer Research Facility, P.O. Box B, Frederick, MD 21701

The met transforming gene was isolated from a tumorigenic derivative of a human osteogenic sarcoma (HOS) cell line and was presumably activated by treating the parental HOS cell line with N-methyl-N-nitronitrosoguanidine (MNNG). The transforming activity of met is contained within 35 kilobase (kb) pairs of DNA and at the level of restriction enzyme analysis is not rearranged. While two classes of met related transcripts are detected in human cell lines [class I (11 kb) or class II (12 kb)] and each appears to be expressed noncoordinately in a cell type specific fashion, both the MNNG HOS cell line and NIH/3T3 met transformants express a new met RNA of 6.5 kb. S1 mapping data indicate that the 6.5 kb met RNA is co-terminal with the 3' end of the class I RNA and shares at its 5' end an exon in common with the 12 kb class II RNA suggesting that the activated met oncogene locus expresses a fused class I, class II hybrid transcript. Sequence analyses of the 3' end of the met locus identifies a region containing 70% amino acid homology with the human insulin receptor indicating that met is a new member of the src tyrosine kinase family. Thus, met activation may result from a mutation(s) which alters an RNA processing site(s) generating a truncated hybrid RNA transcript. The mechanisms of met activation may be analogous to the activation of v-erbB in AMV or c-abl in chronic myelogenous leukemia.

Research sponsored by the National Cancer Institute, DHHS, under Contract No. NO1-CO-23909 with Litton Bionetics, Inc.

L222 Analysis of the Transcriptional Control Domains of human c-myc, C.B.A. Whitelaw and N.M. Wilkie, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD Scotland.

c-myc is the cellular homolog of the transforming gene carried by the avian myelocytomatosis virus. As with other cellular oncogenes, c-myc shows a high degree of evolutionary conservation implying a central role in normal cellular metabolism. Indeed the aberrant deregulated expression of c-myc has been associated with several neoplasias (most notably Burkitt's lymphoma). We have set out to analyse the transcriptional control of c-myc in an attempt to help elucidate its aberrant expression in these neoplasias. Using a transient expression assay (CAT) we have identified an element which suppresses myc transcription. This suppressor has several of the characteristics usually associated with enhancers. Competition experiments have shown this suppression to be regulated by a trans-acting factor (repressor). In addition, two other domains have been identified which have positive transcriptional regulatory functions. These sequences all show good sequence homology between species. We propose that removal or rearrangement of the suppressor element plays an important role in the deregulation of c-myc expression.

L223 ACTIVATION OF c-Ha-ras IN DNA FROM CHEMICALLY-INDUCED HEPATOMAS OF THE MALE B6C3F$_1$ MOUSE, Roger W. Wiseman, S. Jill Stowers*, Elizabeth C. Miller, Marshall W. Anderson*, and James A. Miller, McArdle Lab, Univ. of Wis., Madison, WI 53706 and *National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

Activated Ha-ras proto-oncogenes have recently been identified in DNA of a number of spontaneous hepatocellular adenomas (3/10) and carcinomas (10/13) of the B6C3F$_1$ mouse (S.H. Reynolds, et al., PNAS, in press, 1985). Activation of c-Ha-ras is being examined in DNA from well-differentiated hepatomas initiated by single i.p. dose of a carcinogen to male B6C3F$_1$ mice at 12 days of age. DNA from 32 of 39 hepatomas (7/7 induced by N-hydroxy-2-acetylaminofluorene (HOAAF), 7/7 from vinyl carbamate (VC), 11/11 from 1'-hydroxy-2'3'-dehydroestragole (HODE), and 7/14 from diethylnitrosamine (DEN)) demonstrated transforming activity in the NIH/3T3 transfection assay. Southern analysis of 3T3 focus DNA from 31 of 32 of the positive hepatomas revealed amplified and/or rearranged restriction fragments homologous to a Ha-ras probe. For at least 80% of the positive hepatomas, immunoprecipitation of 3T3 foci with a monoclonal antibody (Y13-259) demonstrated expression of a p21 protein that had increased mobility on SDS-PAGE analysis. An A- T transversion of the 2nd nucleotide of the 61st codon (CAA) of the mouse Ha-ras gene creates a new Xba I restriction site. Xba I digestion of DNA from 3T3 foci revealed a point mutation at this site for 6 of 7 VC, 3 of 6 DEN, 3 of 11 HODE, and 0 of 7 HOAAF-induced hepatomas. This observation is consistent with the hypothesis that c-Ha-ras activation can be a direct result of reaction of ultimate chemical carcinogens with this gene.

L224 ANALYSIS OF THE TRANSACTIVATING FUNCTION OF THE HTLV-III RETROVIRUS

Wright, C.M., Paskalis, H., Felber, B. and Pavlakis, G.
Cell Transformation Section, Molecular Mechanisms of Carcinogenesis Laboratory
National Cancer Institute-Frederick Cancer Research Facility
Frederick, MD. 21701

The sequences of the HTLV-III genome which are responsible for transactivation of the retroviral LTR have been cloned 3' to the mouse metallothionein promoter in a bovine papilloma virus vector. This vector has been used to transfect mouse fibroblasts and stable cell lines selected. These established cell lines overproduce a functional transactivating protein (TA-III).
We have characterized the TA-III protein functionally, as well as by indirect immunofluorescence, immunoprecipitation and western blot analysis.

L225 USE OF CELL VARIANTS TO STUDY THE MOLECULAR AND CELLULAR DETERMINANTS OF TUMOR PROMOTION. H. Yamasaki[1], M. Hollstein[1], E. Hamel[1], L. Giroldi[1], E. Rivedal[2], T. Sanner[2] and T. Kakunaga[3]. [1]International Agency for Research on Cancer, Lyon, France; [2]Norwegian Radium Hospital, Oslo, Norway; [3]Osaka University, Osaka, Japan.

In order to study molecular and cellular determinants of tumor promotion and of cellular susceptibility to tumor promoting agents, we use various genetic cell variants. They include: 1) TPA-sensitive and -resistant murine erythroleukemia cells (MELC) in which cell differentiation can or cannot be inhibited by TPA; 2) TPA-sensitive and -resistant Syrian hamster embryo (SHE) cell lines with respect to TPA enhancement of cell transformation; and 3) BALB/c 3T3 cell variants with high or low susceptibility to induction of cell transformation by chemical carcinogens or UV. Our results from these studies can be summarized as follows: 1. There is no correlation between the number or affinity of phorbol ester binding sites and subsequent actions of phorbol esters. In TPA-resistant SHE many more receptors were detected than in TPA-sensitive SHE. 2. Diacylglycerol inhibits differentiation of TPA-sensitive, but not TPA-resistant MELC, further suggesting that diacylglycerol is a functional endogenous analogue of phorbol esters. 3. TPA inhibits intercellular communication (IC) of only TPA-sensitive SHE, confirming our idea that there is a good correlation between IC block and cell transformation. 4. A transformation sensitive clone of BALB/c 3T3 cells loses its IC at confluence, as if TPA had been added. Thus, this BALB/c 3T3 cell variant has an intrinsic ability to express some of the phenotypes related to tumor promotion. These variant cells are also being used to study the mechanism of tumor promotion at the molecular level. Partly supported by NCI grant No. 1 RO1 CA40534-01.

L226 PROMOTING EFFECT OF VARIOUS CHEMICALS ON THYROID TUMORIGENESIS IN RATS TREATED WITH N-BIS(2-HYDROXYPROPYL)NITROSAMINE, Yoshio Hiasa, Yoshiteru Kitahori, Noboru Konishi, Taketo Shimoyama and Yoshio Murata, 1st Dept. of Pathology, Nara Medical University, Kashihara, Nara, 634, Japan.

The effect of phenobarbital (Pb), 4,4'-diaminodiphenylmethane (DDPM), propylthiouracil (PTU) and 2,4-diaminoanisole (DAA) on thyroid tumorigenesis was studied in male Wistar rats given a single i.p. injection of 280 mg per 100 g body weight of N-bis(2-hydroxypropyl)nitrosamine (DHPN).
Animals were divided into 10 groups. Rats were 7 weeks old at the beginning of the experiment. Groups 1 to 5 (21 rats in each) were given a single i.p. injection of DHPN at 280 mg/100 g body weight and given basal diet supplemented with Pb at 500 ppm (group 1), DDPM at 1000 ppm (group 2), PTU at 1500 ppm (group 3) or DAA at 5000 ppm (group 4) for 19 weeks from week 2 to 20. Rats in group 5 were given basal diet not supplemented with any chemicals. Groups 6 to 10 (21 rats in each) were given the vehicle instead of DHPN and then given the chemical diet or basal diet as described for groups 1 to 5. All rats were killed 20 weeks after the beginning of the experiment. Thyroids from all rats were used for histological studies and serum from some rats were used for radioimmunoassay of TSH, T4 and T3. Pb, DDPM, PTU and DAA promoted the development of thyroid tumors and increased the concentration of TSH in rats treated with DHPN.

Tumor Suppression/Growth or Transformation Inhibitors

L227 PARACRINE AND AUTOCRINE MELANOMA TRANSFORMING GROWTH FACTORS. Marvin D. Bregman, Nancy J. Sipes and Frank L. Meyskens, Jr. Cancer Center, University of Arizona, Tucson, Arizona, 85724.

Human melanoma and platelet extracts contain proteins which have the ability to induce the clonogenic growth of human melanoma cells in soft agar. None of the known growth factors which include EGF, insulin, melanocyte stimulation factor, NGF, PDGF, TGF-α, TGF-β, and combinations of PDGF + TGF-α + TGF-β are able to promote the clonogenic growth of human melanoma. Human platelets contain an acid sensitive melanoma transforming growth factor which has a molecular weight of 55,000. Human melanoma cell extract contains transforming growth factor activity with apparent molecular weights of 200,000, 65,000, 45,000 and 10,000. Information about the biological properties of these unique transforming activities will be presented.

L228 Structural Characteristics of Receptors for Transforming Growth Factor-β. Sela Cheifetz, Betsy Like and Joan Massague, University of Massachusetts Medical School, Worcester, MA 01605

Cell surface glycoproteins which bind transforming growth Factor-β (TGFβ) with the specificity and high affinity characteristic of physiologically relevant receptors have been demonstrated in a number of cell lines by using affinity labeling methodology. The major labeled TGFβ receptor species in many cell lines from human, rodent and avian origin is a 280-330kDa protein which is part of a disulfide linked complex of molecular mass 565-615kDa. In addition to this receptor species, most cell lines also exhibit smaller TGFβ-binding proteins of approximately 65K and 85K (Massague, J. Biol. Chem. 260, 7059; Massague and Like, J. Biol. Chem. 260, 2636). We have been investigating the nature of these minor bands. Competition studies have shown that like the major receptor species, the affinity of the 65K and 85K species for TGFβ is very high (Kd-20-500pM). Comparative peptide maps of the individual affinity labeled proteins indicate a structural relationship between the 280K and 65K proteins; however, the 85K protein gave rise to a distinct peptide map. We have identified a cell line which contains the 65K and 85K TGFβ receptor but lacks the 280K species. Control experiments have indicated that the various receptor species are neither the result of proteolytic degradation during experimental manipulations nor differences in culturing or labeling conditions. Various crosslinking agents added at increasing concentrations did not alter the pattern of the receptor species. These results suggest the possibility that multiple TGFβ receptor forms with distinct functional roles may exist in the target cells.

L229 ONCOGENE EXPRESSION DURING INTERFERON ALPHA THERAPY OF HAIRY CELL LEUKEMIAS, F. Dautry, F. Sigaux, D. Grausz, P. Loiseau, S. Castaigne, L. Degos, G. Flandrin and P. Lehn, Institut Gustave Roussy and Hopital Saint-Louis, Villejuif 94805 VILLEJUIF.

Hairy cell leukemia (HCL), a rare form of chronic leukemia, is a malignancy of a late stage of B cell differentiation[1,2]. Variant HCL is a recently described form of HCL, with intermediate features between typical HCL and B cell prolymphocytic leukemia[3,4]. We report that the variant hairy cells (HC) differ from typical HC by the oncogenes expressed in vivo. They contain both c-fos and c-myc transcripts, whereas the more mature typical HC express c-fos, but not c-myc. While low dose alpha interferon (IFN-) therapy is effective for typical HCL[5-8], the two cases of variant HCL included in our study were not affected by IFN administration. We report also that in typical HCL, c-fos expression is modulated in vivo and c-myc transcripts remain undectectable following IFN treatment, while in variant HCL c-myc expression was unaffected, although c-fos expression varied. This suggests that the failure to modulate c-myc expression in vivo might indicate the limits of low dose IFN therapy.

L230 Mutations in the E1a Gene of Type 5 Adenovirus Alter the Tumorigenic Properties of Transformed Fischer Rat Embryo (CREF) Cells. G.J.Duigou[1], L.E.Babiss[1], W.S. Liaw[1], S.G.Zimmer[2], H.S.Ginsberg[1] and P.B.Fisher[1]., Columbia Univ.[1], New York, NY 10032 and Univ. of Kentucky, Lexington[2], KY 40536. The role of the type 5 adenovirus (Ad5) E1a encoded 289AA and 243AA proteins in regulating tumorigenicity of transformed CREF cells in athymic nude mice and syngeneic Fischer rats has been evaluated. Transformation of CREF cells with wild type Ad5, or the E1a plus E1b transforming gene regions of Ad5, results in epithelioid transformants which grow efficiently in agar, but do not induce tumors in nude mice or syngeneic rats. In contrast, CREF cells transformed by the host-range Ad5 mutant H5hr1, which contains a single base-pair deletion of nucleotide 1055 in E1a resulting in a 28kd protein in place of the wild type 51kd acidic protein, display a cold-sensitive transforming phenotype, an incomplete fibroblastic morphology and induce tumors in nude mice and syngeneic Fischer rats. Tumors develope in both types of animals following injection of CREF cells transformed by other cold-sensitive Ad5 mutants (H5d1101 and H5in106), containing alterations in their 13S mRNA and consequently truncated 289AA proteins, as well as CREF cells transformed with only the E1a gene (0 to 4.5m.u.) from H5hr1 or H5d1101. In addition, tumors develope in both types of animals injected with CREF cells transformed by an Ad5 mutant (H5d1520) and an Ad2 mutant (H2d1500) which do not produce the 289AA E1a protein but synthesize the 243AA E1a protein. Tumor formation did not result in changes in the arrangement of integrated Ad5 DNA or in the expression of Ad5 early genes. These results indicate that the wild type 289AA protein encoded by the 13S mRNA of E1a from Ad5 prevents expression of the tumorigenic phenotype in CREF cells.

L231 EPIDERMAL GROWTH FACTOR RECEPTOR OCCUPANCY INHIBITS VACCINIA VIRUS INFECTION D.A. Eppstein, Y.V. Marsh, A.B. Schreiber, S.R. Newman, G J. Todaro, and J.J. Nestor, Jr. Syntex, Palo Alto, CA and Oncogen, Seattle, WA

Recently it was shown that vaccinia virus encodes an early protein (VGF) which, starting at amino acid 45, is homologous in 19/41 amino acids with epidermal growth factor (EGF) and transforming growth factor-α (TGF-α). [Brown et al., Nature 313:491 (1985); Blomquist et al., PNAS 81:7363 (1984); Reisner, Nature 313:801 (1985)]. As 6/10 amino acids starting at residue 71 are identical with those of the third disulfide loop of EGF or TGF-α [which contains a binding region for the EGF receptor (Nestor et al., 1985)], we speculated that vaccinia virus might utilize the EGF receptor to bind to and infect cells. We have now demonstrated that occupancy of the EGF receptor either by EGF or synthetic decapeptides corresponding to the third disulfide loop of TGF-α or VGF inhibits vaccinia virus infection of cells. The dose response for inhibition of vaccinia virus plaque formation correlated very well with the binding affinity of the peptides and EGF to the EGF receptor, spanning a five $\log_{10}$ concentration range. Single cycle viral replication studies demonstrated that the inhibitory effect was obtained by pre- but not post- infection treatment of the cells with the peptides. These results were obtained both by EGF (an agonist of cellular DNA synthesis) and synthetic decapeptides which are antagonists to the mitogenic effects of EGF. Together, these results suggest that the observed inhibition of vaccinia virus infection occurs by the blocking of the EGF recptor which prevents productive infection by vaccinia virus.

L232 RELATIONSHIP OF 7S NGF AND ITS SUBUNITS TO COLONY STIMULATING FACTORS. C. Erickson-Miller, C.N. Abboud, C.N. Frantz, R.W. Stach, J.K. Brennan and M.A. Lichtman, Univ. of Rochester School of Medicine, Rochester, N.Y. 14642 & SUNY Upstate Medical Center, Syracuse, N.Y, 13210. We set out to investigate the relationship of mouse submaxillary gland nerve growth factor (NGF) to colony stimulating factors (CSFs) in view of the reported human granulocytic CSF activity of mouse NGF (Blood 64,suppl.1,129a, abstr# 425, 1984). High molecular weight 7S NGF, and purified alpha, gamma and beta subunits were tested for mouse and human CSFs as well as for neurite outgrowth activity in a PC-12 differentiation assay. We were unable to detect human active CSF in unfractionated or adherent cell depleted marrow in any of the NGF derived subunits as well as the 7S multimer. Mouse active CSF-1 was readily detected in the gamma chain preparation (1600 units/mg) while only trace amounts were found in the alpha chains and none was present in the 7S multimer and the beta subunit. The similarity of this factor to CSF-1 was further confirmed by: 1) binding to ConA-Sepharose, 2) molecular weight of 68000 by HPLC sizing, 3) neutralization by antibodies to L-cell and human urinary CSF-1 and 4) the presence of a 68kd band in addition to the smaller gamma chain after immunoprecipitation by both antibodies from radiolabelled gamma chains. The gamma chains contained an arginine esteropeptidase while alpha and beta chains as well as purified GCT cell CSF-1 did not, further underscoring that CSF-1 was not part of an NGF subunit. In addition, the PC-12 differention occured only in the beta subunit and the 7S multimer, while none was seen in purified human GCT cell CSF-1. These studies failed to confirm any direct human CSF activity by NGF but indicated that CSF-1 co-purifies with the gamma chain subunit.

L233 **A SOLUBLE BINDING ASSAY FOR TRANSFORMING GROWTH FACTOR TYPE BETA RECEPTORS.** Bradford O. Fanger and Michael B. Sporn, Laboratory of Chemoprevention, NCI, NIH, Bethesda, MD 20892.

A binding assay was developed for the measurement of solubilized receptors for transforming growth factor type beta (TGF-beta). Receptors were solubilized with 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), incubated with radioactive TGF-beta, then the unbound ligand was removed by adsorption to dextran-coated charcoal. The binding of TGF-beta to solubilized receptors was saturable and specific, and increased linearly with the amount of membrane protein added. Furthermore, the cross-linking of radioactive complexes left in the supernatant after adsorptive removal of unbound TGF-beta yielded complexes identical to affinity-labeled TGF-beta receptors from whole cells. Treatment of a 20% charcoal suspension with 0.2-0.4% dextran was optimal to protect receptors from adsorption while allowing free TGF-beta to be removed. Mr ~ 250,000 dextran was more effective in this assay than were dextrans of other sizes. This assay is simple, sensitive, rapid, and inexpensive, and can be used to detect solubilized TGF-beta receptors from purified membranes as well as crude extracts of cells and tissues. Using this assay, we have shown that binding of TGF-beta to its receptor is maximal by 4 h, and that TGF-beta receptors bind to wheat germ lectin, indicating that they are glycoproteins. Further characterization of the TGF-beta receptor will be described.

B. O. F. is supported by a postdoctoral fellowship from BASF Aktiengesellschaft, Ludwigshafen, Germany.

L234 FIBRONECTIN ASSOCIATED TRANSFORMING GROWTH FACTOR, Roy A. Fava and D.B. McClure, W. Alton Jones Cell Science Center,Inc., Lake Placid, N.Y. 12946.

We have observed that purified plasma- and cell-derived fibronectins have the ability to promote anchorage-independent growth of non-transformed rat kidney fibroblasts. This assay operationally defines the family of proteins designated transforming growth factors (TGF). The TGF activity appears to be associated with fibronectin during molecular sieve chromatography at neutral pH in the presence of 4M urea. At extremes of pH however, the TGF activity dissociates from the fibronectins and chromatographs as a 20-30 kDa molecule. The physical-chemical properties, chromatographic behavior and biological activity of the fibronectin associated TGF suggest that it is a type-β transforming growth factor/ growth inhibitor. This activity has been observed to be present in fibronectins isolated from fresh human plasma as well as in fibronectins from several other species obtained from commercial suppliers.

L235 GROWTH AND RECEPTOR ACTIVITY OF TYPE BETA TRANSFORMING GROWTH FACTOR IN RODENT AND HUMAN CELL LINES, J.A. Fernandez-Pol*+, D.J. Klos*, and G.A. Grant#, *VA Medical Center and +Dept. of Medicine, St. Louis University, St. Louis, MO. 63106 and #Dept. of Biological Chemistry, Washington University, St. Louis, MO 63110

Transforming growth factor (TGF) type beta has been purified from serum-free culture fluids of L929 cells which are capable of continual growth in serum-free medium in the absence of any exogenously added polypeptide growth factors. TGF beta has been purified to homogeneity as judged by NH2-terminal amino acid sequence analysis. We show that the anchorage-independent growth of many human carcinoma cell lines -including melanomas, mammary, vulvar, lung and prostatic- is inhibited by TGF beta at concentrations similar to those that stimulate anchorage-independent growth of the rodent cell lines NRK and L929. The peculiar action of TGF beta is further revealed by observations that while epidermal growth factor and TGF beta synergize to induce inhibition of anchorage-independent growth of A431 vulvar carcinoma cells, their effects on anchorage-independent growth of A549 lung and PC3 prostatic carcinoma cell lines are antagonistic. We also show that in these cell lines TGF beta interacts with specific cellular receptors. Further experiments suggest that 1) the expression of both TGF beta and TGF beta receptors by L929 cells and the stimulation of growth of L929 cells in serum-free medium by TGF beta indicates that autocrine TGF beta receptor activation may be important for maintaining the transformed state of L929 cells; and 2) the way in which a cell responds to TGF beta is dependent on the presence or absence of growth factors contained in the serum.

L236 TYPE β TRANSFORMING GROWTH FACTOR STIMULATES DNA SYNTHESIS IN CULTURED PRIMARY HUMAN MESOTHELIAL CELLS, Edward W. Gabrielson[1], John F. Lechner[1], Brenda Gerwin[1], Michael B. Sporn[2], Lalage M. Wakefield[2], Anita B. Roberts[2], and Curtis C. Harris[1], Laboratory of Human Carcinogenesis[1] and Laboratory of Chemoprevention[2], National Cancer Institute, Bethesda, MD. 20892

We have developed a system for identifying specific growth factors for primary human mesothelial cells, obtained from pleural effusions of patients without malignant disease. Transforming growth factor type β (TGF-β) stimulates DNA synthesis in monolayer cultured human mesothelial cells, detected by increased ^{3}H-thymidine uptake. The prereplicative interval for this stimulation is 24-36 hours, which coincides with the prereplicative interval of growth stimulation by epidermal growth factor (EGF), platelet-derived growth factor, and plasma for for these cells. The stimulatory effect on DNA synthesis by TGF-β is independent of and additive to the growth stimulatory effects of EGF and plasma. Interest in TGF-β as a growth factor for mesothelial cells is strengthened by the observation of increased levels of TGF-β in conditioned media obtained from three of four mesothelioma cell lines tested, when compared to conditioned media from normal mesothelial cell cultures. These results suggest that for some human mesothelioma tumors, TGF-β may be an autocrine growth factor.

L237 IRREVERSIBILITY OF RETINOID INHIBITION OF MOUSE SKIN TUMOR PROMOTION, Helen L. Gensler and G. Tim Bowden, University of Arizona College of Medicine, Tucson, AZ 85724

The effect of the time and duration of retinoid treatment on the inhibition of stage II tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA) was studied in CD-1 mice. All mice were initiated with 400 nmoles of benzo[a]pyrene and received stage I tumor promotion (3.2 nmoles of TPA twice weekly for 2 weeks). Animals were then randomized into groups which received 13-cis-retinoic acid (CRA) during early, middle, or late stage II promotion. CRA pretreatments starting on day 1, week 8, or week 23 of stage II promotion resulted in 47%, 28%, or 19% inhibition, respectively, of TPA-induced tumor formation. One half of the mice receiving CRA at day 1 or week 8 were removed from the retinoid treatments at week 23, the time of cessation of TPA promotion. The inhibition of tumor formation remained constant during the 15 week observation period after cessation of retinoid treatment, suggesting that retinoid inhibition of stage II mouse skin tumor promotion is irreversible in the absence of further promotion.

L238 EVIDENCE FOR CELL PROLIFERATION AND CELL DEATH FACTORS REGULATING GROWTH IN PRIMARY ENDOMETRIAL CULTURES. L. E. Gerschenson, M. Lynch and S. Nawaz; Department of Pathology, University of Colorado School of Medicine, Denver, Colorado 80262

Primary cultures of rabbit endometrial cells, grown on floating collagen gels using serum-free medium, exhibit cycles of cell proliferation and cell death. These cycles appear to be regulated by factors produced by the cells, present in the culture medium and detectable also in homogenates of either cultured cells or rabbit uteri.

These factors appear to be proteins and their apparent molecular weights have been determined by column chromatography. Specificity of the factors' effect has also been determined.

Similar cells cultured on plastic appear not to make the Cell Death Factor, but they respond to it.

Our research suggests that growth is regulated not only by Cell Proliferation Factors but also by Cell Death Factors, and that growth substrates regulate growth homeostasis by modulating cellular production of such factors.

This research is supported by NIH grants CA25365 and CA09157.

L239 INHIBITION OF PROTEIN TYROSINE KINASES IN CULTURED CELLS. Allan R. Goldberg, Doris Slate*, Stephen B. Tatter, Czeslaw Radziejewski. The Rockefeller University, New York, New York 10021 and *Pfizer Central Research, Groton, Connecticut 06340.
We have demonstrated previously that several different peptides are able to inhibit the activity of $p60^{v\text{-}src}$. These include the src peptide (residues 412-421)[1] and [Ala^4] angiotensin II[2]. To test if these inhibitors would have an effect in culture, lines of rodent cells transformed by infection with different oncornaviruses whose oncogenes encode protein tyrosine kinases (src, fps, abl, or fgr) were incubated with these compounds at a concentration of 0.1-1.0 mM. Actin is disorganized in the untreated cells because of the kinase activity, but upon incubation of the cells with these peptides for several hours the actin cables became strikingly organized and the cells assumed a more normal morphology. Furthermore, we observed that these same cells were unable to form colonies in soft agar in the presence of the peptides. The peptides at the same concentrations were ineffective when incubated with cells transformed either by Ha-MSV (v-ras) or by an activated ras oncogene. At 1 mM peptide concentrations no cytotoxicity was observed during 48 hour incubations. Substitution of tyr-416 in the src peptide by a phenylalanine residue rendered the molecule inactive. The kinetics of actin cable formation in the presence of the peptides roughly paralleled those observed when cells transformed with temperature-sensitive src or fps oncogenes were transferred from permissive to non-permissive temperatures. (1) Wong, T.W. and Goldberg, A.R. (1983) Proc. Natl. Acad. Sci. U.S.A. 80:2529-2533; (2) Wong, T.W. and Goldberg, A.R. (1984) J. Biol. Chem.

L240 MODULATION OF GROWTH OF MOUSE MAMMARY CELL LINES BY CELL-DERIVED DIFFUSIBLE FACTORS, Steve Hamner and Jean R. Starkey, Montana State University, Bozeman, MT 59717, and Howard L. Hosick, Washington State University, Pullman, WA 99164
The +SA and -SA tumorigenic cell lines, both subcloned from the same WAZ-2T parent cell line isolated from a mouse mammary adenocarcinoma, and the CL-S1 line derived from premalignant mouse hyperplastic alveolar nodules exhibit quite different growth properties when cultured in a collagen gel matrix. +SA cells are stimulated to proliferate by serum but not by epidermal growth factor (EGF), while EGF but not serum elicits a mitogenic response in cultures of -SA cells of late passage. The CL-S1 line grows best in the presence of both serum and EGF, yet still grows quite poorly in comparison to the other two cell lines. Radioiodinated EGF binding analysis reveals that +SA cells bind almost no ligand, while CL-S1 cells bind EGF with about half the affinity displayed by -SA cells. When established in coculture so that paired cell lines grow in separate collagen gels yet share culture medium, growth of each cell line is enhanced to some degree by the presence of the second cell type. The diffusion-mediated enhancement of growth by +SA cells doesn't appear to be related to either alpha- or beta-transforming growth factor (TGF). CL-S1 enhancement of growth doesn't appear to be an alpha-TGF-like activity. Further characterization of the nature of the diffusible activities released by the cell lines is in progress.

L241 MITOGEN MODULATING FACTORS PRODUCED BY RAW117 LYMPHOSARCOMA CELL LINES. S.S. Joshi*, J.G. Sharp#, H.M. Gharpure+ and K.W. Brunson+, *Departments of Pathology & Microbiology, #Anatomy, University of Nebraska Medical Center, Omaha, NE 68105, +Dept.of Microbiology and Immunology, Indiana University School of Medicine, Gary IN 46408.
Serum free culture supernatants from Abelson virus induced lymphosarcoma (large cell lymphoma) cell lines have been tested for their regulatory effects on mitogen induced proliferation of normal Balb/c spleen cells. The serum free culture supernatants had an enhancing effect for Con-A induced proliferation of normal splenocytes. On the other hand, the same serum free supernatant had an inhibitory effect on LPS induced proliferation of normal splenocytes. There was an increase in production of these factors for up to 8 hours after the initiation of the serum free culture, after which the stimulatory or inhibitory activity of the supernatant remained largely unchanged. Serial dilution of the serum free culture supernatant resulted in a gradual decrease in the activity of the factors. These mitogen modulating factors were dialysable indicating that they had low molecular weights. In addition, these factors were heat stable at 95°C for up to one hour. High pressure liquid chromatographic analysis showed two unique and separate peaks and indicated the presence of two molecules in the serum free culture supernatant of the lymphosarcoma cells. Thus Abelson virus induced lymphosarcoma cells secrete factors which have different functions, one enhancing and one suppressing different mitogen induced proliferative activities of normal splenocytes. (This work was supported by Nebraska State Dept. of Health and Lake County Medical Center Development Agency).

L242 GROWTH-STIMULATING FACTOR FOR CANINE MYELOPOEISIS, Thomas G. Kawakami and Gary R. Cain, University of California., Davis, CA 95616

A major hinderance of understanding canine myeloproliferative disease is the difficulty of maintaining normal and neoplastic myeloid progenitor cells in vitro over an extended period to study factors regulating myelopoiesis. In an attempt to study the possible mechanism of radiation-induced myeloid leukemia in dogs, we have undertaken efforts to establish myeloid leukemic cell lines as a source of gene or gene products that regulate canine myelopoiesis. Recently, we established a cell line from a dog with myeloid leukemia which produces a factor that stimulates growth of myeloid progenitor cells. Using the colony forming unit assay in soft agar, conditioned medium from the myeloid leukemic cells stimulated colony formation of myeloblastic cells whereas the addition of conditioned medium from canine lymphocytes or from canine monocytic leukemic cells did not stimulate colony formation. In liquid culture, the factor stimulated the growth of myeloid progenitor cells which differentiated to macrophages and mast cells. Initial study indicates that the growth stimulating factor appears to be a low molecular weight peptide (10-15K).

L243 ENHANCED PRODUCTION OF PLASMINOGEN ACTIVATOR INHIBITORS IN HUMAN LUNG FIBROBLASTS BY TRANSFORMING GROWTH FACTOR BETA. Jorma Keski-Oja, Marikki Laiho and Olli Saksela. Department of Virology, University of Helsinki, SF-00290 Helsinki, FINLAND.

The role of TGFß in the regulation of pericellular proteolysis was studied using cultured human lung fibroblasts (WI-38 and HEL-299) as models. These cells are nontumorigenic and produce plasminogen activators (PAs) into their medium. The cells were grown to confluency, washed with serum-free medium 199 and exposed to TGFß under serum-free conditions for 24 - 48 hrs. The proteolytic activity of the medium was quantified in agarose gels containing plasminogen and casein. TGFß, at nanogram concentrations, induced the secretion into the medium of proteinase inhibitory activity. When the cells were labeled with ^{35}S-methionine for 4 hr after 18 hr exposure to TGFß, specific enhancement of a secreted 49.000 dalton protein was observed in fluorograms of gels. The protein was associated with proteinase inhibitory activity as demonstrated by zymography assays. The 49.000 dalton protein bound to heparin-Sepharose and formed covalent complexes with purified urokinase, thrombin and plasmin. TGFß thus appears to play a role in the control of pericellular proteolysis in cultured human lung fibroblasts. EGF, PDGF and insulin antagonized the effects of TGFß on the net PA-activity of the cells indicating that PA-activity in cultured cells can be modulated by different polypeptide growth factors.

L244 NORMAL AND NEOPLASTIC HUMAN BREAST CELLS PRODUCE TRANSFORMING GROWTH FACTORS, W. R. Kidwell and D. S. Salomon, National Cancer Institute, Bethesda, MD 20892.

Normal and neoplastic human breast were introduced into primary culture in a serum-free growth medium. Release of TFG and TGF into the culture medium was assessed using a radioimmunoassay and a bioassay of anchorage-independent growth stimulating activity with NRK cells, respectively. TGF activity was readily detected in cells from normal, benign and malignant cells. There was no major differences in TGF production as a function of malignancy. On average, the same appears to be the case for TGF production. Tumor tissue biopsies contained from 0.5-6 ng TGF /mg tissue. The normal and benign tissues examined to date contained 2.5-4.5 ng TGF /mg tissue. Conditioned medium from normal cells contained 0.9 ng TGF /ml which compared to a range of 0.02-0.32 ng TGF /ml medium for established mammary tumor cell lines. Production rates of the transforming growth factors may be insufficient to distinguish between normal, benign and malignant mammary tissues.

L245 RETINOIDS INHIBIT THE ANCHORAGE-DEPENDENT AND ANCHORAGE-INDEPENDENT PROLIFERATION OF BLADDER AND PANCREATIC CARCINOMA CELLS. Matthew Knight, Huda Shubeita, and Anne McCormick, Biochemistry, UTHSCD, Dallas, TX 75235.

All-trans-retinoic acid (RA), all-trans-retinol (R), 4-hydroxyphenylretinamide (HPR), and 13-cis-ethylretinamide (ER) reversibly inhibited the anchorage-dependent proliferation of T24 and EJ human bladder carcinoma cells and AT_3A rat pancreatic carcinoma cells. In each of the three cell lines examined, HPR was the most potent inhibitor being an order of magnitude more active than RA. HPR also inhibited the anchorage-independent proliferation of T24, EJ, and AT_3A cells. The cellular uptake of RA by T24 cells was 10-fold lower than HPR uptake indicating that the reduced activity of RA could be related to low uptake. HPR and ER metabolism were examined in T24 bladder carcinoma cells. Both retinamides were converted to RA in situ and this retinoid accumulated in the T24 cells in a time- and concentration-dependent manner. The metabolism data suggested that retinamide action in the inhibition of T24 cell proliferation might be mediated via conversion to RA. T24, EJ, and AT_3A cells contained no detectable cellular retinoic acid-binding protein (CRABP) indicating that the antiproliferative activity of retinoids in these transformed cells was exerted via a CRABP-independent mechanism. Epidermal growth factor is a potent mitogen for T24 and AT_3A cells grown under anchorage-dependent conditions. Retinoid antiproliferative activity in these two transformed cell lines may be mediated through antagonism of EGF action. In an attempt to delineate the mechanism of retinoid action, the effects of retinoids on EGF-stimulated T24 cell proliferation and EGF receptor number and affinity are

L246 SECRETION OF EPIDERMAL GROWTH FACTOR-LIKE MITOGENS BY NORMAL ADULT BOVINE ANTERIOR PITUITARY CELLS IN CULTURE. Jeffrey E. Kudlow, James Samsoondar and Michael Kobrin, Departments of Clinical Biochemistry and Medicine, University of Toronto, Toronto, Canada.

Bovine anterior pituitary cells grow in serum-free defined culture medium with protein requirements of only insulin and transferrin. They secrete mitogenic activity into their culture medium capable of stimulating NRK fibroblasts and bovine adrenal cortical cells to proliferate. The conditioned medium also contains an activity which can compete with EGF for binding to EGF receptors and which can stimulate anchorage independent growth of NRK cells. The secretory rate of EGF-receptor binding activity by the pituitary cells is comparable to the rate by human melanoma cells. The EGF-like activity chromatographs as three peaks on BioGel P60 with apparent molecular weights of 17, 9 and 6 kDa. All three peaks have mitogenic activity and the 17 and 9 kDa also stimulate anchorage independent growth. We have purified the most abundant 9 kDa peak to homogeneity using reversed phase FPLC and HPLC. Amino acid analysis is compatable with a molecular weight of 12 kDa assuming a single tyrosine. The protein cannot be silver stained on a gel. We have iodinated the purified protein and it runs as a single band at 12-14 kDa unreduced and 5.5 kDa reduced. The iodinated protein binds to EGF receptors and the receptor affinity purified material runs identically on the gel. We have raised a high affinity monoclonal antibody to human transforming growth factor (TGF) type I (EGF-like) which does not recognize EGF but which is capable of immunoprecipitating this pituitary growth factor. Binding of iodinated human melanoma TGF to the antibody can be competed equally well by the pituitary material as by human TGF. The studies indicate that normal bovine pituitary cells secrete an EGF-like mitogen related to the TGF secreted by cancer cells.

L247 TRANSFORMING GROWTH FACTORβ ALTERES THE SECRETION OF PLASMINOGEN ACTIVATOR ACTIVITY IN HUMAN SKIN FIBROBLASTS, Marikki Laiho, Olli Saksela, Jorma Keski-Oja, Department of Virology, Univ. of Helsinki, Haartmaninkatu 3, 00290 Helsinki, Finland

Cultured human skin fibroblasts were used as a model to study the effects of certain polypeptide growth factors on the plasminogen activator (PA) activity. PAs play a central role in the regulation of several proteolytic events by activating plasminogen into plasmin, a wide spectrum proteinase. Plasmin degrades known extracellular matrix glycoproteins and is also able to activate latent collagenases. The growth factors were added to the serum-free medium of confluent adult skin fibroblasts, incubated for 48 hrs and the media were collected. The secreted PA-activity was measured by the caseinolysis in agar assay. TGFβ enhanced the secretion of PA-activity at nanogram concentrations. The effect was time-and dose-dependent and was inhibited by cycloheximide. The induced PA-activity comigrated with purified urokinase in polyacrylamide gel electrophoresis and was inhibited with anti-urokinase antibodies. The enzyme accumulated in pro-activator form and was activated after electrophoresis by plasmin added to the indicator gel in the zymography assay. Also the secretion of PA-inhibitors was slightly enhanced, but the net effect was the stimulation of PA-activity. At least two types of inhibitors were enhanced: a protease nexin-like inhibitor and a PA-inhibitor similar to that secreted by endothelial cells. These results suggest a role for TGFβ in the regulation of PA-activity and pericellular proteolysis in fibroblastic cells.

L248 SITE DIRECTED MUTAGENESIS OF A HUMAN TRANSFORMING GROWTH FACTOR -α GENE

E. Lazar*, S. Watanabe*, R. Derynck+, A. Singh+, E. Van Obberghen-Schilling*, L. Dart*, B. Wolff*, and M. Sporn*, *National Cancer Institute, Bethesda, MD 20892 and +Genentech, Inc., South San Francisco, CA 94080

Transforming growth factor-α (TGF-α) is expressed in certain virally, chemically, and spontaneously transformed cells. Although the precise role of TGF-α is not known in vivo, its secretion appears to be strongly associated with transformation. The aim of the present study is to develop peptide antagonists to TGF-α which block its action by preventing the interaction of the growth factor with its cellular receptor. Such antagonists might be made by modifying regions of the molecule that share homologies with epidermal growth factor, since both growth factors apparently bind to the same receptor. A human TGF-α gene has been cloned in the single stranded phage M13 and used to make several TGF-α mutants by oligonucleotide-directed mutagenesis. Selected mutants have been transferred to a yeast plasmid where their expression is under the control of an α-factor promoter. The mutated TGF-α proteins are secreted into the yeast media. The level of expression of the mutated TGF-α by yeast cultures is measured by radioimmunoassay. Biological activity of the modified proteins has been assayed by measurement of competition with 125I-EGF for receptor binding, as well as stimulation of DNA synthesis and soft agar colony formation in NRK cells. Results of these experiments will be presented.

L249 A NOVEL HEMATOPOIETIC INHIBITORY PROTEIN FROM CULTURED AIDS BONE MARROW

Ira Z. Leiderman, Michael L. Greenberg, Bernard R. Adelsberg, Frederick P. Siegal, Mount Sinai School of Medicine (CUNY), New York, NY 10029

Inhibitors of granulopoiesis have been well described in numerous diseases and syndromes. Many of these inhibitors are T-lymphocyte mediated while others are factors released by specific cell populations. Bone marrow (BM) cells from patients with the Acquired Immune Deficiency Syndrome (AIDS) suppress normal proliferation of the granulocyte-macrophage progenitor cell (CFU-GM), whether AIDS BM cells are in direct contact with or in feeder layers beneath the normal cells. A cell free conditioned media (CM) prepared by the liquid culture of BM cells from patients with AIDS or the AIDS related complex (ARC) inhibit CFU-GM growth in a manner similar to the BM cells alone. CM prepared from normal control BM cells had no such effect. Polyacrylamide gel electrophoresis (PAGE) demonstrated a unique band in lanes containing AIDS and ARC CM but not in the control lanes. Periodic acid-Schiff staining revealed it to be a glycoprotein (gp). A molecular weight for this gp was determined to be ≈84 kilodaltons by SDS-PAGE. Eluates of this band from preparative PAGE inhibited hematopoietic proliferation similar to the complete CM. This inhibitory protein did not inhibit mitogenic stimulation of normal control T-lymphocytes. CM from AIDS BM cells depleted of adherent cells did not have the unique band though non-depleted CM from the same patients contained the band. We are presently determining if this gp is related to HTLV-III/LAV infection since serum from all patients studied contained antibodies to this virus.

L250 PURIFICATION OF PROSTATE-DERIVED GROWTH FACTOR

Dean Li, Shuji Maehama, Hiroki Nanri, and Thomas F. Deuel

A novel polypeptide with a potent growth promoting activity has been isolated from rat prostate glands. The purification was carried out by acid extraction of rat prostate glands followed by ion-exchange, reverse-phase, and gel filtration chromatography. The homogeneity of prostate-derived growth factor, PrGF, was assessed by a single silver stained band on SDS-PAGE and by a single symmetrical peak on both a reverse-phase and gel filtration HPLC. Preliminary characterization revealed that PrGF is a glycoprotein with a molecular weight of 25 kDa with an isoelectric point of 5.0. PrGF is acid and heat stable, protease and β-mercaptoethanol sensitive, and without transforming growth activity. Based on this initial characterization, PrGF appears to be a previously undescribed growth factor.

L251 Cyclic AMP Reverses the Transformation of NIH3T3 Cells by the Human H-ras Oncogene
Arthur H. Lockwood, Adam Lazarus, Suzanne K. Murphy and Maryanne Pendergast.
Department of Pediatrics, Albert Einstein Medical Center, Philadelphia, PA 19141.
Human ras oncogenes are responsible for aspects of the transformation of normal tissue cells to cancer cells. The proteins encoded by the ras genes are 21kDa polypeptides called p21. How the oncogenic p21 proteins transform cells is not known. We now report that an increase in intracellular cyclic AMP (cAMP) can reverse most of the morphological and growth changes associated with the malignant phenotype induced in NIH3T3 cells upon transfection with the human H-ras mutant oncogene or its over-expressed cellular progenitor. Various H-ras transformed NIH3T3 clones were exposed to a variety of agents that elevate intracellular cAMP. There was a rapid alteration in cell morphology. The tumor cells became less refractile, less rounded, had a more tranquile membrane, were less adhesive, and spread more on the substratum. Stress fibers were re-established. The culture morphology became typical of normal fibroblasts. Contact inhibition of growth was restored. The saturation density was reduced to that of untransformed cells. There was a reduction in log phase growth rate and DNA synthesis. Flow cytofluorometric analysis showed that growth arrest occured in the G1 phase of the cell cycle. These results demonstrate the elevation of intracellular cAMP can reverse many of the in vitro parameters of oncogenicity associated with expression of the human H-ras oncogene. We conclude that, in certain cells, the ras oncogene functions to reduce cAMP levels. The consequence is a malignant phenotype. We also infer that the human ras protoonocogene is part of a regulatory system that modulates cellular cyclic AMP. (Supported by PHS Grant CA39232).

L252 RESTORATION OF NORMAL GROWTH TO TRANSFORMED CELLS.
Raphael J. Mannino and Lise B. Winters, Albany Medical College, Albany, NY 12208.
Normal growth regulation can be restored to a variety of transformed cell lines, including cells isolated from spontaneous tumors as well as cells transformed by DNA tumor viruses, RNA tumor viruses and chemical carcinogens, using succinylated concanavalin A (succinyl-conA), a non-toxic and non-agglutinating derivative of the plant lectin concanavalin A. RR1022 cells, a Rous sarcoma virus transformed rat tumor cell line, treated with succinyl-conA, exhibit reversible density-dependent growth regulation. Inhibited cells accumulate in the G_1 phase of the cell cycle. In order to understand the mechanism by which normal growth is restored to RR1022 cells, the levels of v-src mRNA and $p60^{v\text{-}src}$ were determined. The percent of v-src mRNA to total cellular RNA was reduced no more than 2-fold in growing versus succinyl-conA inhibited RR1022 cells. The amount of $p60^{v\text{-}src}$ in inhibited cells was reduced no more than 4-fold relative to growing cells. In contrast, the level of $p60^{src}$ in growing RR1022 cells is 100-fold greater than in a growing, non-transformed rat cell line. An analysis of the intracellular localization of $p60^{v\text{-}src}$ kinase activity demonstrated that $p60^{v\text{-}src}$ remains associated with the plasma membrane in inhibited cells. Therefore, growth inhibition is not associated with a significant decrease in the expression, activity, or appropriate intracellular localization of src. In addition, there is no change in the level of c-myc mRNA accompanying growth inhibition. Since succinyl-conA can restore normal growth regulation to a wide variety of transformed cell lines, we are investigating the hypothesis that succinyl-con A works through an ubiquitous mechanism that involves the induction of growth inhibitory functions that can supercede oncogene expression.

L253 SIRS MEDIATED REDUCTION OF C-MYC MRNA LEVELS,
Victoria R. Masakowski and Thomas M. Aune. Washington University School of Medicine, St. Louis, MO 63110
Interferon (IFN) inhibits a variety of cellular functions in addition to its anti-viral properties. These include inhibition of both immune responses and cell division. Many of these functions are shared by soluble immune response suppressor (SIRS), an immunosuppressive lymphokine whose production by suppressor T cells and by some tumor cell lines (MOLT4 and HeLa) can be induced by IFN. SIRS has been shown to inhibit both antibody secretion and tumor cell division.

Recently the inhibition of cell division by IFN has been linked to the specific down regulation of c-myc mRNA levels in several tumor cell lines. IFN-induced decreases in c-myc message levels approach 100% when compared to untreated controls, and have been shown to temporally precede accumulation of cells in the G_1 phase of the cell cycle. Because of the association between IFN-induced SIRS production and inhibition of cell growth, the effects of SIRS on c-myc mRNA levels were investigated in three cell lines, HL-60, MOLT4 and HeLa. The MOLT4 and HeLa lines were chosen because IFN induces SIRS production in these cells within 24 hours. Two hours after direct addition of purified murine SIRS to MOLT4 or HeLa cells, the c-myc mRNA levels were decreased by 60% when compared to untreated controls. After 24 hours of SIRS treatment, the decrement in c-myc mRNA levels was still >50%. IFN decreased c-myc mRNA levels in MOLT4 or HeLa cells by only 20% at 2 hours, but by up to 100% at 24 hours. HL-60 cells, previously shown to be resistant to IFN-mediated modulation of c-myc levels, were also unaffected by SIRS treatment. NIH-AI07163,NSF-PCM8119606,NCI-K04-CA01086.

L254 A-MuLV "TRANSFORMED" PRE-PRE B CELLS RESPOND TO A UNIQUE GROWTH FACTOR, Gregory J. Palumbo, Brad Ozanne, and John R. Kettman, University of Texas Health Science Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235-9048

We have established that A-MuLV "transformed" pre-pre B cells proliferate in a dose dependent fashion to a factor found in long-term bone marrow culture supernatants (Whitlock and Witte cultures). Production of this growth factor in these bone marrow cultures increases through the first 4 weeks with an apparent maximum level attained at 4-8 weeks post-initiation of culture. The level of this factor then fluctuates over the next 12 weeks. Furthermore, this growth factor is non-dialyzable, acid labile, and precipitable with ammonium sulfate. Additional investigations were performed to determine whether the growth factor found in long-term bone marrow culture supernatants was the same as any of the previously identified lymphokines or cytokines. Our studies with a growth factor responsive cell line (52A4.4G19) indicate that it is not induced to proliferate in a dose dependent fashion by either IL-1, IL-2, Il-3, CSF, BSP-1, TGF-alpha, insulin, transferrin, or a series of cell line supernatants including macrophages and fibroblasts. Therefore, we conclude that the growth factor produced in long term bone marrow cultures is unique.

L255 DETECTION OF TWO LARGER POLYPEPTIDES IN MURINE SALIVARY GLANDS STRUCTURALLY RELATED TO EPIDERMAL GROWTH FACTOR (EGF)
Petro E.Petrides, Molecular Oncology Laboratory, Department of Medicine, University of Munich Medical School, 8 Munich 70, Germany
The 53-amino acid EGF is encoded within a 1217 amino acid EGF precursor which is 20 times larger than the mature growth factor. The precursor contains an internal stretch of hydrophobic amino acids which could anchor the molecule in a membrane and would divide it into a large intracytoplasmic domain and a small extracellular domain. EGF is present in the C-terminal region of the extracellular domain. For the generation of EGF from the precursor at least two proteolytic cleavages are necessary. In order to understand how the precursor is processed and how this process is regulated we have searched for high molecular EGF-related molecules in murine salivary glands. Using an EGF radioreceptor assay we have identified two larger polypeptides upon fractionation of tissue extract on size exclusion gels. The molecules were further purified by RPLC on short chain alkyl supports using different mobile phases. SDS-gel electrophoresis revealed molecular masses of 26 and 22 KD for the two proteins. Both molecules also interact with antibodies directed against murine EGF indicating that they contain the epitope responsible for the generation of antibodies against EGF. Elucidation of the N-terminal sequences of these two polypeptides should allow to determine their location within the EGF-precursor and to unravel the pathway of processing of this molecule.

L256 PRE-B CELL AND HEMOPOIETIC SYNERGISTIC ACTIVITY PRODUCED BY A MURINE MARROW CELL LINE, Peter J. Quesenberry, Ken Landreth, Pam Witte, Richard Shadduck, Abdul Waheed, Ellen Kleeman, Elizabeth McGrath and Gwen Baber, University of Virginia, Charlottesville, VA 22908, West Virginia University Medical Center, Morgantown, WVa., Oklahoma Medical Research Foundation, Oklahoma City, OK, and University of Pittsburgh, Pittsburgh, PA
We have previously identified a growth factor (SGF) from the murine marrow adherent cell line, TC-1, which synergizes the effects of colony-stimulating factor 1 (CSF-1) but has no hemopoietic colony inducing activity by itself. Studies utilizing an anti-interleukin-3 (IL-3)-antibody indicate that SGF is not IL-3. The SGF for CSF-1 is nondialyzable (Spectrapor 3500) stable at pH 5-9 and at 56^{o} for 30 minutes. It precipitates at 35-65% ammonium sulfate, binds to DEAE-cellulose, and elutes between 0.2-0.5 M NaCl. It binds to Concanavalin A and has an apparent molecular weight of 61,000 by Sephadex G100 chromatography. The unseparated TC-1 CM interacts with pure IL-3 to give giant multi-lineage colonies including granulocytes (G), macrophages (M) and megakaryocytes. This TC-1 CM is also capable of potentiating the generation of pre-B cells, as determined by cytoplasmic IgM induction, in short term liquid cultures of normal mouse marrow which has been depleted of pre-B cells on 14.8 monoclonal antibody coated dishes. Altogether these data indicate that the marrow cell line, TC-1, gives rise to a growth factor or factors which synergizes megakaryocyte, granulocyte, and macrophage development and which stimulates pre-B cell differentiation

L257 PURIFICATION OF MELANOMA GROWTH STIMULATORY ACTIVITY. Ann Richmond, H. Greg Thomas Robert G.B. Roy and Chris Engel, V.A. Medical Center and Emory University School of Medicine, Atlanta, Georgia 30033.

The Hs0294 human malignant melanoma cell line produces an autostimulatory monolayer mitogen, melanoma growth stimulatory activity (MGSA). MGSA has been purified from acetic acid extracts of lyophilized conditioned medium by gel filtration, RP-HPLC and preparative electrophoresis, resulting in a 400,000 fold purification. The majority of the bioactivity is reproducibily associated with a 16 Kd moiety eluting from RP-HPLC at 35% acetonitrile. ^{125}I-MGSA specifically binds to Hs0294 cells and retains 100% of the growth stimulatory activity. The 16 Kd MGSA stimulates proliferation of Hs0294 cells at concentrations of 0.3-30 pM. Purified MGSA does not promote anchorage independent growth of NRK indicator cells and is therefore different from the previously described transforming growth factors. The amino acid composition of MGSA differs from that of other growth factors, especially in reference to serine content. MGSA is produced by melanoma tumor cells and certain chromosomally abnormal nevus cells, but is not found in human fibroblasts, myocytes, lymphocytes, or a variety of non-melanoma malignant cells. These data suggest that MGSA may represent a separate class of growth factors with biological and biochemical properties different from previously described growth factors. Supported by NCI grant CA 34590 and a V.A. Merit Award.

L258 Fibroblast Growth Factor Behaves as a Transforming Growth Factor. A. Rizzino, E. Ruff and H. Rizzino. Eppley Institute, Univ. Nebraska Med. Ctr., Omaha, NE.

Transforming growth factors (TGFs) are assayed by their ability to induce the anchorage-independent growth of non-transformed cells. Recently, we have determined that PDGF induces the soft agar growth of two unrelated non-transformed cell lines, NRK and NR-6-R (Rizzino et al., J. Cell Biol. 105:115a, 1985). In the same study, it was determined that FGF induces the soft agar growth of NR-6-R cells and potentiates the response of NRK cells to TGFs and to PDGF. To better understand the mechanisms by which growth factors induce soft agar growth, we have examined the effects of FGF in greater detail. We now report that FGF induces the soft agar growth of a second non-transformed cell line, AKR-2B, which is commonly used to assay TGFs. The soft agar growth responses of AKR-2B cells and NR-6-R cells to FGF are significantly reduced by phorbol esters (e.g. TPA) and by retinoic acid. Examination of the effects of TPA on the monolayer growth of NR-6-R cells suggests that TPA selectively inhibits their growth response to FGF. We are in the process of determining whether this is also true for AKR-2B cells. However, the responses of these two cell lines to FGF are not identical. When FGF is added to monolayer cultures of NR-6-R cells, the cells assume a transformed phenotype, which is characterized by overlapping cell borders. This is not the case for AKR-2B cells in monolayer. Overall, our studies demonstrate that several non-transformed cell lines respond to FGF as if it were a TGF. In addition, it appears that the pathways involved in the responses to FGF and TPA may be interrelated. Efforts are underway to determine the mechanisms involved.

L259 GROWTH-SUPPORTING ACTIVITY OF FRAGMENT Ba OF THE ALTERNATIVE COMPLEMENT PATHWAY FOR ACTIVATED MURINE B LYMPHOCYTES, Erik Ruuth[1], E. Lundgren[1], L. Halbwachs-Mecarelli[2] and F Praz[2]. [1]Inst. for Applied Cell and Molecular Biology, Univ. of Umeå, S-901 87 Umeå, Sweden. [2]INSERUM U25 and CNRS LA 122, Hopital Necker, Paris, France.

The complement system has since long been implied in the regulation of immune responses; we have investigated the role of human factor B and its activating enzyme factor D, as well as its fragments Ba and Bb, on the growth of activated murine B lymphocytes, i.e. (i) in vitro LPS-preactivated B-cell blasts, and, (ii) in vivo activated B-cell blasts from unprimed mice of the LPS-non responder C3H/HeJ strain. LPS-preactivated B-cell blasts did not maintain growth when recultured in serum-free medium alone (660 + 110 cpm), but they did so when re-stimulated by LPS (15,238 + 2,101 cpm). The addition of human factor D (5 µg/ml), to serum-free cultures of LPS-preactivated B-cell blasts containing purified factor B (25 µg/ml), increased ^{3}H-thymidine uptake up to 40 times the background. Neither factor D, nor factor B alone, significantly enhanced proliferation, indicating that factor B had to be cleaved by factor D to be mitogenic. We have established that such a growth-supporting activity was mediated by fragment Ba, whereas Bb had no effect on growth rates. Furthermore, Ba has the ability to maintain growth of low density B-cells of unprimed C3H/HeJ mice, whereas it failed to maintain proliferation of TCGF-reactive T-cells. Experiments designed to characterize a Ba receptor on activated B-cells have been undertaken; Preliminary results indicate that Ba binds to a vast majority of the sIg^{+} LPS-preactivated B-cell blasts, as detected in double staining immunofluorescence analysis.

L260 TUMOR SUPPRESSION IN HAMSTER x MOUSE SOMATIC CELL HYBRIDS DOES NOT INVOLVE QUANTITATIVE CHANGES IN TRANSCRIPTION OF CELLULAR ONCOGENES, Reinhold Schäfer*, Sabine Geisse**, and Klaus Willecke**, Ludwig Institute for Cancer Research*, Bern, Switzerland, Institute of Cell Biology (Tumor Research)**, Essen, F.R.G.

The spontaneously transformed Chinese hamster cell lines Wg3-h-o, CI-4, TK 17-o, and E 36-o proliferate without anchorage and form tumors in nu/nu mice after subcutaneous injection of less than 50 cells. Following somatic cell hybridization of these malignant cells with early passage mouse embryo fibroblasts, two sets of hybrids were isolated which exhibited either expression or non-expression (suppression) of tumorigenicity and proliferation in semi-solid agar medium (R. Schäfer et al., Cancer Research 43:2240, 1983). Relative mRNA levels of 10 protooncogenes were determined by dot hybridization of cytoplasmic extracts prepared from hybrids and parental cells using v-onc genes as radioactive probes. Transcripts of c-Ha-ras, c-Ki-ras, c-myc, and c-fos were detectable in these extracts (prepared from 3 x 10^4 cells). Transcripts of the expected size were detected by Nothern blot hybridization using total cellular RNA from representative hybrid clones. By cytoplasmic dot hybridization, transcripts of c-abl, c-erb A and B, c-fes, c-myb, and c-sis were not detectable (extracts prepared from 2.5 x 10^6 cells). Non tumorigenic (suppressed) hybrids and tumorigenic hybrids showed equal levels of mRNAs related to c-Ha-ras, c-Ki-ras, c-myc, and c-fos. Thus, the expression of tumorigenicity was independent of the transcription of these c-onc genes in the hybrids.

L261 MURINE INTERFERON-γ INHIBITS FIBROBLAST TRANSFORMATION BY THE MOS ONCOGENE, Barbara Seliger and Klaus Pfizenmaier, Clinical Research Group "BRWTI", Max-Planck-Society, 3400 Göttingen, F.R.G.

The mouse fibroblast cell line NIH3T3 was used in gene transfer experiments to study the effect of interferon-γ treatment on the transformation by the mos oncogene. The retroviral vector neo^R MPSV, carrying the neomycin resistance (neo^R) gene and the mos oncogene, was transfected into NIH3T3 cells by calcium phosphate precipitation. The mos oncogene induces morphological transformation of cells, whereas the neo^R gene confers resistance of these cells to G-418. The effect of IFN-γ on transformation was determined by soft agar cloning and the appearance of transformed foci in culture. Our results indicate that recombinant murine IFN-γ (400 U/ml) strongly inhibits the transformation of NIH3T3 cells. Similar results were obtained with an established neo^R MPSV transformed NIH3T3 cell line. In the presence of IFN-γ the cloning efficiency of these cells in soft agar was decreased more than 90 %. In addition, changes in morphology and in in vitro proliferation rate indicate that IFN-γ induced reversion to a non-transformed phenotype, but no direct cytotoxic effects of IFN-γ were noted. Although IFN-γ treatment caused reversion to a nontransformed phenotype, the treated cells did not lose the integrated retroviral sequences. Unchenged expression of the neo^R gene and the mos oncogene was detected by Northern blot analysis suggesting that IFN-γ affects the mos oncogene at the post-transcriptional level. The inhibition of 3T3 cell transformation is reversible, as removal of IFN-γ from the culture medium results in reappearance of cells capable to grow in soft agar. The possible influence of v-mos on the expression of cellular oncogenes and their regulation by IFN-γ will be discussed.

L262 THE EFFECTS OF TYPE-β TRANSFORMING GROWTH FACTOR (TGF-β) ON HUMAN MEGAKARYOCYTIC COLONY FORMATION IN VITRO. L.A. Solberg, Jr., R.F. Tucker, R.B. Jenkins, H.L. Moses. Hematology Research, Mayo Clinic/Foundation, Rochester, MN.

Human serum and platelet-rich plasma markedly inhibit human megakaryocytic colony formation. Furthermore, human platelets and serum contain TGF-β, which can stimulate or inhibit growth of different cell types in vitro at picomolar concentrations. Because of these observations we studied the effects of TGF-β on colonies derived from megakaryocytic (CFU-M), multipotent (CFU-GEMM), erythroid (BFU-E), and granulocytic-macrophage (CFU-GM) progenitors. In 3 separate experiments, 2 x 10^5 marrow cells were cultured in 0.9% methylcellulose, 10% PHA-LCM, 5 x 10^{-5} M 2-mercaptoethanol, and 30% plasma from a patient with aplastic anemia (to study CFU-M, CFU-GEMM, and BFU-E) and 30% FCS (to study CFU-GM). TGF-β was added at final concentrations ranging from 0.001 ng/ml (0.04 pM) to 50 ng/ml (2000 pM). TGF-β caused a 50% reduction in colony formation from CFU-GEMM at 1.8 ng/ml, from CFU-M at 2.3 ng/ml, and BFU-E at 9.5 ng/ml (70-380 pM). CFU-GM-derived colonies were not reduced at these concentrations but did contain fewer macrophages and more granulocytes (proportion of 1000 cells, mean ± SEM,%): Controls: granulocytes, 16 ± 4; macrophages, 75 ± 5; 50 ng/ml TGF-β: granulocytes, 55 ± 7; macrophages, 34 ± 8. TGF-β did not suppress DNA synthesis in megakaryocytes as measured by bromodeoxyuridine incorporation (Jenkins, et al., Blood 64:1221, 1984). We conclude that TGF-β potently inhibits megakaryocytic, erythroid, and multilineage colony formation in vitro. The effect on granulocytic-macrophage colonies is more complex with a shift away from macrophage formation. We are proceeding with studies to learn if TGF-β is responsible for the inhibition of CFU-M and BFU-E caused by serum and platelet-rich plasma.

L263 TRANSFORMING GROWTH FACTOR-BETA: RAPID INDUCTION OF FIBROSIS AND ANGIOGENESIS IN VIVO AND STIMULATION OF COLLAGEN FORMATION IN VITRO, Michael B. Sporn, Anita B. Roberts, Richard K. Assoian, Joseph M. Smith, Nanette S. Roche, Ursula I. Heine, and Lance A. Liotta, National Cancer Institute, Bethesda, MD 20892

Transforming growth factor-beta (TGF-beta) was first identified in neoplastic cells and then found in many non-neoplastic tissues, including human blood platelets, human placenta, and bovine kidney. Recently, the human gene for TGF-beta has been cloned, and mRNA transcripts have been found in both neoplastic and non-neoplastic cells; there is a marked induction of TGF-beta mRNA when peripheral blood lymphocytes are activated. The presence of TGF-beta in cells or cell fragments of hematopoietic origin suggests that it may play some intrinsic role in inflammation and tissue repair. We show here that subcutaneous injection of less than 1 microgram of TGF-beta in newborn mice induces angiogenesis and causes rapid activation of fibroblasts to produce collagen. The new tissue formed after injection of TGF-beta is essentially granulation tissue, resembling that found during physiological wound repair. We also show that TGF-beta *in vitro* has marked stimulatory effects on the formation of collagen by a variety of rodent and human fibroblasts.

L264 INHIBITORS OF DNA SYNTHESIS IN HUMAN DIPLOID FIBROBLASTS, Gretchen H. Stein and Laura Atkins, University of Colorado, Boulder, CO 80309.

When normal human diploid fibroblasts (HDF) become crowded or are deprived of serum, they enter a viable, G1-arrested quiescent state. Likewise, when normal HDF reach the end of their proliferative lifespan, they enter a viable, G1-arrested senescent state. Previous studies have suggested that both senescent HDF and quiescent HDF contain inhibitors of entry into S phase. We have now found that both senescent HDF and quiescent HDF have a plasma membrane-associated inhibitory activity that 1) causes 40-50% inhibition of entry into S phase when added to the medium of replicating HDF, 2) is sensitive to heat and trypsin, and 3) is unable to inhibit DNA synthesis in SV40-transformed HDF. Membranes prepared from replicating HDF have negligible inhibitory activity in this assay. These results support our hypothesis that senescent HDF may contain the same inhibitor protein as do quiescent HDF. When quiescent HDF are stimulated with serum, they undergo a wave of DNA synthesis beginning 12-15 hours after stimulation. We have found that the membrane-associated inhibitory activity in quiescent HDF remains high during the G_0 to S transition (0-12 hours after stimulation) but disappears completely by the peak of DNA synthesis at 20 hours. These results suggest that 1) the majority of the inhibitory activity is not eliminated until just before the cells enter S phase, and 2) elimination of the inhibitor is not sufficient for entry into S phase because only 2/5 of the quiescent HDF synthesized DNA after serum stimulation. We are currently investigating whether serum stimulation of senescent cells will eliminate their membrane-associated inhibitory activity. Failure to do so may be a key defect in the ability of senescent HDF to respond to serum.

L265 DIVERGENT EFFECTS OF RECOMBINANT TUMOR NECROSIS FACTOR-ALPHA ON CELL GROWTH *IN VITRO* Barry J. Sugarman, Bharat B. Aggarwal, Philip E. Hass, Irene S. Figari, Michael Palladino and H. Michael Shepard. Departments of Pharmacological Sciences and Protein Biochemistry, Genentech, Inc., South San Francisco, CA 94080.

Modulation of the *in vitro* growth of human and murine cell lines by recombinant human tumor necrosis factor-alpha (rTNF-alpha) and recombinant human interferon-gamma (rIFN-gamma) was investigated. rTNF-alpha had cytostatic/cytolytic effects on only some tumor-cell lines. rTNF-alpha synergized with rIFN-gamma to cause enhanced antiproliferative effects on a subset of the cell lines tested. In contrast to its effects on sensitive tumor cells, rTNF-alpha augmented the growth of normal diploid fibroblasts (e.g. WI-38 or Detroit 551). Variations in the proliferative response induced by rTNF-alpha were apparently not due to differneces in either the number of binding sites per cell (i.e., about 2000) nor their affinity for rTNF-alpha (K_d was about 0.3nM). These observations indicate that the effects of rTNF-alpha on cell growth are not limited to tumor cells, but rather that this protein may have a broad spectrum of activities *in vivo*.

L266 INDUCTION OF SECRETED PROTEINS IN EPITHELIAL CELLS BY TGF-B AND TPA,
Frederic W. Thalacker and Marit Nilsen-Hamiliton, Dept. of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011

To determine the mechanisms by which transforming growth factors exert their influence on cells in culture, five epithelial cell lines, from different species and tissues, were treated with three agents that inhibit DNA synthesis and proliferation: BSC-1 growth inhibitor (GI/TGF-B), platelet Transforming Growth Factor B, and TPA. All cell lines responded to one or more of these agents by synthesizing and secreting a protein or proteins of M_r 48,000-53,000 into the medium. There was little or no effect on other secreted proteins. The pattern of [^{35}S]methionine labeled intracellular proteins as seen by one dimensional SDS-PAGE was unaltered by these treatments. The average increases in amount of [^{35}S]methionine incorporated into the proteins varied from 2-fold to greater than 800-fold compared with the controls. The regulation of the 48,000 M_r protein (IIP48) in CCL 64 cells was investigated in more detail. The maximum increase in induction of IIP48 occurred within 6 hours of addition of the factor to the cells. IIP48 mRNA appears to be very labile. If Actinomycin D, α-amanitin, or DRB was added with the inducing agent both the control and induced levels of IIP48 were selectively decreased even after as little as 6 h incubation. Induction of IIP48 was not correlated with inhibition of DNA synthesis but might be related to the ability of TPA and transforming growth factors to stimulate growth of untransformed cells in soft agar.

L267 ACTIVITIES, PRIMARY STRUCTURE AND HOMOLOGIES OF BRAIN-DERIVED ACIDIC FIBROBLAST GROWTH FACTOR,
Kenneth A. Thomas*, Guillermo Gimenez-Gallego*, Mari Rios-Candelore*, Jerry DiSalvo*, John Rodkey+, and Carl Bennett+, *Merck Sharp & Dohme Res. Labs, Rahway, N.J. 07065 and +West Point, Pa. 19846.

Brain-derived acidic fibroblast growth factor (aFGF) has been purified to homogeneity and shown to be very mitogenic (1/2 max. activity = 2.4 pM) for Balb/c 3T3 cells in culture (Thomas et al., PNAS 81, 357 (1984)). The protein is also a potent mitogen in culture for a variety of types of cells including both large vessel and capillary endothelial cells (1/2 max. activity = 15-40 pM). Pure aFGF (1 ug/egg) induces microvascular proliferation in the presence of heparin (10 ug/egg) on the chick egg chorioallantoic membrane. The complete amino acid sequence of the 16 kDa bovine aFGF has been determined and observed to be homologous to brain-derived basic FGF and interleukin-1s. A neuropeptide-like decapeptide, flanked by lys-lys dipeptides, is contained within the aFGF sequence.

L268 GROWTH AND DIFFERENTIATION FACTORS FROM HUMAN LYMPHOID MALIGNANCIES

C. Uittenbogaart, S. Kanowith-Klein, Y. Ueno, D. Anisman and P. Nishanian
Department of Microbiology & Immunology, UCLA School of Medicine

Mature virus infected T cell lines contitutively produce various growth and differentiation factors. In order to determine if virus negative malignant lymphoid cell lines secrete factors we collected and purified supernatants from these cell lines, which were cultured long term in serum free medium. In addition to the 5-15K autostimulatory growth factor, leukemia derived growth factor (LDGF), which we previously described, we found that the immature T cell lines MOLT-4f, CCRF-CEM and CCRF-HSB-2 make a non IL-2 T cell proliferation factor (TPF) and a B cell differentiation factor (BCDF). The mature T cell line HUT-78 also produces these factors, but the B cell lines, BJAB and 7031-B only produce BCDF. The myeloid cell line KG-1 does not secrete any of these factors. TPF is a 45K factor which promotes the growth of normal stimulated T cells. BCDF from these T and B cell lines is a 40-60K factor which has similar activity as BCDF isolated from PHA-stimulated T cells, but a different molecular weight. Studies to determine the role of these factors for normal and leukemia cells are underway. (Supported by CA-12800, Leukemia Society of America and the Concern Foundation)

L269 Heparin binding endothelial growth factors associated with the subendothelial extracellular matrix. I. Vlodavsky*+, R. Fridman*, J. Sasse+, J. Folkman+, and M. Klagsbrun+. *Hadassah University Hospital, Jerusalem, Israel, and +Children's Hospital Medical Center & Harvard Medical School, Boston, MA.

The subendothelial extracellular matrix (ECM) produced by cultured bovine corneal and vascular endothelial cells induces cell attachment, proliferation and differentiation. Whereas cell attachment is mediated by adhesive glycoproteins the induction of cell proliferation was attributed to a change in cell shape. We now report that the subendothelial ECM contains endothelial cell mitogens that can be released by extraction of ECM with 2M NaCl. Similar results were obtained regardless of whether the ECM producing endothelial cells were removed by lysis or by exposure to 1M urea without cell lysis. The soluble matrix derived growth factors (MDGF) are mitogenic for bovine aortic and capillary endothelial cells. MDGF bind to immobilized heparin with high affinity and cross react with antibodies against two synthetic peptides representing the amino-terminal sequence of basic FGF and a sequence of acidic FGF. Similar growth promoting factors are also found in the lysates of bovine corneal, aortic and capillary endothelial cells. It is suggested that endothelial cell mitogens secreted by endothelial cells are firmly sequestered by the ECM heparan sulfate and are thereby stabilized. These and other factors may participate in the induction of cell proliferation and differentiation by the ECN both in vitro and in vivo.

L270 SUPPRESSION AND REEXPRESSION OF TRANSFORMED PHENOTYPE IN HYBRIDS OF HA-RAS1 TRANSFORMED RAT-1 CELLS AND EARLY PASSAGE RAT EMBRYONIC FIBROBLASTS

Klaus Willecke, Sabine Griegel, Otto Traub and Reinhold Schäfer[1], Institut für Zellbiologie, Universität Essen, Hufelandstr.55, 4300 Essen 1, Fed.Rep.of Germany, [1]present address: Ludwig Institute for Cancer Research, Bern Branch, Inselspital, 3010 Bern, Switzerland.

Rat-1 cells which had been transformed with the activated Ha-ras1 gene from human EJ bladder carcinoma cells were fused with early passage embryonic rat fibroblasts. Four selected cell hybrids expressed the human transforming gene product p21 at the same level as the transformed parental cells. The hybrid cells, however, exhibited normal morphology, anchorage requirement of proliferation, and largely extended latency periods of tumorigenicity in newborn rats. Tumorigenic hybrid derivatives contained reduced numbers of chromosomes compared to the tetraploid parental hybrids. DNA of the non-tumorigenic cell hybrids transformed Rat-1 cells to anchorage independent proliferation as expected for the transforming human Ha-ras gene present in the donor DNA. We conclude that the transforming properties of the activated Ha-ras gene in Rat-1 cells can be suppressed at the posttranslational level by the presence of the genome from diploid embryonic rat fibroblasts. Thus normal cells contain suppressor gene(s) which safeguard these cells against transformation by the product of the transforming Ha-ras1 oncogene.

L271 STRUCTURE FUNCTION ANALYSIS OF SYNTHETIC HUMAN ALPHA TRANSFORMING GROWTH FACTOR (αTGF) David D. L. Woo, Ian Clark-Lewis, David Live, Leroy E. Hood and Stephen B. H. Kent, California Institute of Technology, Pasadena, California 91125

We have optimized an automatic chemical synthesis of human αTGF. The synthetic αTGF was deprotected and cleaved from the resin used in the synthesis, refolded and purified to homogeneity. The resulting molecule competed at equimolar potency with native epidermal growth factor (EGF) for binding to purified EGF receptor and stimulated NRK cells into colony formation in soft agar. We have used the synthetic, fully active αTGF to investigate the location of disulfide bonds. Monoclonal antibodies against the αTGF were obtained to aid in active-site/epitope analysis of the molecule. Nuclear magnetic resonance spectroscopic and x-ray crystallographic studies are underway to investigate the three dimensional structure of human αTGF.

L272 TRANSFORMING GROWTH FACTOR, TYPE BETA EFFECTS ON NON-TUMORIGENIC, NON-ESTABLISHED HUMAN FIBROBLASTS: DIFFERENCES FROM ESTABLISHED CELL LINES. Jeffrey L. Wrana, Jaro Sodek, Rebecca L. Berr and Carlton G. Bellows. Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada.

In NRK-49F and AKR-2B established cell lines, TGFs are able to induce these anchorage-dependent cells to grow under anchorage-independent conditions. Further, TGF-β has been shown to be a potent mitogen for these cells, yet appears to act antagonistically with EGF. However, the response of these cell lines to TGF-β does not necessarily reflect its physiological activity. Analysis of the effects of TGF-β on early passage diploid fibroblasts derived from human gingivae (HGF) demonstrate that the biological effects mediated by TGF-β in these cells differs significantly from those observed in established cell lines. Specifically, TGF-β, whether in the presence or absence of added EGF, did not stimulate the anchorage-independent growth of HGFs, and studies on anchorage-dependent growth have shown that TGF-β alone is unable to induce the proliferation of quiescent HGFs. However, TGF-β acted synergistically with EGF in promoting the proliferation of quiescent, subconfluent HGFs. TGF-β also caused a dramatic cell density-dependent stimulation of protein synthesis in HGFs including a selective stimulation of matrix proteins. These effects of TGF-β, while contrasting results on cell lines, are more likely to represent TGF-β activity in normal tissues and in wound healing.

L273 A HUMAN PRE-B ALL PRODUCES TRANSFORMING GROWTH FACTOR ACTIVITY, Jerome Zack, R. Graham Smith, and Brad Ozanne, University of Texas Health Science Center, Dallas, TX 75235

The human pre-B acute lymphoblastic leukemia cell line, SMS-SB, has been adapted to grow at high cell density in a serum free defined medium. Concentrated conditioned media from these cells contains an activity that allows the anchorage dependent rat fibroblastic cell line NRK-49F to form colonies in soft agar. NRK-49F cells have been shown by others to require both transforming growth factor (TGF) alpha as well as TGF beta to form this type of colony. Competitive receptor binding studies as well as radioimmuno assays have failed to detect the production of TGF alpha by SMS-SB cells. TGF beta alone does not function in our system. Chromatographic separation techniques lead us to believe that the SMS-SB cells are producing a novel growth factor activity. SMS-SB cells are not unique in their capacity to produce this type of activity in that serum free conditioned media from other human leukemia cell lines have a similar effect on NRK-49F cells. This suggests that the activity described here may be involved in the process of leukemogenesis.

Signal Transduction

L274 PHOSPHORYLATION OF THE PHORBOL ESTER RECEPTOR/PROTEIN KINASE C, Curtis L. Ashendel, Linda Hermsen, and Pamela L. Minor, Purdue University, West Lafayette, IN 47907

The phorbol ester receptor can be chromatographically resolved into two forms. These forms were interconverted upon incubation with ATP or acid phosphatase indicating that they differ only by phosphorylation state. The phospho- and dephospho-forms of this protein were extracted from rat brain in nearly equal amounts. These two forms exhibited different binding affinities for phorbol esters. The phospho-receptor bound [^{3}H]TPA with a K_d of 0.6 to 1.4 nM while the dephospho-form K_d was 0.14 to 0.41 nM. The impact of phosphorylation on the functioning of this protein and the regulation of this phosphorylation in relation to signal transduction, cellular transformation, and tumor promotion are currently being investigated.

L275 DEFINING A SUPEROXIDE ANION / DNA STRAND BREAK BIOCHEMICAL PATHWAY IN HUMAN NEUTROPHILS, H.C. Birnboim, Ottawa Regional Cancer Centre, Ottawa, Canada.

Superoxide anion (O_2^-) is a ubiquitous oxygen radical present in low levels in all aerobic organisms. Because of its inherent low reactivity, it is frequently considered important in biological systems only as a precursor to a much more reactive species, the hydroxyl radical (OH·). The study of O_2^- in living cells is hampered because it is fairly rapidly converted to hydrogen peroxide (H_2O_2) which is more stable. Thus, it may be difficult to separate O_2^- effects from H_2O_2 effects. We have been investigating DNA strand breakage in phorbol ester (TPA)-stimulated neutrophils which produce large amounts of O_2^- at their surface. In this system, O_2^- induces breaks by a mechanism which differs from H_2O_2-induced breaks (Birnboim et al., Proc N.A.S., in press). Metabolic processes appear to be involved since metabolic poisons (fluoride, deoxyglucose, A23187) markedly inhibit the damage. A lipophilic chelator (o-phenanthroline) which blocks H_2O_2- strand breakage actually increases O_2^- strand breakage. Other lipohilic chelators inhibit both. Compared to breaks in DNA introduced by damaging agents such as ionizing radiation and bleomycin (which are rapidly rejoined), H_2O_2 breaks are slowly rejoined and O_2^- breaks are not detectably rejoined over a 60 min period. Cell viability is not impaired under the same conditions. Our current working model is that O_2^- is triggering events by a specific metabolic pathway which, in growing cells, may be important in growth regulation and gene expression.

L276 INTERLEUKIN 2 (IL2) STIMULATES TURNOVER OF INOSITOL PHOSPHOLIPIDS. E. Bonvini, F.W.Ruscetti*, M.Ponzoni and W.B.Farrar*. Lab. of Cell Biology, OBRR/FDA, Bethesda, MD 20892 and *Lab. of Molecular Immunoregulation, NCI/FCRF, Frederick, MD 21701.

T-cell proliferation is dependent on the interaction of a T-cell growth factor, IL2, with specific receptors on the surface of T-lymphocytes. The molecular basis by which this extracellular signal initiates cell proliferation is not defined. We have investigated the role of inositol phospholipid turnover as a mechanism of transduction of the IL2 signal. Murine IL2-dependent CT6 cells, rested in G0 phase by starvation from growth factors, were treated with 500 U/ml of recombinant IL2 (Sandoz Pharmaceutical Co., Geneva, Switzerland) and simultaneously labeled with myo-[2-3H]-inositol. A faster rate of incorporation of labeled inositol into chloroform soluble material was observed in IL2-treated cells as early as 5 minutes after IL2 exposure, reaching a 5-6-fold increased labeling after 1 hour. Thin-layer chromatography analysis of the labeled lipids showed most of the label incorporated into phosphatidylinositol, while a small fraction was recovered with phosphatidylinositol 4-monophosphate. Labeling of phosphatidylinositol 4,5-diphosphate was observed exclusively in IL2-treated cells. CT6 cells, prelabeled for 4 hours with myo-[2-3H]-inositol, were subsequently treated with IL2 in the presence of an excess amount of cold inositol and extracted at different times for analysis of water soluble radioactivity. Increased amounts of labeled inositol 1-monophosphate, inositol 1,4-diphosphate and inositol 1,4,5-trisphosphate were observed in IL2-treated cells, suggesting hydrolysis of inositol phospholipids in response to IL2 treatment. These findings indicate the occurence of increased inositol phospholipid turnover in IL2-treated CT6 cells and suggest that phosphoinositide-derived metabolites may constitute a "second messenger" in the IL2-mediated response.

L277 STRUCTURAL INTERACTION BETWEEN EPIDERMAL GROWTH FACTOR-RECEPTOR AND THE CYTOSKE LETON OF A431 CELLS.

J. Boonstra, L.H.K. Defize and F.A.C. Wiegant. Department of Molecular Cell Biology, University of Utrecht and Hubrecht Laboratory, Utrecht, The Netherlands.

The interaction between epidermal growth factor (EGF)-receptor and the cytoskeleton of A431 cells has been studied at the ultrastructural level by means of surface replication and dry-cleavage. Using a monoclonal anti-EGF-receptor antibody in combination with immuno-gold labeling, an apparent homogenous distribution of gold particles on the cell surface was observed by both methods. By providing an image of the membrane-associated cytoskeleton, the dry-cleavage method in addition revealed a preferential localization of EGF receptors superimposed upon these cytoskeletal filaments. The co-localization of gold particles with cytoskeletal filaments was not affected when pre-labeled cells were extracted with the non-ionic detergent Triton X-100, as visualized with dry-cleavage. Using surface-replication this treatment resulted in visualization of the cytoskeleton. Also in these latter preparations, EGF-receptor coupled gold particles remain assocaited with cytoskeletal elements. Moreover, when Triton extraction was performed before immuno-gold labeling of EGF receptors, we were able to show that these isolated cytoskeletons contained binding sites for anti-EGF-receptor antibodies. Using stereo micrographs of replica's obtained from these isolated cytoskeletons, it was shown that gold labeled EGF receptors were only present on the cortical membrane-associated region of the cytoskeleton, whereas gold particles were absent on more intracellular located filaments.

L278 The Synergistic Stimulation by TPA (PKC) and IP_3 (Ca^{2+}) of G1 Development of Serum-Stimulated Ca^{2+}-Deprived Liver Cells. A.L. Boynton[1], L.P. Kleine[2], D. Franks[2], J.F. Whitfield[2]. 1) Cancer Research Center of Hawaii, Honolulu, Hawaii and 2) NRC, Ottawa. Both the $G_0 \rightarrow G_1$ and $G_1 \rightarrow S$ transitions but not intervening events of serum-stimulated confluent cultures of non-neoplastic rat liver epithelial cells require extracellular Ca^{2+}. The usual Ca^{2+} effect on these transitions can be mimiced by addition to the low-Ca^{2+} (0.07mM) medium-serum change PKC activators such as TPA or the synthetic diacylglycerol OAG. TPA or OAG stimulation was dependent on an optimal level of extracellular (and intracellular?) Ca^{2+} because when the Ca^{2+} was reduced to 0.02mM, TPA or OAG could not stimulate $G_0 \rightarrow G_1$ and $G_1 \rightarrow S$. This low (0.02mM)-Ca^{2+} may have reduced the intracellular Ca^{2+} level because the intracellular Ca^{2+} mobilizing agents A23187 or inositol 1, 4, 5-trisphosphate (IP_3) only in conjunction with TPA or OAG enabled T51B cells to transit $G_0 \rightarrow G_1$ and $G_1 \rightarrow S$ in the usually ineffective low (0.02mM)-Ca^{2+} medium-serum. Although TPA/OAG action would appear to be mediated by PKC, a link with cAMP-PK may exist because both TPA and a synthetic diacylglycerol, DiC_8, increase adenylate cyclase activity and cAMP synthesis. These results coupled with our previous findings that the inhibitor of "C" subunits from cAMP-PK blocks TPA-induced DNA synthesis of Ca^{2+}-deprived T51B cells suggests that these two protein kinases are linked to mediate specific cell cycle transitions. Moreover, deregulation or uncoupling of this membrane-based protein kinase system in neoplastic cells is suggested because they require 10-50 fold less Ca^{2+} for these transitions and have an abnormal distribution of PKC.

L279 TYROSINE PHOSPHORYLATION OF 8 IDENTIFIED SUBSTRATES BY $p60^{src}$ LACKING MYRISTIC ACID FAILS TO CAUSE TRANSFORMATION. Jan Buss, Mark Kamps, and Bart Sefton. The Salk Inst. San Diego, CA 92138

Cellular transformation by Rous sarcoma virus is mediated by $p60^{src}$, a tyrosine-specific protein kinase. The amino terminal glycine residue of p60 is covalently modified by a myristic acid (MYR). Using 2 oligonucleotides, the codon for the N-terminal GLY was changed to one encoding ALA or GLU. Both mutant proteins lacked MYR, and, more importantly, failed to induce focus formation, cytoskeletal changes, growth in agar, protease secretion or a reduction in fibronectin. The lack of MYR had only one direct effect on p60. Unlike WTp60, the nonacylated p60's fractionated as soluble proteins. Despite the lack of transformation, the same substrates were phosphorylated in cells infected with either mutant or WT virus. Tyrosine phosphorylation of these substrates by mutant p60 was extensive and differed from WTp60 only quantitatively. Tyrosine phosphorylation of the 36K protein, p81, and LDH decreased 50-80%, while vinculin phosphorylation increased 9-fold. No changes were detected in tyrosine phosphorylation of the p50 protein which forms a complex with the mutant p60's, of p42, phosphoglycerate mutase, or of the soluble protein enolase. These mutants demonstrate that tyrosine phosphorylation of any of these proteins is not sufficient to cause cellular transformation. The soluble p60's apparently fail to phosphorylate as yet unidentified substrates, presumably membrane proteins, which are crucial for transformation. Access of p60 to these substrates requires MYR or the stable association with membranes which the myristic acid induces.

L280 PHOSPHOINOSITIDE HYDROLYSIS BY PHOSPHOLIPASE C IN NRK CELL HOMOGENATES: ROLE OF G PROTEINS. S. B. Chahwala, L. F. Fleischman and L. C. Cantley. TUFTS UNIV. SCHOOL OF MEDICINE, DEPT. PHYSIOLOGY, BOSTON, MA. 02111.

Mitogenic stimulation of quiescent cells activates a transducing mechanism which involves polyphosphoinositide breakdown. Evidence is accumulating to suggest that receptors linked to phosphoinositide breakdown may be modulated by guanine nucleotides. In NRK cell homogenates we demonstrate activation of a phospholipase C that is stimulated by GTPγS and growth factors, including PDGF, thrombin and bombesin. Determination of rate constants via kinetic analysis of various steps of the PI pathway using [^{3}H]inositol or [^{32}P] labelled homogenates suggests that GTPγS and growth factors stimulate the phospholipase C step. Furthermore, GTPγS stimulates formation of both (1,4,5) and (1,3,4) inositol trisphosphate as resolved by HPLC. The activation of phospholipase C by GTPγS can occur at Ca^{+2} concentrations (130 nM) pertaining in resting cells. The results are consistent with a role for guanine nucleotide binding proteins in growth factor induced activation of membrane-bound phosphoinositide-specific phospholipase C.

L281 TYROSYL AND PHOSPHATIDYLINOSITOL KINASES ARE DISTINCT ENZYME ACTIVITIES IN HUMAN RED CELL MEMBRANES, Anna Coco, Mark Vossler, Bimmie Strausser and Ian G. Macara, University of Rochester Medical Center, Rochester, NY 14642

Recent evidence has indicated a possible association between certain oncogene proteins with demonstrable tyrosyl-specific protein kinase (TK) activity and a phosphatidylinositol (PI) phosphorylating activity (Macara, I.G., Marinetti, G.V. and Balduzzi, P.C. (1984) Proc. Natl. Acad. Sci. USA, 81, 2728-2732; Sugimoto, Y., Whitman, M., Cantley, L.C. and Erikson, R.L. (1984) Proc. Natl. Acad. Sci. USA 81, 2117-2121). To determine if such an association is ubiquitous we have partially purified an 'authentic' PI kinase activity from human red cell membranes. Both the PI kinase and TK were found to require detergent for solubilization, were associated with a complex of > 200 kilodaltons, and copurified through an ammonium sulfate precipitation and DEAE ion-exchange step gradient elution. However, the two activities differed with respect to their apparent Km's for ATP and Mg^{2+}; showed different half-lives for temperature inactivation at 37° and 50°C; possessed different relative activities in the presence of Mn^{2+} and Ca^{2+}; and were separable by elution from a DEAE-Trisacryl ion exchange column using a linear NaCl gradient. The kinases could not be autophosphorylated, and PI kinase was not activated by pre-treatment with the TK $p68^{v\text{-}ros}$ or by addition of the phosphotyrosyl phosphatase inhibitor, vanadate, to intact membranes, and was not competitively inhibited by the tyrosyl kinase substrate poly(Glu_4,Tyr). We conclude that the human red cell phosphatidylinositol and tyrosyl kinases are distinct and separable activities. (Supported by NIH Grant CA38888-01.)

L282 PERTUSSIS TOXIN OR PHORBOL ESTERS CAN DISTINGUISH BETWEEN EGF AND ANGIOTENSIN II STIMULATED SIGNALS IN HEPATOCYTES Connelly, P.A., Johnson, R.M., Sisk, R.B., Pobiner, B.F., Hewlett, E.L* and Garrison, J.C. Departments of Pharmacology and *Internal Medicine, University of Virgina School of Medicine, Charlottesville, VA

Epidermal growth factor (EGF) causes rapid increases in free intracellular Ca^{2+} ion and stimulates the phosphorylation of 11 cytosolic proteins in hepatocytes. Ten of the 11 cytosolic proteins altered by EGF are identical to those affected by angiotensin II (AII) or vasopressin, hormones which stimulate the breakdown of phosphatidylinositol 4,5 bis-phosphate. An increase in the phosphorylation of the other protein, spot c (Mr=36,000,pI=5.5), is only observed with EGF. Intact rats were treated with Bordetella pertussis toxin to fully ADP-ribosylate N_i, the inhibitory GTP-binding protein of the adenylate cyclase complex. This treatment abolished the effect of EGF on Ca^{2+} mobilization and on the phosphorylation of the 10 common substrates of EGF and AII, but had minimal effect on the ability of EGF to stimulate the phosphorylation of its unique substrate, spot c. In marked contrast, modification of N_i did not block the ability of AII to stimulate Ca^{2+} mobilization or protein phosphorylation. Pretreatment of normal hepatocytes with 4β-phorbol 12-myristate 13-acetate (PMA) blocked all responses to EGF including the increased phosphorylation of spot c, but had no effect on the responses to AII. These results imply that N_i or a similar pertussis toxin substrate may mediate the effects of EGF on phosphatidylinositol breakdown and that protein kinase C may regulate a site in the transduction pathway proximal to this guanine-nucleotide binding protein. Angiotensin II appears to use a different signal transduction mechanism to stimulate phosphatidylinositol metabolism in hepatocytes.

L283 PMA AND CON A CAUSE OPPOSITE TRANSLOCATION OF KINASE C DURING MONOCYTE ACTIVATION. M.R.Costa-Casnellie, G.B. Segel and M.A. Lichtman. Department of Medicine, University of Rochester, Rochester, NY. 14642

Calcium mobilization and increased phosphoinositide (PI) turnover are two key events in monocyte activation that precede superoxide production. Calcium and diacylglycerol, a by-product of PI turnover, are cofactors of protein kinase C. We have conducted experiments to see if kinase C activation and/or translocation are required for induction of superoxide production. Concanavalin A (CON A) and PMA induce monocyte superoxide production but have opposite effects on the subcellular redistribution of kinase C. Addition of 10nM PMA caused a rapid decrease in the cytosolic enzyme levels (from 725 ±50 to 51 ±30 pmol/min.mg^{-1}) and a parallel increase in the particulate levels (from 719 ±140 to 1133 ±60 pmol/min.mg^{-1}) In contrast, 10ug/ml CON A caused an increase in the cytosolic enzyme levels (to 1044 ±20 pmol/min. mg^{-1}) and a parallel decrease in the particulate levels (to 168 ± 50 pmol/min.mg^{-1}). The concentration dependence and time course of both kinase C translocations are compatible with their involvement in monocyte activation. This hypothesis was further tested by adding 30ug/ml CON A and 2nM PMA together. CON A had no effect on the initial rate of superoxide induced by PMA, but it inhibited PMA induced kinase C translocation. Induction of superoxide by CON A required the addition of 5ug/ml cytochalasin B. However this agent inhibited CON A induced kinase C translocation. Cytochalasin B also inhibited PMA induction of kinase C translocation and superoxide production. Thus, induction of superoxide by Con A or PMA may involve activation of kinase C but this activation is not necessarily associated with enzyme translocation.

L284 PHOSPHORYLATION OF THE ERBB PROTEIN FROM VARIOUS AVIAN ERYTHROBLASTOSIS VIRUS TRANSFORMED CELL TYPES, Stuart J. Decker, Rockefeller University, New York, NY 10021

Phosphorylation of the erbB protein and the effects of the tumor promoter 12-O-tetradecanoyl-13-acetate (TPA) on erbB phosphorylation and on cell proliferation were examined in several avian erythroblastosis virus (AEV) transformed cell types. For chick embryo fibroblasts (CEF) transformed with either the ES4 or H strain of AEV in vivo phosphorylation of the erbB protein was readily detected and occurred primarily on serine and threonine residues. In contrast, almost no phosphorylation of the erbB protein from an AEV-transformed erythroid cell line or an AEV-transformed rat fibroblast cell line was found, even though these cells appeared to possess levels of erbB protein comparable to those in the AEV-transformed CEF. Treatment of cells with TPA caused a 2-fold increase in phosphorylation of the erbB protein from AEV-transformed CEF and inhibited growth of these cells in monolayer and soft agar culture. TPA did not seem to enhance the phosphorylation of the erbB protein from AEV-ransformed erythroid cells or rat fibroblasts, slightly inhibited growth of the AEV-transformed erythroid cells and had no effect on growth of the AEV-transformed rat fibroblasts. The 34k protein known to be a substrate for the src protein tyrosine specific protein kinase was phosphorylated in vivo on tyrosine and serine residues in certain AEV-transformed CEF isolates and TPA treatment of cells preferentially reduced the level of phosphotyrosine in this protein. In vitro autophosphorylation of the erbB protein on tyrosine residues was only detected using erbB protein from AEV-transformed CEF in which erbB was also phosphorylated in vivo and treatment of cells with TPA inhibited in vitro autophosphorylation of the erbB protein.

L285 PHOSPHORYLATION OF DIHYDROFOLATE REDUCTASE AND THE REGULATION OF ITS ACTIVITY DURING THE GROWTH OF HL-60 PROMYELOCYTIC LEUKEMIA CELLS, Shoukat Dedhar, Gerald Krystal, and James H. Goldie, Cancer Control Agency of B.C., Vancouver, B.C., Canada

We have recently demonstrated that the dihydrofolate reductase (DHFR) activity in Methotrexate (MTX)-resistant variants of HL-60 cells is increased 20-fold in the absence of an increase in the amount of DHFR protein, indicating that the enzyme in the resistant cells is catalytically more active (Dedhar et al., Biochem. J. 225:609-617, 1985). To determine whether phosphorylation of DHFR could be correlated with its enzyme activity, both the sensitive and resistant cells were labeled with ^{32}P-orthophosphate, cell extracts prepared, and the DHFR immunoprecipitated with anti-DHFR antiserum. SDS-PAGE demonstrated 21,000-dalton phosphoproteins in the extracts of both cell lines, this protein being much more heavily phosphorylated in the resistant cells. In vitro ^{32}P-labeling of cell extracts using [γ^{32}P]-ATP followed by immunoprecipitation and affinity chromatography on MTX-Sepharose also demonstrated that DHFR in these cells is a phosphoprotein and that it is more heavily phosphorylated in the resistant cells. DHFR activity in both cell lines fluctuated during the growth of these cells, i.e. it was low during the lag phase, peaked ($\sim$10 to 50-fold higher in sensitive and resistant cells, respectively) at mid exponential phase and dropped down to negligible amounts in the stationary phase. The amount of ^{32}P associated with DHFR in both cell lines paralleled this fluctuation. These data indicate that DHFR activity may be controlled by a phosphorylation-dephosphorylation mechanism, possibly in response to external signals such as growth factors.

L286 CHARACTERIZATION OF THE HUMAN GM-CSF RECEPTOR. J.F. DiPersio, P. Eghtesady, D.W. Golde and J.C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Human granulocyte-monocyte colony-stimulating factor (GM-CSF) is a 22,000 dalton glycoprotein hormone which stimulates the growth of normal human bone marrow and several human myeloid leukemic cell lines (HL-60, KG-1) in vitro. GM-CSF enhances both neutrophil chemotaxis and superoxide generation in response to chemoattractant oligopeptides and C5a.

The physiologic responses of leukemic and normal hematopoietic cells to GM-CSF are mediated through specific GM-CSF receptors found on the surface of responsive cells. There appears to be a single class of high affinity binding sites on mature neutrophils (Kd~20 pM, ~300 receptors/cell) and purified human eosinophils (Kd~20 pM, ~600 receptors/cell). KG-1 and HL-60 cells exhibit two apparent classes of GM-CSF receptors, a similar high affinity class (Kd ~ 20 pM, ~ 40 receptors/cell) and a separate low affinity receptor (Kd ~ 3.0 NM, ~ 1100 receptors/cell). DMSO is able to increase the number of high affinity GM-CSF binding sites on HL-60 cells coincident with the induction of neutrophilic differentiation. The significance of these low affinity receptors remains elusive since GM-CSF exerts its maximal biological activities at concentrations of 100 pM. It is not known if the presence of low affinity GM-CSF receptors is a function of the leukemic state or simply a property of cells which are capable of undergoing cell division. Studies delineating the association of low and high affinity GM-CSF receptors and mechanisms of transmembrane signalling are currently underway.

L287 THE ROLE OF PROTEIN KINASE C IN MURINE ERYTHROLEUKEMIA CELL DIFFERENTIATION, Donna L. Faletto and Ian G. Macara, University of Rochester Medical Center, Rochester, NY 14642

Previous studies in this laboratory have demonstrated a significant drop in diacylglycerol (DG) levels within 1 hour of dimethylsulfoxide (DMSO) or hypoxanthine (HX) induction of murine erythroleukemia cell (Friend cell) differentiation. This is the earliest reported change to occur in the Friend cell differentation program. The decrease in DG level precedes decreases in levels of phosphatidylinositol metabolites, c-myc mRNA, and myc-related protein products (Faletto et al., Cell, in press). Since phorbol ester (phorbol 12-myristate, 13-acetate) and two synthetic diacylglycerols effectively block Friend cell differentiation, we have suggested that DG regulation of protein kinase C may play a key role in control of Friend cell differentiation.

To ascertain the functional significance of the decrease in DG levels, we have examined the distribution of kinase C between cytosol and membrane in uninduced and induced Friend cells. A large increase in cytosolic kinase C levels is observed after DMSO treatment, and this increase is blocked by phorbol ester treatment. The effect of HX, and vanadium (another potent inhibitor of Friend cell differentiation) on kinase C localization within Friend cells has also been examined. Our observations are consistent with a regulatory role for kinase C in the Friend cell differentiation program. (Supported by NSF Grant PCM-8304420.)

L288 TUMOR PROMOTERS INCREASE BINDING OF ^{125}I-THROMBIN TO RECEPTORS ON HAMSTER AND MOUSE EMBRYO FIBROBLASTS. E. A Gordon, J. S. Bergmann and D. H. Carney, Division of Biochemistry, University of Texas Medical Branch, Galveston, TX 77550.
Phorbol Myristic Acetate (PMA) attenuates binding of a number of peptide hormones, growth factors and transport molecules. This effect appears to involve a transient loss of high affinity binding sites. With transferrin, this loss of receptors appears to result from an increase in receptor internalization and degradation. In other cases (e.g., EGF), the loss of high affinity binding sites prevents ligand internalization. Thrombin binds to clustered receptors on fibroblasts which are not associated with coated pits or rapid receptor mediated endocytosis. We, therefore, have examined the effects of PMA on ^{125}I-thrombin binding to these cells. Two hour pretreatment of cells with 30 μM PMA causes up to a 2-fold increase in ^{125}I-thrombin binding. This effect is time and concentration dependent for both PMA and ^{125}I-thrombin. A similar effect can be observed on cells preincubated with PMA and then fixed with formaldehyde prior to thrombin binding. Scatchard-type analysis of the binding data suggests an increased affinity rather than an increase in receptor number. PMA has been reported to stimulate release of Protease-Nexin (PN) after prolonged exposure. However, after ^{125}I-thrombin binding to PMA treated cells, SDS PAGE showed that the PMA induced increase in binding could not be attributed to formation of PN-thrombin complexes. Thus, these results demonstrate that PMA effects thrombin binding to its receptors and that this effect appears to be different from that observed in a number of other systems. (Supported by Grants AM-25807 and CA-00805.)

L289 DIFFERENCES IN PRIMARY AND TERTIARY STRUCTURE OF HUMAN AND RAT INSULIN RECEPTOR β-SUBUNIT. H.J. Goren, Department of Medical Biochemistry, University of Calgary.
Molecular weight estimates of the insulin receptor β-subunit (β-sub) are independent of species or tissue source. Whether the primary structure is similarly independent is not known. The primary and tertiary structure of rat hepatoma (FAO) cell and human placenta (pla) β-sub have been compared. Solubilized, wheat germ agglutinin-purified receptor was phosphorylated in the presence of [γ-^{32}P]ATP, $MnCl_2$ and insulin, and then exposed to 5 μg/ml trypsin (T) or staphylococcal protease V8 (SPV8) 30 min, 20°C. ^{32}P-Proteins were separated by SDS-PAGE, excised from the gels, and completely digested with T. ^{32}P-Peptides were separated by reverse-phase HPLC. Phosphorylation occurred in the 95 kDa β-sub and insulin stimulated the reaction. Mild T-digestion decreased both β-sub to 90 kDa and 70 kDa proteins. SPV8 decreased the FAO β-sub to an 85 kDa protein and the pla β-sub to 85 kDa and 60 kDa proteins. Insulin-stimulated phosphopeptides eluted between 10-20% acetonitrile (II) and 20-30% acetonitrile (III) in HPLC. The phosphopeptide analyses were (+, increase; -, absent; +, present):

TISSUE	EFFECTOR	^{32}P-PROTEIN	^{32}P-PHOSPHOPEPTIDE II	III
FAO/Pla	Insulin	β-95	++/+	+/+
FAO/Pla	Trypsin	β-90	-/+	+/+
FAO/Pla		β-70	-/-	+/+
FAO/Pla	SPV8	β-85	-/-	+/+
Pla		β-60	-	+

In conclusion, differences in sites of autophosphorylation and differences in mild protease sensitivity in human and rat insulin receptor β-sub suggest that differences in primary and tertiary structures may exist between human and rat insulin receptors.
(Supported by MRC of Canada.)

L290 $pp60^{src}$ IS A SUBSTRATE OF PROTEIN KINASE C IN VITRO AND IN VIVO; K.L. Gould[1,2], J.R. Woodgett[1], and T. Hunter[1]; [1]MBVL, The Salk Institute, San Diego, CA; [2]Department of Biology, UCSD, La Jolla, CA

$pp60^{v-src}$ is constitutively phosphorylated on Ser 17 and Tyr 416. $pp60^{c-src}$ is also phosphorylated on Ser 17, but at a different tyrosine, Tyr 527. When cells were treated with the tumor promoters, TPA or teleocidin, which bind and activate protein kinase C, new sites of phosphorylation were observed in both $pp60^{c-src}$ and $pp60^{v-src}$. The stoichiometry of TPA-induced phosphorylations was as high or higher than that of Ser 17.

Immunoprecipitated $pp60^{src}$ was phosphorylated in vitro by purified protein kinase C at the same sites induced by TPA in biosynthetically labeled $pp60^{src}$. Five other purified protein-serine/threonine kinases did not phosphorylate $pp60^{src}$ at these new sites.

Protease digestions and Edman degradation of tryptic phosphopeptides and the use of synthetic peptides allowed us to identify the new sites of phosphorylation as Ser 12 in mammalian $pp60^{c-src}$ and Ser 12 and Ser 48 in chicken $pp60^{c-src}$ and $pp60^{v-src}$.

Other agents which result in the activation of protein kinase C, the synthetic diacylgylcerol, OAG, purified PDGF, vasopressin, and bombesin also stimulated phosphorylation of $pp60^{c-src}$ from Swiss 3T3 and/or NIH 3T3 cells at Ser 12. PDGF treatment not only induced Ser 12 phosphorylation but caused the appearance of an additional modified form of the protein. V8 protease mapping showed that the PDGF-specific alteration lay in the extreme N-terminus of the protein and was associated with new phosphorylation sites.

The effects of these new phosphorylations on the kinase activity and other properties of $pp60^{c-src}$ will be discussed.

L291 THE HUMAN B CELL AS A MODEL FOR ANALYSIS OF MITOTIC SIGNALLING MECHANISMS. Graeme R. Guy, John Gordon, Leonie J. Walker, Robert H. Michell and Geoffrey Brown. Birmingham University, Birmingham U.K. B15 2TJ.

The human B cell represents an excellent model system in which to investigate the transducing signals involved in mitogenesis. Complete mitogens include SAC (Staphylococcus Aureus Cowan strain 1), immobilised anti-immunoglobulin and Epstein-Barr Virus (EBV) whilst in the case of soluble $F(ab)_2$ fragments of anti-Ig further addition of T cell derived factors such as B Cell Growth Factor (BCGF) is required for cells to complete their cell cycle. However, both $F(ab)_2$ and SAC crosslink surface immunoglobulin molecules resulting in the hydrolysis of the inositol phospholipid PI 4,5-P_2. The subsequent effects of signals derived from this hydrolysis, calcium mobilisation and diacylglycerol production, can be mimicked by the concurrent addition of diacylglycerol analogues and calcium ionophores at concentrations which are ineffective on their own. In contrast, EBV does not cause hydrolysis of PI 4,5-P_2 thus indicating that cellular signalling events subsequent to EBV stimulation act post PI 4,5-P_2 hydrolysis. Monoclonal antibodies to cell surface antigens have been used to describe the appearance of various putative receptors when B cells are activated. A model of early signal transduction is proposed which focuses attention on the relative membrane and cytoplasmic disposition of protein kinase C and activity status of this enzyme which is a likely focus of mitogenic signal transduction.

L292 EPIDERMAL GROWTH FACTOR RECEPTORS IN HUMAN BREAST, BLADDER AND LUNG CANCER - CORRELATIONS WITH BIOLOGICAL BEHAVIOUR

A.L. Harris, R. Sainsbury, G. Needham, D. Veale, D. Neal, N. Kerr, J. Farndon, G.V. Sherbet, R. Hall. Cancer Research Unit and Department of Clinical Oncology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 4LP, U.K.

EGF receptors (EGFr) have been measured in 104 primary breast cancers, 48 primary bladder cancers and 58 primary non-small cell lung cancers by I^{125} EGF ligand binding. Frozen sections were stained by R_1 (M. Waterfield) anti-EGFr antibody using the immunoperoxidase technique. There was a very high correlation of EGF-r measured by ligand binding with staining intensity. In all tumour types, there were 2 classes of EGFr with high affinity receptors of Kd 10^{-10} - 10^{-9}. In breast cancer there was an inverse relationship to estrogen receptor, ER ($^4/53$ ER+ve tumours had EGFr, $^{31}/51$ ER-ve tumours had EGFr) and a significant correlation with poor differentiation and metastasis. In non-small cell lung cancer, squamous cancers had most EGFr and there was a significant association with high stage tumours. In bladder cancers there was an association with invasive tumours ($^7/24$ superficial tumours EGFr+ve, $^{21}/24$ invasive tumours EGFr+ve). Preliminary analysis of Southern blots from human breast cancer shows rearrangements of EGFr genes.

Thus in 3 common epithelial malignancies, EGFr correlates with high stage, invasion or poor differentiation. EGFr expression may be related to late stages of tumour progression and provides a therapeutic target for the worse prognosis cancers.

L293 SIGNAL TRANSDUCTION MECHANISMS OF T CELL ACTIVATION. D.T. Harris, W.J. Kozumbo, P.A. Cerutti, and J.C. Cerottini. University of North Carolina, Chapel Hill, N.C., Swiss Institute for Cancer Research and Ludwig Institute for Cancer Research, Lausanne, Switzerland. The signal transduction mechanisms involved in lymphokine (LK) production by T cells and IL-2-induced T cell proliferation were investigated. Stimulation of resting cloned murine CTL with either antigen or mitogen resulted in LK production but not proliferation, whereas only the incubation of these CTL with IL-2 resulted in proliferation, but not LK production. Phosphatidylinositol (PI) metabolism was found to be associated only with LK production, but not proliferation. LK production was accompained by poly-PI turnover, diacylgycerol production, and inositolphosphates generation. Further, intracellular Ca^{2+} fluxes were found only upon LK production. These events occurred within minutes of stimulation and persisted for only 8h even in the continuous presence of stimuli. This finding correlated with the observation that only 8h of CTL/stimulus interaction was required for maximal LK production. Both LK production and proliferation were dependent on extracellular Ca^{2+} and seemed to involve protein kinase C activation. LK production was associated with phosphorylation of a 20 kD (non-T3) protein, and proliferation with a 200 kD protein. Artificial manipulations of the systems indicated that PI turnover (with Ca^{2+} flux and phosphorylation) was the signal transduction mechanism responsible for LK production while the mechanism for IL-2-induced proliferation remains unknown. These findings should be applicable to understanding malignant T cell function.

L294 REDUCED MITOGENIC RESPONSES TO EPIDERMAL GROWTH FACTOR DURING HEPATOCARCINOGENESIS IN RATS M.A. Hayes, E. Roberts, L. Harris, V. Preat and E. Farber. Department of Pathology, University of Toronto, Toronto, Ontario Canada, M5S 1A8

In the resistant-hepatocyte model of hepatocarcinogenesis, nodular proliferations of phenotypically altered hepatocytes are generated in Fischer 344 rats initiated with diethylnitrosamine and promoted by a selection regimen of partial hepatectomy (PH) with mitoinhibition by 2-acetylaminofluorene. Some of these nodules persistently proliferate for many months and are a site in which hepatocellular carcinomas may eventually develop. We have examined the role of epidermal growth factor (EGF) in the altered proliferation of persistent nodules and carcinomas. Hepatocytes were isolated by collagenase perfusion from normal livers and also from discrete persistent nodules and carcinomas. In primary monolayer cultures, EGF (0-40 ng/ml) markedly increased S-phase DNA synthesis (by labeling index at 48 hours in ^{3}H-thymidine autoradiographs) of normal hepatocytes from 16 + 8% to 54 + 5%. However the same concentration range of EGF had little influence on the labeling index of hepatocytes from persistent nodules (2 or 8 months) or carcinomas (12-15 mo). Isolated hepatocytes from various stages were incubated at 4°C with ^{125}I-labeled EGF. Saturation binding of EGF to intact hepatocytes was reduced to 7-30% of control values in persistent nodules and to 8% of control values in carcinomas. These results indicate that persistent proliferation of resistant nodules and hepatocellular carcinomas is independent of EGF-mediated stimulation of mitogenesis. Supported by NSERC Canada (A2761, G1494) and MRC Canada (MT-5594).

L295 ACTIVE PHORBOL ESTERS INDUCE PHOSPHORYLATION OF A TRANSFORMATION-SENSITIVE SURFACE GLYCOPROTEIN OF HUMAN FIBROBLASTS WHICH IS ENCODED BY A GENE ON CHROMOSOME 11, Masaru Imada, Meiji Institute of Health Science, Naruda, Odawara, Japan.

We have previously shown that a surface glycoprotein of normal human fibroblasts with an apparent molecular weight of 200,000 daltons is encoded by a gene mapped to the long arm of human chromosome 11 and that its synthesis is induced by an increase of intracellular cyclic AMP concentrations. In this paper, normal and transformed human fibroblasts and human fibrosarcoma cells were examined with specific emphasis on the expression and phosphorylation of this 200,000-dalton protein. Monolayer cell cultures were enzymatically labeled with ^{125}I-iodine or metabolically with ^{14}C-glucosamine or ^{32}P-phosphate and analyzed by two-dimensional polyacrylamide gel electrophoresis. The protein was missing, or at least markedly reduced, in three human fibrosarcoma cell lines and three of four lines of SV_{40}-transformed human fibroblast. This conclusion was drawn from experiments using three different methods of radiolabeling in the presence or absence of phorbol esters or cyclic AMP. Elevated levels of phosphorylation of the protein was induced when ^{32}P-labeled cultures were treated for 30 minutes with active phorbol esters, but not with inactive phorbol esters. The 200,000-dalton protein and a well known transformation-sensitive protein, fibronectin, appeared to be separate molecules because they gave rise to distinct peptides by partial proteolytic digestion. The protein may be a novel receptor for a growth factor which is secreted at higher levels by transformed human fibroblasts.

L296 MODULATION OF p36 PHOSPHORYLATION IN HUMAN CELLS. Clare M. Isacke, Ian S. Trowbridge & Tony Hunter. The Salk Institute, P.O. Box 85800, San Diego, CA 92138

p36 is an abundant plasma membrane-associated protein found in many tissue types and cultured cells. It is a major substrate for protein-tyrosine kinases encoded by viral transforming proteins and some growth factor receptors. To examine the modulation of phosphorylation of this protein in human cells we have used a highly specific monoclonal antibody directed against human fibroblast p36. In both AG1523 fibroblast cells and A431 human epidermoid tumour cells a small proportion of p36 molecules are constitutively phosphorylated on serine residue(s) giving rise to 4 phosphoserine-containing peptides by 2d tryptic peptide mapping. Treatment of AG1523 cells with PDGF or A431 cells with EGF resulted in the appearance of 2 novel phosphotyrosine-containing peptides. p36 from A431 cells transformed with ST-FeSV or RSV, which encode the v-fes and v-src protein-tyrosine kinases, showed a similar peptide pattern with the addition of 3 further phosphotyrosine-containing peptides. Thus, we find that viral transforming proteins phosphorylate p36 on at least one additional tyrosine residue to that phosphorylated in response to growth factors. The treatment of human cells with the phorbol ester, TPA, also resulted in a significant increase in phosphorylation of p36. This occurred at a single serine site, presumably as a result of phosphorylation by activated protein kinase C molecules. Using these monoclonal antibodies we are currently investigating the functions of p36 and its role in viral transformation and mitogenic signal transduction.

L297 A HORMONE-ACTIVATED, GUANINE NUCLEOTIDE-DEPENDENT, PHOSPHATIDYLINOSITOL-4,5-DIPHOSPHATE-SPECIFIC PHOSPHOLIPASE C IN NORMAL AND TRANSFORMED CELLS. S. Jackowski, C.W. Rettenmier, C.J. Sherr and C.O. Rock, St. Jude Children's Research Hospital, Memphis, TN 38101

A variety of hormones and polypeptide growth factors stimulate the formation of inositol-1,4,5-triphosphate ($Ins-P_3$) and diacylglycerol leading to an increase in cytosolic calcium and protein phosphorylation catalyzed by protein kinase C. The regulated step in this cascade is the ligand-promoted cleavage of phosphatidylinositol-4,5-diphosphate ($PtdIns-P_2$). We have biochemically characterized a $PtdIns-P_2$-specific phospholipase C in membranes isolated from human platelets or NIH 3T3 fibroblasts that can be activated by either thrombin or a guanine nucleotide. The same hormone-responsive catalytic unit is present in membranes prepared from a series of mink lung epithelial cell subclones transformed by retroviruses containing either the v-fms, v-fes or v-ras oncogenes. Membranes isolated from the transformed cell lines have significantly higher specific activities of guanine nucleotide-dependent $PtdIns-P_2$ phospholipase C than membranes derived from the nontransformed parental cell line. Nonhydrolyzable GTP analogs are more potent activators than GTP, and GDP is inactive. These data point to enhanced production of $Ins-P_3$ and diacylglycerol second messengers in transformed cells due to the activation of a guanine nucleotide-dependent $PtdIns-P_2$-specific phospholipase C, and suggest that the generation of aberrant, hormonally independent signals is associated with cell transformation by oncogenes encoding tyrosine-specific protein kinases and guanine nucleotide-binding proteins.

L298 ALTERED RESPONSE OF CARCINOGEN TREATED CELLS TO CA2+-MEDIATED INOSITOL LIPID METABOLISM, Susan Jaken, Gwendolyn L. Harms,and Stuart H. Yuspa,Center for Drugs and Biologics, FDA & Lab Cellular Carcinogenesis & Tumor Promotion, NCI,Bethesda,MD

Differentiation of cultured keratinocytes is regulated by the Ca2+ concentration of the culture medium. Below 0.1 mM Ca2+, a monolayer of basal cells is formed which fully differentiate in response to a rise in medium Ca2+. A role for protein kinase C in this differentiation program has been suggested because phorbol esters induce differentiation and exogenously added phospholipase C (which increases cellular diacylglycerol) mimics phorbol ester action. We investigated whether the external Ca2+ signal may lead to C-kinase activation via stimulation of cellular phospholipase C activity. The effect of the external Ca2+ signal on phospholipase C was studied in cultures prelabeled with [3H]inositol. Within 2 minutes after addition of Ca2+ to 1 mM, an increase in inositol phosphates (IPs) was measured. This correlated with a decrease in radiolabeled phosphoinositides suggesting that these were the source of the increased IPs. After 3 hours in 1 mM Ca2+ medium, each of the IPs remained elevated to 140% of control levels. This effect appears to be mediated by a rise in intracellular Ca2+ because the ionophore A23187 causes a similar rise in IP levels. In order to demonstrate functional significance of this pathway in the differentiaton program, we studied the effect of Ca2+ on inositol lipid metabolism in carcinogen-altered cells which are resistant to the Ca2+ signal for differentiation. In 3 of these variant cell lines, Ca2+ did not significantly change IP levels. These results demonstrate that the second messenger system for Ca2+-mediated keratinocyte differentiation may be through a direct effect on phospholipase C activity.

L299 CA2+ REGULATION OF PHORBOL ESTER RECEPTOR AFFINITY, Susan C. Kiley and Susan Jaken Center for Drugs and Biologics, FDA, Bethesda. MD

Protein kinase C (PKC) was purified from rabbit brain cytosol by chromatography on DEAE-Sephacel followed by hydroxylapatite (HAP) and phosphatidylserine (PS). Two peaks of phorbol ester binding activity were eluted from the HAP column. The 2nd peak was eluted with ~190 mM phosphate and cochromatographed with PKC. The 1st peak was eluted with ~100 mM phosphate and appeared to chromatograph with a Ca2+-PS independent kinase. Ca2+ increased the affinity of both receptors for phorbol dibutyrate (PDBu). Subsequent chromatography of peak 2 on PS yielded a nearly homogeneous preparation of PKC (MW = 82 kD). Chromatography of peak 1 on PS also yielded a preparation which contained CA2+-PS dependent kinase of comparable specific activity to that obtained from peak 2. SDS-PAGE showed 1 major band (MW = 82 kD) and additional minor bands. These results are consistent either with 2 phorbol ester receptors, only one of which binds to the PS column, or association of PKC with other proteins which modify its chromatography on HAP. In order to define the difference between the 2 HAP column binding activities, we have begun to characterize the Ca2+ requirements for PDBu binding to purified PKC. Ca2+ increases receptor affinity from Kd = 17 nM (in excess EGTA) to Kd = 1 nM without increasing total binding capacity. The Ca2+ requirement for increased receptor affinity occurred at EGTA:Ca = 1.48, whereas the Ca2+ requirement for kinase activity was significantly higher (EGTA:Ca = 1.15).These results indicate that the high affinity receptor is associated with active PKC. Further characterization of the Ca2+ requirements for binding and kinase activities for the 2 HAP peaks will help to define the coupling between PDBu receptor occupancy and activation of PKC.

L300 **THE ROLE OF POLYPHOSPHOINOSITIDES IN POLYOMA-MEDIATED TRANSFORMATION.** D. Kaplan[1], M. Whitman[1], H. Piwnica-Worms[1], B. Schaffhausen[1], L. Raptis[1], L. Cantley[2], and T.M. Roberts[1]
[1]Dana-Farber Cancer Institute, Boston, MA; [2]Tufts Univ. Med. School, Boston, MA.

Middle T antigen, the transforming gene product of polyoma virus, is a membrane bound protein associated with pp60c-src in polyoma infected cells. We have recently shown that middle T/c-src immunoprecipitates can phosphorylate phosphatidylinositol (PI) in vitro. A number of polyoma mutants defective for transformation show a strong correlation between PI kinase activity in vitro and transforming ability. In addition, polyoma transformation mutants which are wild-type in the tyrosine kinase reaction in vitro are markedly impaired in their ability to phosphorylate PI. Thus, for these middle T mutants, PI kinase activity is more tightly correlated with transformation than tyrosine kinase activity. We have examined in vivo the steady state levels of the two second messengers produced from the breakdown of phosphorylated PI, IP3 and diacylglycerol, and found elevated levels of each in cells infected with wild-type polyoma virus. The products of the phosphorylation reactions, PIP and PIP2, are also elevated in polyoma infected cells. NIH3T3 cells transfected with the middle T gene expressed from the dexamethasone inducable MMTV promoter contain elevated polyphosphoinositide, C-kinase, and intracellular calcium levels as well as enhanced middle T immunoprecipitable PI kinase activity when the cells are treated with dexamethasone. These results suggest that alterations in PI metabolism by middle T may play a role in polyoma transformation. We have also examined middle T and c-src overproduced in bacteria and animal cells and determined that neither protein contains an intrinsic PI kinase activity. We postulate that middle T/c-src activates a cellular PI kinase by tyrosine phosphorylation.

L301 Phorbol Esters Specifically Bind to Isolated Nuclei From Human Promyelocytic Leukemia Cells (HL-60). A.S. Kraft, K. Cunningham, and R.L. Berkow, Department of Medicine and Pediatrics, The University of Alabama at Birmingham, Birmingham, AL.

The mechanism by which phorbol esters induce differentiation of human promyelocytic leukemia cells (HL-60) to macrophages remains unclear. We have demonstrated previously that phorbol ester binding causes the association of a calcium, phospholipid-dependent protein kinase with the plasma membrane. Although this association may explain a number of early effects induced by phorbol esters, it does not explain how phorbol esters induce specific changes in the nucleus which lead to cellular differentiation. To understand this process we examined whether phorbol esters would bind specifically to isolated nuclei. Nuclei were isolated from HL-60 cells in a buffer containing .33m sucrose 20mM Tris pH7.5, 10mM $MgCl_2$, and 2mM $CaCl_2$ by nitrogen cavitation at 750 psi for 5 minutes. Binding of 3H-phorbol dibutyrate (3H-PDBu) to nuclei demonstrated saturation kinetics and was temperature dependent with binding occuring more rapidly at 30 than 4 degrees C and maximal by 20-30 minutes. The potency of a series of phorbol ester analogs in inhibiting 3H-PDBu binding correlates with the activity of these compounds as tumor promoters. Scatchard analysis of the dose response curve demonstrates a one site model with a Kd of 14nM and approximately 50,000 sites per nucleus. This data demonstrates that phorbol esters bind specifically to HL-60 nuclei in vitro, and suggests that nuclear binding of phorbol esters may be an early event in modulating RNA synthesis and inducing differentiation of HL-60 cells.

L302 EVIDENCE THAT PROTEIN KINASE C MEDIATES ADHESION IN HUMAN MYELOMA CELLS, Jeffrey D. Laskin, Joseph A. Dunn, Sean M. O'Connell and Debra L. Laskin. UMDNJ-Rutgers Medical School and Rutgers University, Piscataway, NJ 08854

We have found that rapid cell-cell adhesion of the human myeloma culture GM1500 is dependent on a serum-derived cell adhesion molecule (MyCAM). MyCAM has been purified using gel filtration, concanavalin A affinity chromatography and FPLC and was found to be a high m.w. glycoprotein. Adhesion induced by MyCAM is concentration and time-dependent and requires the presence of magnesium ions in the culture medium. We have also found that phorbol ester tumor promoters can substitute for MyCAM in cell adhesion assays. 12-O-tetradecanoyl phorbol-13-acetate (TPA) induces adhesion in the nmolar concentration range in a dose-dependent fashion. Two other promoters, mezerein and phorbol-12,13-didecanoate (PDD) also induced cell adhesion while two inactive phorbol esters, 4-alpha-PDD and phorbol did not induce cell adhesion. Specific high affinity binding sites for tumor promoters (K_d 3.4 nM, B_{max}, 0.44 pmoles/10^6 cells) on GM1500 cells have been identified and a direct correlation was found between the ability of the various analogs to inhibit specific binding and their activity as inducers of cell-cell adhesion. MyCAM had no effect on specific binding suggesting that it induces adhesion by a mechanism distinct from TPA. Both MyCAM and TPA induce the production of diacylglycerol in GM1500 cells. In addition, diacylglycerol alone, could substitute for TPA or MyCAM in inducing cell adhesion. Since diacylglycerol and TPA are known to activate protein kinase C, taken together, our data suggests that protein kinase C is involved in cell-cell adhesion in human myeloma cells.

L303 EPIDERMAL GROWTH FACTOR AND VANADATE-MEDIATED ACTIVATION OF $^{22}Na/H$ EXCHANGE AND ^{45}Ca INFLUX IN A431 CELLS OCCURS WITHOUT ACTIVATION OF PHOSPHATIDYLINOSITOL TURNOVER, Ian G. Macara, University of Rochester Medical Center, Rochester, NY 14642

Epidermal growth factor (EGF) and vanadate (V_i) activate ^{22}Na and ^{45}Ca uptake by A431 cells without a detectable lag, at 37°C. The activation is partially additive for EGF and V_i, and V_i will still activate uptake after down-regulation of the EGF receptor. The transport systems are therefore independent of the receptor. The activated ^{22}Na and ^{45}Ca influxes are mutually independent. In other cell-types, $^{22}Na/H$ exchange is activated by kinase C either via hormonal stimulation of phosphatidylinositol (PI) turnover and production of diacylglycerol (DG) or by addition of phorbol esters. In A431 cells, ^{22}Na and ^{45}Ca uptake are both partially inhibited by phorbol esters. Equilibrium labeling with [^{3}H]-glycerol showed that no increase in DG concentration accompanied stimulation by EGF or V_i, and pulse-labeling with ^{32}P-phosphate revealed no increase in PI turnover. It seems likely, therefore, that the Na and Ca transport systems are in A431 cells activated by a kinase C-independent mechanism. (Supported by NIH Grant CA38888-01.)

L304 MODIFICATION OF EPIDERMAL GROWTH FACTOR RECEPTOR DURING DIOXIN AND ESTROGEN PROMOTION OF RAT HEPATOCARCINOGENESIS, Karen G. Nelson, Geoffrey I. Sunahara and George W. Lucier, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

We are using the rat two-stage model of hepatocarcinogenesis to study the role of epidermal growth factor receptor (EGFR) in the promotion process induced by two chemically distinct promotors, 17α-ethinylestradiol (EE_2) and tetrachlorodibenzo-p-dioxin (TCDD). Ovariectomized Sprague-Dawley rats were treated with diethylnitrosamine (DEN, 200 mg/kg) or saline (S), then promoted with TCDD (100 mg/kg/day) or EE_2 (s.c. silastic capsules; 90 µg/kg/day). EGFR binding assays performed on liver plasma membranes isolated from untreated animals yielded curvilinear Scatchard plots which suggested the presence of both low and high affinity EGF receptors. At 22 weeks of promotion, TCDD treatment resulted in dramatically different effects on the EGFR in saline treated compared to initiated animals. A 40% reduction in EGFR number (both high and low affinity sites) and phosphorylation occurred in S-TCDD treated rats; while an increase or no change occurred in DEN-TCDD treated rats. Although initiation markedly altered the liver response to TCDD with respect to the EGFR, initiation had no influence on the estrogen induced EGFR changes. After 30 weeks of estrogen promotion, EGFR number was elevated (30% increase) to a similar extent in both saline and DEN-treated animals. Perturbation of the EGFR by EE_2 and TCDD may play an important role in liver tumor promotion by influencing the growth capacity of preneoplastic cells.

L305 PHOSPHOLIPID AND NON-POLAR LIPID METABOLISM DURING DIFFERENTIATION OF U937 AND HL60 CELLS WITH PHORBOL ESTER. M.Ponzoni, E.Bonvini and T.Hoffman. Laboratory of Cell Biology, DBBP, CBRR/FDA, Bethesda, MD 20892.

The human promyelocytic cell line, HL60, and the human promonocytic cell line, U937, acquire characteristics of mature macrophages upon treatment with the phorbol ester, 12-O-tetradecanoate phorbol 13-acetate (TPA). Treatment of HL60 or U937 cells with 30 nM TPA induces increased incorporation of [3H]-glycerol into chloroform extracts after 24 hours. Separation of chloroform extracts by acetone precipitation followed by heptane partition showed proportionally greater labeling of the non-polar lipid fraction compared to the residual phospolipids (PL). Thin-layer chromatography (TLC) analysis of the radiolabeled lipid extracts showed a 30-70% increase in the radioactivity associated with triacylglycerol (TG) as early as 4 hours after treatment with TPA. After 24 hours, there was a 5-6-fold increase in TG labeling. No changes occured in monoacylglycerol, while diacylglycerol showed a 2-fold increase labeling after 24 hours. The pattern of distribution of radiolabeled PL separated by TLC showed only minimal changes after 2, 4 and 8 hours of treatment. After 24 hours of treatment with TPA, the rate of labeling of phosphatidylcholine (PC) was increased 2.5-fold, as in other cellular models where activation of PC synthetic pathway(s) in response to TPA was observed. Limited effects were seen in other PL. These findings indicate similar changes in lipid metabolism in two myeloid cell lines, which occur concomitant with the induction of the differentiated phenotype. The molecular basis of the redistribution of cellular fatty acids, indicated by the increased TG labeling, is currently under investigation to obtain insights into the manner by which changes in lipid metabolism relate to cellular differentiation.

L306 **HIGH AND LOW AFFINITY BINDING STATES FOR T-47D BREAST TUMOR CELL PROGESTERONE RECEPTOR.** K.V.S. Rao, J.C. Sarup, W.A. Coty and C.F. Fox, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.

Cultured T47D breast carcinoma cells displayed two classes of [^{3}H]progesterone binding sites, with K_Ds of 1.5 ± 0.5 and 60 ± 8 nM. These were present at 0.33 ± 0.15 and 2.4 ± 1.0 x 10^6 sites per cell, respectively. In the absence of progesterone, homogenization of cells released the high affinity binding sites quantitatively and in soluble form, while the low affinity sites sedimented quantitatively in the 40,000 g·min fraction. Treatment of the particulate fraction with buffer containing 0.5 M KCl extracted the binding activity in a soluble form; [^{3}H]progesterone binding in this extract had a K_D (2.4 ± 0.2 nM) and ligand binding specificity identical to those of sites in the cytosol fraction. When cells were incubated with varying concentrations of [^{3}H]progesterone and extracted with Triton X-100, occupied high affinity receptor was recovered in the soluble extract, while low-affinity receptor-hormone complexes adhered to the substratum as a nuclear/cytoskeletal/ extracellular matrix fraction. Our results indicate that, in the absence of hormone, a population of progesterone receptors observed in a low-speed particulate fraction exhibited a lower affinity for hormone. In intact cells, saturation of these sites required a higher concentration of hormone and longer incubation times than for soluble fraction sites. However, extraction of low-affinity receptors at high ionic strength resulted in their quantitative conversion to the high-affinity form. Since this conversion occured in the presence of molybdate and glucose-1-phosphate, and in the absence of ATP, the altered affinity is not likely to be the result of phosphatase or kinase action on receptors in the low speed particulate fraction. Supported by ACS Grant No. BC-473 and USPHS Grant No. AM25826.

L307 PURIFICATION OF THE PDGF-RECEPTOR FROM PIG UTERUS AND ITS USE IN THE PREPARATION OF ANTIBODIES AGAINST THE RECEPTOR
Lars Rönnstrand, M. Patricia Beckmann, Bo Ek and Carl-Henrik Heldin
Dept. of medical and physiological chemistry, Biomedicum, Box 575, S-751 23 UPPSALA, Sweden.

Platelet-derived growth factor (PDGF) binds to a 185 kDa receptor on the surface of responsive cells and thereby initiates the sequence of intracellular events leading to cell proliferation.

For the purpose of raising antibodies and for doing protein microsequencing, we have developed a method for the purification of SDS-denatured receptor. The method includes chromatography on wheat germ agglutinin-Sepharose and ion exchange chromatography on a MonoQ column (Pharmacia). As a final step in the purification, the active fractions are run on SDS-gelelectrophoresis. The receptor band is excised, electro-eluted, and injected into a rabbit.

The antiserum raised in this way recognizes bands corresponding to molecular weights of 185, 130 and 55 kDa in protein blotting. These bands coincide with those seen after PDGF-stimulated phosphorylation of partially purified receptor preparations, indicating that the antiserum indeed recognizes the PDGF receptor.

L308 GROWTH FACTORS MODIFY THE EGF RECEPTOR THROUGH MULTIPLE PATHWAYS, Marsha Rich Rosner, BethAnn Friedman, and Stuart Decker*, Massachusetts Institute of Technology, Cambridge, MA 02139 and Rockefeller University*, New York, NY 10021

Previous results have shown that tumor promoters modify properties of the epidermal growth factor (EGF) receptor through the activation of protein kinase C. Diacylglycerol-generating factors such as platelet derived growth factor and p28sis should activate C kinase and alter EGF receptor properties in a similar manner. To test this assumption, we determined whether media from v-sis transformed cells was capable of modifying the EGF receptor. The results indicate that the action of v-sis media mimics the effects of tumor promoters on the EGF receptor in Swiss 3T3 cells by: 1) reducing EGF binding to apparent high affinity EGF receptors; 2) decreasing EGF-stimulated tyrosine phosphorylation; and 3) increasing overall phosphorylation of the EGF receptor at serine residues. To directly test the involvement of C kinase in the action of v-sis media on the EGF receptor, cells were first extensively treated with various concentrations of the tumor promoter phorbol dibuterate (PDBu). This treatment has been shown to reduce levels of active C kinase in cells, making them less responsive to subsequent rechallenge with the tumor promoter. The results indicate that there are at least two components to the action of v-sis media on EGF receptor binding: a labile C kinase-independent factor and a stable C kinase-dependent factor. The action of the second factor is mimicked by PDGF and appears to be partially dependent upon levels of C kinase in the cell.

L309 **RECONSTITUTION OF THE T3-T CELL ANTIGEN RECEPTOR COMPLEX ACTIVATED Ca^{2+} TRANSPORTER FROM HUMAN T CELLS.** **Philip M. Rosoff and Christine M. Hall, Depts. of Pediatrics (Hematology-Oncology), New England Medical Center and Physiology, Tufts University School of Medicine, Boston, MA 02111.**

We have previously shown that stimulation of the human T3-T cell antigen receptor complex present on the leukemic T cell line HPB-ALL by mitogenic monoclonal antibodies leads to rapid activation of a La^{3+}-inhibitible, membrane potential-sensitive Ca^{2+} influx (Oettgen, H.C., Terhorst, C., Cantley, L.C. & Rosoff, P.M. Cell 40: 583-590, 1985). This Ca^{2+} transport function is separable from that stimulated by inositol lipid turnover which serves to release Ca^{2+} from an intracellular store. In addition, the receptor-linked influx is not inhibited by either amiloride or the absence of extracellular Na^+, suggesting that it is not a Na^+/Ca^{2+} antiport. Many other types of cells exhibit similar mitogen or growth factor-stimulated Ca^{2+} influx activity. In order to study this transporter in greater detail, we have reconstituted this activity into plasma membrane liposomes. Purified plasma membranes from HPB-ALL cells were mixed with asolectin and 1% cholate in a lipid:protein ratio of 75:1. Vesicles were formed by gentle vortexing and passage over 2 Sephadex G-50 columns which also removed the detergent. In some batches, Quin2 acid was sealed inside the vesicles as a trapped Ca^{2+} indicator. Vesicles were concentrated by gentle centrifugation over a 5-20% Percoll gradient. Mitogen-stimulated Ca^{2+} transport into the vesicles was detected by determination of vesicle-associated $^{45}Ca^{2+}$ and increases in Quin2 fluorescence after treatment with anti-T3 complex antibodies. Both methods show a very rapid influx of Ca^{2+} into the vesicles on the same time scale as observed in whole cells. Initial experiments have suggested that cross-linking of the mitogen receptor increases the efficiency of transport. Our data would suggest that some component of the T3 heterotrimer complex serves as a receptor-linked, membrane Ca^{2+} transporter. Further work is being done to purify the transporter using this vesicle system.

L310 **GROWTH FACTORS DECREASE PROGESTERONE BINDING IN CULTURED HUMAN BREAST CARCINOMA CELLS.** J.C. Sarup and C.F. Fox, Molecular Biology Institute, UCLA, Los Angeles, CA 90024.
Previous studies from this laboratory (Ghosh-Dastidar et. al, Proc. Nat. Acad. Sci. U.S.A. 81, 1654-1658, 1984; Woo et al., J. Biol. Chem, in press) have shown that the avian progesterone receptor is a high-affinity substrate for the tyrosine protein kinase activities of epidermal growth factor (EGF) and insulin receptors. To extend observations on this phenomenon to intact cells, the effects of EGF and insulin were measured on progesterone binding in the established breast carcinoma cell lines T47D, MCF-7 and ZR75-1. Progesterone receptor was quantified by incubating intact cells with 20 nM [^{3}H]progesterone in the absence or presence of 2 μM unlabeled progesterone, followed by extraction with Triton X-100 and measurement of specifically-bound hormone. As described in a companion abstract (Rao et al.), T47D cells contain high- and low-affinity progesterone receptors; Triton treatment extracts the high-affinity receptors in soluble form, while the low-affinity receptors are recovered as a substratum-adherent insoluble residue. Incubation of T47D, MCF-7 or ZR75-1 cells with 3 nM EGF or insulin for 60 min at 37° resulted in a 30 to 50% decrease of subsequently measured low-affinity progesterone binding in the insoluble residue, but had no effect on high-affinity progesterone binding in the Triton X-100 extract. The effects of EGF and insulin were dose-dependent, with half-maximal responses at 0.1 nM and >1 nM, respectively, indications that EGF acted on EGF receptors, but that insulin may have acted through binding to somatomedin C receptors. The effect of EGF was observed after pre-incubation of 60 minutes and persisted for up to 180 minutes. When EGF-treated or untreated cells were incubated with varying concentrations of [^{3}H]progesterone, followed by extraction with Triton X-100, Scatchard analysis of specifically-bound hormone in the detergent-insoluble fraction revealed a decrease in the number of low-affinity progesterone binding sites, with no effect on binding affinity. These data show that growth factors can down-regulate steroid hormone binding activity in intact cells. This regulatory phenomenon in breast cells could proceed by a phosphorylation reaction mediated by growth factor-stimulated tyrosine kinases. Supported by USPHS Grant No. AM25826 and ACS Grant No. BC-473.

L311 PURIFICATION AND CHARACTERIZATION OF THE HUMAN PDGF RECEPTOR, Subal Bishayee, Alonzo H. Ross, Richard B. Womer, and Charles D. Scher. The Children's Hospital of Philadelphia and The Wistar Institute, Philadelphia, PA 19104

We have purified the platelet derived growth factor receptor (PDGF-R) from a human osteogenic sarcoma cell line (MG-63), which neither expresses c-sis mRNA nor secretes PDGF. Scatchard analysis demonstrates that there are about 20,000 PDGF-R per cell with an affinity constant for the ligand of about $10^{11} M^{-1}$. The PDGF-R (M_r, 180,000) in either whole cells, or in membrane preparations, is phosphorylated in a dose-dependent fashion in response to PDGF. The tyrosine phosphorylated form of the PDGF-R has been purified from MG-63 cell membranes approximately 20,000 fold using two steps: (1) binding to and elution from Sepharose linked to monoclonal antibody directed against phosphotyrosine; and (2)either adsorption to and elution from solid phase wheat germ agglutinin, or anion exchange chromatography. The purification of this PDGF-R was followed by its binding with PDGF in a newly developed soluble receptor assay. Preparations are free of the epidermal growth factor receptor, and are estimated to be 50-80% pure as judged by silver staining and autoradiography of ^{125}I-labeled protein. The purified PDGF-R has ligand-stimulated tyrosine kinase activity as determined by autophosphorylation of the receptor after alkaline phosphatase treatment and by phosphorylation of exogenous substrates, such as angiotensin II and a src related synthetic peptide.

L312 PHOPHATIDYLINOSITOL-4'-PHOSPHATE KINASE FROM FRIEND MURINE ERYTHROLEUKEMIA CELL PLASMA MEMBRANES. John Schulz, Leona Ling, and Lewis Cantley. Department of Physiology, Tufts University School of Medicine, Boston, MA 02111.

The kinases which phosphorylate phosphatidylinositol(PI) and phosphatidylinositol-4'-phosphate(PIP)to phosphatidylinositol-4',5'-bisphosphate(PIP_2) are potential points of regulation in the PI second messenger generating system. Starting with a preparation of Friend murine erythroleukemia cell plasma membranes we have obtained a partially pure PIP kinase activity. The activity is removed from plasma membranes without detergent. It is specific for PIP, prefers ATP over GTP as phosphate donor, and is inhibited by its product, PIP_2.The major protein species in the partially pure kinase migrate on SDS-PAGE gels with apparent M_r's of 105, 75, and 45 kilodaltons. Further purification is in progress.

L313 STRUCTURE AND FUNCTION OF p21 ras PROTEINS: IMMUNOCHEMICAL, BIOCHEMCIAL AND SITE-DIRECTED MUTAGENESIS STUDIES. T. Y. Shih, S. Hattori, D. J. Clanton, L. S. Ulsh and Z. Q. Chen. Laboratory of Molecular Oncology, NCI, NIH, Frederick, MD. 21701.

The highly purified p21 protein overproduced in E. coli exhibits three activities specific for guanine nucleotides, i. e., GTP/GDP binding, GTPase and autokinase. Using a battery of monoclonal antibody probes, we found that Y13-259, previously developed by M. Furth et al., specifically inhibits all p21 in vitro activities. The same antibody when microinjected into cells transformed by ras oncogene, transient reversion to normal phenotype was observed (Mulcahy et al., Nature, 313, 241, 1985). We have constructed point mutations to dissect the structure-function relationship of the GTP binding domain. Mutations at asparagine-116 of p21 to lysine or tyrosine but not the adjacent 117th or 118th positions, abolish in vitro activities associated with binding to GTP as well as in vivo transformation. It is concluded that GTP binding is absolutely required for ras gene function. Contrarily, mutations changing threonine-59 to serine or alanine alter GTPase and autokinase activities, but do not eliminate GTP binding and transformation. In the three dimensional structure of EF-Tu, the homologous asparagine-135 is hydrogen-bonded to the guanine base. These results suggest the GTP binding domain is conserved among G-proteins. Using a specific sulfhydryl reagent, we found that the GTP-modulated reactivity of cysteine-80 of p21 is more similar to EF-G than EF-Tu, suggesting heterogeneity in the fine structure of the GTP binding domain among different G-proteins. These studies indicate the feasibility of designing chemical agents to selectively interfere with p21 switch function in signal transduction.

L314 The Expression of Translocated c-myc Genes in Burkitt Lymphomas as a Function of the Proximity of the Ig Loci. L. C. Showe, L. Sun,* S. Matacic,‡ B. Kramer and C. Croce, The Wistar Institute, Philadelphia, PA; *Present address: Centocor, Malvern, PA; and ‡Haverford College, Haverford, PA.

Several hypothesis have been proposed to explain the deregulation of c-myc expression in Burkitt lymphomas and mouse plasmacytomas including inactivation of sequences for negative regulation and the presence of transcriptional enhancers within the Ig locus associated with the c-myc gene. In order to assess any effects of the distance between the c-myc gene and the Ig loci on c-myc transcription, we have cloned and mapped the 40 kilobases of DNA from the 3' flanking region of the human c-myc gene. Probes derived from this region have been used to map the positions of translocation breakpoints within this region for a number of Burkitt lymphoma cell lines. One breakpoint has been mapped to a position 12 kb 3' of c-myc and another 20-24 kb 3' while the majority have been found to have breakpoints >50 kb 3' of c-myc. The steady state levels of c-myc transcripts as compared to internal standards of β-actin or phosphogycerol kinase showed no relationship to the distance between the myc gene and the Ig locus. A comparison of transcription rates as determined by nuclear run-off assays has been made in order to determine whether steady state levels of c-myc m-RNA are reflected in the transcription rates or are a function of message stability.

L315 PHOSPHORYLATION OF STRESS PROTEIN pp80 IS RELATED TO PROMOTION OF TRANSFORMATION, Bonita M. Smith, Thomas D. Gindhart, Koichi Hirano, and Nancy H. Colburn, NCI/NIH, Frederick, MD 21701. The JB6 mouse epidermal cell system is an in vitro model of late stage promotion, and includes cell lines which are sensitive (P+) or resistant (P-) to TPA-induced anchorage independent transformation. Certain tumor promoter-induced phosphoprotein changes, identified by 1 and 2 dimensional PAGE, are unique to P+ or P- cells and occur only with specific promoters. In particular, an 80Kd phosphoprotein is inversely correlated with phenotype: P- cells contain a constitutively higher level ($p<.001$) of pp80 than P+ cells, and TPA treatment produces a two-fold increase in phosphorylation of pp80 in both cell lines. Transformed cell lines contain barely detectable levels of pp80 and are not sensitive to TPA induction. Progressively decreasing amounts of pp80 are associated with capacity to undergo transformation and the transformed state. pp80 is detectable in cytosolic, not nuclear cell fractions. Maximal induction occurs within 1 to 5 hours after treatment, and returns to basal levels in all phenotypes by 24 hours. TPA treatment had no effect on the rate of synthesis of the 80Kd protein as measured by pulse labelling with ^{35}S-methionine, indicating that the effect of TPA is at the level of phosphorylation, not synthesis, of the 80Kd protein Also, pp80 shares a number of properties with the 80Kd heat stress protein, including molecular weight, relative abundance, and isoelectric point (4.5). Pharmacological analogs of calcium, thelanthanides, are potent promoters of JB6 cells, but have no effect on pp80 phosphorylation. This suggests that if pp80 is on the promotion pathway, it is limited to a specific subset of transformation promoters. BMS is supported by NIEHS NRSA ES05305.

L316 ALTERED EGF RECEPTOR FUNCTION AND C-KI-RAS TRANSCRIPTION IN NEOPLASTIC MOUSE LUNG EPITHELIAL CELL LINES, Garry John Smith[1], Jacqueline M. Bentel[1], Frances A. Bennett[2], John G. Steele[2] and Trevor J. Lockett[2], School of Pathology[1], University of New South Wales, P.O. Box 1, Kensington 2033, CSIRO Division of Molecular Biology[2], P.O. Box 184, North Ryde 2113, Australia

The cell line NAL1A is an epithelial cell type established in culture from normal mouse lung alveolus. NAL1A undergoes spontaneous neoplastic transformation in vitro to line NAL1A^M which exhibits the phenotype of cell line NUL1 cultured directly from urethane induced lung adenomas. NAL1A^M and NUL1 are positive for growth in soft agar and form invasive subcutaneous nodules in immune-suppressed mice producing metastatic lung deposits. These cells represent a series of normal (NAL1A) and related malignant (NAL1A^M and NUL1) cell lines suitable for analysis of the molecular mechanisms of neoplastic transformation.

As compared to the normal cell line, the malignant lines exhibited a 9-fold reduction in cellular binding of [^{125}I] EGF. This reduction did not appear to be due to competition by other growth factors produced by the malignant cell types. Coincidentally, the malignant cell types exhibited alterations to the transcript sizes of poly A$^+$ mRNA homologous to c-Ki-ras. Notably, NAL1A^M and NUL1 had lost the 5.1Kb transcript characteristic of normal NAL1A cell lines and NAL1A^M had acquired two transcripts of 4.6Kb and 4.3Kb.

In summary, the malignant cell lines NAL1A^M and NUL1 exhibit coordinately reduced EGF receptor function and altered transcription of c-Ki-ras coincident with malignant phenotype.

L317 SELECTIVE PHOSPHORYLATION OF HUMAN METHYLTRANSFERASE BY PROTEIN KINASE C Steven S. Smith+, Anna DePaoli Roach*, Peter J. Roach*, and Keith E. Zucker+ Depts. of Thoracic Res. and Molec. Biology+, Beckman Res. Inst. of the City of Hope, 1450 E. Duarte Rd., Duarte, California, 91010; and Department of Biochemistry*, Indiana University School of Medicine, Indianapolis, Indiana 46223.

Transformed cells are often found to possess aberrant patterns of methylation in DNA. Since these patterns can set heritable limits on the trancriptional potential of a cell, they may play a role in the altered patterns of gene expression often observed in transformed cells. We report here that of seven different protein kinases tested, only protein kinase C was effective in recognizing purified human placental DNA methyltransferase. The reaction was stimulated by Ca^{2+}, phospholipid and TPA (12-tetradecanoylphorbol-13-acetate). Phosphorylation to more than 1 mole per mole methyltransferase subunit was observed with modification of both serine and threonine residues. The DNA methyltransferase was a significantly better substrate for protein kinase C than either histone H1 or glycogen synthase. Phosphorylation resulted in 30-50% stimulation of methyltransferase activity. The results suggest a possible mechanistic link between TPA action and the function of DNA methyltransferase.

L318 PROTEIN KINASE C - STRUCTURAL AND FUNCTIONAL CHARACTERISATION, S. Stabel, S. Young, R.M. Marais, M.D. Waterfield, L. Coussens*, T.J. Dull*, A. Ullrich* and P.J. Parker, Imperial Cancer Research Fund, London, WC2A 3PX, * Genentech, Inc., San Francisco, CA 94080.

Protein kinase C is involved in the transduction and modulation of numerous important physiological signals and has been shown to be the major cellular receptor for tumour promoting phorbol esters. As part of a study of the structure and function of protein kinase C and in particular of its role in control of cell proliferation we have purified the bovine enzyme and obtained approximately 100 residues of amino acid sequence from peptide fragments. Using synthetic oligonucleotides of predicted sequence a number of cDNA clones have been characterised and virtually the complete amino acide sequence of the protein obtained. Synthetic peptides based on suitable stretches of amino acid sequence have been used to raise antibodies specific for protein kinase C. Using these antisera and cDNA probes we are currently investigating the expression of protein kinase C in various cell lines.

L319 ACTIVATION OF SURFACE THY-1 DEPLETED LYMPHOCYTES, Janet Stiernberg, Martin G. Low, and Paul W. Kincade. Oklahoma Medical Research Foundation, 825 N.E. 13th Street, Oklahoma City, OK 73104

We recently obtained evidence that the abundant cell surface glycoprotein, Thy-1 is anchored to the membranes of murine T lymphocytes via linkage to phosphatidylinositol. This antigen was efficiently shed in the presence of a phosphatidylinositol specific phospholipase C (PI-PLC) obtained from Staph. aureus (Nature, in press). Lymphocytes remain viable after continuous exposure to the enzyme and while surface Thy-1 is dramatically diminished, the density of Ly-1, L3T4 and immunoglobulin is unchanged on treated splenocytes. The functional capability of PI-PLC treated spleen cells was assessed by stimulation with various mitogens. Responsiveness to lipopolysaccharide was not significantly changed whereas a consistent reduction in Concanavalin A (Con A) stimulated proliferation was found. That Thy-1 removal did not simply prevent Con A binding was demonstrated with fluorochrome labeled Con A. Also, PI-PLC treatment did not protect a T lymphoma cell line from growth inhibition with Con A. A small, but consistent (1.5-3 fold) increase in background proliferation occurred with PI-PLC treatment alone and preliminary experiments indicate that an early transmembrane signaling event may accompany Thy-1 release. (Supported by N.I.H. grant AI-20069 and a Jeane B. Kempner Fellowship to J.S.)

L320 EFFECTS OF TUMOR-PROMOTING PHORBOL ESTER ON MEIOTIC CELL DIVISION IN XENOPUS OOCYTES, Bradley J. Stith and James Maller, Department of Pharmacology, University of Colorado School of Medicine, Denver, CO 80262

Either insulin or progesterone can release Xenopus oocytes from a G2/prophase block and induce resumption of meiotic cell division (meiosis). We have examined whether either of these hormones acts through increasing polyphosphoinositide (PI) breakdown by:

a) injection into oocytes of a PI breakdown product, inositol trisphosphate, which has been shown to release intracellular calcium in many different cells. This injection (final intracellular concentration of 1 uM) resulted in a speeding of meiosis as induced by insulin or progesterone by 25%.

b) addition of a tumor-promoting phorbol ester (TPA) which activates protein kinase C, an enzyme that is stimulated by diacylglycerol produced by PI breakdown. TPA concentrations less than 15 nM inhibited insulin- but not progesterone-induced meiosis, whereas higher concentrations raised intracellular pH and induced meiosis directly with a half-maximal effect at 150 nM. Since induction of meiosis by TPA was inhibited by 10 nM cholera toxin, there appears to be an antagonistic relationship between the cAMP system and the protein kinase C system.

c) addition of neomycin, a putative inhibitor of PI breakdown. Neomycin (1 mM)inhibited insulin but not progesterone-induced meiosis.

Thus, PI turnover and the protein kinase C pathway may not play an important role in progesterone action but may be central to insulin's ability to induce cell division in oocyte. The Xenopus oocyte may be an excellent model system to examine the ability of TPA at different concentrations to either induce mitogenesis itself or affect mitogenesis induced by peptide hormones and growth factors.

L321 THE EFFECT OF SMOKING ON PLACENTAL EGF RECEPTOR AND PHOSPHORYLATION, Geoffrey I. Sunahara, George W. Lucier, Chris P. Miller and Karen G. Nelson, The National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Cigarette smoking causes disturbances in human placental morphology and metabolic function, as well as decreases in fetal birth weight. In this study, we have investigated the effect of smoking on EGF binding and EGF-stimulated phosphorylation in placental plasma membranes. Scatchard analysis of EGF binding to placentae of non-smokers yielded 2 binding sites, a high affinity-low capacity site (app.Kd= 0.14 ± 0.02 nM; app.Bmax= 1091 ± 120 fmol/mg) and a lower affinity-higher capacity component (app.Kd= 0.79 ± 0.11 nM; app.Bmax= 3677 ± 525 fmol/mg). Smoking caused a dramatic decrease in high affinity EGF binding, marked by either a decrease in binding affinity or a reduction in binding capacity (app.Kd= 0.81 ± 0.18 nM; app.Bmax= 2815 ± 585 fmol/mg) compared to non-smokers. Hill coefficients for the non-smokers and smokers were 0.85 ± 0.01 and 0.99 ± 0.01, respectively. Consistent with the binding data, smoking also caused a dramatic decrease in EGF-stimulated phosphorylation compared to non-smokers. These data are consistent with the effects of tumor promoting phorbol esters and polycyclic aromatic hydrocarbons on EGF binding in cells from other tissues. Although the exact role of EGF and its receptor in normal placental and fetal development remains unclear, decreased placental EGF binding and phosphorylation may be involved in some of the alterations in placental and fetal development which are associated with smoking.

L322 **EGF and Oncogene Mediation of an S6 Protein Kinase**
G. Thomas*, I. Novak-Hofer** and B. Friis+, Friedrich Miescher-Institut, *Zentrum für Lehre und Forschung, Kantonspital, Basel, +Ludwig Cancer Institute, Bern, Switzerland.

Addition of EGF to quiescent 3T3 cells in culture leads to a rapid activation of S6 phosphorylation, followed by a burst in protein synthesis and a number of specific alterations in the pattern of translation. Dose response studies with inhibitors or EGF argue that S6 phosphorylation is a prerequisite for the activation of protein synthesis during this stage of cell growth. Extracts prepared from EGF-stimulated 3T3 cells are up to 10 times more potent in phosphorylating S6 than extracts from quiescent cells. To detect maximal kinase activity requires the presence of phosphatase inhibitors during the preparation of extracts, the most efficient being phosphotyrosine. The kinase reaches maximum activation within 5 to 15 min and returns to basal levels by 2 h. Down regulation of EGF receptors and S6 phosphorylation in vivo closely parallel the activity of the enzyme. The results suggest that the kinase or a regulatory component is controlled by phosphotyrosine, and that the level of kinase activation and S6 phosphorylation are coupled to the level of EGF-bound receptors. The kinase acts as a single entity on ion exchange chromatography with an apparent M.W. of 75,000 and pI of 5.5-5.8. All mitogens examined to date including PDGF, Insulin, TPA, and vanadate along with the oncogenes v-sarc and v-ras induce the activation of the kinase.

L323 IDENTIFICATION OF A NOVEL RECEPTOR IN DROSOPHILA FOR BOTH EPIDERMAL GROWTH FACTOR AND INSULIN, Karol L. Thompson, Stuart J. Decker*, and Marsha R. Rosner, Massachusetts Institute of Technology, Cambridge, MA 02139 and *Rockefeller University, New York, NY 010021

The notable amino acid homology among mammalian growth factor receptors with tyrosine-specific protein kinase activity has led to speculation that these receptors derived from a common evolutionary precursor. We report the identificiation of a novel growth factor receptor from Drosophila cell cultures that has dual binding specificity for both insulin and epidermal growth factor (EGF). This 100-kDa protein is also related antigenically to the mammalian receptors for EGF and possibly insulin. The Drosophila protein is recognized by antisera directed against the mammalian receptor for EGF in immunoblot hybridizations. It can be affinity labeled with either 125I-insulin or 125I-EGF after immunoprecipitation with anti-EGF receptor antiserum. Excess unlabeled EGF or insulin will block the affinity labeling with either growth factor, suggesting that both EGF and insulin share a common binding site on the 100-kDa Drosophila receptor. This Drosophila protein, therefore, may be closely related to an evolutionary precursor of the mammalian receptors for insulin and EGF.

L324 GROWTH FACTORS STIMULATE LOCALIZED INCREASES IN FREE CYTOSOLIC CALCIUM IN HUMAN FIBROBLASTS, Robert W. Tucker[1], Harry Loats[2], Johns Hopkins Oncology Ctr, Baltimore, MD[1], Loats Assoc., Westminster, MD[2]

Although changes in free cytosolic calcium (Ca_i) have been implicated in the control of cell proliferation, recent studies with aequorin have shown that the magnitude of increase in total or average Ca_i stimulated by growth factors does not correlate with subsequent mitogenesis. We have investigated whether some spatially localized part of the Ca_i increase may still be related to mitogenesis. Human fibroblasts (FeSin) were loaded with fura 2, a calcium-sensitive probe that has high quantum efficiency and does not bleach or affect mitogenesis. Flourescent images (500 nm emission) of cells excited with 340 or 380 nm were digitized and analyzed using customized software (Loats Assoc.) to correct for background, uneven microscope illumination and spatial variation in camera sensitivity. We found that quiescent fibroblasts (average Ca_i 15-50 nM) had heterogeneous distributions of Ca_i, with highest values (up to 100 nM) in the perinuclear area of the cytosol. PDGF stimulated non-uniform increases in Ca_i (up to 200-400 nM) in both the nucleus and cytosol. By extending these studies to other growth factors, we will be able to determine whether localized increases in Ca_i are related to mitogenesis.

L325 INDUCED DIFFERENTIATION IN CULTURED LEUKEMIA CELLS: METABOLISM OF PHORBOL DIESTER AND DIACYLGLYCEROLS, Clement J. Welsh and Myles C. Cabot, W. Alton Jones Cell Science Center, Inc., Lake Placid, New York, 12946.

Phorbol diesters are poor substrates for a lipase-like activity in rat serum; however, diradylglycerols structurally analogous to the diacyl grouping of TPA (12-O-tetradecanoylphorbol-13-acetate), are hydrolyzed at more than 100-times the rate of TPA [Cabot, M.C. (1985) Biochim. Biophys. Acta 833,330-335]. Because TPA is now thought to act via mimicry of diacylglycerols, the uptake and metabolic fate of phorbol diesters and glycerolipid mediators was examined in cultured cells that undergo TPA-induced differentiation (HL-60 leukemia). After a 60 min incubation with 10 μM TPA, palmitoylacetylglycerol (PaG), or hexadecylacetylglycerol (ePaG, an ether-linked, lipase-stable analog of PaG), 90, 72, and 82%, respectively, of the administered dose was taken up by the cells. When dioleoylglycerol was tested, only 4% of the incubated radiolabel was cell associated. TPA, which induced cell attachment, was poorly metabolized (6% at 24 hr). In contrast, the glycerolipid analogs, PaG and ePaG, that stimulate protein kinase C of mouse brain [Cabot, M.C. and Jaken, S. (1984) Biochem. Biophys. Res. Commun. 125,163-169], were metabolized after 60 min with a >95% conversion and did not cause cell attachment. Details of the intracellular metabolism and the in vitro enzymatic hydrolysis of the diacyl lipids will be presented. Diacylglycerols are of current interest in cell research; inasmuch, many questions will arise regarding the stability and cellular fate of these mediators.

L326 INSULIN RAPIDLY STIMULATES TYROSINE PHOSPHORYLATION OF A MR=185,000 SOLUBLE PROTEIN IN INTACT CELLS. MF White, R Maron, CR Kahn, Boston, MA.

One of the earliest detectable responses to insulin binding is activation of the insulin receptor kinase and tyrosine autophosphorylation of the β-subunit. In vitro, the purified receptor incubated with (γ-^{32}P)ATP, Mn^{2+} and insulin phosphorylates various proteins on tyrosine residues. However, thus far, no proteins other than the insulin receptor have been identified to undergo tyrosine phosphorylation in response to insulin in the intact cell. Using anti-phosphotyrosine antibodies, we have identified a novel phosphotyrosine-containing protein of Mr=185,000 (pp185) which occurs during the initial response of FAO cells to insulin binding. Tyrosine phosphorylation of pp185 is maximal within seconds after exposure of cells to insulin and exhibits a similar dose-response as receptor autophosphorylation. By phosphoamino acid analysis, the β-subunit and pp185 contain about equal amounts of phosphotyrosine and phosphoserine. In contrast to the insulin receptor, pp185 can be extracted from cells without Triton X-100, and does not bind to wheat germ-agglutinin agarose or anti-insulin receptor antibodies. Thus pp185 is not the precursor of the insulin receptor and is not the receptor for EGF or PDGF, but is a unique protein that is weakly membrane associated or located in the cytoplasm. Tryptic digestion of pp185 and separation of the phosphopeptides by RP-HPLC suggests that several phosphorylation sites exist in the protein which do not correspond to those in the β-subunit. These characteristics suggest that pp185 represents the endogenous substrate for the insulin receptor kinase which could transmit the insulin signal from the plasma membrane to other intracellular sites. Further characterization of this protein should provide important clues for our understanding of insulin action and diabetes.

L327 BIOCHEMICAL CHARACTERIZATION OF POLYOMA MIDDLE T ASSOCIATED PHOSPHATIDYLINOSITOL KINASE ACTIVITY. M.Whitman*, D.R.Kaplan+, B.S.Schaffhausen**, B.*, L.C.Cantley.*, and T.M.Roberts+. Department of Physiology and Department of Biochemistry,Tufts University School of Medicine; +Laboratory of Neoplastic Disease Mechanisms and Department of Pathology,Dana Farber Cancer Institute,Harvard Medical School;

Products of phosphatidylinositol (PI) turnover have recently been implicated as regulators of cell growth and differentiation in a variety of systems. We have previously reported that polyoma middle T immunoprecipitates can catalyze two possible regulatory steps in the PI turnover pathway, the phosphorylation of PI to phosphatidylinositol 4 phosphate (PIP), and the phosphorylation of PIP to phosphatidylinositol 4,5 bisphosphate (PIP_2). We have recently succeeded in extracting PI kinase activity from middle T immunoprecipitates and away from the bulk of the middle T-c-src present in the complex, indicating that neither middle T nor c-src is directly catalyzing the PI kinase reaction. We are currently purifying and characterizing this extracted PI kinase activity. We have also examined PI kinase activity in whole cell lysates and in subcellular fractions, and have found two functionally distinct types of PI kinase. One type appears to be strongly stimulated by the addition of detergent to the PI kinase reaction, the other type is detergent independent. Only the detergent independent type of PI kinase specifically associates with the middle T-c-src complex. These two types of PI kinase activity are physically separable and appear to have different pH optima and subcellular localizations. We are currently working to identify the proteins responsible for these activities and to determine their relative importance in phosphoinositide metabolism in vivo.

L328 ISOLATION AND CHARACTERIZATION OF PLATELET DERIVED GROWTH FACTOR (PDGF) RECEPTOR. David D.L.Woo,Leroy E. Hood, Biology Division, CALTECH, Pasadena, CA. 91125
Russel Ross, Pathology Dept, Univ. of Washington, Seattle, 98195
C.Fred Fox, Microbiology Dept. U.C.L.A. California, 90024

A 185kDa glycoprotein with PDGF dependent tyrosine protein kinase and PDGF binding activity has been purified to homogeniety from Triton X100 extracts of NR6 cells employing galactose specific lectin affinity chromatography followed by non-ideal gel permeation HPLC on TSK-4000. This protein is presumed to be the cell surface receptor for PDGF based on the above criteria. Two dimentional phosphopeptide mapping analysis resolved three distinct tryptic phosphopeptides. Two of these peptides were specifically induced by PDGF. Phosphoamino acid analysis of the individual peptides indicated that tyrosine is the sole amino acid phosphorylated. cDNA clones have been obtained from a λgt10 library of NR6 using synthetic oligonucleotide probes derived from peptide sequence information obtained from sequencing the isolated protein.

L329 ENDOGENOUS 34,000 DALTON PROTEIN PHOSPHORYLATED IN RESPONSE TO EPIDERMAL GROWTH FACTOR. P. Woost and G. Schultz, Dept. of Biochemistry, Univ. of Louisville, Louisville, KY 40292.
The epidermal growth factor-receptor (EGF-R) possesses an intrinsic EGF-stimulated kinase activity. Identification of endogenous cellular substrates which are phosphorylated by the EGF-R and the elucidation of their biological functions may improve our understanding of how EGF transmits its biological signal within the cell. To this end, we have identified a 34 kDa protein whose phosphorylation was stimulated by EGF in three distinctly different biological systems: purified human placental microvillus membranes, and crude membrane fractions prepared from human MDA-MB-231 breast cancer cells and human A431 epidermoid carcinoma cells. The 34 kDa protein was identified in autoradiographs of polyacrylamide gels of phosphorylation assays in which membranes were incubated with ^{32}P-ATP in the presence and absence of EGF. The 34 kDa protein was alkali stable when polyacrylamide gels were treated with 1N NaOH, suggesting the presence of phosphotyrosine. When MDA-MB-231 membranes were incubated with EGF, ^{32}P-ATP, and 5-p-fluorosulfonylbenzoyl adenosine, an affinity label shown previously to irreversibly modify the ATP-binding site within the EGF-R of A431 cells, ^{32}P-labeling of the EGF-R and the 34 kDa protein was inhibited. Tryptic digests of phosphoproteins from Triton X-100 solubilized MDA-MB-231 membranes yielded a fragment of 33-34 kDa from the EGF-R. Since a tryptic fragement similar in size to the 34 kDa phosphoprotein was identified, the two proteins were analyzed by peptide mapping with Staphylococcal V-8 protease. However, phosphopeptide maps indicated the proteins were structurally dissimilar. Sequence analysis of the 34 kDa protein is currently being determined. Funded in part by NIH grant EY-05587.

L330 TRYPTIC PHOSPHOPEPTIDE ANALYSIS OF FRAGMENTS GENERATED FROM THE EGF RECEPTOR BY CALCIUM-ACTIVATED PROTEASE, Robin W. Yeaton[1], David D-L. Woo[2], Michael T. Lipari[3] and C. Fred Fox[1], (1)Molecular Biology Institute, University of California, Los Angeles, CA 90024, (2)Biology, California Institute of Technology, Pasadena, CA 91125 and (3)Genentech, South San Francisco, CA 94080.
Epidermal growth factor (EGF) binds to an extracellular domain on its transmembrane 170 kDa receptor, stimulating a series of events which culminate in cell division. Although both the hormone and its receptor have been sequenced and many phenomena related to EGF reception have been characterized in detail, the precise methods by which EGF's signal is transduced remain a mystery. Two interrelated events of particular interest are the EGF receptor's tyr kinase activity (increased in transformed cells) and Ca^{2+} activated proteolysis of the EGF recptor. Ca^{2+} activated protease has also been implicated in activation of C-kinase which phosphorylates a thr residue on the EGF receptor which inhibits EGF receptor tyr kinase activity, and in intracellular processing of steroid hormone receptors, including progesterone receptor which is phosphorylated on tyr by EGF receptor. To study the exchange of energy catalyzed by Ca^{2+} activated proteolysis of the EGF receptor, from A431 human epidermoid carcinoma cells we purified tyr kinase-active, undegraded EGF receptor (radiophosphorylated by ^{32}Pi in the cells or *in vitro* by $^{32}\gamma$ ATP) and Ca^{2+} activated protease, combined them *in vitro*, and characterized by phosphamino acid mapping, the peptides released from the 170 kDa EGF receptor in the presence of Ca^{2+}, as well as the phosphorylated domains on the remaining 145 kDa EGF receptor.

Late Additions

L331 Activation of oncogenes in more progressive sublines of human mammary cancer cell lines
R.Michalides and J.Hilkens.Dept.of Tumorbiology,Netherlands Cancer Institute,Plesmanlaan 121,1066CX Amsterdam,The Netherlands.

In order to mimic tumorprogression in vivo,we selected more progressive variants from established human mammary cancer cell lines by selection for anchorage independent growth in soft agar,and for invasive growth in the chicken myocardial infiltration assay and growth in nude mice.Sublines established from single colonies in soft agar showed a variable oncogene expression.A subline of a newly established human mammary cancer cell line,MPL13,which was selected for growth in nude mice,showed an altered morphology,altered cell surface markers and oncogene expression.The original MPL13 cell line grows as a monolayer and forms organoid structures.The subline which was able to grow in nude mice has lost this differentiation feature and does not form organoids any more.The MPL13 cell line contains an activated c-Ki-ras oncogene,as was demonstrated in a nude mouse tumorgenicity assay with NIH 3T3 cells as acceptor cells for the MPL13 DNA.
These findings indicate that,in case of the MPL13 cell line,progression proceeds through differential activation of oncogenes and coincides with a loss of the ability to differentiate.

L332 PROLIFERATION INHIBITORY AND DIFFERENTIATION EFFECTS ON MYELOID LEUKEMIC AND NORMAL CELLS BY T-LYMPHOCYTE DERIVED DIFFERENTIATION INDUCING FACTOR (DIF), Inge L. Olsson, Urban Gullberg and Eva Nilsson, Department of Medicine, University of Lund, Sweden

A differentiation inducing factor (DIF) for the promyelocytic HL-60 cell line is constitutively produced by the T-lymphocyte line HUT-102. DIF was purified from HUT-102 conditioned media utilizing DEAE-chromatography, gel filtration, high resolution ion exchange chromatography on a Mono Q column and SDS-PAGE. In addition to inducing differentiation of wild type HL-60 into monocyte-like cells, DIF at a ten-fold lower concentration inhibited clonal growth of the monoblastic U-937 cell line as well as of subclones of HL-60 (50% inhibition at approx. 10 pM DIF). Growth of normal granulocyte macrophage colonies was inhibited at a similar concentration while early erythroid colonies were rather resistant. DIF and Interferon-γ were shown to be separate molecules in as much as a neutralizing antibody for Interferon-γ did not abolish the DIF effect. Our results indicate that the differentiation effect on wild type HL-60 and the proliferation inhibitory effect on leukemic and normal myeloid cells are exhibited by identical polypeptides since both activities cochromatographed during all purification steps. DIF was purified 100,000-fold and found to have a molecular weight of approximately 45,000. However, high resolution ion exchange chromatography revealed a considerable charge heterogeneity, which was confirmed by results from chromatofocusing.

L333 INDEPENDENTLY ALTERED ONCOGENES IN HUMAN TUMORS, Horacio G. Suarez, Pierre Nardeux and Yannick Andéol, IRSC, 94802 Villejuif, France.

We investigate, in 10 cell lines derived from human tumors other than hematopoietic malignancies and bearing an activated c-ras oncogene, the presence of an independently altered oncogene. The genomic level and the transcripts of 7 oncogenes in the tumor cells, have been compared with that of normal human cells. Data showed that in 3 tumors : RD (fibrosarcoma), SHAC (stomach sarcoma) and SW 480 (colon carcinoma) a second oncogene (c-myc) is altered. In RD and SHAC the activated transforming gene is ras-N ; in SW480 it is ras-Ki. RD and SHAC present a c-myc genomic amplification of approximately 10 times, while in SW480 the genomic level is similar to that of normal control cells. However, while in the c-myc gene of the two former cell lines no apparent rearrangements were observed, in the latter cell line this gene is highly polymorphic. Northern blot showed that in the 3 tumors there are significantly higher levels of the 2.4 Kb c-myc transcript, than in the normal control. The dot blots indicate that RD, SHAC and SW480 exhibit respectively about 8, 5 and 20 times more c-myc poly A+ RNA than normal cells. When foci are induced in mouse 3T3 cells by RD, SHAC or SW480 high molecular weight DNAs, only ras-N or ras-Ki are present in the transformants. Karyotype analysis of RD and SHAC cell lines does not show a translocation or another abnormality involving chromosome 8. Work is in progress to : 1) establish the karyotype of SW480 cells and 2) more precisely determine the structure of its c-myc gene and the relationship between its polymorphism and the high level of transcription.

L334 MECHANISM OF ACTION OF PROOXIDANT PROMOTERS, Peter A. Cerutti, Department of Carcinogenesis, Swiss Institute for Experimental Cancer Research, 1066 Epalinges/Lausanne, Switzerland.

The establishment of a prooxidant state in the initiated target cell appears to exert a promotional (progressional) effect. Prooxidant states can result from the exposure to bona fide oxidants, from the metabolism of xenobiotics or indirectly from the infiltration of inflammatory cells (1).

In mouse skin inflammation appears to be necessary for promotion. Phorbol-myristate-acetate (PMA) causes the infiltration of monocytes/macrophages which release a complex mixture of components with paracrine and clastogenic (chromosome-breaking) activities. We have studied the composition and mode of action of clastogenic factor (CF) from PMA stimulated human monocytes. It contains TXB_2, $PGF_2\alpha$, PGE_2, HHT, HETES, arachidonic acid (AA), H_2O_2 and probably Fe. The hydroxyl-derivatives of AA in CF (i.e. PG and HETE) are formed from hydroperoxy-precursors which possess sufficient stability in the promoter-treated tissue to reach neighboring epidermal cells. Insight in the mode of action of monocyte CF was obtained in experiments with isolated rat liver mitochondria. We found that hydroperoxy-AA (HPETE) and, less efficiently, HETE induced the release of Ca^{2+} in a reaction which was accompanied by the oxidation and hydrolysis of intramitochondrial pyridine nucleotides. It is suggested that HPETE and HETE act as Ca^{2+} mobilizers during signal transduction by prooxidant promoters. HPETE and HETE were clastogenic (i.e. they induced DNA single strand breaks) in mouse fibroblasts in reactions which

required the presence of Ca^{2+} and Fe. Ca^{2+} dependent breakage may involve the activation of nucleases while Fe-dependent breakage is probably caused by radicals formed in Fenton-type reactions.

Prooxidant promoters may affect gene expression of initiated cells via the post-translational modification of chromosomal proteins. We found that bona fide oxidants (with promotional activity) and PMA induced poly ADP-ribosylation of numerous chromosomal proteins, including certain histones and the auto-modification of ADPR-transferase. The reaction could be inhibited by antipromotional antioxidants, the protease inhibitor antipain and the ADPR-transferase inhibitor 3-aminobenzamide (2,3).

(1) P. Cerutti, Science 227, 375 (1985).
(2) N. Singh et al, EMBO J. 4, 1491 (1985).
(3) N. Singh et al, Carcinogenesis 6, 1489 (1985).

L335 GENES FOR GROWTH FACTORS AS ONCOGENES, Stuart A. Aaronson, National Cancer Institute, Bethesda, MD 20892

The first direct link between oncogenes and proteins with known function came from the discovery that the v-sis oncogene encodes a protein closely related to one of two major polypeptide chains of PDGF (human platelet-derived growth factor). The v-sis transforming protein has been shown to be structurally and immunologically related to PDGF. Moreover, it functions in an analogous manner in that it binds and triggers the PDGF receptor and is specifically mitogenic for cells possessing such receptors. Finally, evidence that the v-sis product mediates its transforming activity by direct interaction with the PDGF receptor comes from findings that this transforming gene only induces growth alterations of cells possessing PDGF receptors. By sequence analysis, the human sis proto-oncogene has been shown to encode PDGF chain 2. Moreover, the normal PDGF-2 coding sequence can be activated as a transforming gene simply by causing it to be expressed in a cell responsive to the growth promoting activity of PDGF. These findings imply that activation of normal sis/PDGF-2 expression may play a role in tumors of connective tissue origin which abnormally express this gene. Mechanisms affecting sis/PDGF-2 expression in human normal and tumor cells as well as functional domains within the sis/PDGF-2 molecule will be discussed.

L336 TRANSFORMING GROWTH FACTORS, Harold L. Moses and Edward B. Leof, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

Transforming growth factor-α (TGFα), a 5.6 kD polypeptide, shows sequence homology with EGF and appears to mediate all of its biological effects through binding to the EGF receptor. TGFß is a larger molecule (25 kD) 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. TGFß stimulates growth of mouse AKR-2B cells in soft agar with serum without other added growth factors while the stimulation of growth of NRK cells in soft agar requires EGF addition to the serum-containing medium. TGFß also stimulates growth of AKR-2B cells in serum-free monolayer culture, but with delayed kinetics of DNA synthesis relative to stimulation with serum or other growth factors. We have demonstrated that TGFß induces c-sis and the release of PDGF-like activity. TGFß also induces PDGF-inducible genes, including c-fos and c-myc, but with delayed kinetics relative to induction by PDGF. The data suggest that stimulation of proliferation by TGFß in this circumstance is indirect through induction of c-sis and autocrine activity by endogenous PDGF. Although TGFß has been demonstrated to be stimulatory for certain fibroblastic cells, it is a growth inhibitor for most cell types tested. TGFß is very similar (and perhaps identical) to the BSC-1 cell-derived growth inhibitor described Holley. We, in collaboration with a number of groups, have demonstrated that TGFß is inhibitory for growth of secondary cultures of human keratinocytes and human mammary epithelial cells and primary cultures of rat hepatocytes and human bone marrow precursor cells. TGFß is likely to play a role in several disease states involving abnormal proliferation including neoplasia. In chemically-transformed mouse embryo-derived 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. In a squamous cell carcinoma cell line a lack of inhibition by TGFß was observed. It is hypothesized that autocrine stimulation by endogenous TGFß (fibroblastic cells) or loss of sensitivity to the 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. Additionally, processing of the inactive TGFß precursor may be an important regulatory step in TGFß action and could be a step at which important changes occur in neoplastic transformation.