NEGATIVE GROWTH CONTROL

Organizers: Harold L. Moses and Allan Balmain

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Plenary Sessions Page

Poster Sessions

The Cell Cycle (Joint)

G 001 G1/S-PHASE CONTROL IN YEAST AND HUMAN CELLS, S.I. Reed, V.Dulic, J. Ghiara, M. Hentze, D. Lew, N. Marini, H. Richardson, C. Stueland, K. Sugimoto, C. Wittenberg, Department of Molecular Biology MB-7, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037

The *S. cerevisiae* gene *CDC28* encodes a 34 kilodalton protein kinase catalytic subunit with highly conserved couterparts in all eukaryotic organisms. In both *S. cerevisiae* and fission yeast, *S. pombe*, genetic analysis indicates a role for this protein kinase, p34, both in regulating the *G1 to S-phase transition and the G2 to M-phase transition. In S. cerevisiae* our investigations suggest that these diverse regulatory activities are achieved by assembling active heteromultimeric complexes with G1- and G2-specific subunits. G1 regulation depends upon accumulation of a class of unstable cyclin-like proteins that are essential for the assembly of active Cdc28 protein kinase complexes. These proteins (G1 cyclins), designated Cln1, Cln2 and Cln3, normally accumulate late in G1 and are prevented from accumulating by signals that restrain cell division in G1. Furthermore, mutational analysis suggests that the accumulation of these proteins is rate limiting for Cdc28 protein kinase activity and passage through G1. Passage through G2 depends upon accumulation of four different cyclins, Scb1, Scb2, Scb3 and Scb4, that show a high degree of similarity to cyclin B, and assembly of a different Cdc28 protein kinase complex. In this case, however, cyclin accumulation, although periodic, is not rate limiting. The action of a regulatory network that controls the kinase by phosphorylation and dephosphorylation of the catalytic subunit appears to be rate limiting for activity in G2. This is analogous to the mitotic regulation of p34 and cyclins in animal cells. We have found, surprisingly, that B-type cyclins in budding yeast have a role in the initiation of S-phase in addition to their mitotic role. Genetic experiments place this cyclin function downstream of the major G1 regulatory point, START, but prior to DNA replication. This function may be analogous to that ascribed to cyclin A in animal cells.

In order to address the issue of whether G1 cyclins and p34 control the G1/S-phase transition in animal cells, human cDNAs that can rescue deletion of the G1 cyclin genes in yeast were isolated. Three novel human cyclins have been identified that have been designated cyclins C, D and E. The mRNAs corresponding to cyclins C and E show G1 periodicity in HeLa cells. *The encoded protein and associated protein kinase activity* corresponding to cyclin E exhibit maximal levels at the G1/S-phase boundary. In addition, cyclin E shows a complex pattern of intracellular localization that is dynamic through the cell cycle. Although difficult to assay, the protein kinase activity associated with cyclin C appears to be periodic through the cell cycle. Immunofluorescence staining suggests that cyclin C is primarily nuclear. We are attempting to determine the targets of the cyclin C- and E-associated kinases. In addition, we are determining the specific members of the human Cdc2 kinase superfamily (p34s) that are activated by each cyclin.

G 002 Mos PROTO-ONCOGENE AND CELL CYCLE REGULATION. George F. Vande Woude, Renping Zhou, Richard S. Paules[1], Ira Daar, Nelson Yew, and Marianne Oskarsson. ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702; [1]National Institute of Environmental Health Sciences, Mammalian Molecular Genetics Group, Research Triangle Park, NC 27709.

The mos proto-oncogene product, $pp39^{mos}$, is required for meiotic maturation in vertebrate oocytes and is an active component of cytostatic factor (CSF), an activity in unfertilized amphibian eggs believed to be responsible for their arrest at metaphase II. First described 20 years ago, CSF was shown to arrest egg development at M-phase by stabilizing high levels of maturation promoting factor (MPF). Thus, the mos product functions at a major cell cycle control point, and its activity is directly or indirectly responsible for the stabilization of MPF. This link between proto-oncogene function and M-phase cell cycle regulation could be responsible for certain phenotypes of transformed cells.

We have found that mos product is associated with and phosphorylates tubulin in vitro. Our analyses have also shown that β-tubulin is preferentially associated with and phosphorylated by the Xenopus mos product from either transformed cells or unfertilized eggs. Immunofluorescence and immunoelectron microscopy analyses have shown that the mos product in transformed cells co-localizes with tubulin in microtubules as well as in the metaphase spindle pole and early telophase mid-body and asters. We speculate that the mos product may contribute to the formation of the spindle pole as well as the spindle and thereby contribute (as CSF) to metaphase arrest. Constitutive expression of mos in somatic cells is sufficient for morphological transformation, but only cells expressing low levels of $pp39^{mos}$ can grow as transformed cells. We postulate that this amount of product is not sufficient to cause mitotic arrest but is enough to impart M-phase phenotypes during interphase. Altered cell morphology and loss of contact inhibition during interphase could be due to cytoskeletal changes that normally occur during mitosis.

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Negative Regulation By TGF-Beta

G 003 GROWTH INHIBITION BY TGFß, Harold L. Moses, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

TGFß1 is the prototype of a large family of genes involved in growth control, extracellular matrix production, and development. TGFß1 has marked stimulatory effects on connective tissue formation. It is chemotactic for fibroblasts, an indirect mitogen for certain mesenchymal cells and a stimulator of extracellular matrix deposition. The TGFßs are also potent inhibitors of proliferation of most cell types in culture. *In vivo* studies have indicated that the predominant effect of TGFß1 on cell proliferation is inhibition. We have investigated the mechanism of TGFß1 inhibition of skin keratinocyte proliferation. Earlier studies demonstrated that TGF-ß1 inhibition of keratinocyte proliferation involves suppression of c-*myc* transcription and indirect evidence suggested that pRB may be involved in this process. Skin keratinocytes transformed by SV40 and human papilloma virus-16 (HPV-16) or HPV-18 were demonstrated to be resistant to the growth inhibitory effect of TGFß. TGFß was unable to suppress c-*myc* mRNA levels in these DNA tumor virus transformed cells. Transient expression of HPV-16 E7 gene, adenovirus E1A, and SV40 large T antigen (TAg) blocked the TGFß1 suppression of c-*myc* transcription in a transient assay using human *myc*/CAT constructs. These DNA tumor virus oncoproteins have been demonstrated to bind the protein product of the retinoblastoma gene (pRB). Mutants of E1A and TAg that do not bind pRB are transformation defective and expression of these proteins in the keratinocytes failed to block the TGFß1 suppression of c-*myc*. These studies suggested that a cellular protein(s) that interacts with a conserved domain of the DNA tumor virus oncoproteins blocks TGFß1 suppression of c–myc transcription and keratinocyte growth. Several cellular proteins, in addition to pRB, bind to the same conserved domain in the viral oncoproteins, and any of these proteins could be necessary for suppression of c-*myc* transcription in response to TGFß1 treatment. More recently, transient expression of pRB in skin keratinocytes was shown to repress human c-*myc promoter/CAT transcription as effectively as TGF-ß1. The same c-myc* promoter region, termed the TGFß Control Element (TCE), was required for regulation by both TGFß1 and pRB. Oligonucleotides containing the TCE bound to several nuclear factors in mobility shift assays and a cellular protein of approximately 106 kD in Southwestern assays. Binding of these factors could be demonstrated in cells with or without normal pRB, and the binding of some factors was rapidly inhibited by TGFß1 treatment of TGFß-sensitive but not TGF-ß-insensitive cells. These data indicate that pRB can function to inhibit c-*myc* transcription and suggest the involvement of cellular factor(s) in addition to pRB in the TGFß1 pathway for suppression of c-*myc* transcription and growth inhibition. The possible involvement of pRB in the TGFß1 pathway for suppression of c-*myc* transcription has a number of implications. Tumor suppressor genes may function in the response pathway for diffusible growth inhibitors analogous to nuclear protooncogene involvement in the growth factor pathway. This predicts that one mechanism for loss of the growth inhibitory response to TGFß would be inactivation of the retinoblastoma gene.

G 004 REGULATION OF TRANSCRIPTION OF THE TGF-β ISOFORMS, Anita B. Roberts, Seong-Jin Kim, Lalage M. Wakefield, Michael A. O'Reilly, Andrew G. Geiser, Su Wen Qian, James K. Burmester, Klaus Busam, Keunchil Park, David Romeo, and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

Three distinct isoforms of TGF-β are expressed in mammalian tissues. Although the biological effects of the mature forms of each of these isoforms are often indistinguishable *in vitro*, their patterns of expression are often distinct as in embryonic development, tissue differentiation, and in response to treatment with members of the steroid/ retinoid family of compounds. These effects probably involve differential control of both transcription and translation of the isoforms. To study transcriptional regulation, the 5′ flanking sequences of each of these 3 genes have been cloned and characterized with respect to known transcription factor binding sites. Expression of the TGF-β1 gene is upregulated by several oncogenes including *ras*, *src*, *abl*, *jun*, and *fos*. It is also induced by the Tax transactivator protein of the HTLV-1 virus. Together, the action of these factors probably is responsible, in part, for the elevated level of TGF-β1 expression often seen in carcinoma and leukemia cells. Expression of TGF-β1 is also upregulated by the product of the retinoblastoma gene, Rb, through a response element called RCE. Although RB has been shown to act indirectly, recent studies implicate several known transcription factor binding sites, including Sp1, in the TGF-β1 RCE. Whether this regulation by Rb constitutes an important feature of cell-cycle control is not known at present.

The control of transcription of the TGF-β2 and TGF-β3 genes is less well understood. In contrast to the TGF-β1 promoter which lacks a TATAA box, each of these genes contain classic TATAA boxes just upstream of the transcriptional start sites and a CRE site approximately 30-40 bp 5′ of the TATAA box. Studies using *Drosophila* Schneider cells have demonstrated that Sp1 sites in the TGF-β1 and TGF-β3 promoters are essential for their activity; in the TGF-β3 promoter, AP-2 enhances the promoter activity in the presence of Sp1, but not alone. The TGF-β2 promoter is not responsive to Sp1 under similar conditions. The CRE sites in the TGF-β2 and 3 promoters are essential for basal promoter activity, since mutation in these sites abolishes the activity of upstream promoter elements. The TGF-β2 and 3 genes are induced in response to differentiation signals; the sequences involved are presently being characterized, but appear to represent novel transcription factor binding sites. In additional to transcriptional regulation, translation of the TGF-β isoforms is also differentially regulated. Studies of translational regulation of the TGF-β isoforms suggest that regions of the 5′ UTR's of the TGF-β mRNAs can form stable stem-loop structures, and that control of translation can be localized to these domains.

Gene Loss in Tumor Progression

G 005 CELL CYCLE REGULATION AND CELLULAR SENESCENCE, J. Carl Barrett, Cynthia A. Afshari, Lois A. Annab, Barbara A. Burkhart, Jeff Boyd, Russell D. Owen, P. Andrew Futreal, Gloria Preston, and K. Hartmut Richter, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, National Institutes of Health, P.O. Box 12233, Research Triangle Park, N.C., 27709

We have proposed the hypothesis that cellular senescence is controlled by normal genes that are activated or whose functions become manifest at the end of the life span of a cell. Defects in the function of these genes can allow cells to escape the program of senescence and become immortal. This hypothesis is based on our ability to map senescence genes to specific chromosomes using somatic cell hybrids and microcell-mediated chromosome transfer. Candidate genes on human chromosomes 1, 4, and X have been mapped by these techniques, and we are currently attempting to clone a gene on chromosome 1q25. In order to understand the mechanisms of growth arrest in senescence, we examined the status of the RB protein in senescent cells. Senescent cells failed to phosphorylate the RB protein in response to serum. Since a central role for the $p34^{cdc2}$ protein kinase is postulated in control of the cell cycle and RB phosphorylation, we examined the status of this kinase in senescent cells and other growth-arrested cells. In senescent cells, which failed to incorporate [^{3}H]-thymidine, there was no $p34^{cdc2}$ mRNA. Young cells maintained for 48 h in 0.5% serum also retained only marginal cdc2 expression. After stimulation of low serum-arrested cells by addition of 10% serum, the quiescent cells showed a time-dependent increase of cdc2 mRNA, which was not seen when senescent cells were serum stimulated. In other growth-inhibited states, brought about by isoleucine deficiency in G1, by aphidicolin at G1/S, by etoposide in G2, or by colcemid in M-phase of the cell cycle, cdc2 mRNA was expressed at high levels. Following transfection of a plasmid containing the human cdc2 gene into hamster cells, expression of cdc2 mRNA failed to overcome the block to DNA synthesis in senescent cells. Taken together, these data support the concept that a chain of events leads to senescence, where $p34^{cdc2}$ kinase is one of the critical elements but other cell cycle controls are also affected.

G 006 APC (ADENOMATOUS POLYPOSIS COLI) GENE, MCC (MUTATED IN COLORECTAL CANCER) GENE, AND COLOLECTAL TUMOR, Yusuke Nakamura[1], Isamu Nishisho[1], Yasuo Miyoshi[1], Keneth Kinzler[2], Bert Vogelstein[2], Yoshio Miki[1], Akira Horii[1], Hiroshi Ando[1], [1]Department of Biochemistry, Cancer Institute, 1-37-1, Kami-Ikebukuro, Toshima, Tokyo 170, Japan; [2]Oncology Center, Johns Hopkins University, Baltimore, Maryland 21231, USA

Familial polyposis coli (FAP) is an autosomal dominant hereditary disease characterized with hundred to thousand of benign adenomatous polyps in colon, some of which progress to colon carcinomas if left without surgical treatment. Recently we have isolated the APC (Adenomatous Polyposis Coli) gene responsible for FAP by the positional cloning method and identified germ-line mutations of the APC gene in more than 50 FAP patients. Most of them were disrupted the gene product due to point mutation, or new termination due to frame shift by 1 ~ 5 bp insertion or deletion. Amino acid changes (Arg to Cys and Ser to Thr) due to a single base mutation were observed in only two cases. Mutations are scattered throughout the gene, which codes 2843 amino acids, and no hot spot of mutation was identified. Furthermore, we have examined somatic mutations in multiple colorectal tumors in four FAP patients. In one case, loss of heterozygosity (LOH) on chromosome 5q was detected in five adenomas and one colorectal cancer. In this case, as the region lost in six independent tumors was identical, it was suspected that a somatic interstitial deletion may be caused not by random mechanism, but by a specific mechanism. In contrast to 5q, LOH on chromosome 17p (the p53 locus) was observed only in colon cancers, but in none of 15 adenomas in these patients. LOH on chromosome 18q (the DCC locus) was not detected in any of tumors. This result has confirmed the model that the gene on chromosome 17p has a critical roll for malignant transformation of adenoma to carcinoma of colorectal tumor.

In one colon cancer developed in a FAP patient, we found the disruption of the APC gene by the insertional mutation of LINE-1 (L1) sequence which composed of 600 bp of 3′ portion of L1 sequence and 150 bp of poly A residues. In addition, an 8 bp target-site duplication was observed. Hence, the mechanism of this insertional event was suspected as retro-transcriptional insertion of a human mobile genetic element.

G 007 BIOCHEMICAL BASIS FOR p53 ACTION, Jennifer Pietenpol[1], Scott Kern[1], Wafik El-Deiry[1], Paula Friedman[2], Carol Prives[2], and Bert Vogelstein[1], [1]The Johns Hopkins Oncology Center, Johns Hopkins School of Medicine, Baltimore, MD 21231 and [2]The Department of Biological Sciences, Columbia University, New York, NY 10027.

Although p53 is the most commonly mutated gene yet identified in human cancers, little is known about its normal function(s) or the effect of mutations on such function(s). Recent data suggest the hypothesis that p53 binds specifically to DNA and that this binding might mediate transcriptional activation or suppression of replication. To test this hypothesis, we have used various techniques to define human DNA sequences to which p53 can specifically bind in vitro. Using "whole genome PCR", a technique in which the complete human genome can be amplified by the polymerase chain reaction, we have identified over 15 different sites in the human genome which have the capacity to bind to p53. Each of these sites is currently being tested to determine (i) the critical nucleotides required for binding; (ii) whether mutant p53 proteins consistently lose the ability to bind to these sites; (iii) whether p53-associated proteins are required for this binding; and (iv) whether these sites can mediate biologic effects in vivo on transcription or replication.

p53

G 008 p53-DEFICIENT MICE DEVELOP NORMALLY BUT ARE SUSCEPTIBLE TO TUMORS

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Mutations in the p53 tumor suppressor gene are the most frequently observed of the genetic lesions found in human cancers. Germ line p53 mutations in humans have been recently associated with an inherited cancer syndrome (Li-Fraumeni syndrome). To examine the role of p53 in mammalian development and tumorigenesis, a null mutation was introduced into mouse embryonic stem (ES) cells by homologous recombination. An ES cell clone containing the null mutation was used to generate mice heterozygous and homozygous for the disrupted p53 allele. Germ line heterozygotes and homozygotes are normal by gross physical examination and histopathology. The homozygote mice do not express intact p53 mRNA in any of three tissues examined. Embryo fibroblasts derived from homozygote mice contain no detectable intact or truncated p53 protein. Wild type controls and heterozygotes have not developed tumors (up to 9 months of age). However, homozygote mice show an early susceptibility to an array of different tumors by the age of four months. The tumor types most frequently observed include lymphomas, testicular carcinomas, hemangiosarcomas, and undifferentiated sarcomas. Five of the first eleven animals with tumors had multiple primary tumors of different cell type origin. Our results indicate that while p53 is dispensable for normal development, its loss may predispose mice to a variety of spontaneously arising neoplasms.

G 009 LESIONS DISRUPTING GROWTH CONTROL IN LUNG CANCER. John D. Minna[1], Domenic D'Amico[1], Scott Bader[1], David P. Carbone[1], Michael Chia[2], Adi F. Gazdar[1], Josef Geradts[2], Rhoda F. Maneckjee[1], Marion Nau[2], Tamar Unger[2], Stefan Winter[1]. Simmons Cancer Center[1], University of Texas Southwestern Medical Center, Dallas, TX 75235-8590, and NCI-Navy Medical Oncology Branch[2], Bethesda, MD 20889

Lung cancer cells exhibit many mutations inactivating "tumor suppressor" genes. These mutations are indicated by the large number of chromosomal deletions, unbalanced translocations, and RFLP loss of heterozygosity for multiple sites including chromosomes 1, 3p14, 3p21, 3p24-25, 11p, 13q (*rb* gene), & 17p (p53 gene), as well as other chromosomes such as 9p and 5q. The large number of genetic lesions (estimated to be 10-20 per cancer cell) raises the question of whether or not the mutations arose independently. Candidate genes assigned to 3p include retinoic acid receptors and phosphatase genes and lung cancer cells appear to have greatly dimished or absent growth regulation by retinoic acid. The *rb* gene is mutated in nearly all cases of small cell lung cancer (SCLC) and in ~ 20-30% of non-SCLCs. Absent expression of *rb* protein is usually seen, presumably allowing the uncontrolled function of nuclear proteins usually bound by *rb* that participate in growth regulation. *p53* is mutant in 50% or more of all lung cancers, and is nearly always found in a mutated form in SCLC. However, we have not yet found an inherited p53 mutation in patients with sporadic lung cancer. Most mutant p53 proteins found in lung cancer have greatly diminished transactivation capacity, a finding consistent with the hypothesis that p53 participates in the transcription of other genes regulating cell growth. We have identified a new autocrine/paracrine regulatory systems involving opioid and nicotine receptors where the expression of opioid peptides and their cognate receptors represent a system of "tumor suppression" whose function can be inactivated in some cancer cells by nicotine. Lung cancer cell lines of diverse histologic types express multiple, high affinity membrane receptors for μ, d, and k opioid agonists and for nicotine and a-bungarotoxin. Lung cancer cells also express various combinations of immunoreactive opioid peptides. μ, d, and k opioid agonists at low concentrations (1-100 nM) inhibit lung cancer growth *in vitro* . While nicotine at concentrations (~100 nM) found in smokers has little effect on the *in vitro* growth of most lung cancer cell cell lines, it can partially or totally reversed opioid induced growth inhibition in several lung cancer cell lines.

Growth Regulation By RAS and GAP (Joint)

G 010 ACTIVATION AND SUPPRESSION OF RAS, Douglas R. Lowy[1], Ke Zhang[1], Jeffrey E. DeClue[1], Hui Cen[1], Maureen R. Johnson[1], and Berthe M. Willumsen[2], [1]National Cancer Institute, Bethesda, MD and [2]University Institute of Microbiology, Copenhagen, Denmark.

Ras proteins (Ras) bind guanine nucleotides with high affinity, possess intrinsic GTPase activity, and cycle between an active GTP-bound state and an inactive GDP-bound state. The GTP-bound form of mammalian Ras can be negatively regulated by GAP or NF-1, which accelerate conversion to the inactive GDP-bound form. We have been studying regulation of mammalian Ras activity by GAP, NF-1, and stimulated guanine nucleotide exchange, which may be mediated by a mammalian homolog of CDC25.

Ras activity is required for the induction of DNA synthesis by mitogens such as serum and PDGF, and treatment of NIH 3T3 cells with these mitogens induces a rapid increase in GTP-bound Ras. Using Ras mutants, we have found that this higher concentration of GTP-bound Ras may be induced by stimulation of guanine nucleotide exchange, rather than by a decrease in the activity of GAP and/or NF-1. We have also identified a region of Ras that is more critical for the biological activity of normal Ras than for the activity of a GAP and NF-1 resistant activated Ras. This region of Ras may be involved in nucleotide exchange, since the activity of normal Ras is more dependent on stimulated exchange than mutant Ras.

Using Ras/Rap chimeric proteins, we have determined that sensitivity to GTPase acceleration by GAP is mediated by the effector region and loop 4 of Ras, presumably by stabilizing Ras in a conformation that fosters hydrolysis of bound GTP. Chimeric proteins that are sensitive to GAP may be resistant to NF-1, which suggests that additional regions of Ras are required for NF-1 sensitivity.

From in vivo results obtained with chimeras that are sensitive to GAP but resistant to NF-1 in vitro, we infer that endogenous GAP in NIH 3T3 cells is sufficient to negatively regulate Ras, although the cells contain NF-1 protein. By contrast, each of two malignant Schwannoma cell lines from NF-1 patients display abnormally high concentrations of GTP-bound, normal Ras at low cell density. Abnormal regulation of Ras activity in these cells correlates with a reduction of NF-1 protein in one line and its absence the other. Even in the cell line that lacks NF-1 protein, the concentration of GTP-bound Ras is reduced to normal levels when cells become confluent. A qualitatively similar, but less dramatic, reduction of GTP-bound Ras has also been noted in confluent NIH 3T3 cells. These changes correlate with increased GAP activity in confluent cells. In the cell line that lacks NF-1 protein, overexpression of the GAP catalytic domain can induce morphological reversion and reduce GTP-bound Ras at low cell density. The results suggest that NF-1 may function as a tumor suppressor gene product by negatively regulating Ras in Schwann cells.

G 011 REGULATION OF RAS p21 AND K-rev1 PROTEINS BY GTPase ACTIVATING PROTEINS (GAP)s, Frank McCormick[1], George Martin[1], Gail Wong[1], Paul Polakis[1], Robin Clark[1], Gideon Bollag[1], Bonnee Rubinfeld[1], Atsuko Yatani[2], and Arthur Brown[2], [1]Cetus Corporation, Emeryville, CA 94608 and [2]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Ras p21 is regulated by at least three distinct GTPase Activating Proteins (GAP)s, each of which converts active, GTP-bound ras p21 to the inactive, GDP-bound form. p120-GAP and p100-GAP are generated from the same gene by alternative splicing events. These proteins contain regions of homology with src oncogenes (SH2 and SH3 regions) which allow GAP to interact with specific proteins containing phosphotyrosine. PDGF-receptor is the best known of these proteins. The ability of p120-GAP and p100-GAP to interact with ras p21 and tyrosine kinase substrates suggests that GAP connects ras and tyrosine kinase signalling pathways. We have studied the nature of this connection using a cell-free system in which recombinant GAP has a measureable biochemical activity. In this system, GAP uncouples a muscarinic receptor from its G-protein. This effect of GAP is ras dependent. We have found that the SH2/SH3 region of GAP is sufficient for this effect, and that this isolated domain acts in a ras-independent manner. Based on these data, we propose that the ras-binding region of GAP is a regulatory domain that normally prevents GAP's SH2/SH3 region from binding its target. This constraint is relieved by binding ras p21, or, in the cell-free system by removal of the domain. We are currently testing whether ras regulates binding to PDGF-receptor, and other target proteins. One of these targets is p62, a major tyrosine phosphoprotein in many transformed cells. We have cloned this protein, and find it has strong homology to hnRNP and snRNP proteins, suggesting a role in mRNA metabolism.

The product of the gene responsible for neurofibromatosis type 1 (NF1) is also a GAP for ras p21. NF1-GAP has a very high affinity for ras p21: the binding constant for the oncogenic leucine-61 H-ras protein to NF1 is 2 nM. Normal ras p21 also binds tightly to NF1-GAP, and this interaction is stabilized by signalling lipids, such as PIP2 and phosphatidic acid. These compounds inhibit the GTPase effect of NF1-GAP on ras p21, but allow tight binding. A model will be presented in which GAP and NF1 are dual effectors of ras p21 action, that allow ras p21 to co-ordinate two signalling pathways. One of these pathways includes tyrosine kinases (the p120-GAP pathway) and other relates to lipid metabolism (the NF1-GAP pathway).

The biological activity of ras p21 is inhibited by K-rev1, a protein 50% identical to ras p21. The GTPase activity of K-rev1 is regulated by at least one GAP. We have cloned K-rev1 GAP, and examined its biological and biochemical activities. K-rev1 GAP is unrelated to p120 ras GAP or NF1 ras GAP, but has some interesting structural properties, such as sites for phosphorylation by kinase A and p34. We will discuss the possible relationship between K-rev1 GAP action and ras p21.

G 012 STUDY ON THE *Krev*-1 TRANSFORMATION SUPPRESSOR GENE, Makoto Noda[1], Hitoshi Kitayama[2], Naoki Nishino[2], Tomoko Matsuzaki[1], and Yoji Ikawa[2, 3], [1]Cancer Institute, Japanese Foundation for Cancer Research, Kami-Ikebukuro, Toshima-ku, Tokyo 170, [2]Tsukuba Life Science Center, The Institute of Physical and Chemical Research, Koyadai, Tsukuba, Ibaraki 305, [3]Tokyo Medical and Dental University School of Medicine, Yushima, Bunkyo-ku, Tokyo 113, Japan.

Krev -1 cDNA was originally recovered from a "flat" revertant (R16) derived from Kirsten sarcoma virus-transformed NIH3T3 cells (DT line) after transfection of a pcD2-based human fibroblast cDNA expression library followed by enrichment for cells with increased attachment to the plastic dish . The *Krev* -1-encoded protein was found to be identical to *Rap* 1a and *smg* p21, independently discovered by other groups through different approaches, and it shares around 50% amino acid identity with Ras proteins. Besides the conserved motifs commonly found in G proteins, Krev-1 and Ras share an identical amino acid sequence in the region know as the effector-binding domain. The carboxy terminal region of the *Krev* -1 protein contains two characteristic structures playing roles in membrane association: lysine-repeats and a CAAL box. The latter serves as the signal for geranylgeranylation on the cysteine residue. The serine residue adjacent to the CAAL box has been shown to serve as a target for A-kinase. Two types of proteins modulating the biochemical activities of the *Krev* -1 protein (i. e. GDP/GTP-binding and GTPase) have been found: GTPase activating proteins (rap1 GAPs) and GDP-dissociation stimulator (*smg* GDS). The *smg* GDS has an additional activity to remove *smg* p21 from the membrane and this process is accelerated upon the serine-phosphorylation of *smg* p21 by A-kinase. The *Krev* -1 gene and/or its close homologue (*rap*1B) is expressed ubiquitously in various tissues and especially abundant in neurons, platelets and neutrophils. Association of the *Krev* -1 protein with the Golgi apparatus in fibroblasts has been demonstrated.

When *Krev* -1 expression plasmid is transfected into DT cells, a few percent of the transfectant colonies show flat morphology, and only a subtle morphological changes could be found in the rest of the transfectants. A part of the reason for this low efficiency of revertant-induction may be explained by the model in which the Krev-1 protein is switched-on in response to an unknown upstream signal. Although certain point mutations in the *Krev* -1 gene, that are expected to impair the intrinsic GTPase activity of its product ("activating" mutation), increased the efficiency of revertant-induction up to 5 fold, more than 80% of the transfectants still remain transformed. These findings may suggest the existence of additional regulatory mechanisms for the Krev-1 protein, besides the GDP/GTP-exchange reaction, and/or the existence of clonal beterogeneity among the recipient DT cells in the levels of molecules essential for the Krev-1 protein in exerting its biological effects. To understand the molecular basis underlying the apparently opposing biological activities between Ras and Krev-1, we previously analyzed the transforming and the revertant-inducing activities of a series of chimeric H-*ras*-*Krev*-1 genes, and found that small clusters of divergent amino acid residues surrounding the putative effector-binding domain are probably responsible for the difference in the activities. Recently, we have examined the *Krev*-1 as well as H-*ras* genes carrying amino acid substitutions at these divergent residues, and found that substitutions of two amino acid residues in H-Ras protein to Krev-1 type amino acids (D30E and E31K) are sufficient to convert the oncogenic H-Ras protein to a transformation suppressor. To examine whether alterations of the *Krev* -1 gene is involved in the process of human carcinogenesis, we are trying to: 1) develop a retrovirus-based *Krev* -1 expression system to test its effects on various types of tumor cells, and 2) to determine the structural of the genomic *Krev* -1 gene to facilitate the survey of possible gene alterations in tumors samples. In the first line of experiments, we have found that the "activated" *Krev* -1 gene exibits growth suppressing or tumor suppressing activity on certain cell lines derived from fibrosarcoma and colorectal carcinoma. In the second, we have learned so far that the *Krev* -1 gene is split by at least 6 introns (3 in the coding region), and there is at least one pseudogene exists in the human genome.

The Retinoblastoma Gene

G 013 THE FUNCTION OF *RB1* AS A TUMOR SUPPRESSOR GENE DEPENDS ON INTERACTING FACTORS, Brenda L. Gallie, Brenda L. Cohen, Monty Gill, Paul A. Hamel, Michelle Muncaster, and Robert A. Phillips. Division of Immunology and Cancer, Hospital for Sick Children Research Institute, and Departments of Ophthalmology and Molecular and Medical Genetics, University of Toronto, Canada M5G 1X8.

The observed general role of the *RB1* gene in the cell cycle is inconsistent with the highly tissue specific oncogenic effect of *RB1* mutations. Germline *RB1* mutation imposes a 40,000-fold relative risk (RR) of retinoblastoma on the infant, a 500-fold RR of osteosarcoma in the second decade, but no increase in RR for hematopoietic and some other malignancies. Thus, the role of *RB1* as a strong tumor suppressor is restricted to critical stages in specific differentiative pathways. On the other hand, once malignancy is initiated in a broad spectrum of tissues, mutation of *RB1* contributes a selective growth advantage.

In comparison to results of others, we have found that reconstitution with wild-type pRB has only a subtle effect on growth of tumor cell lines with multiple mutations in other tumor suppressor genes and oncogenes in addition to *RB1*. Thus, mutation of genes regulated by pRB, or whose products interact with pRB, may abrogate the tumor suppressive effect of replacement of *RB1*. Important growth-related genes are transcriptionally regulated by pRB in complexes with other proteins. We have shown that the pRB weakly represses transcription from the c-*fos* promoter, but strongly represses transcription from the c-*myc* promoter, dependent on both intact Large T binding domains and certain 5' sequences. Furthermore, the modulation of these important oncogenes is dependent on the state of differentiation of the cells.

Understanding the role of *RB1* mutations in oncogenesis will require analysis of the transcriptional activity of pRB in the cells most at risk, the developing retina and bone. Cancer may be the consequence of a tissue- and developmental-specific function of pRB related to the cellular decision to cease proliferation and terminally differentiate.

G 014 MOLECULAR BASIS OF CANCER SUPPRESSION BY THE HUMAN RETINOBLASTOMA GENE, Wen-Hwa Lee, PhD, Center for Molecular Medicine/Institute of Biotechnology, University of Texas Health Science Center, 15355 Lambda Drive, San Antonio, Texas , 78245

A class of cellular genes in which loss-of-function mutations are tumorigenic has been proposed. The RB gene (RB) appears to operate in exactly this fashion. The RB gene contains 27 exons dispersed over more than 200 kb and expresses a 4.7 kb mRNA ubiquitously. From the sequence analysis of the RB cDNA, the predicted RB protein has 928 amino acids with m.w. about 110 kd. The RB protein is a nuclear phosphoprotein capable of binding to DNA and to form a complex with oncoproteins of several DNA tumor viruses.

RB was found to be mutated in many other cancers such as osteosarcoma, breast carcinoma, small cell lung carcinoma, and prostate carcinoma. In order to develop a biological assay for tumor suppressor genes, and to determine the significance of these mutations in adult cancers, a cloned RB gene was introduced, via retrovirus-mediated gene transfer, into retinoblastoma, osteosarcoma and prostate carcinoma cells that have inactivated endogenous RB genes. Expression of the exogenous RB gene consistently suppressed their tumorigenicity in nude mice, suggesting that RB may act as a general tumor suppressor gene.

How RB acts to bring about this suppression is not completely clear. The RB gene product undergoes changes in phosphorylation in synchrony with the cell cycle. The phosphorylation state of the protein fluctuates with the unphosphorylated form existing in the G1 phase and distinct phosphorylation events occurring in late G1 phase, early S phase, and at the G2 to M transition point. The parallel between this cyclical phosphorylation pattern and the cyclical activity of the CDC2 protein kinase complex has engendered the hypothesis that this kinase is responsible, at least in part, for RB phosphorylation. The presence of multiple CDC2 consensus target sites in the RB protein sequence and the ability of MPF to phosphorylate RB protein on these sites in vitro provided further evidence that RB serves as a substrate of CDC2 kinase.

To test whether RB regulates cell cycle progression directly, purified RB proteins, either full-length or a truncated form containing the T-antigen binding region, have been injected into cells and the effect on entry into S phase determined. Synchronized cells injected early in G1 with either RB protein exhibit blocked progression into S phase. This effect is antagonized by co-injection with either antibodies directed against RB or with T antigen peptide. Injection of RB protein into cells arrested at the G1/S boundary or 6-10 hours before the end of G1 has no effect on BrdU incorporation suggesting that RB protein does not directly inhibit DNA synthesis in S phase. These results further indicate that RB may regulate cell proliferation by restricting cell cycle progression at a specific point in G1.

Two regions of RB essential for forming complexes with oncoproteins of several DNA tumor viruses frequently contain mutations in tumor cells. These observations suggest that endogenous cellular proteins may bind to the same regions of RB and thereby mediate RB's function. By adding exogenous, purified RB protein to cell lysates to drive complex formation, we have identified a cellular 46kd protein that specifically binds to RB. To purify this 46 kdprotein, an affinity column consisting of a truncated 56kd Rb protein was used. In addition to the 46kd protein, about 8 different proteins were reproducibly eluted from this column after stringent washings. These proteins failed to bind the column when T peptide, but not K peptide (a mutant version of T peptide) was used as competitor for binding to the column, suggesting that these proteins specifically bind to the Rb protein.

Using purified RB protein, we have extended our characterization of the interactions of this molecule. As predicted by sequence similarity to the intermediate filament family of proteins (of which the nuclear lamins are members), RB protein can polymerize into microscopic filaments in vitro. Further, the RB protein forms stable complexes with two distinct members of the CDC2 protein family. Based on these findings and on previous discoveries, a model in which the RB protein acts as a negative regulator by sequestering or "corralling" a variety of growth-promoting proteins into an inactive subnuclear structure or compartment, which may disassemble upon phosphorylation of the RB subunits, was proposed to describe how RB functions in the cells..

DNA Viral Oncoproteins and Growth Control

G 015 THE HPV ONCOPROTEINS AND THEIR MECHANISMS OF ACTION, Peter M. Howley, Martin Scheffner, Jon M. Huibregtse, Karl Münger, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, Maryland 20892

Insight into the mechanisms by which DNA tumor viruses transform cells has come from the recognition that oncoproteins encoded by these viruses interact specifically with important cell regulatory proteins. The E7 proteins of the ano-genital specific HPVs, the large tumor antigens of the polyomaviruses, and the adenovirus E1A proteins can bind the product of the retinoblastoma tumor suppressor gene. The ano-genital human papillomaviruses can be divided into a "high risk" group such as HPV-16 and HPV-18 and "low risk" group such as HPV-6 and HPV-11 based on their association with human cancer. The "high risk" HPVs are more efficient in their immortalization and transformation capacity in tissue culture than the "low risk" HPVs. In correlation with their transforming potential, the E7 proteins of the "high risk" HPVs bind pRB with a higher affinity than the E7 proteins of the "low risk" HPVs (1).

Like SV40 large T antigen and the adenovirus 5 E1B 55kD protein, the E6 proteins of HPV-16 and HPV-18 can complex with the tumor suppressor protein p53, whereas binding of p53 to the E6 proteins of the "low risk" HPVs has not been demonstrated (2). Recent studies suggest that an additional cellular protein of 100 kD is required for the interaction of p53 with HPV-16 and HPV-18 E6. Further evidence that p53 and pRB are relevant targets in cervical carcinogenesis has come from studies examining the state of p53 and RB genes in HPV-positive and HPV-negative carcinoma cell lines (3). These studies revealed that in the HPV-negative cases both the p53 and RB genes are mutated, whereas in the HPV-positive cases no mutation could be detected. These data indicate that in these cancers, the normal function of these tumor suppressor genes is abrogated either by mutation or as a consequence of the interaction of their products with the HPV oncoproteins.

A possible mechanism by which the "high risk" HPV E6 proteins abrogate the normal function of p53 has been provided by the finding that the E6/p53 interaction results in the accelerated degradation of p53 *in vitro* (4). The E6 promoted degradation was specific for p53 in that other proteins such as pRB or SV40 large T antigen which do not interact with E6 remain stable in the presence of E6. Recent studies indicate that the ability of the "high risk" HPV E6 proteins to promote the degradation of associated proteins seems to be a function common to the "high risk" and "low risk" ano-genital specific HPVs.

(1). Münger et al, EMBO J. 8:4099-4105, 1989.
(2). Werness et al, Science. 248:76-79, 1990.
(3). Scheffner et al, Proc. Acad. Sci. USA. 88:5523-5527, 1991.
(4). Scheffner et al, Cell. 63:1129-1136, 1990.

G 016 FUNCTIONAL ANALYSIS OF THE RB GENE PRODUCT AND RELATED PROTEINS. Mark E. Ewen, William G. Kaelin, Thomas Chittenden, Suman Shirodkar, James DeCaprio, Eric Flemington, James D. Griffin, Florence Ajchenbaum, Charles Sherr*, and David M. Livingston. Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA; and *St. Jude Hospital and Research Institute, Memphis, TN.

RB and p107 are related members of the nuclear family of 'pocket'-containing proteins. Each can form specific complexes with a defined set of nuclear proteins of unknown function in cell-free assays. Each can also form a complex with the 'immediate-early' transcription factor, E2F. Such complexes can be detected in crude cell extracts, implying that they exist in vivo. RB and p107 are both suspected of having cell cycle regulatory function(s).Recent evidence suggests that E2F-RB and E2F-p107 complexes form in a cell-cycle dependent manner. The timing of RB and p107-E2F complex formation is different, and major aspects of the timetable are reproducible in different human cells. Moreover, at least one such complex (p107-E2F) contains a third component, cyclin A. In in vitro assays, Cyclin A can bind specifically to a ~200 aa segment of the p107 'pocket' domain, while other 'pocket' structures are responsible for E2F binding. These discrete structure-function relationships underscore the potential significance of detecting such a tripartite complex in crude cell extracts. In addition, in cycling and primary, non-cycling cells driven into cycle with a suitable mitogen, p107-E2F-cyclin A complexes appear first in S. The suspected role of cyclin A in promoting cellular DNA replication and its synthesis near the G1/S boundary are both consistent with when in the cycle the aforementioned tripartite complex appears. From the available data, one can hypothesize that release of E2F from RB occurs at a time in G1 when this factor is needed for activation of certain, important 'immediate early' genes. One could also argue that it is brought back under control in S by p107. There is also reason to suspect that release from, or failure to bind to RB at the appropriate time in G1 may be linked to RB phosphorylation. If true, it will be interesting to determine whether these findings contribute to the mechanism which underlies Go/G1->S progression.

G 017 Tumor suppressor activities of wt p53: biological manifestations and molecular mechanisms. D. Ginsberg[1], E. Yonish-Rouach[1], D. Michael[1], J. Lotem[2], L. Sachs[2], A. Kimchi[2], M. Yaniv[3] and M. Oren[1], Departments of Chemical Immunology[1] and Molecular Genetics and Virology[2], The Weizmann Institute, Rehovot 76100, Israel, and Department of Biotechnology, Institut Pasteur, Paris, France[3].

In an attempt to understand the basis for the tumor suppressor activity of wt p53, a temperature-sensitive p53 mutant was used in order to induce the overexpression of functional wt p53. The consequences varied greatly among cell systems. In rat fibroblasts, excess wt p53 activity caused a reversible growth-arrest. On the other hand, the overexpression of wt p53 in cells of the M1 mouse myeloid leukemia line led to the induction of apoptosis (active cell death). This effect was inhibited by Interleukin-6 (IL-6). IL-6 has two effects on M1 cells: induction of growth arrest and induction of differentiation into monocytes. To determine which of these activities of IL-6 underlies the interference with p53-mediated apoptosis, we tested the effect of various growth-inhibitory and differentiation-promoting agents on this process. It was found that the mere inhibition of cell proliferation, induced by various cytokines (interferon, TGF-β) or by hydroxyurea, did not provide any protection against p53-mediated apoptosis. Some, but not all, inducers of M1 differentiation had a similar effect to that of IL-6. Hence, it is not differentiation per se but rather the activation of particular signalling pathways that inhibit p53-mediated apoptosis.

It has been suggested that p53 is a transcriptional modulator. To determine whether such property of wt p53 could account for its antiproliferative activities, we assessed the ability of p53 to affect the expression of potentially relevant genes. It was found that the steady-state levels of c-myc mRNA, as well as those of serum-inducible c-fos mRNA, decreased rapidly after the activation of wt p53. In cotransfection experiments, wt p53 effectively reduced transcription from a series of promoters derived from growth-related genes, including c-fos and c-myc, but not from a major histocompatibility (MHC) gene. Attempts to map the sequences responsible for p53 responsiveness, as well as to define the molecular mechanism underlying p53-mediated transcriptional repression, will be described.

Regulation of Gene Expression (Joint)

G 018 RETINOID RECEPTORS IN DEVELOPMENT AND DISEASE, Ronald M. Evans[1], Akira Kakizuka, Steve Kliewer David Mangelsdorf, and Kazuhiko Umesono[1], Howard Hughes Medical Institute[1], The Salk Institute, La Jolla, CA 92037.

The cellular responses to RA are mediated by two families of transcription factors, which include the RA receptors (RARs) and the retinoid X receptors (RXRs). Although both RAR and RXR respond specifically to RA, they differe substantially from one another in primary structure and ligand specificity. A major question raised by the discovery of two retinoid-responsive systems is whether their functions are independent, interactive, or redundant. One approach to answer this question is to determine whether they share common or distinct downstream target genes. In regard to target sequences we have recently described properties of direct repeats (DRs) of the half-site AGGTCA as hormone response elements. According to our results, spacing of the half-site by 3, 4, or 5 nucleotides determines specificty of response for vitamin D3, thyroid hormone and retinoic acid receptors, respectively. This so-called "3-4-5" rule suggests a simple physiologic code exists in which half-site spacing plays a critical role in achieving selective hormonal response. As part of these studies, we have also identified that the RXR, but not the RAR, is able to activate through a direct repeat spaced by one nucleotide. In contrast, both RAR and RXR are able to commonly activate through a DR with a spacing of 5. Evidence that RXR heterodimers modulate the RA response will be presented.

Finally we will discuss the isolation and characterization of a fusion product produced as a consequence of a t(15;17) translocation characteristic of human acute promyelocytic leukemia. This translocation which occurs in the retinoic acid receptor gene generates a unique mRNA which encodes a fusion protein between the retinoic acid receptor alpha (RARα) and a myeloid gene product called PML. Structural analysis reveals that the PML protein is a member of newly recognized protein family that includes a variety of putative transcription factors as well as the recombination-activating gene product (RAG-1). The abberant PML-RAR fusion product, while typically retinoic acid responsive, displays both cell type and promoter specific differences from the wild type RARα. Because patients with APL be induced into remission with high dose RA therapy, we propose that the non-liganded PML-RAR is a new class of dominant negative oncogene product.

G 019 ACTIVATION AND ANTIREPRESSION OF TRANSCRIPTION BY PROMOTER- AND ENHANCER-BINDING FACTORS, Paul J. Laybourn, Rohinton T. Kamakaka, Glenn E. Croston, and James T. Kadonaga, University of California, San Diego.

Transcription by RNA polymerase II involves the basal transcriptional machinery, sequence-specific DNA binding factors that interact with promoter and enhancer elements, and the template DNA, which is packaged into chromatin. In addition, it appears that there may be another class of factors, which have been named coactivators, mediators, or adapters, that are required for activation of transcription by the promoter- and enhancer-binding factors. The basal transcriptional apparatus comprises RNA polymerase II as well as several auxiliary factors, which are generally referred to as the general factors. Several of the general factors, including the TATA-box binding polypeptide of TFIID, TFIIA, TFIIB, TFIIE, and TFIIF, have been purified and cloned from various organisms, but the exact number of general factors that are required for basal transcription has not yet been elucidated. Furthermore, it is not known whether all promoters require the same set of general factors. The first portion of the lecture will describe our recent studies on the purification, characterization, and cloning of the general transcription factors from Drosophila. The remainder of the talk will be devoted to the antirepression hypothesis and the use of chromatin templates for transcription in vitro. Briefly, it can be imagined that promoter- and enhancer-binding factors may activate transcription by either or both of the following two mechanisms. First, the sequence-specific factors may facilitate the inherent transcription reaction -- we refer to this as "true activation." Alternatively, the promoter- and enhancer-binding factors may counteract a general repression of basal transcription by a nonspecific DNA binding entity (*i.e., chromatin*) -- we have designated this effect as "antirepression." In our earlier studies on transcriptional activation in vitro, we had found that Sp1 (which contains a glutamine-rich activation region) and the GAL4-VP16 fusion protein (which contains an acidic activation region) were capable of both true activation and antirepression. The antirepression effect was unexpected, however, because chromatin assembly did not occur under the reaction conditions. We thus purified the DNA binding repressor and identified the protein as histone H1. A number of studies, both in vivo and in vitro, have suggested that H1 is involved in transcriptional repression. We have extended these studies with chromatin templates, and it presently appears that promoter- and enhancer-binding factors function both for true activation and antirepression and that under certain circumstances, there is a competition for promoter binding between the sequence-specific factors and histone H1 that leads to either gene activation or repression.

G 020 STRUCTURE AND FUNCTIONAL INTERACTIONS OF GENERAL INITIATION FACTORS, REGULATORY FACTORS AND COFACTORS, Robert G. Roeder, Ananda Roy, Micheal Meisterernst, Philippe Pognonec, Yan Luo, and Hiroshi Fujii, Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10021.

Continued studies of the purification and characterization of general factors, which are ubitquitous and commonly required by most class II genes, have implicated factors IIA, IIB, IID, IIE, IIF, IIG, and II-I in transcription initiation from minimal (core) promoters by RNA polymerase II. Amongst these factors, TFIIA and TFII-I are functionally interchangeable and form alternate preinitiation complexes on the adenovirus ML promoter, which suggests the possibility of selective responses to different regulatory factors. Unlike TFIIA, TFII-I binds stably to a number of initiator elements, but, like TFIIA, interacts cooperatively with TFIID. TFII-I also has been shown to interact cooperatively with the upstream activator USF (a helix-loop-helix protein), suggesting a novel mechanism for communication between regulatory factors and the general transcriptional machinery. The cloning of TFII-I has revealed structural relationships with helix-loop-helix proteins.

Studies of the AdML and HIV promoters have identified a novel cofactor (USA) that is required for physiological levels of promoter induction in purified systems (with general factors) by USF (AdML), Sp1 (HIV) and NFkB (HIV). The action of USA involves both a large net increase in promoter activity in conjunction with the activator and a repression of activator-independent activity. Fractionation and mechanistic studies indicate the involvement of both a negative cofactor (NC1) which competes with TFIIA for binding to TFIID, leading to basal repression, and a positive cofactor (PC1) which, with the activator, reverses the action of NC1 and effects a large positive promoter response. These results, along with the identification of other negative and positive factors interacting with TFIID, suggest novel promoter regulatory mechanisms that differ from previous models involving simple adaptors. Although the action of USF appears general, the possibility of activator-specific components has not been ruled out.

Related studies have revealed that the ubiquitous Oct1 and the lymphoid-restricted Oct2 are indistinguishable with respect to their intrinsic abilities to activate immunoglobulin (Ig), H2B, or snRNA promoters but that they interact with additional factors in a promoter-specific way to enhance transcription by the common factors. The isolation and characterization of a novel B cell-specific accessory factor (BAF) that specifically and markedly enhances Ig promoter activity via Oct1 or Oct2 suggests the existence of a new class of tissue specific cofactors/coactivators that may play a major role in the determination of promoter specificity and activity in vivo.

Wilms Tumor and Neurofibromatosis Genes

G 021 MOLECULAR ANALYSIS OF THE NEUROFIBROMATOSIS GENE AND ITS PROTEIN PRODUCT, Francis Collins[1], David Gutmann[1], Douglas Marchuk[1], Lone Andersen[1], Paula Gregory[1], Margaret Wallace[1], Susan Wilson-Gunn[1], Anna Mitchell[1], Deborah Wood[1], Roxanne Tavakkol[1], Manju Swaroop[1], Roymarie Ballester[2], Michael Wigler[2], [1]University of Michigan, The Howard Hughes Medical Institute, Ann Arbor, MI 48109-0650, [2]Cold Spring Harbor Laboratory, NY 11724

Type 1 neurofibromatosis (NF1) is a common autosomal dominant condition characterized by café-au-lait spots, multiple neurofibromas, Lisch nodules of the iris, and a variety of other variable complications including seizures, learning disabilities, and an increased risk of malignancy, particularly of the nervous system. The NF1 gene was successfully identified in the summer of 1990 using a positional cloning strategy. The complete coding region of the gene has now been cloned and sequenced using a cDNA walking strategy. The gene extends across approximately 300 kilobases of chromosome 17, and encodes a protein of 2,818 amino acids. The entire locus has been cloned in a series of yeast artificial chromosomes (YACs), and these have been reconstructed by homologous recombination into a single large YAC containing the entire gene and extensive flanking sequences. Southern analysis using various subfragments of the gene indicate that there are other homologous loci on chromosomes 14, 15, and 22, although at least some of these appear to represent pseudogenes. Analysis of the NF1 gene in patients with NF1 has uncovered several examples of mutation. Only a small minority of patients have grossly apparent rearrangements by Southern blotting. The vast majority appear to have more subtle sequence alterations, including single nucleotide substitutions and small insertions or deletions.

The predicted amino acid sequence of the NF1 protein product has homology in its mid-portion to the mammalian GAP protein and the *IRA1* and *IRA2* genes of Saccharomyces cerevisiae. Gene transfer experiments show that this structural homology is reflected by the ability of a central domain of the NF1 protein to complement ira1$^-$ ira2$^-$ mutants in yeast. Biochemical evidence of GTPase activity of this domain against human and yeast RAS genes has also been obtained. Gene transfer experiments in yeast document that this homology is reflected by GTPase activity of a central domain of the NF1 protein. Preliminary mutagenesis analysis of this domain suggests that it may be possible to separate the GTPase activity from the ability to complement the heat shock sensitivity phenotype of *ira1*$^-$ *ira2*$^-$ yeast strains, which is an unexpected finding. Using a series of fusion proteins and synthetic peptides, it has been possible to raise several antisera which detect the NF1 protein in a multitude of species and tissue sources. As was suggested by previous RNA analyses, it appears that the protein is ubiquitously expressed. Immunofluorescence experiments have revealed unexpectedly that the NF1 protein appears to be associated with microtubules. A careful reanalysis of the amino acid sequence indicates the presence of a domain which may be responsible for this association. The role of the NF1 protein, which we suggest denoting NF1GRP for "NF1 GAP-Related Protein," in the pathways of signal transduction in the cell is under active investigation.

G 022 WILMS TUMOR: RECONCILING THE GENETICS WITH THE BIOLOGY, Veronica van Heyningen, Melissa Little, Kathryn Pritchard-Jones, Jane Armstrong, Jonathan Bard, Jane Prosser, Nicholas Hastie and Wendy Bickmore, MRC Human Genetics Unit, Edinburgh EH4 2XU, UK

Wilms tumor (WT) is genetically and biologically more complex than retinoblastoma. The existence of at least three tumor-predisposition genes is predicted: at chromosome 11p13, p15 and elsewhere in the genome. The WAGR-associated zinc finger gene, WT1, (11p13) has been identified Spatial and temporal expression suggests an important role in developmental regulation in both kidneys and gonads, at a basic level in control of proliferation and differentiation. Mutations in WT1 are being observed in about 8-10% of "sporadic" tumors analyzed. The nature of the alterations observed may give clues to function Most mutations so far described have led to premature chain termination, but we have observed two independent point mutations (one constitutional), which, from predicted zinc finger structure, are expected to lead to altered DNA binding capacity. Reduced binding is indeed suggested when protein expressed from one of these mutant sequences *shows a reduced ability to bind to the zinc finger target sequences which we have* found to be very similar to consensus sequences defined by a different approach. However identification of real physiological targets is much more difficult. Partly with this aim in mind we are making antibodies to the WT1 protein. A second gene at 11p15 is implicated in WT, through loss of heterozygosity confined to this chromosomal region. Whether loss of function mutations in both copies of WT1 are necessary for malignancy (ie whether the gene behaves as a tumor suppressor) is not clear. Dominant negative mutations may exist. Or perhaps interaction between heterozygous altered genes at two different loci can lead to uncontrolled growth. Unravelling WT1 function will clarify this. Manipulation of kidney differentiation in in vitro organ culture, with antisense probes, growth control molecules and antibodies should give insight into function. We have achieved knockout of WT1 *by homologous recombination in ES cells and Chimaeric mice* are being produced for further study of the developmental role of this gene. The statistically high association of gonadal anomalies in male WT patients led us to suggest pleiotropic effect of the WT gene on gonadal development. Deletion studies supported this hypothesis and now constitutional WT1 mutations have been described in two patients. In one case the mutation was transmitted from father, without genital abnormality but with WT, to son with both afflictions. When allele loss is seen, >95% is of maternal origin. This suggests involvement of *imprinting phenomena in tumorigenesis.* However, we have shown that both parental alleles of WT1 are expressed in normal and tumor tissue. Therefore imprinting may be confined to the p15 region, where it has been demonstrated in Beckwith Wiedemann Syndrome (BWS), itself associated with high WT incidence. Two genes which map to this region, IGF2 and H19, are known to be imprinted in the mouse. Upstream IGF2 regions contain WT1 target sequences - is this of physiological significance?.

Cell Membrane and Extra-Cellular Matrix

G 023 STRUCTURE AND FUNCTION OF RECEPTOR-LINKED PROTEIN TYROSINE PHOSPHATASES

Haruo Saito, Michel Streuli, Neil X. Krueger, Michiyasu Itoh, and Alex Y.M. Tsai,
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Protein tyrosine phosphorylation by protein tyrosine kinases (PTKases) plays an important role in the regulation of cell proliferation. The positive signals for cell proliferation transmitted by PTKases must, however, be counterbalanced by protein tyrosine phosphatases (PTPases). Given that hyper-phosphorylation of protein tyrosine residues can cause cell transformation, it is plausible that lack of dephosphorylation *resulting from loss of a PTPase function may be oncogenic. Indeed, the* human hPTPα and LAR genes have been mapped to chromosomal regions that are frequently implicated in several types of human tumors. As was the case with PTKases, there are two types of PTPases: soluble cytoplasmic PTPases and transmembrane receptor-linked PTPases. By using consensus oligonucleotide probes or cross-hybridization to previously identified PTPase cDNAs, we have isolated and determined the structures of cDNA clones that encode eight distinct human receptor-linked PTPases. Receptor PTPases are composed of one or two cytoplasmic catalytic domain(s) and an extracellular receptor region connected by a transmembrane peptide. The structures of these PTPases suggest an evolution by gene reshuffling. The extracellular receptors of human LAR and hPTPδ, and *Drosophila* PTPases DLAR and DPTP are all composed of several immunoglobulin-like domains and Fibronectin type-III domains. This organization is similar to that of cell adhesion molecules such as N-CAM. Thus, LAR, hPTPδ, DLAR, and DPTP might be themselves cell adhesion-type receptors. This raises a possibility that their function is to suppress cell growth upon cell-cell contact.. Immunohistochemistry on human tissues demonstrated LAR expressed by various cell lineages, including epithelial cells, smooth muscle cells and cardiac myocytes. LAR is expressed on the cell surface as a complex of two non-covalently associated subunits (extracellular E-subunit and cytoplasmic P-subunit) derived from a precursor protein. The E-subunit is shed during cell *growth, suggesting that receptor-shedding may be a part of the regulation* of the LAR PTPase. Other human receptor-linked PTPases have receptors of various sizes and structures. For example, the very large extracellular receptor of hPTPβ is composed of 16 fibronectin type-III domains but contains no Ig domain. Although no ligands have been unequivocally identified to these receptor-linked PTPases, the diverse receptor structures suggests that there also exist diverse types of ligands, which may include cell surface molecules, extracellular matrix, and soluble factors. Thus, it is likely that receptor PTPases are involved in the regulation of a wide variety of cell activities. One of the interesting features of the receptor PTPases is the presence of two tandemly duplicated PTPase domains in their cytoplasmic regions. Deletion and site-directed mutagenesis analyses indicated that only the membrane proximal domain 1 is enzymatically active, even though the C-terminal domain 2 is highly homologous to other PTPases. *In vitro* characterization of LAR PTPase suggested that the domain 2 regulates the activity of the domain 1.

The Cell Cycle; Negative Regulation by TGF-Beta

G 100 ANALYSIS OF THE INVOLVEMENT OF PROTEIN PHOSPHATASE-2A IN G0/G1 AND G1/S TRANSITIONS, Arthur S. Alberts, Andrew M. Thorburn, Marc Mumby+ and James R. Feramisco, Departments of Pharmacology and Medicine, University of California at San Diego, Cancer Center, La Jolla, CA 92093-0636; +Department of Pharmacology, University of Texas, Southwestern Medical Center at Dallas, Dallas, Texas 75235-9041

We have studied the effects of the microinjection of serine-, threonine-specific protein phosphatase-2A (PP2A) on gene expression in a series of mammalian cell lines containing an integrated lacZ gene under the control of different enhancers, *i.e.*, serum responsive (SRE), phorbol ester responsive (TRE), and cAMP responsive (CRE) elements. In addition, endogenous c-fos expression was monitored by immunofluorescence. Cells were arrested in G0 by serum deprivation, injected with PP2A or other proteins and were refed with medium containing serum. β-galactosidase activity was measured *in situ* at various times thereafter. It was found that injection of PP2A catalytic subunit inhibited serum activated SRE-lacZ, but potentiated serum induced TRE-lacZ expression. Following serum stimulation, c-*fos* expression was greater in fibroblasts injected with PP2A than those injected with a marker antibody alone as a control. PP2A catalytic subunit had no effect on the CRE-lacZ reporter following stimulation by 8BrcAMP. We have begun to examine the role of PP2A in events in the cell cycle. Purified catalytic subunit of protein phosphatase markedly inhibits serum-stimulated DNA synthesis when microinjected into synchronized cells during late G1, but not earlier. Currently, we are studying the effects of microinjected PP2A in DNA synthesis in cells previously injected with the oncogenic form of ras (T24), which like serum, initiates entry into the cell cycle. It has recently been shown that the RB protein is phosphorylated during the G1/S transition and becomes extractable from the nucleus. We are currently examining whether microinjection of PP2A effects either the localization of p105-RB or the expression of p53 in the nucleus during the cell cycle.

G 101 ROLE OF BCL-2 IN THE PROTECTION AGAINST APOPTOSIS INDUCED BY DEPRIVATION OF GROWTH FACTORS OR CROSSLINKING OF mIg RECEPTORS IN IMMATURE B CELLS, J. E. Alés-Martínez, E. Cuende, C. Martínez-A. and L. Ding† and G. Nuñez†, Centro de Biología Molecular, C.S.I.C. and U.A.M, 28049 Madrid, Spain, and †Dept. of Pathology, Ann Arbor, Michigan 48109-0602, USA

mIg cross-linking induces a cell-cycle block in G1, followed by apoptosis in immature B cells. In other studies, it has been shown that increased expression of the bcl-2 gene confers resistance against growth-factor deprivation induced apoptosis. Also, in a bcl-2 transgenic model, the B-cell population is expanded and this expansion could be due to interference with the normal processes which regulate the life-span of B cells. In this work, we investigate first, whether forced hyper-expression of bcl-2 interferes with apoptotic cell death induced through the mIg receptor. If this is the case, it could provide a rationale for the development of autoreactive B-cells and antibodies in certain lymphoproliferative disorders. Secondly, we intend to ask, on the basis of the effect of bcl-2 expression, whether serum deprivation and mIg crosslinking provoke apoptosis by at least partly separate pathways.

G 102 BIOLOGICAL PROPERTIES OF BETAGLYCAN, A BIFUNCTIONAL GROWTH FACTOR BINDING PROTEOGLYCAN, J.L. Andres, M. Noda, S. Cheifetz, F. López-Casillas, and J. Massagué. Department of Cell Biology and Genetics, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10021.

Betaglycan is a heparan sulfate/chondroitin sulfate proteoglycan which binds TGF-ß through its core protein with an affinity in the picomolar range. It exists in both a membrane-bound and soluble form. Betaglycan has now been purified to near homogeneity, cloned, and expressed in eukaryotic cells. We report here that purified betaglycan can also bind a second growth factor, fibroblast growth factor, through its glycosaminoglycan chains and that in some TGF-ß and FGF-responsive systems this may be a regulated function. In addition, expression of the cDNA for betaglycan indicates that the soluble form of betaglycan is produced by cleavage of the mature molecule from the cell surface, and sequence analysis reveals that betaglycan contains a sequence identical to the regulated cleavage site for TGF-α. This finding suggests an additional site for regulation of the growth factor binding function of betaglycan. We suggest that betaglycan, through its unusual ability to bind one hormone through its core protein and another through its glycosaminoglycan chains, may provide a very specific mechanism for regulating the information available to signalling receptors at the cell surface.

G 103 CHARACTERIZATION OF A PUTATIVE SERINE/THREONINE KINASE RECEPTOR, Liliana Attisano, Jeffrey L. Wrana and Joan Massagué, Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Centre, New York City, New York, 10021.

Using a PCR-based approach we have isolated a putative serine/threonine kinase receptor from Balb/c 3T3 fibroblasts. A unique PCR-generated DNA clone was used to screen a Balb/c 3T3 fibroblast cDNA library and several partially overlapping clones were identified. Sequence analysis of these isolates identified a contiguous nucleotide sequence of 1636 bp with an open reading frame encoding a 512 amino acid polypeptide. The predicted product of this cDNA has the characteristics of a transmembrane protein including a N-terminal hydrophobic signal sequence, a short extracellular region (116 amino acids), a transmembrane region and a large cytoplasmic region. The cytoplasmic domain has features typical of a serine/threonine kinase. This polypeptide is similar to the mouse activin receptor in terms of both amino acid sequence and domain structure. The extracellular domains share approximately 50% amino acid identity with all 10 cysteines and 2 N-linked glycosylation sites conserved. In addition, the cytoplasmic domain is approximately 75% identical. This receptor exists in two alternatively spliced forms, and their mRNAs are present in approximately equal abundance in Balb/c 3T3 cells. Mammalian cell transfections are presently underway in order to identify the ligand for this receptor and to determine the functional relevance of these two forms.

G 104 DIFFERENTIAL IMMORTALIZATION POTENTIAL OF HPV TYPES 16 AND18 AND THE ROLE OF TGF-ß RESPONSIVENESS. Jane L. Brokaw, Karl Münger, and Peter M. Howley, Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892

The growth of normal human keratinocytes and cervical epithelial cells is markedly inhibited by TGF-ß. Human keratinocytes immortalized by human papillomavirus type 16 (HPV-16) are also susceptible to the growth-suppressive effect of TGF-ß, and display a reduction in transcription of viral E6 and E7 early genes. In contrast, HPV-18-immortalized lines are resistant to the growth suppressive effects of TGF-ß, and the transcription of the the early viral genes remains relatively unaffected. It has been reported that cervical carcinoma lines are also insensitive to the inhibitory effect of TGF-ß. While both HPV-16 and HPV-18 are associated with invasive squamous cell carcinomas and precancerous lesions, HPV-18 is predominantly associated with invasive carcinoma and is less frequently associated with premalignant cervical intraepithelial neoplasia. Moreover, HPV-18 is several-fold more efficient than HPV-16 in the in vitro immortalization of human keratinocytes. Therefore, it has been suggested that HPV-18 may behave more aggressively in the carcinogenic process. The enhanced transforming capacity of HPV-18 may be related to its resistance to inhibition by TGF-ß. Studies are currently underway to delineate the sequence within the HPV-18 long control region (LCR) that confers HPV-18 with its heightened transforming efficiency and distinguishes it from HPV-16, and to define the TGF-ß-responsive element in the HPV-16 LCR .

G 105 ISOFORM-SPECIFIC TGFß BINDING PROTEINS SENSITIVE TO PIPLC, Sela Cheifetz and Joan Massagué, Cell Biology and Genetics Program and Howard Hughes Medical Institute, Memorial Sloan Kettering Cancer Center, New York, NY 10021

Novel cell surface proteins that bind TGFß in an isoform specific manner were identified on FBHE fetal bovine heart endothelial and MG-63 human osteosarcoma cell lines. These include three proteins of 90, 100, and 180 kDa and two proteins of 60 and 140 kDa that preferentially bind to TGFß1 and TGFß2 respectively. The 180-kDa TGFß1 binding proteins and the 60- and 140-kDa TGF-ß2 binding proteins were released from the cell surface by treatment with phosphatidylinositol-specific phospholipase C, suggesting the presence of a phosphatidylinositol anchor. The 90- and 100-kDa TGF-ß1 binding proteins are components of a 190-kDa disulfide linked complex. The structural properties and the selective affinity of these proteins for the different TGFß isoforms defines them as distinct from TGFß receptors I and II or the TGFß binding proteoglycan betaglycan.

G 106 IN VITRO APOPTOSIS IN THE HUMAN HEPATOMA CELL LINE INDUCED BY TRANSFORMING GROWTH FACTOR-BETA (TGF-β), Chen-Kung Chou and Jen-Kou Lin , Deparment of Medical Research and Department of Surgery, Veterans General Hospital, 201, Section 2, Shih-Pai Road, Shih-Pai, Taipei, Taiwan, R.O.C.

The effect of transforming growth factor-beta (TGF-β) on human hepatoma cells (Hep 3B) was studied. Cell death was observed when the serum starved Hep3B cells were exposed to a very low dose of TGF-β . The half maximal cytocidal concentration of TGF-β was around 20 pM. Cell death began approximately 24 hours following treatment, with more than 80% of the cells dying after 48 hours. In contrast, the control cells, which were cultured in serum free condition still gradually proliferated. Furthermore, the cytocidal effect of TGF-β on Hep 3B cells was not altered by either cycloheximide or actinomycin D. It was discovered, using diphenylamine assay, that TGF-β induced DNA fragmentation in Hep 3B cells, with a time course prior to that of cell death. Using gel electrophoresis, the fragmented DNA, could be displayed, and showed a characteristic step ladder pattern. Thus, it appeared that TGF-β induced a particular pathway in Hep 3B cells in which de novo protein synthesis was not actively involved, but endogenous nuclease was activated which cleaves cellular DNA and induces cell death.

G 107 DNA-DEGRADATION (APOPTOSIS) OR DNA REPLICATION: DEFINITION OF A CONTROL POINT FOR INTERLEUKIN-2 DEPENDENT CELLS IN THE LATE G1 PHASE, Ge Deng and Eckhard R. Podack, Department of Microbiology and Immunology, University of Miami School of Medicine, Miami, FL 33101

DNA degradation (programmed cell death, apoptosis) is a biologically important mechanism to eliminate superfluous cells in multicellular organisms. In the immune system, growth factor (Interleukin-2, IL-2) availability controls the extent of clonal expansion and the elimination by apoptosis of T-cell clones during and after an immune response, respectively. IL-2 deprivation from unsynthronized IL-2 dependent cells cause cell death within 16-24 h by DNA degradation. Our studies define a critical point in the cell cycle of CTLL2, an IL-2 dependent cell line, in late G1 phase, where IL-2 controls the decision between DNA replication (IL-2 present) and DNA degradation (IL-2 absent). Once the cells pass the commitment point for DNA degradation in the absence of IL-2, readdition of IL-2 does not prevent apoptosis. Death committed cells degrads their DNA within 1.5 h at 37°C to fragments of 200 bp or multiple intergers thereof by internucleosomal cleavage. Uncommitted cells after 1-2 h begin to replicate their DNA without a requirement for continued IL-2 presence. Prior to the commitment point in late G1, CTLL2 can be rescued from apoptosis by the readdition of Il-2 or, alternatively, of cycloheximide. The rescue effect of IL-2 is dependent on gene transcription, since actinomycin D abolishes the rescue from apoptosis by IL-2. The rescue by cycloheximide is dependent on its ability to block protein synthesis. These data indicate that IL-2 dependent CTLL2 contain a long lived mRNA whose translation causes directly or indirectly, DNA degradation and programmed cell death (pcd-RNA). IL2 protects, via gene transcription in late G1, CTLL2 from the effect of the pcd-RNA. We postulate that CTLL2 survival is dependent on the continuous protection of its DNA from the killer (pcd) mRNA by IL-2 dependent, cell cycle dependent synthesis of a protecting factor.

G 108 THREE CELL CYCLE CHECKPOINT CONTROLS ARE DEFECTIVE IN TRANSFORMED RODENT CELL LINES

C. Stephen Downes and Robert T. Johnson, Cancer Research Campaign Mammalian Cell DNA Repair Research Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK.

The mechanisms of cell cycle control are highly conserved in eukaryotes. A normal feature is that completion of each cell cycle stage is monitored by checkpoint mechanisms, which allow progression to the next stage only when the essential previous activities have been completed. Recently, however, Kung et al. (Proc Natl. Acad. Sci. 87, 9553, 1990) it have reported that transformed rodent cell lines evade one of these normal cell cycle checkpoints: they can proceed into the next cell cycle when spindle assembly and cytokinesis are blocked. We here show that rodent cell transformation allows two other cell cycle checkpoints - controlling progression from S-phase into G2, and preventing entry into S-phase without passage through G2 and mitosis - to be bypassed, by different mechanisms. Transformation of human cells does not normally affect these checkpoints.

Rodent transformation allows cycle controls to be altered in S-phase arrest induced by hydroxyurea, so that treatment with caffeine produces rapid S-phase condensation. It also causes cells treated with doxorubicin to omit the subsequent mitosis, and to endoreduplicate. Such behaviour is not characteristic of transformed human cells. However, transformed or diploid human cells lose their coordination of mitotic controls, and behave in this respect like transformed rodent cells, when treated in mitosis with aminopurine protein kinase inhibitors. The human response to doxorubicin is not affected by aminopurines. The transformed rodent S-phase response to hydroxyurea can be suppressed with purine deoxyribonucleosides; these do not affect the response of mitotic human cells to aminopurines, or of rodent cells to doxorubicin. The loss of coordination of cycle controls produced by rodent transformation is therefore a complex phenomenon.

G 109 CELL CYCLE-DEPENDENT NEGATIVE REGULATION OF $p34^{cdc2}$ BY TRANSFORMING GROWTH FACTOR ß1, Scott T. Eblen, Prashant Joshi, Rebekah Burnette-Greene and Edward B. Leof, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232

The ability of Transforming Growth Factor ß1 (TGFß1) to act as a cell-type specific activator or suppressor of cell growth is an interesting and perplexing phenomenon. It has been established by our lab that TGFß1 arrests quiescent restimulated epithelial cells at the G1/S phase border. Inhibition of DNA synthesis occurred through a post-transcriptional action of TGFß1. The mechanism of this growth arrest has not been conclusively established. To further address this problem we are currently investigating the effects of TGFß1 in cycling CCL64 cells, a mink lung epithelial cell line growth-arrested by TGFß1. Release of sparse CCL64 cultures from TGFß1 growth inhibition similarly indicated a G1/S phase cell cycle arrest. A potential regulator of both G1/S as well as G2/M traverse in all eukaryotic systems studied is the $p34^{cdc2}$ protein kinase. Addition of TGFß1 to cycling cells decreased the synthesis of $p34^{cdc2}$. Protein levels decreased within 3-6 hours following TGFß1 addition and continued to decline to only 10% of control levels by 24 hours. The kinetics of TGFß1 action on $p34^{cdc2}$ synthesis preceded any effect on cell division and paralleled the observed decrease in DNA synthesis. These effects of TGFß1 are cell cycle-dependent in that they are G1 specific. Moreover, this decrease in $p34^{cdc2}$ synthesis by TGFß1 is not simply reflecting a general decrease in protein synthesis since other growth regulatory proteins are unaffected. TGFß1 also decreased both the phosphorylation and histone H1 kinase activity of $p34^{cdc2}$. Western analysis indicated that 24 hour treatment with TGFß1 did not significantly affect total $p34^{cdc2}$ levels; the half-life was approximately 18-24 hours in either the presence or absence of TGFß1. Understanding the control of $p34^{cdc2}$ activity may provide insights into the biochemical mechanism(s) of TGFß1 growth inhibition.

G 110 CYCLIN A FORMS COMPLEXES WITH SEVERAL CELLULAR PROTEINS, Barbara Faha, Li-Huei Tsai , Matthew Meyerson, and Ed Harlow, Molecular Oncology, MGH Cancer Center, Bldg. 149, 13th Street, Charlestown, MA 02129.

The adenovirus E1A proteins associate with a group of cellular proteins in virus transformed or infected cells. One of these proteins has been identified as human cyclin A (Pines and Hunter, 1990). We have been studying cyclin A as a means to understand the functional implications of E1A binding to cyclin A. To do this, we have investigated the cellular proteins that co-immunoprecipitate with cyclin A. One such protein is the E1A-associated p107 protein. The association between cyclin A and p107 is not dependent upon the presence of E1A or the retinoblastoma protein (pRB) and occurs in all cell lines tested. p107 has recently been cloned (Ewen et al., in press) and was shown to have homology with pRB within the E1A binding region. The association between E1A and p107 has been shown to be important for oncogenic transformation by adenoviruses. In addition, p107 has been shown to associate with the large T antigens of SV40 and JC viruses. The significance of cyclin A's association with p107 has yet to be determined. We are currently investigating the cell cycle regulation of this association.

We have also shown that cyclin A associates with both $p34^{cdc2}$ and p33, a protein originally described by Pines and Hunter (1990). V8 partial proteolytic analyses demonstrated that these proteins are distinct, however, N-chlorosuccinimide digestion suggested that they were related. Based on these analyses, we hypothesized that p33 might be encoded by a cdc2-related gene. Using degenerate oligonucleotides derived from conserved regions of the cdc2 gene family, fragments were amplified by PCR from cDNA made from two cell lines, HeLa and Nalm-6. These fragments were used to screen cDNA libraries for full length clones. Eight novel human cdc2-related genes were obtained. One of these clones showed 65% identity with human cdc2 and 89% identity with Xenopus Eg-1 at the amino acid level. In addition, by several criteria, we have shown that this clone encodes the cyclin A-associated p33. This clone has been named cdk2 for cyclin-dependent kinase. We also show that $p33^{cdk2}$ is found in anti-E1A immune complexes. Since we have been unable to detect $p34^{cdc2}$ in these complexes, it appears that E1A may target the cyclin A/$p33^{cdk2}$ complex.

G 111 INCREASED EXPRESSION OF TGF-ß ISOFORMS BY MALIGNANT GLIOMAS, Leslie I. Gold, Babita Saxena, David Zagzag, Douglas C. Miller, Maxim Koslow, Leslie Brandeis, and Jean-Pierre Farmer, Departments of Pathology, Neuropathology, and Neurosurgery, New York University Medical Center, New York, NY 10016.

TGF-B is a potent biregulator of cellular growth and differentiation, is involved in angiogenesis, and also is an immunosuppressive cytokine. Because of these characteristics, it was our interest to examine the expression of the three mammalian isoforms of TGF-ß (TGF-ß1, TGF-ß2, TGF-ß3) in gliomas, ranging in grade of malignancy, to understand the role TGF-ßs might play in the behavior of these tumors. The expression of TGF-ßs in gliomas compared to normal cerebral cortex and cerebellum was examined by immunohistochemistry using peptide affinity purified anti-peptide antisera specific for each isoform. TGF-ß2 and TGF-ß3, but not TGF-ß1, were expressed by normal astrocytes and oligodendrocytes of both grey and white matter; TGF-ß1 only showed immunostaining of myelin. Also, in the cerebellum, TGF-ß2 and TGF-ß3, but not TGF-ß1, specifically stained the cell bodies and dendrites of the purkinje cells. Conversely, all three isoforms of TGF-ß stained both reactive and tumor astrocytes in all grades of astrocytomas (I-IV; IV = Glioblastoma multiforme), pilocytic astrocytomas, PNETs, and gliosarcomas ($n = 5$ of each). In mixed gliomas, the oligodendrocytic constituent demonstrated higher immunoreactivity than in normal brain tissue. Although TGF-ß expression was generally much greater in the astrocytes of tumor tissue compared to normal, the presence of a specific isoform of TGF-ß and the apparent intensity of expression did not correlate with the grade or tumor type. There was very striking expression of TGF-ß2, and especially TGF-ß3 in the smooth muscle cells of the blood vessels in all the gliomas examined and all three isoforms were expressed by the endothelial cells in most of the tumors. In all cases, the normal tissue adjacent to the tumor demonstrated intense immunostaining of TGF-ßs that progressively decreased distally to the tumor. This may indicate a paracrine effect of the tumor on the surrounding normal tissue. Further studies will examine the biological significance of the increased expression of TGF-ß in gliomas and the specific activation of the expression of the TGF-ß1 isoform.

G 112 MULLERIAN INHIBITING SUBSTANCE (MIS) SLOWS THE CELL CYCLE PROGRESSION OF HUMAN EPIDERMOID CARCINOMA, A431, CELLS, *‡P.L.Hudson, †F.Preffer, †D.Domkowski, ‡D.T.MacLaughlin, *J.K.Biusztajn, and ‡P.K.Donahoe, *Department of Pathology, Boston University School of Medicine, Boston, MA 02118, †Department of Pathology and ‡Pediatric Surgical Research Laboratories, Massachusetts General Hospital, Boston, MA 02114

MIS is an embryonic regulator of morphogenesis which causes the regression of growth of the mullerian ducts in males. It is included within the Transforming Growth Factor β group due to it's C terminus amino acid homology and similar growth regulating traits. Previous work has shown MIS to have antiproliferative activity against human ovarian and endometrial tumors as well as a human ocular melanoma in *in vivo* and *in vitro* studies. We have found that an early indication of inhibition of growth by MIS is a slowing of G1 cells in their transition to S phase. We used Flow Cytometry to assess the ability of MIS to prevent cell cycle progression. A431 cells grown to 100% confluency were found to be primarily in the G1 phase (e.g., 87.7% G1, 1.4% S, 10.9% G2/M phase). When replated at a subconfluent density the cells began to divide and at 24 hours 39.1%, 52.3% and 8.6% were found to be in G1, S and G2/M phases respectively. In contrast cells replated in media containing 20 μg/ml human recombinant MIS (143 nM) did not progress from G1 arrest and at 24 hours 73.9% were still in G1 whereas 23% were in S phase. The human ocular melanoma, OM431 cells, found to be growth inhibited by MIS, also exhibited this cell cycle effect. Cells exposed to MIS do slowly leave G1 phase and replicate, however, the slowing of cell cycle kinetics results in 50% inhibition of growth at 72 hours in comparison to untreated controls. Ion exchange (DEAE), dye affinity (Matrex® Green Gel A) purified MIS was consistently more effective in inhibiting growth of A431 cells than immunoaffinity purified MIS (IAP MIS). The decreased inhibition of growth by IAP MIS could reflect : 1. molecular latency due to incomplete enzymatic processing, 2. aggregation of the molecule preventing processing or 3. absence of an essential cofactor. Experiments are underway to characterize these molecular events.

G 113 INTERLEUKIN-6: A POSSIBLE MEDIATOR OF SOME OF THE ACTIONS OF TGF-β1. Ronald A. Ignotz and Chris Bombara. Dept. of Cell Biology, Univ. Massachusetts Med. School, Worcester MA 01655.

Transforming growth factor-β1 elicits a variety of responses in many cell types. In some instances, these responses may be mediated through the induction of secondary factors such as c-sis in proliferative responses. Here, we report that antibodies which neutralize Interleukin-6 (IL-6) can block the growth inhibitory effects of TGF-β1 on human THP-1 promonocytes. Interestingly, IL-6 itself is capable of inhibiting the proliferation of promonocytes suggesting that TGF-β may elicite some effects through the induction of this cytokine. TGF-β1 is also capable of inducing the expression of IL-6 mRNA 8-10 fold in human diploid fibroblasts. Preliminary studies with promoter sequences for the IL-6 gene linked to a reporter gene and transfected into cell lines also indicate that TGF-β1 is capable of activating IL-6 gene transcription. In contrast, others have reported that TGF-β suppresses IL-6 expression in endothelial cells. Our results are in agreement with the report of Guerne el al. on induction of IL-6 in chondrocytes. Thus, the regulation of IL-6 expression by TGF-β1, both positive and negative, is an attractive model for examining tissue specific effects of TGF-β1. In addition, this comparison may give insight into the complex pattern of responses occuring in tissues. Some effects attributed to TGF-β1 may be indirect and the result of other induced cytokines which show a higher level of cell type discrimination.

G 114 Macrophage cell cycle arrest by cAMP occurs in mid-G1 and overrides constitutive c-*myc* expression, Suzanne Jackowski, John L. Cleveland and Charles O. Rock, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.

A murine macrophage cell line (BAC1.2F5) was employed to investigate the inhibition of colony-stimulating factor 1 (CSF-1)-dependent proliferation by elevation of intracellular cAMP via prostaglandin E_2 (PGE_2). BAC1.2F5 cells were synchronized in early G1 following transient removal of CSF-1 from the medium and required the presence of CSF-1 throughout G1 to obtain the maximum mitogenic response. PGE_2 was an effective inhibitor of [^{3}H]thymidine incorporation when added up to 4 h after CSF-1, but addition at later times did not block the onset of S phase. The time required to enter S phase following release from cAMP arrest was midway between the G1/S boundary as defined by arrest with aphidicolin and the time to S phase entry following CSF-1 addition to CSF-1-starved cells. BAC1.2F5 cells released from aphidicolin block entered S phase in the absence of CSF-1, but cells arrested with a cAMP analog required the presence of CSF-1 to initiate DNA synthesis. PGE_2 or cAMP analogs did not block the initial induction of c-*myc* mRNA by CSF-1, but did abolish the CSF-1-dependent expression of c-*myc* mRNA in the mid-G1 stage of the cell cycle. PGE_2-mediated reduction in c-*myc* RNA levels required protein synthesis and, based on nuclear run-on experiments, the reduction in c-*myc* mRNA levels occurred at the level of transcription. BAC1.2F5 cell lines in which c-*myc* expression was independent of CSF-1 were derived using a Moloney murine leukemia virus-derived vector encoding c-*myc*. Stable clones constitutively producing c-*myc* mRNA still required CSF-1 for growth and survival. These cell lines were also growth-arrested by PGE_2 and cAMP analogs in mid-G1 supporting the view that the reduction in endogenous c-*myc* mRNA levels by PGE_2 was a consequence rather than the cause of growth inhibition and demonstrating that constitutive expression of c-*myc* can not overcome cAMP growth stasis. Taken together, these results suggest that cAMP-mediated growth arrest and the inhibition of c-*myc* mRNA transcription is mediated by a cAMP-inducible gene product(s) that arrests macrophage cells in the middle of the G1 phase of the cell cycle. (Supported by ACS BE-121B, GM45737 and DK44158)

G 115 **IDENTIFICATION AND CHARACTERIZATION OF THE CHICKEN TRANSFORMING GROWTH FACTOR-β3 PROMOTER, Sonia B. Jakowlew, Robert Lechleider, Andrew G. Geiser, Seong-Jin Kim, Tomas Santa Colona, Michael B. Sporn and Anita B. Roberts, Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892**

The promoter regions of the three mammalian transforming growth factor-beta genes (TGF-βs 1, 2 and 3) have been cloned and characterized. The sequences show little similarity suggesting different mechanisms of transcriptional control of these genes. As an initial step in understanding transcriptional regulation of chicken TGF-β, we have cloned and sequenced the 5'-flanking region of chicken TGF-β3. Characterization of the 5'-flanking region showed a 94 base pair 5'-untranslated region, a TATA box 352 bp upstream from the transcriptional start site, and cAMP-responsive element (CRE) and AP-2 binding site consensus sequences starting at 12 and 28 bp, respectively, upstream from the TATA box. Moreover, 14 additional putative AP-2 sites and 10 binding sites for the transcription factor Sp1 were also identified as well as one potential 12-O-tetradecanoylphorbol 13-acetate (TPA) consensus binding site. Except for 32 bp of identity centered around the TATA box and CRE site, and other relatively small regions of identity, the chicken TGF-β3 promoter was found to be structurally very different from the human TGF-β3 promoter. Promoter fragments were cloned into a chloramphenicol acetyltransferase reporter plasmid to study functional activity. Basal transcriptional activity of the promoter was regulated by multiple upstream elements including the TATA, CRE and AP-2 sites in human adenocarcinoma A375 cells and in quail fibrosarcoma QM7cells. Our results indicate a complex pattern of regulation of the chicken TGF-β3 gene.

G 116 POSITIVE REGULATION OF CELL CYCLE PROGRESSION BY TGFß ON MOUSE FIBROBLASTS, Tae A. Kim, Alan J. Kinniburgh, and Charles E. Wenner, Experimental Biology and Human Genetics, Roswell Park Momorial Institute, Buffalo, NY. 14263

In mouse C3H 10T1/2 fibroblasts, TGFß (5 ug/ml) exerts a growth stimulatory effect and a somewhat delayed EGF-, PDGF-induced S-phase entry. This positive effect of TGFß involves several possible parameters including (1) a slower induction of c-myc expression by TGFß, (2) autocrine/paracrine induction of PDGF by TGFß and (3) induction of AP-1 related "immediate early gene" expression such as c-jun, c-fos and junB.

Moreover, we have recently observed that TGFß upregulates the expression of retinoblastoma gene (Rb) within 3 hr. The mRNA levels of Rb increase 5-8 fold between 3 to 12 hrs after TGFß treatment. The induction of Rb gene expression was also detected by stimulation of EGF (20 ng/ml) and tumor promoter, TPA ($2X10^{-7}$M), in quiescent, postconfluent C3H 10T1/2 cells. Interestingly, the significant induction (5 fold) of Rb gene by TPA occurs within 1 hr. The upregulation of Rb mRNA by growth factors or tumor promoters can generate the high level of Rb gene product (pRb). Since underphosphorylated pRB is known to be a growth suppressor, we propose that growth stimulation leads to the hyper-phosphorylation of pRB that is generated by growth factors in mouse embryonic fibroblasts.

G 117 MECHANISMS OF KERATINOCYTE GROWTH INHIBITION BY CALCIPOTRIOL AND VITAMIN D_3: ENHANCEMENT OF TGFβ EXPRESSION AND DECREASE OF PLASMINOGEN ACTIVATOR ACTIVITY. Katri Koli and Jorma Keski-Oja, Departments of Virology and of Dermatology and Venereology, University of Helsinki, SF-00290 Helsinki, Finland.

Vitamin D_3 and its metabolites regulate the growth and differentiation of several cell types. $1,25(OH)_2D_3$ and its analogue calcipotriol (MC 903) inhibit the proliferation of cultured human and mouse keratinocytes and induce keratinocyte differentiation. Since TGFβ is a very potent inhibitor of keratinocyte growth we investigated the effect of vitamin D_3 on the secretion of TGFβ1 and TGFβ2. We found that vitamin D_3 and calcipotriol [10^{-8}M] inhibited the DNA-synthesis of EGF stimulated mouse keratinocytes by 40 %. The inhibition was time and dose dependent. Keratinocytes secreted into their medium activity that inhibited the growth of Mv1Lu cells. Activation of latent forms of TGFβ by heat-treatment revealed even more growth inhibitory activity in vitamin D_3 and calcipotriol treated than in control cultures indicating that a fraction of TGFβ was in a latent form. Antibodies to TGFβ1 and TGFβ2 decreased, and when used together, prevented the observed inhibition of growth. These results suggest that TGFβ1 and TGFβ2 mediate the growth inhibitory effects of vitamin D_3.

Calcipotriol is effective for the treatment of psoriasis where increased plasminogen activator activity has been reported. We analyzed the effects of calcipotriol and vitamin D_3 on the production of plasminogen activator activity by mouse keratinocytes. Caseinolysis-in-agar assays indicated that vitamin D_3 decreases total plasminogen activator activity in murine keratinocytes. Zymographic analyses of the medium indicated that the activator was of the urokinase type (u-PA). Unlike in TGFβ treated keratinocyte cultures decreased secretion of plasminogen activator inhibitor-1 (PAI-1) was observed. The results suggest that vitamin D_3 and calcipotriol decrease plasminogen activator activity independent of TGFβ induction, in association with growth inhibition of keratinocytes, which is mediated via TGFβ1 and TGFβ2.

G 118 EXPRESSION OF HUMAN RETINOBLASTOMA GENE PRODUCT IN MOUSE FIBROBLASTS: REGULATION BY MOUSE CELL FACTORS AND EFFECTS ON CELL GROWTH. Marikki Laiho and Kimmo Pitkänen, Department of Virology, University of Helsinki, 00290 Helsinki, Finland.

Expression of human retinoblastoma cDNA in human retinoblastoma and osteosarcoma cells defective of the retinoblastoma gene (*RB1*) almost fully arrests the growth of the cells and prevents tumor formation *in vivo*. To evaluate the characteristics and functions of the human retinoblastoma gene product in a heterologous system we have attempted to stably express human full-length RB cDNA in normal mink epithelial cells or mouse fibroblasts using a mammalian expression vector. Whereas high levels of RB expression was readily observed in mouse fibroblasts, no stable epithelial cell lines expressing human RB were obtained. The mouse fibroblast produced human RB protein appears to have similar properties as in human cells. It is phosphorylated and dephosphorylated in a cell cycle dependent manner indicating that the rodent and human kinases and phosphatases are closely related. Moreover, human RB protein expressed in mouse cells is able to form complexes with SV 40 large T antigen, suggesting that it is functionally active. In contrast to tumor cells and the mink epithelial cells the expression of normal RB does not affect the growth properties of mouse fibroblasts. In epithelial cells RB may mediate the growth inhibitory signals generated by transforming growth factor-ß1 (TGF-ß1). Although TGF-ß1 prevents the proliferation of most epithelial cells, it weakly stimulates mitogenic responses in mouse fibroblasts. The growth responses of RB expressing cells to TGF-ß1 were, however, comparable to those of RB^- fibroblasts. Thus the expression of RB in mouse fibroblasts did not alter the growth regulatory circuits of TGF-ß1.

G 119 AN EPIGENETIC MECHANISM MAY COORDINATELY REGULATE EXPRESSION OF SEVERAL HUMAN BRONCHIAL EPITHELIAL CELL GENES. J.F. Lechner[1], K.A. Elliget[2], V.L. Wilson[3], R. Modali[4], B.I. Gerwin[5] and C.C. Harris[5], [1]Inhalation Toxicology Research Institute, Albuquerque, NM 87185, [2]Dept. Path., Univ. MD, Baltimore MD 21201, [3]Mol. Genetics/Path., Univ. CO, Denver CO 80281, [4]Bioserve, Inc., College Park, MD 20742, and [5]Lab. Human Carcinogenesis, NCI, Bethesda, MD 20892.

Two isogeneic subclones of the SV40 T-antigen immortalized human bronchial epithelial cell line (BEAS-2B) exhibit several dissimilar characteristics. S.6 cells undergo squamous differentiation when exposed to TGF-β_1, but R.1 cells continue replicating. In addition, S.6 cells have relatively higher steady state levels of TGF-α & EGFR mRNAs. Also, only in S.6 cells do TGF-β_1 and epinephrine repress and induce, respectively, the steady state level of c-*myc* mRNA in S.6 cells. We have now found that the TGF-β_1 receptor binding kinetics and physical characteristics of S.6 & R.1 cells are indistinguishable. Thus, gene regulation of these expressions by TGF-β_1 is distal to ligand binding. On the other hand, the genomic level of 5-methyl cytosine of R.1 DNA is twice that of the S.6 cell DNA. This observation suggests that the differential expressions of S.6 and R.1 cells could be due to an epigenetically based mechanism that coordinates a global change in gene regulation that includes TGF-β_1 signal transduction. Since lung squamous cell carcinomas contain both squamous differentiating S.6-like and continuously replicating R.1-like cells, understanding the mechanisms that govern the epigenetic interconversion of these phenotypes could possibly provide novel clinical intervention modalities.

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G 120 EXPRESSION CLONING OF THE TYPE II TGF-β RECEPTOR. Herbert Y. Lin, Xiao-Fan Wang, Elinor Eaton, Robert A. Weinberg, and Harvey F. Lodish. Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have succeeded in isolating the cDNA encoding the type II TGF-β receptor by expression cloning in COS cells. A cDNA mammalian expression library was constructed from LLC-PK1 porcine kidney epithelial cells and plasmid miniprep pools representing ~10,000 independent cDNA clones were transfected into COS cells grown on glass slide flaskettes. 48-60 hours after transfection, cells were incubated with iodinated TGF-β1, then extensively washed, and autoradiographic analysis was performed. After screening 400,000 recombinants, one positive pool of 6000 recombinants was identified and a single clone with an insert of 0.9 kb was isolated through serial subpooling.

Northern analysis using this cDNA as a probe shows that the corresponding message is a 5 kb message which is expressed in different tissues and cell lines. A full length cDNA was subsequently isolated by high stringency hybridization from a human HepG2 cell cDNA library. Sequence analysis indicates that the cDNA has an open reading frame encoding a core 573 aa residue protein with a single putative transmembrane domain, several consensus glycoslyation sites, and a putative intracellular serine/threonine kinase domain. Crosslinking experiments using iodinated TGF-β1 in transiently transfected and stably transfected cells indicates that the expressed protein has all the characteristics of the type II receptor: binding to TGF-β1, correct expressed protein size and expression pattern. Surprisingly, the ability of the type I receptor to bind TGF-β1 is increased by the expression of the type II receptor.

G 121 DOMINANT NEGATIVE MUTANTS OF TRANSFORMING GROWTH FACTOR-B1 INHIBIT THE SECRETION OF DIFFERENT TRANSFORMING GROWTH FACTOR-B ISOFORMS, Alfredo R. Lopez[1,2], Prescott L. Deininger[4], and Rik Derynck[1,3] [1]Department of Developmental Biology, Genentech Inc. South San Francisco CA 94080. Departments of [2]Medicine, [3]Growth and Development, and Anatomy, University of California San Francisco, San Francisco CA 94143, and [4]Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70121.

Transforming growth factor-β (TGF-β) is a secreted polypeptide factor that is thought to play a major role in the regulation of proliferation of many cell types and various differentiation processes. Several related isoforms have been structurally characterized, three of which, TGF-β1, -β2 and -β3, have been detected in mammalian cells and tissues. Each TGF-b form is a homodimer of a 112 amino acid polypeptide which is encoded as a larger polypeptide precursor. We have introduced several mutations in the TGF-β1 precursor domain using site-directed mutagenesis, resulting in an inhibition of the TGF-β1 secretion. The most potent of these mutants ▲ RK, lacks the highly charged sequence that follows closesly the signal peptide at amino acids 41 to 62 and is identical in the precursor polypetides for all three TGF-β sequences. Coexpression of these mutants with wild type TGF-β1, -β2 and -β3 results in a competitive and specific inhibition of the secretion of different TGF-b forms, indicating that these mutated versions act as dominant negative mutants for TGF-β secretion. Overexpression of dominant negative mutants can thus be used to abolish endogenous secretion of TGF-β and structurally related family members both in vitro and in vivo, and to probe in this way the physiological functions of the members of the TGF-β superfamily.

G 122 STRUCTURE AND EXPRESSION OF THE MEMBRANE PROTEOGLYCAN BETAGLYCAN, A COMPONENT OF THE TGF-ß RECEPTOR SYSTEM, Fernando López-Casillas, Sela Cheifetz, Jacqueline Doody, Janet L. Andres, William S. Lane and Joan Massagué, Cell Biology and Genetics Program and Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, and Harvard Microchemistry Facility, Harvard University, Cambridge, MA 02138.

The primary structure of rat betaglycan, a polymorphic membrane-anchored proteoglycan with high affinity for transforming growth factor-ß (TGF-ß) is described. As deduced from its cDNA sequence, the 853 amino acid core protein of betaglycan has an extracellular domain with clustered sites for potential attachment of glycosaminoglycan chains. These chains are dispensable for TGF-ß binding to the core protein. The transmembrane region and the short cytoplasmic tail of betaglycan are very similar to these regions in human endoglin, an endothelial cell membrane glycoprotein involved in intercellular recognition. The ectodomain of betaglycan can be released as a soluble proteoglycan; a potential cleavage site near the transmembrane region is identical to the highly regulated cleavage site of the membrane-anchored transforming growth factor-a precursor. The unique features of betaglycan suggest important roles in cell interaction with TGF-ß.

G 123 EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA IN THE CENTRAL NERVOUS SYSTEM, Ursula Malipiero, Daniel Constam, Jeannette Philipp, and Adriano Fontana, Section of Clinical Immunology, University Hospital Zürich, 8044 Zürich, Switzerland.

In inflammatory diseases of the central nervous system (CNS), activation of infiltrating T- and B- cells from the blood may not only depend on presentation of antigen by glial cells in a MHC dependent manner, but also on local production of cytokines that modulate the immune response. Intrathecal synthesis of some cytokines such as IL-6 and IFN-γ, which regulate B-cell differentiation, can be measured in the cerebrospinal fluid in infectious diseases of the CNS. A negative regulator of the immune response that inhibits the antigen dependent growth of T-cells was found to be produced by human glioblastoma cells and belongs to the family of the transforming growth factors-β (TGF-β). The factor was isolated, cloned and the sequence is termed TGF-β2. In the present study, we show that not only transformed glial cell lines but also mouse astrocytes express TGF-β2 mRNA and protein but no TGF-β1 and -β3 protein. In contrast, TGF-β rotein was found to be produced by microglial cells ne TGF-βs have multiple effects on immunoregulation, embryogenesis, cell division as well as differentiation. In order to investigate the nervous system for the regulation of activation of the TGF-β genes, we cloned and sequenced the promoter region of the human TGF-β2 gene and determined the transcription initiation sites. A comparison with the TGF-β1 promoter reveals considerable differences. Putative TATA boxes and AP-2 sites were found in TGF-β2 but not in TGF-β1. By footprinting analysis, we further investigated the promoter region.

G 124 CONVERSION OF A HUMAN COLORECTAL ADENOMA CELL LINE TO A TUMORIGENIC PHENOTYPE IS ACCOMPANIED BY A REDUCED RESPONSE TO THE INHIBITORY EFFECTS OF TRANSFORMING GROWTH FACTOR β. Anna M Manning[1], Ann C Williams[1], Angela Hague [1], Stephen M Game [2] and Christos Paraskeva[1], [1]Department of Pathology & Microbiology, University of Bristol, University Walk, Bristol BS8 1TD, [2] Department of Oral Medicine, Lower Maudlin Street, Bristol BS1 2LY, United Kingdom.

The growth of three non-tumorigenic human colonic adenoma cell lines at early passage, designated AA/C1, RG/C2 and RR/C1 was significantly inhibited by TGFβ (50% inhibition of DNA synthesis after 24 hours of TGFβ at 0.05-0.5ng/ml). A fourth adenoma cell line, BR/C1 although inhibited by TGFβ was relatively resistant (30% inhibited by 10ng/nl TGFβ after 24 hours) compared to the other three adenoma cell lines. Five human colon cancer cell lines under identical conditions, however, were resistant to high concentrations of TGFβ (2-10ng/ml) This is the first report of well characterised premalignant human colonic cells showing sensitivity to TGFβ.

The TGFβ sensitive cell lines AA/C1 and RG/C2 were derived from relatively large adenomas and have either a k-ras or p53 gene mutation indicating that loss of response to TGFβ occurs at a relatively late stage in colorectal carcinogenesis and the presence of these mutations do not necessarily confer resistance. The conversion of the AA/C1 adenoma cell line to a tumorigenic phenotype (Williams et al., 1990, Cancer Research, 50, 4724) is accompanied by a reduced response to the inhibitory effects of TGFβ up to 10 ng/ml. Of further interest continuous in vitro passage alone results in a significant increase in resistance to TGFβ by both the AA/C1 and RG/C2 cell lines. Both the TGFβ sensitive adenoma cell line AA/C1 and its TGFβ resistant transformed derivatives have similar numbers of TGFβ receptors indicating a post-receptor mechanism is involved in the development of TGFβ resistance in this system. Using Western blotting techniques we have found no clear correlation between the expression of the retinoblastoma gene product and sensitivity to TGFβ. Reduced responsiveness to the inhibitory effects of TGFβ may be an important event in the loss of growth control in colorectal carcinogenesis.

G 125 ISOLATION OF MAMMALIAN HOMOLOGS OF YEAST CELL CYCLE INHIBITORY GENES *FAR1* AND *FUS3*, Andrew R. Mendelsohn and Roger Brent, Department of Molecular Biology, Massachusetts General Hospital Boston, MA 02114

We are in the process of identifying human homologs of yeast genes that are critical in negatively regulating the cell cycle. When *S. cerevisiae* haploid cells are exposed to mating factor from cells of the opposite mating type, FAR1 inhibits CLN2, a yeast cyclin that promotes transit from G1 to S, and FUS3 inhibits CLN3, a cyclin that also promotes transit through the G1/S boundary. Thus, FAR1 and FUS3 can be considered negative regulators of the cell cycle. If the mechanics of cell cycle regulation are conserved between yeast and vertebrate cells, then it should be possible to identify mammalian homologs of *FAR1* and *FUS3*.

We are attempting to isolate human homologs of *FAR1* and *FUS3* by complementation of *S. cerevisiae far1* and *fus3* mutant strains. Our scheme takes advantage of the fact that haploid *far1* yeast mate far more poorly with a *far1* strain of opposite mating type than with a wild type *FAR1* strain of opposite mating type. This same pattern also holds true for *fus3* strains. We hope to isolate a *FAR1* human homolog by transforming a *far1 trp1 S. cerevisiae* strain with a human cDNA library in a galactose inducible expression vector. In the presence of galactose the cells are mated to another *far1 trp1* strain of opposite mating type. Only cells in which *far1* is complemented should mate efficiently and form diploids. A second round of screening will be used to confirm potential homologues. We will use a similar strategy for the isolation of *fus3* complementors.

To expedite this work we have obtained a *MATα,ura3,leu2,trp1,met1,far1-1* strain and constructed a *trp1* derivative of a *MATa,ura3,leu2,his4,far1-1* strain, which can be transformed with our cDNA libraries that contain the *TRP1* gene. In addition we have constructed *fus3* strains from a *MATα,ura3,trp1,his3,leu2* strain and a *MATα,ade,his3,lys2,ura3* strain by integration of a *FUS3* gene interrupted in its coding region by a *hisG-URA3-hisG* construct. We are trying to use this strategy to clone the putative *FUS3* and *FAR1* human homologs.

G 126 KINETICS AND MECHANISM OF TGFß MEDIATED GROWTH ARREST IN EPITHELIAL CELLS, Karl Münger[1], Jennifer A. Pietenpol[2], Peter M. Howley[1] and Harold L. Moses[2], [1]Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892; [2]Department of Cell Biology, Vanderbilt University, Nashville TN 37232

Transforming growth factor ß (TGFß) excerts strong growth inhibitory activity on a variety of cells including keratinocytes. TGFß inhibits keratinocyte growth when added at any time during G1 but not in the S phase of the cell cycle. The synthesis of the c-*myc* protein is also downregulated by TGFß treatment even when added after G1/S progression. This suggests that the c-*myc* protein may be important for the initiation of S phase in epithelial cells but is not absolutely required for progression in S phase. In TGFß treated primary human keratinocytes and a series of human papillomavirus and SV40 immortalized human keratinocyte cell lines the phosphoryla- tion status of pRB strictly correlated with cell growth in that there was an accumulation of hypophosphorylated pRB in G1 arrested cells. No evidence was found for a direct effect of TGFß on the pRB phosphorylation status in these cells.

G 127 EVIDENCE FOR THE INVOLVEMENT OF PROTEIN KINASE ACTIVITY IN TGF-ß SIGNAL TRANSDUCTION; Masahiko Ohtsuki and Joan Massagué#, Cell Biology and Genetics Program and #Howard Hughes Medical Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Transforming growth factor-ß1 (TGF-ß1) rapidly increases the expression of *junB* transcription factor and plasminogen activator inhibitor-1 (PAI-1), and prevents the cell cycle-dependent phosphorylation of the RB protein during late G1 phase in Mv1Lu lung epithelial cells. We show that these responses are blocked when the potent serine/threonine protein kinase inhibitor, H7, is added together with TGF-ß1. Added alone, H7 does not alter the basal *junB* or PAI-1 mRNA levels, the deposition of PAI-1 into extracellular matrix or the phosphorylation of RB in late G1 phase. The analogues H8 and H9, which are preferential inhibitors of cyclic nucleotide-dependent protein kinases, are 5-fold less potent than H7 as inhibitors of the TGF-ß response. The PAI-1 response to TGF-ß1 is not affected by simultaneous addition of the protein kinase C inhibitor, staurosporin, or by prolonged preincubation of cells with phorbol 12-myristate 13-acetate which down-regulates protein kinase C. We conclude that H7 and its analogues block various early TGF-ß responses by inhibiting a serine/threonine kinase(s) that mediates TGF-ß action.

G 128 IDENTIFICATION OF DIFFERENT LATENT FORMS OF TGF-ß1, -ß2 AND -ß3 SECRETED BY A HUMAN GLIOBLASTOMA CELL LINE, Anders Olofsson, Pascal Colosetti, Tetsuto Kanzaki, Kohei Miyazono, and Carl-Henrik Heldin; Ludwig Institute for Cancer Research, Box 595, Biomedical Center, S-751 24 Uppsala, Sweden.

Transforming growth factor beta-1 (TGF-ß1) is secreted as latent high molecular weight complexes, with or without an associated component denoted latent TGF-ß1 binding protein (LTBP). We have found that a human glioblastoma cell line (U-1240 MG) secretes all isoforms of TGF-ß found in mammalian cells (TGF-ß1, -ß2 and -ß3). Approximately 26% of the secreted TGF-ß was in active form. Latent TGF-ß:s were partially purified from medium conditioned by the U-1240 MG cell line. Anion exchange chromatography followed by immunoblotting using antibodies against precursor parts of the different TGF-ß isoforms, and against LTBP, revealed that all three isoforms of TGF-ß occured in high and small molecular weight forms. The high Mr forms occured both in complexes asociated with LTBP and in complexes associated with a protein(s) distinct from LTBP. LTBP has several EGF-like repeats and another repeat containing eight cysteine residues; fibrillin, a protein responsible for Marfan syndrome was recently found to have a similar structure. The EGF-like repeats have been implicated in the binding of Ca^{2+}. Structural and functional properties of the second form of latent TGF-ß binding protein is under investigation.

G 129 EGF and TGF- ß PROFILES IN NORMAL AND MALIGNANT HUMAN ORAL KERATINOCYTES, Stephen S. Prime, Andrew J. Silverthorne, Stephen M. Game, Mary J. Donnelly, Andrea M. Stone, Andrew Yeudall and Crispian Scully, Centre for the Study of Oral Disease, Department of Oral Medicine, Surgery and Pathology, University of Bristol, UK.

This study examined the response of normal and malignant human oral keratinocytes to exogenous EGF and TGF- ß and correlated the findings to cell surface receptor expression and the autocrine production of growth factors.

Normal (n=3) keratinocytes were stimulated by EGF (0.1-10 ng/ml); 5/8 malignant cell lines were stimulated at higher EGF concentrations only (>0.5 ng/ml) whilst the remaining 3 lines were refractory to EGF. Malignant cell lines predominantly over-expressed both high ($\bar{x}$ Bmax 61,941, K_D 0.23 nM; n=4) and low ($\bar{x}$ Bmax 524,366, K_D 17.1 nM; n=8) affinity EGF receptors compared to normals ($\bar{x}$ HA:Bmax 17,685, K_D 0.27; LA:Bmax 534,938, K_D 16.4). Malignant keratinocytes produced more TGF- α ($\bar{x}$ 37.4 pg/10^6 cells/48 hr) than normals ($\bar{x}$ 15.2 pg/10^6 cells/48 hr). All of the malignant cell lines were inhibited by exogenous TGF-ß (>0.1 ng/ml) but, by contrast to normals, malignant cells showed a loss of response to TGF- ß at lower concentrations (<0.1 ng/ml). 6/8 malignant cell lines expressed more TGF-ß receptors of lower affinity ($\bar{x}$ Bmax 4014, K_D 22.8pM) than normals ($\bar{x}$ Bmax 1125, K_D 13.3 pM); 2 malignant cell lines had < 100 TGF- ß receptors per cell. TGF-ß autocrine production was less in 5/8 malignant cell lines ($\bar{x}$ 6.5 pg/10^6 cells/48 hr) than normals ($\bar{x}$ 16.0 pg/10^6 cells/48 hr), but in 3 cell lines TGF- ß production was increased (x 22.4 pg/10^6 cells/48 hr).

This study demonstrates that EGF and TGF-ß growth factor control is not always lost in human oral squamous carcinoma cell lines. The response to exogenous ligand correlated directly to the expression of the specific cell surface receptors (EGF $p < 0.05$; TGF-ß trend only) and indirectly with the autocrine production of growth factors (TGF-α $p < 0.05$; TGF- ß $p < 0.05$). There was no evidence, however, for a down-regulation of either EGF or TGF- ß receptors by the autocrine production of ligand.

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G 130 REVERSAL OF RESISTANCE TO TRANSFORMING GROWTH FACTOR-ß1 IN STABLE SOMATIC CELL HYBRIDS OF HUMAN SQUAMOUS CARCINOMA CELLS AND NON-NEOPLASTIC KERATINOCYTES, Michael Reiss and Vincent F. Vellucci, Section of Medical Oncology, Dept. of Medicine and Yale Comprehensive Cancer Center, Yale University School of Medicine, New Haven, CT 06510 and Janet M. Cowan, Dept. of Pediatrics, East Virginia Medical School, Norfolk, VA 23507.

Based on our previous observation (Cancer Commun. 2:363, 1990) that human squamous carcinoma cell lines (SqCCs) are frequently refractory to the anti-proliferative action of Transforming Growth Factor-ß1 (TGFß1) *in vitro*, and that this phenomenon cannot be attributed to an altered expression of cell-surface receptors for TGFß1, we wished to determine whether resistance to TGFß1 is a dominant or a recessive property of SqCCs. The TGFß1-resistant aneuploid human SqCC line, FaDu, was used to generate a Hygromycin B-resistant subclone (FaDu-Hyg®), by infection with pSV2-HYG, packaged in a defective amphotropic retroviral vector. In addition, an HPV16-immortalized, TGFß1-sensitive, diploid human foreskin keratinocyte cell line, R12 HKc/HPV16, was used to generate a G418-resistant subline (HKc-neo®) by infection with pC6M-neo, contained in a defective amphotropic retroviral vector. These 2 parental cell lines were fused in the presence of polyethylene glycol, and the resulting hybrids selected, ring-cloned, and expanded in culture in the presence of both Hygromycin B and G418. Two individual hybrid cell lines (FaDuXHKc.1 and FaDuXHKc.2) have now been passaged in culture continuously for 10 months. Cytogenetic analysis of these lines revealed chromosome numbers consistent with hybrid formation (FaDuXHKc.1: 120 and FaDuXHKc.2: 110 chromosomes). Each of the 2 hybrids have retained different sets of marker chromosomes from the parental FaDu-Hyg® cells, as well as many normal chromosomes, presumably contributed by HKc-neo® cells. These findings indicate that these cell lines are true hybrids. TGFß1 maximally inhibited the incorporation of [^{3}H]-thymidine into DNA of HKc-neo®, FaDuXHKc.1 and FaDuXHKc.2 cells by 70 to 85%, with an IC_{50} between 2 and 5 pM. In contrast, DNA synthesis of parental FaDu-Hyg® cells was not inhibited by TGFß1 at concentrations up to 100 pM. Thus, TGFß1-resistance of FaDu cells appears to be recessive and presumably due to the loss of post-receptor elements of the signaling pathway. Cytogenetic differences between the 2 hybrid cell lines may aid us in localizing the recessive gene(s) that play a role in this pathway.

G 131 TGF-β PRODUCTION AND NEGATIVE GROWTH REGULATORY EFFECTS OF TGF-β ON HUMAN MELANOMA CELLS. Ulrich Rodeck, Csaba Kari, Anna Bossler, and Ullrich Graeven. The Wistar Institute, Philadelphia, PA 19104.

We have shown previously that cultured human normal melanocytes and metastatic melanoma cells express TGF-β1 mRNA. Bioactive platelet-derived TGF-β, however, reversibly inhibited melanocyte and melanoma cell growth associated with an incomplete cell cycle block at the G1/S boundary. As determined in Mv1Lu assays, conditioned media of melanoma cells in culture (8/8) but not normal melanocytes (4/4) contained biologically inactive, latent TGF-β which could be converted into the bioactive form by acid-, and, to a lesser degree, heat-treatment. Neutralizing antibodies to TGF-β abolished the growth-inhibitory activity of acid-activated, melanoma-derived TGF-β in the Mv1Lu assay. However, no effect of TGF-β neutralizing antibodies on melanoma cell proliferation was detected consistent with the notion that only latent TGF-β is secreted by these cells.

Although all melanocyte and melanoma cultures tested so far were growth-inhibited by exogenous bioactive TGF-β (ED_{50} 0.5 - 2 ng/ml) immortalized human melanocytes transfected with the SV40T antigen were not responsive to treatment with TGF-β even at high concentrations (10 ng/ml). Together with our finding that TGF-β inhibited entry of melanoma cells into S-phase this result suggests that SV40T antigen interactive nuclear proteins such as the retinoblastoma protein (pRB) or p53 are potential targets for TGF-β mediated growth inhibition of melanocytic cells. We are currently testing this possibility using antisense strategies that suppress pRB and p53 protein synthesis.

G 132 THE ROLE OF TRANSFORMING GROWTH FACTOR-β (TGFβ) IN THE GROWTH REGULATION OF PRIMARY RAT TRACHEAL EPITHELIAL (RTE) CELLS DURING DIFFERENT STAGES OF GROWTH. Joyce E. Rundhaug, Ronald W. Steigerwalt, Thomas Gray, and Paul Nettesheim. Laboratory of Pulmonary Pathobiology, Natl. Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.

The purpose of our studies was to determine whether RTE cell cultures produce TGFβ, what isoforms of TGFβ are being produced and whether the autocrine growth factors play an important role in restraining growth. Northern analysis of RNA obtained from the cultures indicated that TGFβ1 transcripts were expressed at early as well as late stages of growth, but TGFβ2 transcripts were not detected. Media obtained from cultures in late exponential growth and through the plateau phase of growth contained TGFβ, as measured by radioreceptor assay. Using TGFβ1 and TGFβ2 specific antibodies, we demonstrated that active TGFβ1 was already being produced during the early exponential growth phase of the cultures, while TGFβ2 was produced only after plateau was reached. This was shown by adding the respective antibodies to the media during different stages of growth and measuring DNA synthesis. In spite of these results, it is not certain that the autocrine TGFβ's are the crucial factors triggering the onset of plateau, which occurs at cell densities of 5-10 X 10^4 cells/cm^2, since we found that preplateau and plateau phase cultures have a markedly reduced responsiveness to the growth inhibitory effects of TGFβ compared to cultures in early exponential growth phase.

G 133 ANTI-IMMUNOGLOBULIN TREATMENT OF MURINE B-CELL LYMPHOMAS CAN CAUSE UNDERPHOSPHORYLATION OF pRB INDEPENDENT OF TGF-β. David W. Scott, Garvin L. Warner, Arti Gaur, Deirdre O. Nelson and John Ludlow*. Divisions of Immunology and Immunotherapy and of Tumor Biology*, University of Rochester Cancer and Dept. of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Treatment of the WEHI-231 and CH31 B-cell lymphomas with anti-immunoglobulin (Ig) causes growth arrest at G_1/S, leading to apoptosis and cell death. We previously found that these lymphomas produce active transforming growth factor β (TGF-β) when treated with anti-Ig, and that their hierarchy of sensitivity to TGF-β correlates with their growth inhibition by anti-Ig. Since TGF-β interferes with the phosphorylation of the retinoblastoma gene product, pRB, we determined the state of phosphorylation of pRB in WEHI-231 B-cell lymphomas treated with anti-Ig for 24 hours. Interestingly, pRB protein was found to be predominantly in the underphosphorylated form in these cells, as previously reported for cells arrested by the exogenous addition of TGF-β. Surprisingly, neutralizing antibodies to TGF-β were unable to prevent growth inhibition by anti-Ig in WEHI-231 and CH31. Moreover, in WEHI-231 lymphoma cells selected for growth in TGF-β, the majority of the TGF-β-resistant clones remained sensitive to anti-Ig-mediated growth inhibition; pRB in these clones was in the underphosphorylated form after 24 hours treatment with anti-Ig, but not with TGF-β. Our results suggest that anti-Ig treatment of murine B-cell lymphomas stimulates the production of *active* TGF-β, but that a TGF-β-independent pathway may be responsible for the pRB underphosphorylation and cell cycle blockade. [Supported by grants from the ACS (IM-495) and NIH (CA11198).]

G 134 TRANSFORMING GROWTH FACTOR BETA (TGFß) SUPPRESSES THE *RAS*-TRANSFORMED PHENOTYPE, R.Serra, M.F.Verderame, H.C.Isom, Department of Microbiology and Immunology, Hershey Medical Center, Hershey, PA 17033

We have developed a series of simian virus 40 (SV40)-immortalized (Mol. Cell Biol. 7: 3740-3748,1987) and *ras*-transformed, SV40-immortalized hepatocyte cell lines. One of the SV40-immortalized cell lines, CWSV1, has been well characterized. CWSV1 cells are not transformed; the cells will not grow in soft agarose and will not form tumors when injected into newborn syngeneic rats. Tumorigenic cell lines, NR3 and NR4, were derived from the CWSV1 cell line by transfection with the activated c-Ha-*ras* and neomycin resistance genes followed by selection in G418 containing media. Control cells, N1, containing only the neomycin resistance gene and selected in G418 containing media were also derived. In this study, we examined the effects of TGFß on primary hepatocytes, CWSV1, N1, NR3 and NR4 cells. TGFß inhibited DNA synthesis in EGF stimulated primary hepatocyte cultures but did not inhibit DNA synthesis or anchorage dependent growth of CWSV1,N1 and NR4 cells. Even though TGFß did not inhibit DNA synthesis in the cell lines the NR3 and NR4 cells did respond to TGFß. TGFß caused NR3 and NR4 cells to revert to a more CWSV1-like morphology. The change in morphology was accompanied by a dramatic increase in the organization of the actin cytoskeleton. The ability of NR3 and NR4 cells to demonstrate anchorage-independent growth in soft agarose was inhibited by TGFß in a dose dependent manner. TGFß also altered expression of specific genes in NR4 cells. The expression of mRNA for the ß1 subunit of integrin increased two to three fold, the α1 integrin transcript increased by three to ten fold while the α5 integrin transcript increased less than two fold. Fibronectin mRNA levels were increased 2.5 to 3.5 fold in response to TGFß. The levels and profile of integrins on the cell surface as determined by cell surface protein iodination of NR4 cells treated with TGFß were altered so that the integrin profile on treated NR4 cells resembled that for CWSV1 cells. No changes were detected in the cell surface integrin profile of CWSV1 cells treated with TGFß. c-Ha-*ras* and c-*myc* expression were not altered in NR4 cells treatmented with TGFß. We conclude that TGFß can suppress the *ras*-transformed phenotype. These experiments suggest that TGFß acts through multiple pathways: a pathway blocked by SV40 T antigen and a pathway which is not blocked by SV40 T antigen. We are in the process of testing the possibility that TGFß may act to suppress transformation by altering integrin expression.

G 135 NEGATIVE REGULATION OF PANCREATIC BETA-CELL GROWTH, Åke Sjöholm, Department of Endocrinology, Karolinska Institute, Karolinska Hospital, Box 60500, S-104 01 Stockholm, Sweden.

A common denominator of all forms of diabetes mellitus is insufficient extent of pancreatic beta-cell replication needed to expand the beta-cell mass in order to compensate for the decreased insulin output by the pancreas resulting in hyperglycemia. While a number of stimulators of beta-cell replication have been identified, considerably less attention has been paid to inhibitors of this process despite the possibility that they may be of regulatory importance. In the present study I have utilized a system of fetal rat pancreatic islets enriched in rapidly proliferating beta-cells in order to study the putative antiproliferative actions of a variety of different factors. It was found that among peptides known to be produced by islet cells, pancreastatin and diazepam binding inhibitor exerted inhibitory effects on beta-cell growth in a dose- and glucose-dependent manner, while somatostatin, glucagon and galanin were without effect. Interestingly, pancreastatin, but not diazepam binding inhibitor, also lowered the islet contents of polyamines and insulin and suppressed their secretion of insulin. TGF-beta was found in the same system to block the mitogenicity of glucose, but not that of GH, while at the same time stimulate insulin secretion. The effects of TGF-beta were obviously not effected through changes in polyamine synthesis, because the islet content of these amines were not altered by the peptide. A lack of autocrine/paracrine control of islet cell function by TGF-beta is suggested by the finding that neutralizing TGF-beta antibodies did not modify beta-cell DNA synthesis or secretion rates. The islets form a highly innervated organ, implicating a possible regulatory role of neurotransmittors released from nerve terminals. While it was found that carbachol did not influence DNA synthesis, the alpha-adrenergic agonists phenylephrine and clonidine and the beta-adrenoceptor antagonist propranolol potently inhibited proliferation and lowered the islet cAMP content. When islets were exposed to the stimulatory cAMP analog Sp-cAMP[S] or pretreated with pertussis toxin, which enhanced DNA synthesis, the repressive actions of the adrenergic factors were blocked, indicating that they suppress beta-cell replication at least in part through interference with an inhibitory GTP-binding protein connected to adenylyl cyclase. Preliminary findings indicate that a similar pathway is affected by prostaglandins of the E-series.

G 136 LOSS OF MITOGENIC RESPONSIVENESS DURING ADIPOCYTE DIFFERENTIATION IN A31T6 CELLS, Miriam J. Smyth and Walker Wharton, Cellular and Molecular Biology Group, LS1, Los Alamos National Laboratory, Los Alamos, NM 87545.

The differentiation of A31T6 (BALB/c 3T3) murine fibroblasts is being studied using a multiparameter flow cytometer. When medium containing insulin, dexamethasone, and indomethacin is added, the cells convert into adipocytes and accumulate copious amounts of lipid. By day 12, for example, a control sample contains 18% differentiated cells whereas a sample treated with insulin, dexamethasone, and indomethacin contains 73% differentiated cells. We present data showing both the progress and extent of differentiation by use of fluorescent intensity spectral analysis. We find that exposure to indomethacin alone is sufficient to achieve maximal lipid accumulation in individual cells. In control samples, 13% of the cells have high extent of differentiation and the value is 50% in response to treatment with indomethacin. Addition of indomethacin in combination with any of the other agents promotes the rate of differentiation. By day 6, 58% of the cells are differentiated in response to exposure to indomethacin and dexamethasone whereas either indomethacin alone or the combination of insulin and dexamethasone reaches only 20%. Our investigations of the role of protein kinases show that the phorbol ester, TPA, inhibits acquisition of the highly differentiated phenotype but does not inhibit early differentiation. By day 15, 32% of the cells in samples treated with insulin and dexamethasone are highly differentiated whereas the value is 19% in samples treated with TPA at confluence. Cholera toxin, in contrast, inhibits differentiation. The rate at which these cells lose the ability to respond to mitogens is proportional to the rate at which they acquire the differentiated phenotype. TPA is not preventing differentiation by stimulating cell cycle traverse. Differentiation-promoting agents, instead, cause loss of mitogenic responsiveness to TPA.

G 137 Expression of the ryanodine receptor, an endoplasmatic Ca^{2+} release channel, is controlled by TGFß. Is the intracellular Ca^{2+} concentration involved in TGFß signaling ?

Giuseppe Giannini, Roberta Ceci and Vincenzo Sorrentino, European Molecular Biology Laboratory, Heidelberg, F.R.G.

As a step toward a more complete understanding of the molecular basis of TGFß actions we have screened a cDNA library from TGFß-treated CCL64 cells and identified clones corresponding to mRNAs whose levels are either induced or repressed by TGFß.

One of these clones corresponds to the sarcoplasmatic Ca^{2+} channel gene, ryanodine receptor. Expression of the ryanodine receptor mRNA is specifically stimulated by TGFß while does not respond to EGF, TPA or fetal calf serum, and does not reflect the growth state of the cells. Induction by TGFß of the ryanodine receptor expression does not require on-going protein synthesis, but may depend on phosphorylative events not involving Protein Kinase C.

Transduction of many extracellular stimuli, including hormones and neurotransmitters is mediated by an increase in the level of cytoplasmic Ca^{2+} which is released, through endoplasmatic Ca^{2+} release channels (like the ryanodine receptor or the Ins(1,4,5)-P3-binding protein), from specialized regions of the endoplasmatic reticulum.

The finding that ryanodine receptor gene expression is stimulated by TGFß suggests a possible link between Ca^{2+} regulation and TGFß signaling. Work is in progress to verify whether modifications in Ca^{2+} homeostasis may be involved in long term TGFß signal transduction.

G 138 EGF/TGFα AND TGFβ CONTROL OF NORMAL AND IMMORTALIZED HUMAN MAMMARY EPITHELIAL CELL GROWTH IN CULTURE, Martha Stampfer, Junko Hosoda, C.-H. Pan and Paul Yaswen, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

In order to examine and compare cell cycle events in finite lifespan vs. immortally transformed human mammary epithelial cells (HMEC), we have been exploring potential methods for obtaining reversible growth arrest. Our HMEC culture system permits active long term growth (45-80 pd) of finite lifespan cells derived from individual reduction mammoplasty tissues. Previous studies have indicated that normal HMEC have a stringent requirement for EGF for clonal growth, but grow in mass culture in the absence of exogenously added EGF due to the endogenous production of TGFα. A rapid, efficient, and reversible growth arrest can be achieved by addition of a blocking antibody to the EGF receptor (MAb 225). Overall protein synthesis remains depressed in the presence of the antibody, and DNA synthesis is sharply decreased by 24hr. Removal of MAb 225 leads to a rapid increase in protein synthesis; DNA synthesis increases only after 10hr and peaks around 18hr. A 1hr exposure to EGF is sufficient to allow the majority of cells which are capable of cycling to subsequently enter S phase. High levels of synthesis of mRNA for the early response genes c-myc, c-fos, c-jun, and MGSA are observed within 1hr of antibody removal. It thus appears that blockage of EGF receptor signal transduction is sufficient by itself to cause normal HMEC to enter a Go-like resting state. A Go-like growth arrest similar to that observed with MAb 225 in normal HMEC was obtained in the immortal cell line 184B5, derived from normal HMEC specimen 184 following exposure to benzo(a)pyrene, by removal of EGF from the culture medium. TGFβ is growth inhibitory to normal HMEC. In this case, growth arrest occurs during the late G1 phase of the cell cycle and is correlated with increased protein synthesis, particularly components of the extracellular matrix. Reversal of TGFβ growth inhibition does not produce the level of synchronous re-entry into the cell cycle observed with reversal of MAb 225 inhibition. The phenotype observed with MAb 225 is dominant in most aspects to that seen with TGFβ, except in the level of synthesis of secreted proteins. Addition of MAb 225 to cells growth arrested at late G1 with TGFβ can at least partially alleviate the G1/S block, suggesting that EGF/TGFα is required for this action of TGFβ. 184B5 cells maintain active growth in the presence of TGFβ; however, they show similar induction of secreted protein synthesis. Further studies will examine possible differences in expression, activity, and phosphorylation patterns of cell cycle related proteins in normal HMEC vs. immortally transformed HMEC cell lines.

G 139 EXPRESSION CLONING OF THE TYPE III TGF-β RECEPTOR. Xiao-Fan Wang, Herbert Y. Lin, Elinor Eaton, Harvey F. Lodish, and Robert A. Weinberg. Whitehead Institute for Biomedical Research, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have succeeded in isolating the cDNA encoding the type III TGF-β receptor by expression cloning in COS cells. A cDNA mammalian expression library was constructed from A-10 rat embryonic aortic smooth muscle cells and plasmid miniprep pools representing ~10,000 independent cDNA clones were transfected into COS cells grown on glass slide flaskettes. 48-60 hours after transfection, cells were incubated with iodinated TGF-β1, then extensively washed, and autoradiographic analysis was performed. After screening 860,000 recombinants, one positive pool of 7500 recombinants was identified and a single clone with an insert of 2.8 kb was isolated through serial subpooling.

Northern analysis using this cDNA as a probe shows that the corresponding message is a 6 kb message which is widely expressed in different tissues and cell lines. A full length cDNA was subsequently isolated by high stringency hybridization. Sequence analysis indicates that the cDNA has an open reading frame encoding a 853 aa residue protein with a single putative transmembrane domain, several N-glycosylation sites and a consensus proteoglycan site. Crosslinking experiments using iodinated TGF-β1 in transiently transfected and stably transfected cells indicates that the expressed protein has all the characteristics of the type III receptor: binding to TGF-β1, proteoglycan modification, correct expressed size and mRNA expression pattern. Not suprisingly, the sequences of two peptides, previously obtained by microsequencing of purified type III TGF-β receptor, can be identified in the coding region of the cDNA. Surprisingly, the ability of the type II receptor to bind TGF-β1 is increased by the expression of the type III receptor in L6 myoblasts.

G 140 CLONING AND EXPRESSION OF NOVEL MEMBERS OF THE SERINE/THREONINE KINASE RECEPTOR FAMILY UTILIZING PCR-BASED STRATEGIES. Jeffrey L. Wrana, L. Attisano and J. Massagué, Cell Biology and Genetics Program, Memorial Sloan-Kettering Cancer Centre, New York City, New York, 10021.

The recent cloning of the activin receptor and the *C. elegans* gene *daf-1* has identified novel transmembrane signalling mechanisms involving serine/threonine kinase receptors. In order to identify new members of this family of receptors, a PCR-based cloning strategy was developed. Comparison of the sequence of the kinase domains of the activin receptor and *daf-1* has revealed several regions of identity which were used for the design of PCR amplification primers. Degenerate sense and antisense oligonucleotides that corresponded to the peptide sequence within two of these areas of high conservation were synthesized and used as primers in a polymerase chain reaction utilizing cDNA from Balb/c 3T3 fibroblasts as the template. Amplified products from the PCR were subsequently subcloned into pBluescript and random clones sequenced. Sequence analysis of several of these clones revealed that they encoded novel cDNAs with many of the conserved features of serine/threonine kinase domains. Two of these clones, designated D22 and D51 also displayed, respectively, 75% and 30% identity to the activin receptor. To further characterize D22 and D51, a Balb/c 3T3 cDNA library was screened with the PCR-derived probes in order to obtain sequence encompassing their entire open reading frames. Transient expression of D22 in COS cells is currently being utilized to identify the ligand for this novel member of the serine/threonine kinase receptor family.

Gene Loss in Tumor Progression; p53

G 200 SPECIFIC CHROMOSOMAL ABNORMALITIS IN Eμ-myc TRANSGENIC MICE, Kiwamu Akagi and Ken-ichi Yamamura, Institute for medical genetics, Kumamoto University Medical School, Kumamoto 862, Japan

c-myc is a nuclear proto-oncogene that, when activated, induced malignancies in a variety of tissue. But not only the activation of c-myc but also the other events are necessary for the development of tumor. We produced transgenic mice bearing the human c-myc gene activated by an immunoglobulin heavy chain enhancer(Eμ) to study the multistep process of tumorigenesis and the influence of genetic background. C57BL/6 transgenic mice bearing Eμ-myc gene mostly developed B cell lymphomas, but C3H/HeJ transgenic mice bearing Eμ-myc gene developed T cell lymphomas. We carried out cytogenetic studies to see whether specific chromosomal abnormality was observed in lymphoma cells derived from B6 and C3H transgenic mice. Interestingly, most of lymphoma cells from C3H transgenic mice had an abnormality of band C-G in chromosome 6 where T cell receptor ß chain, K-ras, CD4, CD8 are mapped. In addition, Ishizaka et al reported the existence of tumor suppressor gene in this region from the studies on 3-Methylcholanthrene induced T cell lymphoma. It is possible that the same gene is disrupted by chromosomal aberration leading to T cell lymphoma. No specific chromosomal aberration was found in B cell tumors of B6 transgenic mice.

G 201 THE DCC GENE IN NERVE GROWTH FACTOR-INDUCED NEURONAL DIFFERENTIATION, Jeff Boyd and John I. Risinger, Gene Expression Section, Laboratory of Molecular Carcinogenesis, NIH/National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

The DCC gene was recently identified as a candidate tumor suppressor gene by virtue of its localization to a region of chromosome 18q that is subject to allelic deletion in human colorectal carcinomas; in addition, mutations and reduced expression of the gene occur in colon tumors and cell lines (Fearon et al. Science 247:49-56, 1990). We have obtained evidence that DCC mutations also occur in human endometrial carcinomas (Boyd et al., unpublished). Since DCC mRNA levels are extremely low in most epithelial tissues, with highest levels in brain, we examined the hypothesis that cells of neuronal origin would facilitate studies on the regulation of DCC expression. Furthermore, since DCC encodes a novel protein with greatest homology to neural cell adhesion molecule, we addressed the possibility that DCC expression is associated with cell and/or surface adhesion, and possibly the morphologic differentiation of neuronal cells. While examining cells of neuronal origin for DCC expression, we observed that the IMR-32 human neuroblastoma cell line exhibited unusually high levels of DCC mRNA, rendering this cell line a potentially powerful tool in the characterization of DCC expression and function. Among a number of growth factors tested with these cells, nerve growth factor (NGF) was found to reproducibly up-regulate DCC expression at the mRNA level (serum was found to inhibit DCC mRNA levels). When cultured on an extracellular matrix surface, this stimulation of DCC mRNA levels by NGF was associated with the induction of neurite outgrowth. Current studies are designed to determine the molecular basis of DCC regulation by NGF, as well as to demonstate a direct role for the DCC gene product in cell-surface adhesion and neuronal differentiation.

G 202 ALTERATION OF THE *WT1* TUMOR SUPPRESSOR GENE IN WILMS' TUMOR. Keith Brown, Jo Watson, Veronique Poirier, Jem Berry, Martin Mott & Norman Maitland. Dept. Pathology, Medical School, University Walk, Bristol BS8 1TD, U.K.

A candidate gene (*WT1*) has recently been isolated for the Wilms' tumor suppressor gene at 11p13. This gene codes for a zinc finger protein, which can bind to a specific transcriptional control sequence. Twenty Wilms' tumors have been examined for alterations in this gene. No homozygous deletions were detected by Southern blotting, using a cDNA probe to the 3' region, and no abberent RNAs were detected by Northern blotting, although expression levels showed great variation. The entire coding region was amplified by RNA-PCR, in 4 fragments ranging in size from approximately 200bp to 600bp. In a single tumor a deletion of 226bp was observed in the 3' region. The deletion causes a frameshift which leads to a premature termination of translation, completely deleting the zinc finger region. A small deletion was detected in the genomic DNA from the tumor, using the normal cDNA from the deleted region as a probe. This deletion was in a tumor from a WAGR patient, who had a constitutional, cytogenetically visible deletion of 11p13. The small intragenic deletion therefore represents the second hit at the *WT1* gene, and provides strong evidence that loss of function of *WT1* is an important factor in the development of at least some Wilms' tumors. The other tumors are now being examined for the presence of small deletions and point mutations, by separating the RNA-PCR products on SSCP gels.

This work was supported by the Cancer & Leukaemia in Childhood Trust.

G 203 p53 MUTATIONS IN BREAST AND LUNG TUMORS, Graham Casey, Martha E. Lopez, Jane M. Arboleda and Dennis J. Slamon. Department of Pediatrics, University of California, Irvine, Irvine CA 92717 (GC, MLL); Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024 (JLA, DJS)

Recent studies suggest that many common malignancies involve mutations in the same genes. The most frequent genetic alteration identified in human cancers are mutations of the p53 gene. Mutations are clustered in regions of the gene which are most highly conserved between species, and some are known to result in functionally different mutant proteins. This functional difference suggests that there may be a correlation between tumor progression and specific mutations. Further, there is a suggestion that the type and distribution of p53 mutations may be influenced by the cancer type or cellular origin. Clearly environmental factors strongly influence the development of certain tumors, for example lung tumors. Are certain types of p53 mutations more frequent in these malignancies?

To answer some of these questions we have undertaken a comparative analysis of p53 mutations in two malignancies, breast and lung, in which alterations of the p53 gene are strongly implicated. We have analysed over 200 tumor samples by reverse-transcriptase PCR amplification followed by direct sequencing and have identified mutations of the p53 gene in approximately 45% of lung tumors, and 30% of breast tumors. Mutations occur primarily in the conserved regions of the gene, and there are tumor-specific hot spots. Interestingly the type of mutations differs between the two tumor types. In lung tumors the most frequent (greater than 30%) mutation involves a G to T transversion, which is consistent with mutations induced by benzo(a)pyrene exposure, a component of tobacco smoke. Therefore there is a strong correlation in lung cancer between environmental exposure and mutations of the p53 gene. The clinical significance of p53 mutations will be discussed.

G 204 IDENTIFICATION OF ALLELIC LOSS IN LIVER TUMORS FROM THE B6C3F1 MOUSE William J. Caspary[1], Lisa Davis[2], John Hozier[2], Sameer Sakallah[2], J. Carl Barrett[3], Roger Wiseman[3] and Robert Maronpot[1]. [1]Experimental Toxicology Branch, NIEHS/NIH, RTP, NC, [2]Applied Genetics Laboratory, Melbourne, FA and [3]Laboratory of Molecular Carcinogenesis, NIEHS/NIH, RTP, NC.

Loss of alleles at heterozygous loci in tumors is considered an indication of involvement of a tumor suppressor gene. Spontaneous and chemically induced tumors in the B6C3F1 mouse offer a unique opportunity to identify such allelic losses because the parental strains, C3H/He and C57BL/6, are laboratory inbred strains and are globally homozygous. Genetic differences that exist between the two strains will appear as heterozygous loci in the F1. The identification of consistent loss of heterozygosity over a number of independent tumors but identical in their etiology would suggest the location of a putative tumor suppressor gene on that chromosome. We have identified three different types of allelic differences between the two parental strains: a) restriction fragment length polymorphisms at known mapped genes or anonymous DNA sites, b) the presence or absence of unique restriction fragments created by the evolutionarily ancient and stable integration of nonecotropic murine leukemia viruses into the mouse genome and c) amplification by the polymerase chain reaction of microsatellite repeat sequences which vary in length between the two parental strains. Of the first 60 microsatellite sequences we have examined, 31 are identical between the two parental strains and 11 reproducibly identify heterozygous differences between the parental strains. These eleven microsatellites are located on chromosomes 1, 3, 4, 6, 7, 11, 12, 14 and 15. Among the three types of polymorphisms, we have identified a total of 116 allelic differences between the parental strains with a chromosome distribution ranging from as few as one marker (chromosome 10 and 18) to as many as fifteen on chromosome 11. We are examining a series of spontaneous and tetrachloroethylene, methylene chloride and chlordane induced liver tumors for losses at these loci. Our preliminary examination of microsatellite loci reveal no losses on chromosomes 1, 4, and 11 but suggest a loss of heterozygosity at the *LY-3* locus on chromosome 6 in a small number of tumors.

G 205 MUTATION OF p53 BY HOMOLOGOUS RECOMBINATION. Clarke AR, Purdie CA, Peter A, Dobbie L, Hooper ML, Wyllie AH. To investigate further the possible role of P53 as a tumour suppressor gene, we have sought to use homologous recombination in mouse embryonic stem cells (ES cells) to both inactivate the gene by creating exonic deletions and also to generate point mutations within exons. Inactivation of the gene has been attempted with both insertion and replacement type vectors. Data will be presented on recombination frequencies and attempts to obtain germ line colonization.

G 206 USE OF TRANSGENIC MICE TO STUDY TUMOR SUPPRESSOR GENE ALTERATIONS, Charles Cochran[1], Peter Söderkvist[1], Mary Moore[2], Arnold Levine[2], and Roger Wiseman[1], Laboratory of Molecular Carcinogenesis[1], National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and Princeton University[2], Princeton, NJ 08544

Recent studies by our group indicate that losses of heterozygosity occur frequently on mouse chromosome 4 and 11 in chemically induced lung and mammary carcinomas, respectively. Likewise, mutations in the p53 tumor suppressor gene have been identified in a number of tumors with allele loss on chromosome 11. We have extended these findings to tumors of transgenic mice to test the hypothesis that tumor suppressor gene inactivation represents a secondary genetic alteration during malignant progression. Mammary carcinomas from three transgenic lines (MMTV-ras, MMTV-neu, and MMTV-myc) have been screened by immunoblotting with p53 antibodies. Although the p53 protein was undetectable in normal mammary tissue or tumors from MMTV-ras and MMTV-neu mice, each MMTV-myc tumor exhibited p53 overexpression. Since examination of p53 cDNA/PCR products from the MMTV-myc tumors by SSCP analysis and direct sequencing has failed to reveal point mutations, we are currently investigating the possibility that p53 overexpression results from myc binding at a helix-loop-helix motif in the p53 promoter. We have generated nine transgenic lines containing mutated alleles of the mouse p53 gene under its own promoter that develop lung tumors and lymphomas after one year. Tumors are being generated in heterozygous mice from p53 X MMTV-myc crosses as well as a variety of crosses between transgenic lines and Mus spretus, Mus Castaneous, or C3H/HeJ. Analysis of these tumors for allele loss near known tumor suppressor genes using RFLPs and microsatellite length polymorphisms will be presented.

G 207 DNA POLYMORPHIC DIFFERENCES BETWEEN NORMAL AND TUMOR SAMPLES CAN BE DETECTED USING POLYMERASE CHAIN REACTION (PCR) WITH ARBITRARY PRIMERS, Peter Economou[1], Gregory Kelly, Jonathan Samet[1] and John F. Lechner, Inhalation Toxicology Research Institute, Albuquerque, NM 87185. [1]Department of Pulmonary and Critical Care Medicine, University of New Mexico at Albuquerque, NM 87131.

Williams, et al., NAR 18:6531('90) and Welsh, et al., NAR 19:303('91) have reported that simple and reproducible fingerprints of complex genomes can be obtained using single arbitrary primer PCR (AP-PCR). We have found that the AP-PCR technique can also be used to detect polymorphic differences between normal and tumor DNA samples from human donors with lung cancer. Specifically amplified DNA sequences are obtained when the PCR protocol consists of first, two low stringency cycles of 94°C for 5 minutes, 48°C for 5 minutes and 72°C for 5 minutes and second, 30 standard high stringency cycles. The arbitrary primers used to date have been the 5' lambda GT10 and 3' lambda GT10 cloning sequences of 21 and 24 nucleotides in length, respectively and AP2, a 10 nucleotide sequence of CGGCCCCGGC. These primers yield distinctive fingerprints consisting of more than 50 bands. Polymorphic differences between normal and tumor DNAs have been found with all primers. In addition, donor specific polymorphisms have also been detected. The results of these preliminary observations suggest that AP-PCR may be a useful technique to reveal polymorphisms between human normal and tumor tissues that may be seminal in transformation. AP-PCR may also be useful in revealing animal tumor/normal polymorphisms where restriction fragment length polymorphism analysis is impractical.

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G 208 CHANGES IN THE ABUNDANCE AND CELLULAR LOCALIZATION OF THE MURINE C-REL PROTEIN IN P75 DURING B-CELL DIFFERENTIATION, Steve Gerondakis and Raelene J Grumont, The Walter and Eliza Hall Institute of Medical Research, PO, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia.

v-rel, the oncogene carried by the avian retrovirus ReV-T causes an acute B cell leukemia in infected birds. The cellular homologue, c-rel a member of the NF-κB family of transcription factors is expressed in a wide range of cell types. However, only in the B cell lineage is c-rel constitutively expressed, with the level of gene expression in B cell lines presenting different stages of development varying in a differentiation stage specific manner.

We have analyzed murine c-rel expression and the sub-cellular localization of the c-rel protein p75 in cultures of normal splenic B cells undergoing proliferation and differentiation when stimulated with mitogens and cytokines. While c-rel is constitutively expressed in normal B cells, it exhibits a biphasic 5-10 fold induction of expression due to increased transcription that peaks 2 and 72 hours after initiating these cultures. Western blot analysis of the nuclear and cytoplasmic fractions reveal quantitative changes in p75 levels in these cellular compartments during the time course of B-cell stimulation. These findings suggest that c-rel is implicated in the genetic regulation of a number of cellular events that occur during B cell proliferation and differentiation.

G 209 **CHANGES IN EXPRESSION OF TGF-α and TGF-β IN MULTISTAGE CARCINOGENESIS OF THE SKIN,** ADAM B. GLICK, HENRY HENNINGS, KATHLEEN C. FLANDERS*, MICHAEL B. SPORN*, and STUART H. YUSPA, Laboratory of Cellular Carcinogenesis and Tumor Promotion, and *Laboratory of Chemoprevention, National Cancer Institute, Bethesda MD 20892

Normal mouse epidermis and cultured keratinocytes express low levels of TGF-α. TGF-α increases in initiated cells due to $\underline{ras}^{Ha}$ gene activation, and elevated expression is maintained in benign and malignant tumors. In vitro sensitivity to TGF-β1 growth inhibition is similar among normal, initiated, papilloma and carcinoma cells. However neoplastic progression is associated with changes in expression of the TGF-β's. TGF-β2 is expressed by differentiating cells of normal keratinocytes both in culture and in vivo. In contrast, keratinocytes infected with a $v\text{-}ras^{Ha}$ retrovirus express predominantly TGF-β1. $v\text{-}ras^{Ha}$ and chemically-induced papillomas express high levels of TGF-β1 in the basal and spinous layers and TGF-β2 and TGF-β3 in the differentiating cell layers. Treatment of chemically-induced papillomas with TPA results in an increase in basal and spinous expression of TGF-β1 and a loss of expression of TGF-β2 and TGF-β3. In benign tumors with a high risk for malignant conversion, loss of expression of all TGF-β isoforms is common. Furthermore in chemically and oncogene-induced carcinomas, expression of TGF-β protein is not detectable. Matched malignant and benign tumor cell lines express similar amounts of TGF-β1 mRNA suggesting that post-transcriptional mechanisms regulate the loss of TGF-β1 protein. Basal and spinous co-expression of TGF-α and TGF-β1, as well as loss of TGF-β2 and TGF-β3 following TPA treatment, may be important for generation of the benign tumor phenotype. Loss of TGF-β expression in benign tumors may be relevant to outgrowth of malignant cells.

G 210 THE IDENTIFICATION OF MUTATIONS IN THE GENE FOR ADENOMATOUS POLYPOSIS COLI IN PATIENTS WITH FAMILIAL POLYPOSIS, Joanna Groden, Lisa Spirio, Larry Gelbert, Andy Thliveris, Geoff Joslyn, Wade Samowitz, Mary Carlson, Mark Leppert and Ray White, Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84103

Familial adenomatous polyposis (APC), an inherited syndrome of colon cancer, is characterized by hundreds to thousands of adenomatous colonic polyps. The gene for this disorder recently has been isolated from a region of DNA deleted in two APC patients and was more precisely identified by the presence of inactivating mutations in the coding region of four other affected individuals (Joslyn et al, 1991; Groden et al, 1991). Further study of the APC gene, by genomic PCR and single strand conformation polymorphism (SSCP) analysis of DNAs from the original set of 60 APC patients, has revealed other inactivating mutations. These include a single base pair change that introduces a stop codon into the coding region and base pair deletions that introduce frameshifts. One mutation, a five base pair deletion, is present in more than one, unrelated, affected individual. In the kindred of one sporadic patient, this is a new mutation, as both normal parents and a normal sibling do not exhibit the novel SSCP conformer. Also, the position of this deletion is within 50 base pairs of a stop codon found in another APC patient. Both mutations occur in a region of the gene that encodes an amino acid repeat. Mutations now have been observed throughout the APC gene; all are inactivating mutations.

G 211 ALLELIC LOSS DURING METHYLENE CHLORIDE-INDUCED LUNG CARCINOGENESIS IN B6C3F1 MICE, Monika E. Hegi, Marshall W. Anderson and Roger W. Wiseman[1], Laboratories of Molecular Toxicology and Molecular Carcinogenesis[1], National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Tumor suppressor gene inactivation appears to play an important role in carcinogenesis, especially during tumor progression. Loss of specific chromosomal regions can unmask mutations in the remaining allele of tumor suppressor genes. To localize potential suppressor genes in B6C3F1 mice, we have examined methylene chloride-induced lung tumors for losses of heterozygosity at loci on eight different chromosomes using length polymorphisms in restriction fragments and microsatellites. Allelic losses on most chromosomes were infrequent. However, allelic losses were observed on chromosome 4 in twenty of forty chemically induced lung carcinomas. One of six spontaneous lung carcinomas also lost chromosome 4 markers, but no alterations of chromosome 4 were detected in five lung adenomas. There was a strong bias for the loss of the maternal C57BL/6J chromosome (17 of 20 cases). With the exception of three tumors that retained both copies of the Mos proto-oncogene near the centromere and another that retained a distal marker (Pnd), most tumors showed losses at each of six chromosome 4 markers analyzed. This suggests that nondisjunction events are more common than somatic recombination or deletion. No correlation between allelic loss on chromosome 4 and K-ras activation, carcinoma type, or tumor size was observed. A tumor suppressor gene was previously mapped to mouse chromosome 4 in somatic cell hybrid studies and homozygous deletions and frequent allelic losses have been reported in a homologous region of human chromosome 9p in various human cancers, including lung tumors. Allelic losses were also detected in five of forty tumors with markers surrounding the p53 tumor suppressor gene on chromosome 11. Further examination of the p53 gene in these tumors by single strand conformation polymorphism analysis suggested the presence of mutations in exon 5, 6, and 7. Characterization of these p53 mutations by direct sequencing and analysis of allelic losses on other chromosomes are under investigation.

G 212 TUMOR SUPPRESSION IN HUMAN HEAD AND NECK SQUAMOUS CELL CARCINOMA (SCC) CELL LINES FOLLOWING INTRODUCTION OF CHROMOSOME 11, Carol Bova Hill and Tona M. Gilmer, Glaxo Research Institute, RTP, NC 27709

Several lines of evidence suggest that the accumulation of genetic changes in both oncogenes and tumor suppressor genes is responsible for many adult cancers. The location of tumor suppressor genes to specific human chromosomes has been facilitated by the microcell transfer technique whereby a single "normal" human chromosome is introduced into tumorigenic cells. Suppression is then analyzed by tumorigenicity in nude mice. A number of genetic alterations have been identified in human head and neck SCC. Our laboratory and others have found amplification of chromosome 11q13 sequences in approximately 50% of head and neck tumors. Since tumor suppressor genes have been mapped previously to chromosome 11 in other cancers, we wanted to ask if chromosome 11 also contains tumor suppressor gene(s) relevant to head and neck SCC. We therefore introduced a chromosome 11 tagged with a neomycin resistance gene into three established head and neck SCC cell lines, FaDu, SCC-4, and SCC-25 by microcell transfer. The cell lines contain approximately 10X, 6X, and 3X amplification of 11q13 sequences, respectively. Tumor latency was altered in the FaDu microcell hybrids. The SCC-4 hybrids showed changes in cell morphology and loss of tumorigenicity. Also a significant number of the hybrids appeared to senesce with time. The transfer experiments with SCC-25 cells rarely gave G418 resistant colonies, and all colonies appeared to senesce after a limited number of population doublings. We conclude that the introduction of chromosome 11 does appear to influence growth and tumorigenicity in head and neck SCC cell lines.

G 213 A CELLULAR PROTEIN MEDIATES ASSOCIATION OF p53 WITH THE E6 ONCOPROTEIN OF HUMAN PAPILLOMAVIRUS TYPES 16 OR 18. Jon M. Huibregtse, Martin Scheffner, and Peter M. Howley. Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda, MD 20892.

The E6 protein of human papillomavirus types 16 and 18 (HPV-16 and HPV-18) can stably associate with the p53 protein in vitro. In the presence of rabbit reticulocyte lysate, this association leads to the specific degradation of p53 through the ubiquitin-dependent proteolysis system. We have examined the E6-p53 complex in more detail and have found that association of E6 with p53 is mediated by an additional cellular factor. This factor is present in rabbit reticulocyte lysate, primary human keratinocytes, and in each of five human cell lines examined. The factor is designated E6-AP, for E6-associated protein, based on the observation that the E6 proteins of HPV-16 and 18 can form a stable complex with the factor in the absence of p53, whereas p53 association with the factor can be detected only in the presence of E6. Gel filtration and coprecipitation experiments indicate that E6-AP is a monomeric protein of approximately 100 kd.

G 214 ALTERATIONS IN p53 IN TRANSPLANTABLE PRENEOPLASTIC MAMMARY OUTGROWTHS AND TUMORS,

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Point mutations in the tumor suppressor gene p53 are the most frequently observed alteration in breast cancer. However, it is unclear whether mutation of p53 is an obligate change found in preneoplastic lesions or is acquired during progression to overt tumors. In mice, hyperplastic alveolar nodules (HAN) represent a preneoplastic intermediate in mammary tumorigenesis allowing the status of p53 to be analyzed at the earliest stages of tumor development. Transplantable HAN lines that give rise to tumors at a predictable frequency have been established (designated the DIM and TM series). These HAN lines and HAN-derived tumors have been analyzed for alterations in p53 by immunoprecipitation with monoclonal antibodies followed by immunoblot detection with a polyclonal anti-p53 antiserum. In 5 of 6 HAN lines, p53 was below the limit of detection. In tumors derived from these HAN, the incidence of detectable p53 ranged from 0% in TM2 to 67% in TM10. The DIM3 HAN line differed in that the majority of the p53 appeared to be mutant (PAb240$^+$). Tumors arising from DIM outgrowths maintained this pattern of PAb240$^+$ p53 expression. The absence of detectable accumulation of p53 in any TM2 tumors whereas p53 accumulation and mutation were evident in nearly all DIM tumors suggests that TM2 tumors and DIM tumors represent distinct pathways by which mammary tumors can develop. The strong PAb240-reactivity of p53 from DIM outgrowths and tumors was also in stark contrast to the very low level of PAB240$^+$ p53 found in the parental COMMA-D cell line from which the DIM lines were derived. Sequence analysis of p53 exons 4-10 of DIM tumor cDNA revealed a single C to G transversion causing a replacement of a valine with a glycine at codon 271. Surprisingly, this mutation was also present in the parental COMMA-D cells. These data suggest that DIM cells have acquired additional mutations in p53 that lie outside the region analyzed or other factors that influence p53 conformation are altered in DIM cells.

G 215 DEFINITION OF A TUMOR SUPPRESSOR LOCUS AT 3p21-p22

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Cytogenetic abnormalities and high frequency allele losses involving the short arm of human chromosome 3 have been found in a variety of histologically different neoplasms. These findings suggest that a tumor suppressor gene(s) may be located in the region of 3p14-p25 although definitive functional proof for the involvement of a particular region of 3p has not been generated. We report a rapid genetic assay system which has allowed the functional analysis of defined regions of 3p in the suppression of tumorigenicity in vivo. Interspecific microcell hybrids containing fragments of chromosome 3p were constructed and screened for tumorigenicity in athymic nude mice. Hybrid clones were obtained which showed significant tumor suppression and contained only the region 3p21 near the interface with 3p22 in a murine cell background. With these hybrid clones we have defined a genetic locus at 3p21-p22 intimately involved in tumor suppression.

G 216 NEGATIVE GROWTH REGULATION BY OVER-EXPRESSED RPI1 IN Saccharomyces cerevisiae,

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RPI1, a novel down-regulator of Ras, has recently been identified in S. cerevisiae (MCB11:3894 '91). The activity of the RPI1 gene product is suggested to operate upstream of Ras on the Ras/cAMP signal transduction pathway and also to require the presence of one of the two Ras GTPase activators, IRA1 and IRA2. These results prompted us to propose that RPI1 acts as a positive regulator of IRA activity.

Unexpectedly, when overexpressed under the GAL10 inducible promoter, RPI1 induced a strong growth suppression. This growth suppression activity was demonstrated on all the mutant strains tested except the ira1ira2 double disruption strains. This suggests that the growth suppression activity by RPI1 again requires the presence of one of the two Ras GTPase activators, IRA1 and IRA2. Surprisingly, the growth suppression by RPI1/IRA seems not to require Ras activity, indicating that the negative growth regulation by RPI1 acts independently to its activity as a down-regulator of the Ras/cAMP pathway.

G 217 ISOLATION OF TUMOR SUPPRESSOR GENES FROM BCR/ABL TRANSFORMED CELLS

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The Philadelphia chromosome, t(9;22) (q34;q11) is found in the leukemic cells of more than 90% of patients suffering from chronic myelogenous leukemia (CML). The molecular consequence of the reciprocal translocation leading to the formation of the Philadelphia chromosome is the synthesis of a 210-kd bcr/abl chimeric protein (P210). Secondary genetic events may be involved in the progression to the blast crisis phase. Stable expression of P210 in Rat-1 cells causes a distinct morphological change, capacity for anchorage-independent growth and tumorigenicity.

In our work somatic cell hybrids between P210-transformed rat-cells (Rat-1/P210) and normal diploid rat-cells (208F YP1) were produced. The hybrids gained a fibroblast-like morphology and required anchorage for growth.

Rat-1/P210-cells were transfected with a candidate human tumor suppressor gene. The transfectants showed a reversion of the transformed phenotype, needed higher concentrations of FCS in the culture medium to grow, had lower growth rates, were unable to grow in semisolid agar and showed a significantly reduced tumorigenicity in nude mice. The transfected cells still contain the transforming oncogene as verified by Southern blots and express the transforming oncogene product P210 at an unchanged level as verified by Northern blots.

G 218 ELEVATED EXPRESSION OF MITOCHONDRIAL GENES IN SENESCENT HUMAN FIBROBLAST CELLS, Seiji Kodama, Hideto Yamada and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, NIEHS/NIH, Research Triangle Park, NC 27709

In order to isolate cDNAs expressed differentially in senescent cells, we constructed a λZAP cDNA library from polyA$^+$ mRNA of senescent human fibroblast cells and screened 10^5 cDNAs by the +/- differential screening method. mRNAs were isolated from cultured human fibroblasts at early and late culture stages and used to prepare cDNA probes. After two cycles of screening, we isolated five cDNA clones expressed preferentially in senescent cells. These five clones were divided into two groups by cross-hybridization. Sequence analysis revealed that one of two clones was homologous to human mitochondrion cytochrome b gene and the other was homologous to human mitochondrion ND4/4L gene. These two mitochondrion genes were expressed at levels three- to five-fold higher in late passage, senescent cells than in early passage cells. However, the expression of mitochondrion cytochrome oxidase I gene was not changed during in vitro culture, suggesting that the elevated expression in senescent cells was selective for certain mitochondrion genes, specifically mitochondrion cytb and ND4/4L genes. This result implies that upregulation of these two genes may be involved in the process of cellular senescence.

G 219 MOLECULAR CLONING OF cDNA SEQUENCES DIFFERENTIALLY EXPRESSED DURING MALIGNANT PROGRESSION IN MOUSE SKIN CARCINOGENESIS, Robert Kösters, Peter Krieg, Gerhard Fürstenberger and Friedrich Marks, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Germany

We have used the model of multistage carcinogenesis in mouse skin to identify and study genes the expression of which changes during tumor progression from benign papillomas to malignant squamous cell carcinomas. In order to isolate genes which are transcriptionally activated or repressed during this step of malignant progression subtractive cDNA cloning was applied. Mouse skin tumors were induced by combined DMBA/TPA treatment and cDNA libraries were constructed from polyadenylated RNA extracted from either pooled papillomas or one individual squamous cell carcinoma. Subtraction of these libraries was performed in both directions yielding a total number of 49 cDNA-clones, expected to be diffentially expressed during malignant progression. So far three different genes specifically overexpressed in the carcinoma state and two genes overexpressed in the papilloma state have been identified. Sequence analysis revealed that one of the carcinoma specific clones is identical to mouse lipocortin I. The second carcinoma specific clone shows strong homology to human and chicken alpha-1 type three collagen and represents probably the respective mouse homologue. The third carcinoma specific clone shows extensive homology to the 3´untranslated region of human ADP-ribosylation factor 4 (hARF4), a member of a family of highly conserved small GTP-binding proteins. Of the two different cDNA clones representing papilloma specific sequences one has been identified as mouse keratin KI, whereas the other one displays no sequence similarity to any known gene. Other cDNA clones obtained in our cloning approach are currently under further investigation results of which will be presented at the meeting.

G 220 MALIGNANT TRANSFORMATION OF A HUMAN FIBROBLAST CELL STRAIN, MSU-1.1 BY THE CARCINOGEN BENZO[A]PYRENE-7-8-DIOL-9,10-EPOXIDE, J. Justin McCormick, Dajun Yang, Calvert Louden, David Reinhold, Suzanne Kohler, and Veronica M. Maher, Departments of Microbiology and Biochemistry, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824

We have found that treatment of cells of the infinite life span human fibroblast-derived cell strain, MSU-1.1, induces focus formation in a dose dependent manner. Cells were isolated from eight independently derived foci. As expected these eight cell strains all grew to a higher density in medium containing 1% serum than did MSU-1.1 cells they were derived from. Three of the 8 cell strains were able to form low to high grade sarcomas in athymic mice in 2-3 weeks. These three cell strains had the following properties: 1)they grew rapidly in serum-free medium without added growth factors, 2)they formed anchorage independent colonies >120 μm in diameter at a frequency of 5-19%, 3) they exhibited the loss of 2-3 chromosomes, from the following group, chromosomes 13, 16, 17, 19, and Y, 4) they were round or spindle shaped. One of the other five strains formed a high grade sarcoma after 11 weeks. The cells derived from that tumor had the same growth properties as the three focus-derived strains described above. In addition to loss of 1 or 2 chromosomes, these cells showed a chromosome rearrangement. Since the pre-tumor focus-derived cells of this strain had *in vitro* characteristics like the non-tumorigenic, focus-derived strains, it appears that the cells injected into mice contained a small sub-population of highly tumorigenic cells. Three of the four non-tumorigenic strains grew moderately well in serum-free medium without growth factors, one grew rapidly. These four non-tumorigenic stains 1)formed anchorage independent colonies > 120 μm in diameter at a frequency of only <0.03 to 0.3% and 2) were chromosomally the same as the MSU-1.1 cells, except for the presence of a minority of chromosomally abnormal cells in one strain. The *in vitro* characteristics of the four tumorigenic strains are the same as those of MSU-1.1 cells malignantly transformed by transfection of the *ras* oncogene. Supported by DHHS Grant CA21289, DOE Grant ER60524 and NIEHS Contract NO1-65152.

G 221 GROWTH FACTOR STIMULATION OF NADH OXIDASE ACTIVITY OF RAT LIVER PLASMA MEMBRANES IS REDUCED OR ABSENT WITH HEPATOMA PLASMA MEMBRANE, D.J. Morré, M. Bruno, A.O. Brightman, J. Lawrence, D.A. Werderitsh and D.M. Morré, Department of Medicinal Chemistry and Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907

NADH oxidase activity (electron transfer from NADH to molecular oxygen) of plasma membranes purified from rat liver was studied. The enzyme was stimulated (2- to 3-fold) by growth factors (diferric transferrin and epidermal growth factor) and hormones (insulin and pituitary extract) to which liver cells were known to respond. Additionally NADH oxidase was inhibited up to 80% by several agents known to inhibit growth or induce differentiation (retinoic acid, calcitriol, and the monosialoganglioside, G_{M3}). The growth factor-responsive NADH oxidase of isolated plasma membranes was not inhibited by KCN or common inhibitors of oxido-reductases of endoplasmic reticulum or mitochondria. As well, NADH oxidase of the plasma membrane was stimulated by concentrations of detergents which strongly inhibited mitochondrial NADH oxidases and by lysolipids or fatty acids. Taken together, the evidence suggests that NADH oxidase of the plasma membrane is a unique oxido-reductase and may be important to the regulation of cell growth. In striking contrast, hepatoma plasma membranes demonstrated an intrinsically increased level of NADH oxidase activity which was not stimulated further by the addition of growth factors. The results suggest that the NADH oxidase of the hepatoma plasma membrane is no longer correctly coupled to hormone and growth factor receptors. This biochemical defect may parallel the loss of growth control that is characteristic of neoplastic transformation in hepatocarcinogenesis and other transformation systems.

G 222 FUNCTIONAL SIGNIFICANCE OF PHOSPHORYLATION OF p53 BY cdc2 KINASE, Michael Mowat and Nancy Stewart, Manitoba Institute of Cell Biology, University of Manitoba, 100 Olivia Street, Winnipeg, Canada, R3E 0V9

The functional significance of phosphorylation of p53 by the cell cycle protein cdc2 kinase is not known at present. It has been previously shown that genes mutated at the cdc2 phosphorylation site (serine 312) retain many of the wild type characteristics of the p53 gene product, including a short half life, recognition by wild type specific p53 antibody (pAb 246), and inhibition of SV40 replication (1). To test the role of cdc2 phosphorylation of p53 we have transfected these plasmids into the REF52 cell line. We have previously shown that transfection of wild type p53 expressing plasmids into REF52 cells prevents colony formation. Preliminary results indicate the cdc2 phosphorylation site mutants were as efficient at colony formation as cells expressing mutant p53 val^{135}. The cdc2 phosphorylation site mutant colonies maintained the appearance of the parental cell type, and expressed p53 protein at lower levels than mutant p53 val^{135} colonies. As well, we are interested in whether these cdc2 phosphorylation site mutants have acquired the ability to rescue REF52 cells from ras-induced growth arrest. Cotransfection along with a plasmid expressing ras gave a similar efficiency of colony formation as cells cotransfected with p53 val^{135} and ras. The cdc2 phosphorylation site mutant/ras cells did not appear transformed. These results suggest cdc2 phosphorylation may be required for p53's negative growth regulation properties. Lack of phosphorylation, however, may not be sufficient to block endogenous p53 negative growth functions. In addition, we have transfected the p53 cdc2 phosphorylation site mutant genes into a Friend erythroleukemia cell line. This cell line does not express p53 due to rearrangement of both alleles of the p53 gene. Preliminary results indicate that half of the clones express p53 protein at very low levels compared to cells expressing mutant p53 val^{135}. This is in contrast to transfection of a wild type gene into this cell line in which expression of wild type p53 is not seen. We will be reporting on further characterization of these cell lines.

1. Meek, D.W. and Eckhart, W. (1990) J. Virol. 64, 1734.

G 223 p53 Changes in Murine Mammary Preneoplasia.

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Mutations in the p53 gene are a commonly identified alteration in human cancer. To study the potential role of p53 mutations in early stages of breast cancer, we are using a mouse model system in which hyperplastic alveolar nodules (HAN) represent a clear preneoplastic lesion. Mammary epithelial cell lines have been established that display dissimilar outgrowth characteristics when transplanted into cleared mammary fat pads. The FSK-7 cells give rise to only normal ductal outgrowths, while COMMA-D and FSK-3 cells produce only HAN. FSK-2, FSK-4, and FSK-6 cells give rise to both ductal and HAN outgrowths *in vivo*. MOMA-1 cells produce no outgrowths. The status of p53 has been analyzed in these different cell populations using monoclonal antibodies that distinguish between the wild-type (PAb 246-reactive) and mutant (PAb 240-reactive) forms of p53. MOMA-1 and FSK-7 cells express only wild-type conformations of p53 having a short half-life of ~30 min. Conversely, both COMMA-D and FSK-3 cells contain approximately equal levels of wild-type and mutant p53. The other cell lines express wild-type p53 and variable amounts of mutant forms of p53, as evidenced by an extended half-life or reactivity with PAb240. The areas known to be hot-spots for mutation (exon 5-9) in p53 are being analyzed at the nucleic acid level, using PCR-based techniques. Initial data indicate that FSK-7 cells express only wild-type p53. These observations suggest that mutant p53 is not essential for mammary epithelial cells to be established in culture. While primary mammary epithelial cells and cells that generate ductal outgrowths *in vivo* fail to express mutant forms of p53, cell lines that grow out as hyperplasias *in vivo* contain mutant p53. It appears that changes in p53 may be involved in the early stages of mammary tumorigenesis.

G 224 GERMLINE MUTATIONS IN THE WILMS' TUMOR GENE, WT1, CONTRIBUTE TO ABNORMAL GENITAL SYSTEM DEVELOPMENT AND HEREDITARY WILMS' TUMOR. Jerry Pelletier, Wendy Bruening, and David Housman. McGill Cancer Center, McGill University, Montreal, Canada, H3G 1Y6 and M.I.T. Cancer Center, Boston, MA 02139.

Wilms' tumor, a malignancy of embryonal kidney cells, is found in association with three other cardinal features: aniridia, genitourinary abnormalities, and mental retardation in patients with hemizygous deletions of DNA sequences in band 11p13 (WAGR syndrome). A key issue raised by the association of these phenotypes is whether some or all of these are due to hemizygosity at a single locus or whether several independent genetic loci are involved in the etiology of the WAGR syndrome. The tumor suppressor gene for Wilms' tumor (WT1) residing in band 11p13 is expressed at high levels in the glomeruli of the kidney during organogenesis and is likely to play a significant role in controlling the program of kidney differentiation. WT1 is also expressed in the gonadal ridge of the developing gonad, the Sertoli cells of the testis, and the epithelial and granulosa cells of the ovary. These results suggest the possibility that the WT1 gene might play an equally important role in controlling the development of the urogenital system. We have sought to test the hypothesis that hemizygous expression of the WT1 gene is directly responsible for the urogenital abnormalities in the WAGR syndrome. To address this question, we chose to study a rare class of patients, those with Wilms' tumor and urogenital abnormalities without other stigmata of the WAGR syndrome. We hypothesized that these patients would have constitutional point mutations or deletions in the WT1 gene which would lead to reduced expression levels of the WT1 transcript as well as providing the first hit in the process of Wilms' tumorigenesis. We have characterized two such individuals. One patient (PG) had bilateral Wilms' tumor, hypospadias, and an undescended left testicle. The second patient (TS) was born with hypospadias and bilateral cryptorchidism and recently developed a Wilms' tumor. A detailed examination of constitutional DNA from PG revealed a 17 bp deletion within exon 4 of one allele of WT1; whereas DNA from TS revealed a single nucleotide deletion within exon 6 of one allele of WT1. Both of these alterations result in translational frameshifts leading to early termination of translation. All mutated copies of WT1 have undergone reduction to homozygosity in tumors from both individuals. These results provide genetic evidence that germline inactivation of the WT1 gene can cause abnormalities in genitourinary development as well as being implicated in some familial cases of Wilms' tumor.

G 225 *Abstract Withdrawn*

G 226 FUNCTIONAL EVIDENCE FOR A TUMOR SUPPRESSOR GENE FOR RENAL CELL CARCINOMA. Y. Sanchez and A.M. Killary, Division of Laboratory Medicine, University of Texas M.D. Anderson Cancer Center, Houston, Texas.
Non-papillary renal cell carcinoma is an adult cancer of the kidney which has been characterized by specific cytogenetic deletions and high frequency allele losses on the short arm of chromosome 3. Two critical regions on 3p have recently been identified which may encode tumor suppressor loci, one or more of which may be intimately involved in the genesis of this cancer. To assay for the function of genetic loci on chromosome 3 and subsequently regions of 3p, we constructed monochromosomal microcell hybrids containing a normal intact chromosome 3 in the renal cell carcinoma cell line SN12C. These hybrids showed significant tumor suppression when injected into athymic nude mice. To precisely define the regions of 3p involved in tumor suppression, neo-marked fragment-containing microcell hybrids were constructed in the mouse A9 cell background. Specific fragments of 3p are being introduced into the renal cell carcinoma line to define the region or regions required for the suppression of tumorigenicity in this system.

G 227 CHARACTERIZATION OF PROTEIN KINASE ACTIVITIES INVOLVED IN PHOSPHORYLATION OF THE CELLULAR PROTEIN P53. Karl H. Scheidtmann, Elke Bartsch, Doris Hecker, and Birgit Jaitner, Dept. of Molecular Genetics, University of Bonn, FRG.
P53 is involved in cellular growth control. Recent data suggest that its subcellular localization, its conformation, and its activities vary and are regulated during the cell cycle, but the mechanisms underlying these variations are unknown. P53 is phosphorylated at multiple sites, presumably by three or four different kinases. Two of these have been identified as cdc2 kinase and casein kinase II. These kinases are probably constitutively expressed in all proliferating cells but their activities are regulated during the cell cycle. In rat cells, there appear to be additional kinases that are induced or activated upon infection and transformation of cells by SV40 large T antigen. All these kinases seem to be assembled in p53 immunecomplexes from SV40-transformed, but not from normal cells as revealed by phosphopeptide analyses of in vitro phosphorylated p53. We are trying to dissect the individual kinase activities by cell cycle experiments and by inhibition/competition experiments in vitro and to elucidate the functional significance of individual phosphorylation events by biochemical analyses of differentially phosphorylated p53 species.
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G 228 p53 GENE STRUCTURE AND EXPRESSION IN LYMPHOID TUMORS INDUCED IN *c-myc* TRANSGENIC MICE
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Alterations in the p53 gene are repeatedly found in human colon and lung carcinomas. More recently mutations in p53 have also been found in several human lymphocytic leukemias. We have analyzed the expression of the p53 gene in B cell-lineage tumors arising spontaneously in transgenic mice carrying the *c-myc* gene under the control of the IgH μ enhancer. A significant fraction of these tumors express little, if any, p53 RNA as measured by quantitative PCR. Additional experiments to determine the nature of these loss-of-expression "mutations" will be presented. This loss of p53 expression was found in tumors with both preB and mature B phenotypes. Alteration of p53 expression in a relatively large proportion of tumors derived from these transgenic mice suggests that p53 can effectively collaborate with myc in transformation. In addition, these experiments demonstrate that the investigation of the expression patterns of known oncogenes in spontaneously arising tumors is a useful model for identifying genes involved in transformation.

G 229 SPATIAL DISTRIBUTION OF TRANSFORMING GROWTH FACTORS IN PAPILLOMAS AND CARCINOMAS: ENHANCED EXPRESSION DURING MALIGNANT PROGRESSION OF MOUSE SKIN CARCINOGENESIS
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Experimental evidence from various animal models suggests that wounding acts as a promoter of tumor formation in tissues upon preceding initiation with chemical carcinogens or viruses. This indicates that the post-initiation stages of tumor development may be entirely controlled by endogenous factors, which are released at high levels upon wounding. Among these factors strongly accelerating wound healing are TGF-α and TGF-β. Indeed, the intracutaneous injection of both factors into initiated mouse skin has been shown to induce tumors upon subsequent chronic growth stimulation. Thus, the elaboration of endogenous TGF-α and TGF-β in wounds may be responsible for the promoting effect of wounding and of phorbol ester tumor promoters, which are known to induce the expression of both factors in the epidermis. Northern blot analysis of RNA from tumors arising along the initiation-promotion-protocol revealed slightly increased levels of TGF-β_1 mRNA in papillomas but constitutive overexpression in carcinomas as compared with normal epidermis. In situ hybridization localized this TGF-β mRNA expression to the epithelial compartment of the tumors, immunocytochemical analyses using TGF-$\beta_{1\text{-}3}$ subtype-specific antibodies and antibodies against the protein precursor demonstrated the mature protein in epithelial areas of the tumors, predominantly in the suprabasal regions. There was no drastically difference in the expression of the different protein subtypes. Additionally, these tumors also produced TGF-α in high levels, shown by immunocytochemical studies, but with a localization predominantly in stromal elements.
These data suggest that the endogenous generation of TGF-α and TGF-β is involved in both, early and late stages of tumor development in initiated mouse skin. TGF-α may function as an autocrine stimulator of epithelial and mesenchymal growth, whereas TGF-β affects stromal elements by the induction of tumor stroma and keratinocyte growth. It is assumed that TGF-β inhibits proliferation of normal keratinocytes rather than the initiated cells which would result in a clonal selection of those cells culminating in the development of tumors.

G 230 CHARACTERIZATION OF p53 MUTATIONS AND ALLELIC LOSSES ON CHROMOSOME 4, 11, AND 14 IN BUTADIENE-INDUCED TUMORS OF B6C3F1 MICE, Peter Soderkvist[1], Tamra Goodrow[2], Charles Cochran[1], and Roger Wiseman[1], Laboratory of Molecular Carcinogenesis[1], National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709 and Merck, Sharp, and Dohme Research Labs[2], West Point, PA 19486
As a means of identifying the potential involvement of tumor suppressor genes during carcinogenesis in B6C3F1 mice, we examined DNA from butadiene-induced lung and mammary carcinomas and lymphomas for losses of heterozygosity. Both traditional RFLP analysis and a novel PCR protocol based on microsatellite length polymorphisms between mouse strains were utilized. Analysis of five markers flanking the p53 gene on mouse chromosome 11 revealed allelic losses in thirteen of seventeen mammary tumors as well as three of ten lymphomas and two of eight lung tumors. Nondisjunction was responsible for the majority of chromosome 11 losses but at least six examples of somatic recombination or deletion were also detected. To determine its relationship to the chromosome 11 alterations the p53 tumor suppressor gene was examined by direct sequencing of PCR products from cDNA or genomic DNA. To date seven point mutations have been identified in highly conserved regions of the gene. These mutations were confirmed by analysis of single strand conformation polymorphisms (SSCP) in multiplex PCR reactions. Altered mobility, consistent with mutations in exons five through eight, was detected by SSCP for each of these tumors. Southern analysis also revealed a homozygous deletion including exons 5 through 9 of the p53 gene in another mammary tumor. RFLP analysis of endogenous virus loci indicated that allelic losses were relatively common on chromosomes 4, 12, and 14. Allelic losses of the Rb-1 tumor suppressor gene and a distal marker on chromosome 14 (Hpg locus) were observed in 7 of 17 mammary tumors. Of six markers on chromosome 4 analyzed, the Ifa-2 locus was lost most frequently. Six of eight butadiene-induced lung tumors, and four of ten of lymphomas exhibited allelic losses. A tumor suppressor gene was previously mapped to this chromosomal region in somatic cell hybrid studies by Harris and coworkers. In addition homozygous deletions and frequent allelic losses have been reported in a homologous region of human chromosome 9p for a variety of human cancers including lung tumors. Current studies are directed at the generation of a physical map of this chromosome 4 region using a panel of microdissection clones (generously provided by J. Freidman, Rockefeller University) that are tightly linked to the Ifa gene cluster.

G 231 ANALYSIS OF GENETIC CHANGES IN NON MALIGNANT BRONCHIAL EPITHELIUM, PRENEOPLASTIC LESIONS AND LUNG CANCER. Gabriella Sozzi, Monica Miozzo, Tania C. Cariani, Ugo Pastorino*, Silvana Pilotti° and Giuseppe Della Porta. Division of Experimental Oncology A, *Division of Chest Surgery and °Division of Anatomical Pathology and Cytology - Istituto Nazionale Tumori, Via G. Venezian 1 - 20133 Milan, Italy.

To identify and characterize the early genetic changes occurring in lung tumor development, we performed a cytogenetic and immunohistochemical analysis on non-malignant bronchial tissues and in lung tumor specimens. In tumor cells of 11 cases comprising different histotypes, that showed heavily rearranged karyotypes, deletions or rearrangements at 3p were the most frequently observed chromosomal changes. The analysis of non-malignant bronchial samples of 41 patients, showed chromosomal abnormalities, in particular deletions, in 13 cases. Interestingly, the deletions involved chromosomal regions where tumor-suppressor genes have been previously located: chromosome 3p (3 cases), 11p (1 case) and 17p (3 cases). In two cases with a 17p- chromosome, the histopathologic examination revealed the presence of a severe dysplasia while an immunohistochemical analysis, with anti-p53 antibodies, indicated the presence of a mutated form of the dominant tumor-suppressor gene product p53. Ongoing molecular studies on the same specimens are aimed to confirm these previous results and to associate the occurrence of cytogenetic alterations in non-malignant and malignant lung lesions with the inactivation of putative tumor-suppressor genes. The present results indicate that specific genetic abnormalities are present both in the non-malignant bronchial epithelium and in preneoplastic lesions and can represent early changes in the multistep lung carcinogenetic process useful for the diagnosis of premalignant lesions and early cancer.

G 232 MOLECULAR AND GENETIC ANALYSIS OF VARIABLE PHENOTYPE ADENOMATOUS POLYPOSIS COLI (APC) PATIENTS, Lisa Spirio, Mark Leppert and Ray White, Department of Human Genetics and the Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT 84112
Familial adenomatous polyposis coli (APC) is an autosomal dominant disorder characterized by multiple adenomatous, colonic polyps that eventually progress to carcinomas. A subset of this disorder exists in which affected individuals have a variable phenotype, with the number of colonic polyps varying markedly in affected family members who have inherited a predisposing allele. Additionally, the average age of colon cancer onset is older than that of classical polyposis patients. The same set of DNA markers used to establish linkage in APC families has been applied to five variable phenotype colon cancer families. This includes a prototype family, a large Utah kindred which has previously been shown to segregate a defective APC allele (Leppert et al., 1990). Multilocus linkage analysis was performed on the four additional families and the disease phenotype mapped to the APC locus with a combined LOD score of approximately 6.0 (Spirio et al.; unpublished data). Recently the APC gene has been identified and found to contain inactivating mutations in the coding sequence of APC patients (Josyln et al., 1991; Kinzler et al., 1991; Groden et al., 1991; Nishisho et al., 1991). The polymerase chain reaction (PCR) followed by single strand conformation polymorphism (SSCP) analysis has been used to screen for mutations in the APC gene in the five variable phenotype families. A four base pair deletion in the five prime end of the APC gene has been found in one of the families. This mutation creates a downstream stop in the new reading frame. Therefore, we have found an apparent null allele in a family that has an attenuated polyposis phenotype.

G 233 REINSERTION OF CHROMOSOME 10 INTO GLIOBLASTOMA CELLS; BIOLOGICAL AND MOLECULAR ANALYSIS. Peter A. Steck, Mark A. Pershouse, Azra Hadi, Ann M. Killary, Elton Stubblefield, and W. K. Alfred Yung, Departments of Neuro-Oncology and Molecular Genetics, U.T. M.D. Anderson Cancer Center, Houston, TX 77030

The loss of alleles on chromosome 10 has been observed to be the most common genetic alteration to occur during the development of malignant glioblastomas (GBMs). We have reinserted an intact chromosome 10 into two independent glioblastoma cell clones by microcell-mediated chromosomal transfer. The presence of the new chromosome 10 or control chromosomes (2 & 15) were documented by karyotypic and molecular methods. The hybrid cells containing an new chromosome 10 showed a reversion of their oncogenic phenotype as compared to the parental cells, with a dramatic decrease in their ability to growth under anchorage-independent conditions and a loss of tumorigenicity in nude mice. The cloned hybrid cells also showed a more "astrocytic" morphology, particularly at low cell densities. The exponential growth rate of the individual hybrid clones was variable, however, all the hybrids exhibited lower saturation densities. The presence of chromosome 10 also did not effect the differentiation status of the cells as assessed by GFAP, or the expression of tumor suppressor genes (p53) and proto-oncogenes (TGF-α or EGF-receptor) that have been implicated in glial tumorigenesis. To further examine the effects of reinsertion of chromosome 10, subtractive hybridization was performed between cDNA from a hybrid cell with an intact chromosome 10 and mRNA from the parental GBM cells which are devoid of intact chromosomes 10s. Presently, about forty cDNA clones are being analyzed to determined the nature of genes which are differentailly expressed as a result of the reinsertion of chromosome 10 into GBM cells.

G 234 IS THE GENESIS OF ANEUPLOIDY RELATED TO LOSS OF KINETOCHORES ?

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Some chromosomes in rat fail to display the presence of kinetochore proteins (K^-, Vig and Sternes, 1991), which may constitute a mechanism for the genesis of genomic loss. We have analyzed the metaphase ring arrangements in three mouse cell lines (A9, LA9, immortalized; MBT, brain tumor) and a human tumor cell line (HeLa) for the presence of kinetochore proteins, detected by antikinetochore antibody. In the tumor cell lines with a high degree of variation in chromosome number (HeLa, MBT) we observed that 5-10 % of the cells carried a dislocated K^- chromosome. In the immortalized cell lines with less variation (A9, LA9) only 5-6 % of the cells contained K^-. Even though the frequency of such akinetochoric chromosomes was not high enough to account for the known variability in chromosome number, this phenomenon may contribute to this variability. In addition, we have analyzed dislocated K^- chromosomes in HeLa cells for the presence of centromeric DNA (in situ hybridization). Our results indicate that the observed loss of kinetochores is not necessarily due to a loss of centromeric DNA. - Nevertheless, a number of kinetochore bearing chromosomes (K^+) was also observed to be dislocated in metaphase. It is still an open question whether these are also associated with the genesis of aneuploidy since they still may be or may become attached to the spindle.

G 235 p53 INDUCES CATHEPSIN L GENE TRANSCRIPTION,

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Cathepsin L is a lysosomal protease whose synthesis is induced by malignant transformation, tumor promoters (TPA), and second messengers (cAMP). Because *ras*-transformed and colon carcinoma cells express high levels of cathepsin L, cathepsin L gene expression may be a model system for studying mechanisms of transcriptional regulation by the *ras* and p53 gene products. Therefore, we have cloned the gene for cathepsin L from NIH 3T3 (NIH) cells and constructed a series of transcriptionally active chloramphenicol acetyl transferase (CAT) plasmids containing various regulatory regions of the cathepsin L gene. Deletional mutations of the cathepsin L-CAT plasmids and transient transfection analysis have allowed us to localize cAMP responsive sequences to the first 81 base pairs upstream of the transcriptional initiation site. Furthermore, our results demonstrate that a minimum of three distinct regions within base pairs -273 to -28 mediate basal activity. Sequence analysis of the promoter region reveals multiple putative transcriptional factor binding sites, including 3 AP-2 sites and 1 EGR-1 site. Co-transfection of wild-type p53 with cathepsin L-CAT plasmids containing either 4000 or 300 base pairs upstream of the transcriptional inititation site stimulates CAT expression. A mutant p53 (Arg→His, a.a. 175) does not appear to stimulate cathepsin L-CAT expression. In contrast, co-transfection of the K-*ras* oncogene reduces expression of the same cathepsin L-CAT plasmids. Primer extension analysis of transiently transfected plasmids that also contain the first three introns and exons of the cathepsin L gene demonstrates that sequences downstream of the transcriptional inititation site are necessary for stimulation by TPA. Of note is that the first intron contains 2 AP-1 and 2 AP-2 sites. Therefore, cathepsin L gene expression appears to be regulated in a complex manner where upstream regions mediate p53 responsiveness but are not sufficient to mediate stimulation by *ras*. Conversely, downstream elements mediate tumor promoter stimulation of cathepsin L gene expression. These cathepsin L-CAT constructs will permit us to localize the p53 responsive element within the cathepsin L gene and to assess whether downstream elements also mediate the response to *ras* .

G 236 INCREASED FREE INTRACELLULAR CALCIUM (Ca_i) AND ANCHORAGE-INDEPENDENT GROWTH: EFFECT OF TUMOR SUPPRESSOR GENES. Jennifer Luzietti*, Cindy Afshari*, J. Carl Barrett*, and Robert W. Tucker*. *Johns Hopkins Oncology Center, Baltimore, MD, and *National Institutes of Science; Environmental Health, Research Triangle Park, NC.

Anchorage-independent growth is an important phenotype of many tumor cells. We previously reported that a novel tumor suppressor gene for fibrosarcomas negatively regulates growth in agar. Nontumorigenic subclones of an immortal Syrian hamster cell line were isolated that either retained (supB+) or lost (supB-) the ability to suppress tumorigenicity of a fibrosarcoma in cell hybrids. Because blockage of early signals (e.g. Ca_i and pH_i increases) have been implicated in the failure of non-neoplastic cells to grow in suspension (anchorage-dependence), we used digital image analysis of Fura 2-loaded SupB+ and SupB- cells to investigate the relationship between Ca_i increases and cell growth in suspension. Combinations (EPI) of growth factors (EGF + PDGF + Insulin) stimulated colony growth of SupB-, but not SupB+, cells suspended in agar. EPI stimulated different Ca_i increases in two paired SupB+/SupB- cell lines: In one pair (10W), changes in Ca_i were similar, but basal Ca_i was elevated in SupB- compared to SupB+ cells; in another pair (DES), the magnitude of Ca_i increases was less in SupB- cells than in SupB+ cells in suspension. Thus, SupB- cells growing in suspension have altered Ca_i signalling, either with higher peak Ca_i increases (10W), or with absence of induced Ca_i increases reminiscent of ras-transformed fibroblasts (DES). Ca_i changes were measured in suspension culture on Hydron (non-adhesion polymer) without any supporting matrix, while colony growth was measured in agar. However, recent measurement of DNA synthesis in 10W cells (+ and -) on Hydron revealed no differences in EPI-stimulated DNA synthesis, in contrast to dramatic differences in agar. Experiments are in progress to determine whether EPI-induced Ca_i transients are different in 10W(-) and 10W(+) cells in agar. Suspended SupB- cells may have decreased dependence on early mitogenic signals (e.g. Ca_i increases), thereby contributing to their anchorage-independent growth in agar in the presence of EPI.

G 237 INVOLVEMENT OF THE TUMOR SUPPRESSOR GENES IN ESOPHAGEAL SQUAMOUS CELL CARCINOMAS, Takashi Wagata, Kanji Ishizaki, Masayuki Imamura, Yutaka Shimada, Mituo Ikenaga and Takayoshi Tobe. First Department of Surgery and Radiation Biology Center, Kyoto University, Kyoto, Japan.

To know whether tumor suppressor genes are involved in the development of esophageal squamous cell carcinomas (ESC), we have analyzed allelic deletion at 23 loci on 18 different chromosomes in 35 ESC tissues by using RFLP markers. Loss of heterozygosity was detected on chromosomes 2,3,6,7,11-14,16-18,21,and 22, while no loss was detected on chromosomes 1,4, and 8-10. Only the loss of chromosome 17p was detected with high frequency(45%) and losses on other chromosomes had frequencies less than 22% which might be caused by random loss of chromosome in the course of tumorigenesis. These results suggest that the loss of 17p might play an important role in the development of ESC, such as inactivation of a tumor suppressor gene. Since the p53 gene is located on 17p and mutations of this gene are observed in variety of tumors, we have supposed that the p53 gene is also changed in ESC. We have analyzed these samples for mutations of the p53 gene by using SSCP analysis. The p53 mutations were detected in 53%(17/32) cases. Most of these mutations of the p53 gene are observed in tumors which showed loss of heterozygosity on chromosome 17p.

G 238 INVOLVEMENT OF RB AND p53 IN TUMORIGENESIS IN A LI-FRAUMENI FAMILY, Sally Warneford, Leah Witton, Megan Townsend, Antony Braithwaite, Luciano Dalla-Pozza, Geoff Symonds, Leukaemia Research and Viral Pathology Unit, Children's Medical Research Foundation, Sydney, Australia, 2050

Li-Fraumeni syndrome is a rare autosomal dominant susceptibility to a variety of cancers including breast, adrenal, brain, muscle and leukemia. Affected individuals develop cancer at a young age and often at multiple primary sites. This laboratory has been conducting a study into the genetic basis of cancer in a particular Li-Fraumeni family. Because of the lack of consistent chromosomal abnormalities in this syndrome, the tumor suppressor genes RB and p53 were selected for study on the basis of their involvement in the genesis of sporadic tumors of the same type as those occurring in Li-Fraumeni families. RB mRNA and protein levels were examined in tumor and normal tissue from two related individuals in this family and were found to be elevated in an adrenocortical carcinoma from a young boy when compared to controls. The RB gene was found not to be rearranged or amplified in this tumor. RB expression was not elevated in the breast carcinoma from the other individual examined, the implication being that the RB over-expression was involved in the progression of the adrenal tumor but not in the family's susceptibility to cancer. When p53 was examined as a candidate susceptibility gene it was found that in affected individuals there was an aberrant larger transcript present in both tumor and constitutional material in addition to the normal-sized transcript. Sequencing revealed a point mutation in a splice donor site in one allele in affected individuals, which could account for the appearance of the larger transcript. The demonstration of a germline splicing mutation in affected individuals from a Li-Fraumeni family provides for a novel mechanism of p53 inactivation not seen previously in other affected families.

G 239 p53 MUTATION AND PRESENCE OF HPV-16 DNA IN HUMAN ORAL SQUAMOUS CARCINOMA CELL LINES, Andrew Yeudall, Kathryn A. Elsegood, Andrea M. Stone, Crispian Scully and Stephen S. Prime, Centre for the Study of Oral Disease, Department of Oral Medicine, Surgery and Pathology, University of Bristol, UK.

Viral and cellular oncogenes and tumour suppressor genes are closely associated with tumour development as a consequence of their vital role in control of cellular proliferation. The molecular events which occur in oral cancer are largely unknown. Previous studies have shown that ras gene mutations are rare in this tumour type; this study examines the presence of HPV-16 and p53 mutation and gene loss in eight newly described oral squamous cell carcinoma lines.

Polymerase chain reaction (PCR) and sequencing of amplified DNAs was used to locate p53 mutations. PCR was used to identify HPV-16 DNA. Eight of eight malignant cell lines harboured HPV-16 early region (LCR-E6-E7) sequences. One cell line (H157) had a single C to T point mutation at the end of exon 8 resulting in the substitution of an arginine residue with a stop codon. Another (H103) had a G to T point mutation in exon 7 which changed a glycine to a cysteine residue. A third (H191) showed complete absence of p53 sequences. Currently we are undertaking further genetic and functional analysis of these cell lines. The results indicate the frequent occurrence of HPV-16 in oral cell lines and that p53 may be a common target for DNA damage during oral carcinogenesis.

Growth Regulation by RAS and GAP; The Retinoblastoma Gene

G 300 INDUCTION OF c-fos FAILS TO INDUCE DNA SYNTHESIS IN SENESCENT HUMAN FIBROBLASTS. Mark.J. Adler*, David W. Rose*+, Greg McCabe*,and James R. Feramisco*+. *Departments of Medicine* and Pharmacology+, and UCSD Cancer Center*, University of California San Diego, La Jolla, CA 9093.

The demonstration that serum does not induce c-fos transcription in senescent fibroblasts has raised the issue of a central role for fos in the G1/S blockade of cellular senescence. Since fos is produced in cycling cells,and required for re-entry of serum starved Go cells into G1, its absence could explain persistent non-proliferative states such as senescence and terminal differentiation. Moreover, evidence for a retinoblastoma control element (RCE) within the fos promoter, suggested that fos repression might mediate the anti-proliferative activity of the retinoblastoma (Rb) gene product. We sought in the present experiments a more direct test of the role of fos by inducing its expression in senescent cells by microinjection of oncogenic ras (T24). While ras induced marked nuclear fos expression and functional AP-1 activity this did not lead to DNA synthesis in senescent fibroblasts. The absence of fos, therefore, does not sufficiently account for senescence, and if RB plays a critical role, its effect is apparently mediated through alternate mechanisms.

G 301 DOWNREGULATION OF WILDTYPE RAS ACTIVITY BY P120-GAP, Nadia Al-Alawi, David W. Rose, Judy L. Meinkoth, Vickie LaMorte, Robin Clark*, George Martin*, Frank McCormick*, and James R. Feramisco, Departments of Pharmacology and Medicine, Univ. of California at San Diego, Cancer Center, La Jolla, CA 92093-0636; * Department of Molecular Biology, Cetus Corporation, Emeryville, CA 94608.

Mutations of cellular ras proteins are frequently associated with several types of human cancers. The GTP-bound forms of ras, members of the GTPase superfamily, are involved in signal transduction pathways. Despite the importance of ras proteins, their mechanisms of action are not fully understood. p120-GAP is a GTPase activating protein which binds ras proteins and stimulates the hydrolysis of GTP bound to wildtype (wt) ras. Previous studies have shown that the number of foci produced by cells transfected with wt ras can be decreased by cotransfection with p120-GAP. Similarly, the transformed morphology of wt ras-transformed cells was reverted to a normal phenotype following transfection with p120-GAP. These results suggest that p120-GAP may act as a negative regulator of wt ras. To directly test this hypothesis, we investigated the effects of p120-GAP on wt ras by microinjection techniques. Microinjection of ras proteins into fibroblasts that contain the reporter gene lac Z under the regulation of AP-1 promoter elements leads to the rapid induction of expression of B-galactosidase. We injected wt ras and p120-GAP proteins into this reporter cell line to assess the role of p120-GAP. As expected, injection of wt ras alone into the reporter cell line induced expression of the lac Z gene. Injection of p120-GAP did not induce expression of this reporter gene. Coinjection of p120-GAP with wt ras abolished the induction of the reporter seen with ras alone. These experiments strongly support the notion that p120-GAP can downregulate wt ras activity. To map the regions of p120-GAP responsible for downregulation we are currently investigating the effects of specific p120-GAP domains on wt ras activity.

G 302 Cloning and analysis of the human tyrosine kinase controlling c-*src* activity, a potential anti-oncogene Juha Partanen, Elina Armstrong, Mathias Bergman, Tomi P. Mäkelä, Harri Hirvonen, Kay Huebner and **Kari Alitalo**

We have cloned the cDNA for a cytoplasmic tyrosine kinase (TK) which contains amino terminal SH2 and SH3 domains and an unusually short carboxy-terminal tail. One distinctive feature distinguishes this TK from all other known members of the TK gene family: it lacks a tyrosyl residue in the position corresponding to the autophosphorylation site in the catalytic domain of the *src* TK (Y416), which is conserved among other known TKs. The novel gene is located in chromosome 15q22-qter and is expressed as mRNAs of approximately 2.5 and 3 kb in several human leukemia cell lines and in a variety of human fetal tissues. Human embryonic thymus and spleen contain similar amounts of the two mRNA forms, whereas predominantly the 3 kb form is found in brain, choroid plexus, kidney and cardiac muscle. Also, K562 leukemia, A549 alveolar carcinoma and Tera-2 teratocarcinoma cell lines express only the 3 kb mRNA, indicating independent regulation of the two mRNA forms. Recent data indicates that this TK is the human homologue of the recently cloned rat CSK TK which phosphorylates c-*src* in the Y527 position. As this site is lost in all oncogenic pp60src proteins and the *crk* and mT oncogenes apparently transform by interference with the phosphorylation of this site, one may speculate that the CSK TK is a candidate anti-oncogene. Experiments to test this hypothesis are underway.

G 303 HA-RAS ONCOGENE ACTIVATION IN MAMMARY GLANDS OF N-METHYL-N-NITROSOUREA-TREATED RATS GENETICALLY RESISTANT TO MAMMARY ADENOCARCINOGENESIS, Michael C. Archer and Shi-Jiang Lu, Department of Medical Biophysics, University of Toronto, Ontario Cancer Institute, 500 Sherbourne St, Toronto, Canada M4X 1K9

A single dose of N-methyl-N-nitrosourea given to sexually immature, female Buf/N rats, produces a high incidence of mammary adenocarcinomas. A large percentage of these tumors contain the Ha-ras oncogene, activated by a G to A transition at the second nucleotide of codon 12. Copenhagen rats, on the other hand, are completely resistant to mammary tumor induction by a number of carcinogens including N-methyl-N-nitrosourea. Here we show, using a sensitive method involving the polymerase chain reaction, that codon 12 Ha-ras mutations occur in the mammary glands of both Buf/N and Copenhagen rats 30 days after N-methyl-N-nitrosourea treatment. These mutations were evenly distributed amongst individual mammary glands, and were present in purified mammary epithelial cells. In Buf/N rats, the fraction of cells containing a mutated Ha-ras allele increased by a factor of 10-100 between 30 and 60 days, whereas in Copenhagen rats, there was no such increase during this time period. We conclude that the resistance of the Copenhagen rat to mammary carcinogenesis is not due to a defect in initiation, but rather appears to be due to the action of a suppressor gene that prevents cells containing a mutated ras allele from undergoing sustained clonal expansion.

G 304 IDENTIFICATION AND CHARACTERIZATION OF HUMAN cDNA SEQUENCES WHICH ARE PROBABLY CONNECTED WITH TUMOR SUPPRESSION, Sergei Arsenian, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-5660

Retinoblastoma (Rb) is a prototype model for the study of recessive oncogenes, the inactivation of retinoblastoma susceptibility gene is correlated with the development of a subset of human neoplasias. After introduction, via retroviral mediated gene transfer, into retinoblastoma, prostate and bladder carcinoma cells, the intact Rb gene suppressed the neoplastic phenotype of the target cells. Using the subtractive cDNA technique we had identified cDNA sequences, which differentially expressed in wild type WERI-Rb-27 retinoblastoma cells compared to the reconstituted cells. The cloned cDNA sequences are in the process of characterization. Their possible role in the reversion of tumorigenic phenotype will be discussed.

G 305 MECHANISM OF NEGATIVE GROWTH CONTROL BY CYCLIC AMP IN A HUMAN PROSTATE CARCINOMA CELL LINE: CYCLIC AMP INDUCES PRODUCTION OF BIOACTIVE TGF-β2 AND UNDERPHOSPHORYLATION OF THE RETINOBLASTOMA PROTEIN, Yung-Jue Bang,[1] Jane B. Trepel,[1] Michael A. O'Reilly, [2] David Danielpour, [2] Won-Ki Kang,[1] Charles E. Myers,[1] and Seong-Jin Kim[2], [1]Clinical Pharmacology Branch, and [2]Laboratory of Chemoprevention, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

While studying the signal transductions that regulate the growth of androgen-independent prostate carcinoma cells, we found that elevation of intracellular cyclic AMP (cAMP) through addition of cAMP analogs or phosphodiesterase inhibitors induces G_1 synchronization and growth arrest in the human androgen-independent prostate carcinoma cell line PC-3. Although it is known that cAMP can inhibit the expression of growth stimulatory genes such as c-*myc* and the transferrin receptor, the regulation of negative growth regulatory peptides or tumor suppressor genes by cAMP is an unexplored area. The most potent negative growth regulatory peptides for most epithelial cells are the transforming growth factor beta (TGF-β) polypeptide family. We found that growth inhibition induced by dibutyryl (db)cAMP was associated with the concentration-dependent production of bioactive TGF-β2 and a marked increase in TGF-β2 messenger RNA. The level of TGF-β1 messenger RNA and TGF-β1 secretion were unchanged. PC-3 growth was inhibited by TGF-β in the same concentration range as that induced by dbcAMP. TGF-β has been shown to regulate the level of phosphorylation of the retinoblastoma gene product RB, and RB has been shown to regulate TGF-β1 gene expression. To assess the role of RB in cAMP-induced growth arrest, we examined the effect of dbcAMP on RB phosphorylation. When confluent, growth inhibited PC-3 cells were released into logarithmic growth in the presence or absence of dbcAMP, dbcAMP-treated cells showed a marked underphosphorylation of the RB protein compared to cells in the absence of dbcAMP, for up to 48 hours after dbcAMP addition. These data demonstrate that in the PC-3 *prostate carcinoma cell line, in which* cAMP is highly growth inhibitory, addition of dbcAMP induces the expression of bioactive TGF-β2, and the underphosphorylation of the RB protein. These data suggest that TGF-β2 and the RB protein can act as transducers of the growth arrest signal initiated by elevation of intracellular cAMP.

G 306 EPINEPHRINE SUPPRESSES RAP1B.GAP ACTIVATED GTPASE ACTIVITY. Kim Bencke Marti and Eduardo G. Lapetina. Division of Cell Biology, Burroughs Wellcome C., Research Triangle Park, N.C. 27709

Platelet lysate from quiescent cells promotes rapid hydrolysis of [γ^{32}P]GTP bound to Rap1B. Various platelet agonists including platelet-activating factor, phorbol 12,13-dibutyrate, α-thrombin, epinephrine, ADP and iloprost which affect platelet metabolism by different signal transduction pathways were used to stimulate intact platelets to study their effects on Rap1B.GAP activated GTPase activity. Only epinephrine was found to dramatically decrease not only the hydrolytic rate, but also the amount of hydrolysis of Rap1BGTP activated by Rap1B.GAP.This effect is dose dependent and occurs rapidly. The suppression of GTPase activity is specific for Rap1B.GAP, in that, Ras.GAP and Rap2B.GAP activated GTPase activity were not affected by epinephrine stimulation. This effect appears to be mediated by the α_2-adrenoceptor, as evidenced by a similar suppression of GTPase activity by stimulating platelets with the synthetic α_2-adrenoceptor agonist, UK14304 (bromoxidine). Furthermore, the selective α_2-adrenoceptor antagonist, yohimbine, blocked the suppression of GTPase activity expressed in epinephrine stimulated cell lysates. No apparent changes in the patterns of protein expression or tyrosine phosphorylation were observed. Although the migration characteristics upon anion exchange chromatography of Rap1B.GAP, as well as Ras.GAP activities were unaffected by epinephrine stimulation, the relative specific activity of Rap1B.GAP was noticeably decreased with 250μM and 500μM epinephrine. These results suggest a possible role for Rap1B and Rap1B.GAP in epinephrine-stimulated signal transduction.

G 307 THE MECHANISM OF P21ras ACTIVATION BY INSULIN J.L. Bos[1], R.H. Medema[1], B.M.Th. Burgering[1], A.M.M. de Vries-Smits[1], G.J. Pronk[1], F. McCormick[2], A.Osterop[3] and J.A. Maassen[3]. [1]Laboratory for Physiological Chemistry, University of Utrecht, Utrecht, The Netherlands; [2]Cetus Corporation, Emeryville, CA.; [3]Department of Medical Biochemistry, Sylvius Laboratory, Leiden, The Netherlands.

Previously, we have suggested a possible involvement of p21ras in the insulin signal transduction pathway in rat fibroblasts. Recently, we obtained firm evidence for this hypothesis by measuring the effect of insulin stimulation on the activation of p21ras in NIH/3T3 cells overexpressing the insulin receptor. A conversion of rasGDP into rasGTP (20 to 70% GTP as percentage of total nucleotides bound) was found within 1 min after insulin stimulation. Using cells overexpressing high levels of EGF- or PDGF-receptors, we were not able to detect a significant shift in the GTP/GDP ratio after stimulation with EGF or PDGF, stressing the specificity of insulin in the activation of p21ras (Burgering et al., EMBO J. 10, 1991, 1103). Using dominant inhibitory mutants of p21ras we have investigated which cellular effect of insulin stimulation is mediated by p21ras. We found that these mutants inhibit insulin-induced activation of the c-fos promoter. We conclude from our results that p21ras is involved in insulin-induced signal transduction, in particular in the induction of gene expression. Our results do not exclude a role of p21ras in other signal transduction pathways.

To study the mechanism of insulin-induced activation of p21ras in more detail, we used the putative phosphatase inhibitor phenylarsine oxide. This compound both inhibited insulin-induced p21ras activation and stimulated insulin-induced phosphorylation of GAP. These and other results suggest a model in which GAP mediates insulin-induced activation of p21ras.

G 308 CHARACTERISATION OF RB BINDING PROTEINS, Rod Bremner, Brenda Gallie and Robert A. Phillips, Department of Immunology and Cancer, Hospital for Sick Children, Toronto, Ontario, Canada, M5G 1X8.

The product of the retinoblastoma tumour suppressor gene plays a critical role in the regulation of a number of promoters. It is assumed that these effects, and their resultant cellular consequences, are mediated by Rb's interaction with other regulatory proteins. By using different parts of Rb fused to glutathione-S-transferase we are determining important interactions in a variety of cellular contexts. Thus, the pattern of Rb binding proteins (a) during differentiation of embryo carcinoma and hematopoietic lineages; (b) during passage through the cell cycle; and (b) before and after transformation of cell cultures with oncogenes will be reported. Differences in the binding patterns in these systems will give insight into critical points of regulation by Rb. To complement this work, we are using the same cell systems to monitor the interactions of Rb binding proteins which have already been identified; namely, c-myc and 2 newly cloned genes RBP-1 and 2.

G 309 Isolation of *rsp-1*, a Novel cDNA Associated with Reversion of v-Ki- *ras* Transformation. L. Zanoni, N. Talbot, R. Bassin, and M.L. Cutler. Laboratory of Tumor Immunology and Biology, National Cancer Institute, Bethesda, Md.

To isolate genes capable of suppressing the v-Ki-*ras* transformed phenotype a vector primed cDNA expression library was constructed using polyadenylated RNA from the *ras* revertant cell line, CHP 9CJ. CHP 9CJ cells were isolated from the v-Ki-*ras* transformant, DT, and contain v-Ki-*ras* RNA and high levels of viral p21, but are phenotypically non transformed. Following transfection of the cDNA library into the transformed DT cell line, and selection in G418, a small percentage of the resulting colonies appeared phenotypically flat. The cDNA recovered from one of these flat transfectants, referred to as *rsp-1*, suppressed growth of DT cells in agar. In addition, *rsp-1* specific RNA is overexpressed in the ras revertant cell line, C11, and underexpressed in *ras* and *mos* transformed NIH3T3 cells in comparison to NIH3T3 cells . The DNA sequence was determined following isolation of a full length *rsp-1* cDNA from an NIH3T3 cell library. Analysis revealed that *rsp-1* is a novel gene with no significant homologies to any other reported sequences at the nucleic acid level. It contains a long open reading frame which, when translated, encodes a 277 amino acid protein. The major features of the protein include a series of eight leucine rich repeats which match the consensus for the leucine repeats in the regulatory region of the yeast adenylyl cyclase and a pair of cyclic AMP dependent protein kinase phosphorylation sites. Moreover, the mouse *rsp-1* cDNA hybridizes equally well to mouse, rat, cat and human DNA at high stringency, suggesting that the *rsp-1* gene is very highly conserved. Our current efforts are aimed at determining the biological activity of the full length cDNA and deletion mutants in both transformed and normal cells in order to elucidate the normal function of this gene.

G 310 CHARACTERIZATION OF A PROTEIN P37 THAT INTERACTS WITH THE RETINOBLASTOMA GENE PRODUCT, Elizabeth Fortunato and Suresh Subramani, Dept. of Biology, University of California San Diego, La Jolla CA 92093

The retinoblastoma gene product (RB) is believed to act as an important cell cycle regulator. The early proteins of several DNA tumor viruses complex with RB. Sequence comparison between the viral proteins within the regions that bind to RB has shown strong similarities. Prompted by the notion that this conserved region may be used by the viruses to mimic a cellular protein with which RB normally interacts, we designed a peptide to the conserved region in the BK virus T antigen sequence and have generated a polyclonal antibody.

The antibody recognizes a primary band with a molecular weight of 37 kd by western blot analysis. This band can be competed to an appreciable extent with peptide conjugated to BSA. Immunofluorescence data indicate that the antibody recognizes a protein or proteins that colocalize with RB to the nucleus during G1, S, and G2 phases of the cell cycle, but localize to the spindle pole during mitosis. Immunoprecipitation data indicates a specific interaction between p37 and RB that can be competed by the presence of a functional SV40 T antigen (in a temperature sensitive cos cell line). The normal cytoplasmic localization of p37 is indicated by immunofluorescence in mutated and RB minus cell lines. Functional studies, as well as the cloning of p37, are underway.

G 311 CLONING, EXPRESSION AND CHROMOSOMAL LOCALIZATION OF A NEW PUTATIVE RECEPTOR-LIKE PROTEIN TYROSINE PHOSPHATASE,

Martijn F.B.G. Gebbink, Ingrid van Etten, Guus Hateboer, Ron Suijkerbuijk, Roderick L. Beijersbergen, Ad Geurts van Kessel and Wouter H. Moolenaar, Division of Cellular Biochemistry, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Protein tyrosine phosphorylation plays a major role in the regulation of eukaryotic cell proliferation and differentiation and is regulated by the activities of both protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). We have isolated a mouse cDNA of 5.7 kb, encoding a new member of the family of receptor-like protein tyrosine phosphatases, termed mRPTPμ. The cDNA predicts a protein of 1432 amino acids (not including signal peptide) with a calculated Mr of about 162 k. In addition, we have cloned the human homolog, hRPTPμ, which shows 98% amino acid identity to mRPTPμ. The RPTPμ protein is composed of a 722 amino acid extracellular region, containing 13 potential N-glycosylation sites, a single transmembrane domain and a 688 amino acid intracellular part containing two tandem repeats homologous to the catalytic domains of other tyrosine phosphatases. The N-terminal extracellular part consists of a 160 amino acid region with no sequence similarities to known proteins followed by one Ig-like domain and four fibronectin type III-like domains. RNA blot analysis reveals a single mRNA transcript, showing highest expression in lung and much lower expression in brain and heart. Transfection of the mRPTPμ cDNA into COS cells results in expression of a protein with an apparent Mr of 195 k, as detected in immunoblots using an antipeptide antibody. The human RPTPμ gene is localized on chromosome 18 band 11p2. To further characterize the role of RPTPμ we have made chimeric constructs containing the extracellular part of the EGFR and the intracellular part of hRPTPμ. Transfection studies using EGF as a ligand are planned.

G 312 Transcription regulation by the retinoblastoma protein. Ullrich Graeven, Nobuo Horikoshi, Meenhard Herlyn and Roberto Weinmann. The Wistar Institute, Philadelphia, PA 19104.

In addition to hereditary human retinoblastoma, deletions or mutations of the retinoblastoma susceptibility gene, which result in a loss or aberrant production of the retinoblastoma protein (pRB), have been detected in several human tumors, including osteosarcoma, breast carcinoma and small cell lung carcinoma. Although the normal pRB has been shown to be phosphorylated in a cell cycle specific fashion and to form complexes with transforming proteins of DNA tumor viruses, like SV40 T, adenovirus E1A and papillomavirus E7, the exact function of this tumor suppressor remains to be determined. Recent results showing that pRB is a component of a complex (E2Fi) that inhibits DNA binding of the transcription factor E2F (Bagchi, Weinmann & Raychauduri 1991,Cell 65:1063-72) and that this inhibition can be competed with purified E1A suggest an important function for pRB in transcription regulation. To study the potential effect of pRB on gene expression, we have co-transfected RB-sense and -antisense expression vectors together with promoter-reporter constructs for E2early, c-fos and RB into several cell lines. Expression of RB-antisense mRNA resulted not only in activation of the heterologous promoters of E2early and c-fos but also in activation of the autologous RB promoter. These results suggest that pRB modulates transcription of cellular and viral genes and that pRB expression might be autoregulated.

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G 313 THE NEUROFIBROMATOSIS TYPE I GENE PRODUCT ENCODES A SIGNAL TRANSDUCTION PROTEIN WHICH CO-LOCALIZES WITH MICROTUBULES, David H. Gutmann, Paula E. Gregory, Deborah L. Wood and Francis S. Collins, The Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI 48109-0650

The gene for von Recklinghausen neurofibromatosis type (*NF1*) was recently identified by positional cloning and found to code for a protein with sequence similarity to a family of GTPase activating proteins (GAPs). In an effort to identify the *NF1* gene product, antibodies were generated against both synthetic peptides and fusion proteins. A panel of rabbit antisera directed against the NF1 protein all identified a unique protein migrating at 250 kDa by immunoprecipitation and immunoblotting. The difference between the predicted molecular size (327 kDa) and the size seen on SDS-polyacrylamide gels does not appear to be due to protein processing, as antibodies directed against the amino terminal, carboxy terminal and various epitopes in between all recognize a 250 kDa protein. This protein, termed *NF1*-GAP-related protein (NF1GRP) to underscore the known relationship between *NF1* and these GAP-related proteins, was detected in a wide variety of cultured cell lines. Immunoblotting of homogenates from adult mouse tissues demonstrated that NF1GRP was expressed in all tissues examined. As was predicted from the amino acid sequence, NF1GRP was detected predominantly in the cytoplasm and is not heavily N- or O- glycosylated. Indirect immunofluorescence confirmed these findings and suggested that NF1GRP was expressed in a fibrillar pattern, reminiscent of cytoskeletal elements. NF1GRP and tubulin appear to co-localize by immunofluorescence and treatments which disrupt microtubules also destroy the NF1GRP fibrillary staining pattern. Rat brain homogenate fractions enriched in microtubules by repeated cycling demonstrate the presence of NF1GRP by immunoblotting. There was no cross-reactivity between the NF1GRP antisera and other microtubule associated proteins (MAP1, MAP2, and tau) as determined by immunofluorescence, immunoprecipitation and immunoblotting. After stimulation with growth factors, there is no tyrosine phosphorylation of NF1GRP, as observed with mammalian GAP, in keeping with the lack of SH2 and SH3 domains in NF1GRP. Changes in NF1GRP subcellular localization were observed with growth factor stimulation, suggesting that NF1GRP, like GAP, participates in signal transduction pathways. The finding that NF1GRP localizes to microtubules underscores its role in signal transduction pathways and may relate to growth factor-mediated changes in the cytoskeleton, as microtubule assembly and disassembly are GTP-dependent processes.

G 314 Transcriptional Repression of E2-containing Promoters by the Murine Retinoblastoma Protein.

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The murine retinoblastoma gene product, p110^{RB1}, has recently been demonstrated to be present in protein complexes which modulate transcription from the E2 element. In order to examine the role of p110^{RB1} as a transcription factor, we have assessed the ability of p110^{RB1} to suppress activity of the adenovirus EIIaE, *c-fos* and *c-myc* promoters in undifferentiated and retinoic acid (RA) induced P19 cells. Similar to previous reports, we consistently observe a 1.5 to 2 fold reduction of *c-fos* promoter activity when co-transfected with wild type p110^{RB1}. However, p110^{RB1} was observed to consistently suppress EIIaE activity 8 fold in differentiated P19 cells. CAT constructs containing 2.0 kb of the *c-myc* promoter, including both initiation sites in exon 1 (P1 and P2), p110^{RB1} were suppressed transcriptional activity 5 to 8 fold by p110^{RB1}. Only 100bp 5' of P2 was sufficient for p110^{RB1} suppression of *c-myc*.

As expected, Large T-binding domains were required for transcriptional repression of the EIIaE promoter. A panel of RB proteins with intact Large T domains but containing deletions in the flanking regions or mutant in p34^{cdc2} consensus sequences were also studied. Although mutation of a single p34^{cdc2} site was observed to prevent the shift in molecular weight of hyper-phosphorylated p110^{RB1} observed in SDS-PAGE gels, and mutation of 8 p34^{cdc2} sites prevented hyper-phosphorylation of p110^{RB1}, strong repression of the EIIaE promoter was retained. These results indicate that repression of promoters by p110^{RB1} is not dependent on the integrity of the regions flanking the Large T-binding domains.

G 315 CELL CYCLE-DEPENDENT EXPRESSION OF THE RETINOBLASTOMA GENE. Tomoko Hashimoto[1)], Jun-ichi Furuyama[1)], Hideki Shindo[2)], Eiichi Tani[2)], Hidekazu Nakabayashi[3)], Taiki Tamaoki[3)], and Rei Takahashi[4)], [1)]Department of Genetics, [2)]Department of Neurosurgery, Hyogo College of Medicine, Nishinomiya, 663, Japan, [3)]Department of Medical Biochemistry, Faculty of Medicine, University of Calgary, Calgary, AB T2N 4N1, Canada, [4)]Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381

The retinoblastoma (RB) gene is inactivated in various human tumor cell lines and RB protein has been considered as one of the key factors which suppress cell growth. However, little is known about the level of expression of the RB gene during cell cycle. In this study, we analyzed the expression of the RB gene and factors that control the expression of the RB gene in relation to cell cycle.

Normal human fibroblasts arrested in a quiescent stage with serum deprivation were stimulated by adding FCS to the medium. Within 1 hr, significant expression of the RB gene was observed as analyzed by dot blotting. At the same time, the RB protein was detected in nuclei by immunostaining, whereas it was not detectable before stimulation by FCS. 1These results suggest that RB gene expression might be controlled by growth factors in an early stage of the G1 phase.

To examine transcriptional control element(s) of the RB gene, we isolated genomic sequences upstream of the RB gene. In CAT assays, 0.8kb 5'-flanking region exhibited promotor activity. However, the CAT expression supported by this sequence was different among cell lines, suggesting that RB gene expression could also be controlled by cell-specific transcription factor(s).

G 316 Characterization of PTPase Monoclonal Antibodies David E Hill[1], Karen A Johnson[1], Arthur Bruskin[1], Sheryl Brown-Shimer[1], Andrea Schiavelli[2], Ray Erikson[2], Norbert Zander[3], Carolyn Dadaby[3], and Deborah Cool[3].

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Phosphotyrosine phosphatases (PTPases) appear to perform a critical role in signal transduction mediated by tyrosine kinases. We have recently shown that PTPase 1B and the T cell PTPase are capable of suppressing oncogenic transformation by tyrosine kinase oncogenes. In order to further characterize the roles of PTPases in cellular processes, we have developed a panel of monoclonal antibodies to both PTPase 1B and the T cell PTPase. Two of these antibodies appear to be specific for the full length forms of either PTPase 1B or T cell PTPase presumably recognizing unique epitopes in each protein. Other antibodies show pan reactivity toward PTPases. The antibodies are capable of immunoprecipitating enzymatically active PTPase. Furthermore, using the antibodies in immunofluorescence experiments suggests that both PTPases are localized to the endoplasmic reticulum. The availability of both monospecific and pan reactive antibodies will facilitate determining the role of PTPases in signal transduction and cell cycle regulation.

G 317 DELETION OF THE 3' REGION OF THE RB GENE WHICH WAS OBSERVED IN SOMATIC CELLS OF AN INHERITED RB PATIENT AND IN A CELL LINE ESTABLISHED FROM THE TUMOR TISSUE, Kanji Ishizaki, Mitsuo Kato, and Masao S. Sasaki, Radiation Biology Center, Kyoto University, Kyoto, 606, Japan.

Many of mutations of the RB gene observed in tumors involve the domains of the RB protein which are required for binding to the SV40 T antigen. However, we recently found a deletion of the 3' region of the RB gene which is outside of the binding domains in an inherited RB patient. The somatic cells are heterozygote of this mutation and the cell line established from the tumor tissue is hemizygous for this mutation. RT-PCR analysis have shown that mRNA in somatic cells dose not contain mutant type suggesting some mechanisms to suppress the expression of a mutant allele. On the contrary, mRNA of the tumor cell is deleted exon 24 and this deletion gave rise a stop codon in exon 25. Since this mutant gene is supposed to produce RB protein with truncation of COOH-terminal, this mutation may be useful to know the function of COOH-region of the RB protein.

G 318 CHARACTERIZATION OF THE CATALYTIC DOMAINS OF HUMAN RECEPTOR-LINKED PROTEIN TYROSINE PHOSPHATASES. Michiyasu Itoh, Michel Streuli, Neil X. Krueger, Alex Y.M. Tsai and Haruo Saito, Division of Tumor Immunology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

The recent identification and characterization of a family of protein tyrosine phosphatases (PTPases) suggests that dephosphorylation as well as phosphorylation of tyrosine residues of proteins plays an essential role in regulating diverse cell activities. Given that hyper-phosphorylation of protein tyrosine residues can cause cell transformation, it is possible that loss of a PTPase function may also be oncogenic. Indeed, the human gene encoding the receptor-linked PTPase, hPTPγ, was mapped to the chromosomal region 3p21, which is frequently deleted in renal cell carcinomas and lung carcinomas. Thus, it seems likely that PTPases are tumor suppressor genes. Human hPTPβ, LCA, and LAR are transmembrane receptor-linked PTPases whose cytoplasmic regions contain either one (HPTPβ) or two (LCA and LAR) domains that are homologous to PTPases. Whereas the membrane-proximal domain 1 has enzymatic activity, the membrane-distal domain 2 of both LCA and LAR has no detectable catalytic activity. In order to understand the physiological role of these receptor-linked PTPases, it is important to characterized the properties of the catalytic domains. Therefore, we expressed the cytoplasmic regions of HPTPβ, LCA, and LAR in *E. coli*, and purified to greater than 90% purity. Modulatory effects of various low molecular weight compounds and homo- and co-polymers of amino acids were examined. Several polypeptides that contain a high proportion of tyrosine were strongly inhibitory to these PTPases. To determine a possible role for the catalytically inert LAR domain 2, the properties of recombinant LAR PTPases containing both domains 1 and 2 (LAR-D1D2) or only the domain 1 (LAR-D1) were compared. In nearly all aspects examined, LAR-D1 and LAR-D1D2 were indistinguishable. However, polycationic polypeptides strongly stimulated the PTPase activity of LAR-D1D2 but not LAR-D1. Thus, basic polypeptides seem to indirectly stimulate the catalytic activity of the domain 1 by interacting with the domain 2, suggesting that the presence in human cells of a basic protein that regulates PTPase activity by interacting with the domain 2.

G 319 MULTIPLE PATHWAYS FOR THE REGULATION OF p21^{ras} IN T LYMPHOCYTES. M. Izquierdo, J.D. Graves, J. Downward and D.A. Cantrell. Lymphocyte Activation Laboratory. I.C.R.F. Lincoln's Inn Fields. London WC2A3PX.

T lymphocyte activation via the antigen receptor complex (TCR) and CD2 antigen results accumulation of p21^{ras} in the active GTP bound state. Stimulation of protein kinase (PKC) can also activate p21^{ras} and we have proposed a model that the TCR regulating p21^{ras} occurs as a consequence of TCR regulation of PKC. The T cell growth factor Interleukin-2 (IL-2) does not activate PKC in T cells and yet IL-2 can induce p21^{ras} activation. These data imply that both PKC and non PKC mediated pathways for activation of p21^{ras} exist in T cells. To test the role of PKC in TCR regulation of p21^{ras} we have used a permeabilized cell system to examine TCR regulation of p21^{ras} under conditions where TCR activation of PKC is blocked i.e. 1) use of a PKC pseudosubstrate inhibitor , 2)use of ionic conditions (0nM Ca2+ in the permeabilisation buffer) that block diacylglycerol production by phospholipase C. TCR stimulation of p21*ras* was much less sensitive to PKC pseudosubstrate inhibition comparing to that exerted by PDBu. On the other hand, when PLC-induced PKC activation was blocked the stimulation of the TCR was still able to activate p21*ras*. The results obtained suggest that there are two main pathways for TCR regulation of p21^{ras} in T cells, one involving PKC activation and one that does not.

G 320 E1A Domains that Bind p105Rb and p300 are Required to Block NGF-induced Differentiation of PC12 Cells. Daniel Kalman+ , J. Michael Bishop+ and Paul H. O'Lague*
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Nerve Growth Factor (NGF) causes PC12 cells to cease division and undergo sympathetic neuron-like differentiation including neurite outgrowth. We are testing whether differentiation and division share overlapping control mechanisms in these cells. To do this we are perturbing the activity of proteins known to participate in cell cycle regulation by introducing the E1A oncogene or its mutant forms via microinjection. E1A acts in part through specific binding domains to titrate putative cell cycle control proteins including, for example, p105Rb (the product of the retinoblastoma susceptibility gene) and p60 Cyclin A, as well as others of unknown function such as p107 and p300 (Whyte et al. 1989; Cell 56:67). Similar to previous results (Maruyama et al.1986; Oncogene 1:361), we find that wild type E1A, detected immunohistochemically, blocks NGF-induced neurite extension. However NGF does cause neurite outgrowth in the presence of E1A mutants known to have greatly reduced binding to either p105Rb or p300. These results suggest that cell cycle regulatory proteins such as p105Rb and p300 might play an overlapping role in the NGF signal transduction pathway. Biochemical experiments with Rb isolated from untreated or NGF-treated PC12 cells using immunoprecipitation techniques suggest that NGF affects Rb phosphorylation. Rb from untreated PC12 cells migrates as a hyper- and under-phosphorylated form on SDS-PAGE (~110kD and 105 kD respectively). However, Rb isolated from NGF-treated cells that are growing neurites migrates only as an underphosphorylated species (~105kD). Similar effects on Rb phosphorylation have been observed with other growth factors that inhibit cell proliferation such as TGF-β (Pietenpol et al 1990; Cell 61:777). Experiments are currently under way to determine cellular mechanisms by which underphosphorylated Rb contributes to NGF-induced morphological differentiation.

G 321 THE RETINOBLASTOMA PROTEIN IS PHOSPHORLYATED BY CDC2 OR A CDC2-RELATED KINASE IN VIVO, Jacqueline Lees, Qianjin Hu, Karen Buchkovich and Ed Harlow, Molecular Oncology, MGH Cancer Center, Building 149, 13th St., Charlestown, MA02129.

Recent work from a number of laboratories has shown that the transforming proteins of small DNA tumor viruses often form protein/protein complexes with the products of tumor suppressor genes. The best studied example of this is the interaction found between the E1A proteins of adenovirus, the large T antigens of polyomavirus or the E7 proteins of papillomavirus and the retinoblastoma gene product, p105-RB. Genetic studies of these three viruses have shown that any mutation that abrogates the binding of p105-RB drammatically reduces the transformation potential of these viruses. This suggests that these virus may stimulate cellular proliferation by binding to, and therefore sequestering, p105-RB in a manner that mimics the loss of p105-RB in naturally occuring retinoblastomas.

Although p105-RB is postulated to act to inhibit cellular proliferation, it continues to be expressed in actively dividing cells. However, a number of studies have shown that p105-RB becomes phosphorylated on transition from G1 to the S phase of the cell cycle, suggesting that the tumor suppressor function of the retinoblastoma protein is regulated by phosphorylation and that the unphosphorylated form of p105-RB is the active repressor. Using a number of monoclonal antibodes we have shown that p105-RB forms a complex with human cdc2. Moreover, cell staging experiments show that there is a direct correlation between the association of this kinase and the timing of p105-RB phosphorylation. Purified cdc2 is also able to phosphorylate p105-RB in vitro, and 2D tryptic maps show that the pattern of phosphorylation is very similar to that found in vivo. By analysing the phosphorylation of pRB peptides we have been able to identify five of these phosphorylation sites, each of which correlates with an in vivo phosphorylation event. In each case sequencing has confirmed that these correspond to the concensus cdc2 phosphorylation site. Taken together these results suggest that either cdc2 or a cdc2-related kinase acts to regulate p105-RB function in vivo.

G 322 EJ-RAS INDUCES MORPHOLOGIC TRANSFORMATION AND GROWTH ARREST IN A HUMAN COLON ADENOMA CELL LINE CONTAINING A MUTANT P53 ALLELE. Sanford Markowitz, Louis Myeroff, Bin Yang, Kay Molkentin, Margaret Lyon, James Willson, Case Western Reserve University, and University Hospitals of Cleveland, Cleveland, OH 44106. This study tests the transforming acitvity in human colon epithelium of combined expression of mutant ras and mutant p53 alleles. Combined expression of mutant ras and mutant p53 alleles transforms primary rat embryo fibroblasts. Roughly half of colon cancers bear combined mutations in both ras and p53 alleles. We have coexpressed mutant ras and mutant p53 alleles by transfecting into a human colon adenoma cell line, VACO-330, a transforming EJ-ras allele. VACO-330, a nontransformed cell line established from a benign human colon polyp, bears one mutant p53 allele (codon 248 val to trp), one wild type p53 allele, and only wild type ras alleles. Control VACO-330 were transfected with an empty expression vector bearing only sequences for G418 resistance. The parent VACO-330 cell line exhibits epithelial morphology, growing as islands of cells with apical polarity and tight junctions, and exhibits neither tumorigenic growth in the nude mouse nor anchorage independent growth in soft agar. Control transfectants 1) form G-418 resistant colonies which grow as islands of epithelial cells with morphology identical to parental VACO-330; and 2) are easily expanded into daughter cell lines. In contrast, G418 resistant colonies bearing the EJ-Ras allele 1) grow as large individual cells lacking cell to cell contacts and 2) cannot be carried past 7 to 8 passages. PCR analyses confirms to presence of EJ-ras in the morphologically altered clones. Coexpression in VACO-330 of EJ-ras and a codon 248 mutant p53 allele induces altered morphology, but the altered cells rapidly growth arrest. We conclude that 1) EJ-ras expression is growth inhibitory to the VACO-330 cell line; 2) Full transformation of the VACO-330 adenoma cell line likely requires genetic events additional to the expression of transforming ras alleles; 3) In colon epithelium, complementation of ras and p53 transforming activities may require interaction of only certain mutant ras and p53 alleles.

G 323 NOT AN ANTI-ONCOGENE? A STRUCTURAL/FUNCTIONAL ANALYSIS OF Krev-1 p21. Mark S. Marshall, Elizabeth A. Eklund and Theodore G. Gabig. Department of Medicine,Hematology/Oncology Section, Indiana University School of Medicine, Indianapolis, Ind. The Krev-1 gene is able to suppress ras-mediated transformation at low frequency in vitro when expressed at high levels. Both the ras and Krev-1 proteins have identical "effector" domains (residues 32-40), which are required for biological activity and for interaction with Ras GTPase Activating Protein (GAP). It has been demonstrated that the differing Ras p21 and Krev-1 p21 "activities" are determined by amino acids 18-60. We have identified five specific amino acid residues flanking the Ras p21 effector domain (N26,H27,D30,E31 and V45) which promote *activation* of the ras target. The substitution of Krev-1 p21 residues 26,27,30,31 and 45 with the corresponding amino acid residues from Ras p21 resulted in a Krev-1 protein which had ras function in both mammalian and yeast biological assays. Substitution of corresponding Krev-1 p21 residues into Ras p21 resulted in biologically defective proteins.

Although structural and functional comparison of Ras p21 vs Krev-1 p21 has yielded significant information, it is conceivable that suppression of the ras-transformed phenotype by overexpression of Krev-1 is not based on a physiological pathway. Krev-1 p21 is physically associated with the oxidase cytochrome of neutrophil NADPH oxidase, suggesting a functional interaction. The neutrophil NADPH oxidase can be reconstituted in vitro and requires a GTP-binding protein for O_2- generation. We have identified the regulatory GTP-binding protein as a small 21 kDa protein. When the 21 kDa protein was immuno-depleted from the reconstitution mix, no O_2- was generated. We were able to restore NADPH oxidase activity in a GTP-dependent manner by adding back Krev-1 protein but not ras protein. These results suggest that a physiological function of Krev-1 p21 is the regulation of NADPH oxidase and not control of Ras p21 function.

G 324 THE HUMAN RETINOBLASTOMA GENE PRODUCT (Rb) DISSOCIATES FROM THE CYCLIN A COMPLEX BY PHOSPHORYLATION IN THE NORMAL CELL CYCLE,

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Rb labeled with [γ-^{32}P]ATP by *in vitro* phosphorylation was detected in the supernatant of the cyclin A immunocomplex after an *in vitro* kinase reaction, but not in the binding fraction. Our result demonstrates that Rb is present in the cyclin A complex and dissociates from it as a consequence of phosphorylation. Dissociation from the cyclin A complex is found only at the G1/S and S phase in the cell cycle. These findings suggest that growth suppressing function of an underphosphorylated form of Rb in G1 is inactivated by dissociation as a consequence of phosphorylation of Rb. Furthermore, when bacterially expressed Rb proteins were used as a substrate for *in vitro* kinase reaction, the domain of Rb necessary to bind SV40 large T or adenovirus E1A was found to be necessary for phosphorylation of Rb by the cyclin A complex.

Recently, several other studies have revealed that the Rb and cyclin A are involved in the cellular transcription factors DRTF and E2F. Furthermore, the association of Rb and these transcription factors is regulated by E1A. E1A is presumed to inactivate the growth suppressing function of Rb by its dissociation from the transcription factor complex.

Our results represent a direct evidence of activation mechanism of the transcription factors by dissociation of transcription suppressing factor, Rb. The G1/S block of cell proliferation by Rb may therefore be liberated by cyclin A in normal cell cycle control.

G 325 ACTIVATION OF $p21^{ras}$ BY TRANSFORMING GROWTH FACTOR β IN EPITHELIAL CELLS, Kathleen M. Mulder and Sheila L. Morris, Department of Pharmacology, Pennsylvania State University, College of Medicine, Hershey, PA 17033

The transforming growth factor β (TGFβ) family of polypeptides are ubiquitously expressed and control a variety of cellular processes by interacting with at least two types of high affinity cell surface receptors. However, the primary signal transduction mechanism of the receptors is unknown. The ras-encoded 21 kDa GTP binding proteins have recently been shown to mediate the effects of other polypeptide growth factors. Here we show that both $TGF\beta_1$ and $TGF\beta_2$ (200 pM) result in a rapid (within 6 min) stimulation of GTP bound to $p21^{ras}$ in TGFβ-sensitive intestinal epithelial cells, but not in TGFβ-resistant cells derived from the same parent cell line (IEC-18). The CCL64 epithelial cell line (extremely sensitive to growth inhibition by TGFβ) displayed a concentration-dependent increase in GTP bound to $p21^{ras}$ by $TGF\beta_1$, and a rapid activation of $p21^{ras}$ by $TGF\beta_2$. The results provide the first evidence for a direct receptor coupling event for TGFβ signal transduction in epithelial cells.

G 326 RB GENE INACTIVATION IN HUMAN BREAST CANCER SPECIMENS AND NUDE MOUSE XENOGRAFTS

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Inactivation of the retinoblastoma susceptibility gene (RB) has been implicated in the development of a number of human malignancies. In order to define more precisely the role that RB gene mutations may play in human breast cancer, we undertook a comprehensive analysis of RB gene structure and expression in resected breast cancer tumor specimens and in human breast cancers propagated as nude mouse xenografts. RB gene structure was studied by Southern analysis, and expression was studied by Northern analysis of the RB transcript and immunohistochemical analysis of the RB protein (p105RB). Structural alterations of RB gene were detected in only 2/109 tumor specimens, but were found in 4/17 nude mouse xenografts. Absence or alteration of the RB transcript was found in 2/49 primary tumor specimens, and in 6/15 nude mouse xenografts. Immunohistochemical analysis of tumor specimens revealed 10/34 (29%) to lack the normal expression of the RB protein. These data confirm that RB gene inactivation occurs in a significant fraction of human breast cancers. Propagation of human breast cancer in nude mice may select for tumors which have RB gene mutations and/or may facilitate the detection of these alterations by molecular techniques.

G 327 EXPRESSION OF RETINOBLASTOMA PROTEIN IS REGULATED IN NORMAL HUMAN TISSUE,

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The retinoblastoma protein (pRB) has been implicated in the control of cell cycle progression and differentiation in a wide variety of cell types. Inactivation of RB gene predisposes to the development of hereditary and sporadic retinoblastoma. RB defects have been observed in cell lines and tumor tissue derived from patients with small cell carcinoma, breast cancer, osteogenic sarcomas and bladder carcinoma, suggesting that loss of RB function results in lack of growth control. We have examined the expression of pRB in normal human tissue using immumohistochemical techniques on frozen tissue sections. pRB is expressed in all tissues examined, however, the expression within certain tissues is limited to specific cell types. In cells of the stratified epithelium from skin, cervix, and esophagus, pRB is barely detectable in the highly proliferative basal layers, but expressed at much higher levels in the differentiating suprabasal layers. In lymph node, pRB is easily detected in the germinal center, composed of proliferating B lymphoctyes, but barely detectable in the surrounding B and T cells. In testes, pRB is restricted to the Sertoli cells, with little or no expression of pRB in the spermatogonia and spermatozoid cells. In thyroid, prostate, brain, liver, adrenal and kidney pRB is expressed in only a subset of cells. Therefore, the regulation of expression of pRB is complex and appears to be dependent upon several factors including the proliferative and developmental state of the cells.

G 328 REGULATION OF SP1, E2F, AND ATF TRANSCRIPTIONAL ACTIVITY BY THE RETINOBLASTOMA ANTI-ONCOGENE PRODUCT,

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The retinoblastoma anti-oncogene product has been shown to positively and negatively regulate transcription from several promoter elements. We have identified a similar motif (RCE) within the TGF-ß1, c-*fos*, c-*myc*, and IGF-II promoters that is responsible for conferring positive activation by Rb. Using several different methodologies, we have demonstrated that the transcription factor Sp1 binds to and activates transcription through the RCE. Furthermore, we have demonstrated directly that Rb can stimulate Sp1 transcription *in vivo* using a vector expressing a GAL4-Sp1 fusion protein and a GAL4 reporter plasmid. Moreover, we have mapped the regions of Rb responsible for conferring stimulation of GAL4-Sp1 activity to the T-antigen/E1a binding pocket as well as to a region in the amino terminus of the protein. In a related set of experiments, we have demonstrated that Rb is able to negatively regulate transcription of the Adenovirus E2 promoter through both an E2F binding site and an ATF binding site. Although the E2 promoter contains two E2F binding sites, only the most proximal binding site is affected by Rb. Analysis of the Rb domains involved in the negative regulation of E2F activity suggests that similar, but not identical regions of Rb, are responsible for negative regulation of E2F and positive regulation of Sp1. The involvement of the ATF site in the regulation of the E2 promoter by Rb has led us to examine the ability of Rb to regulate ATF-1 and ATF-2 transcriptional activity. Results of experiments examining the ability of Rb to regulate ATF-1 and ATF-2 transcriptional activity will be presented. Moreover, results of experiments analyzing the effect of SV40 T-antigen on the regulation of GAL4-Sp1 and GAL4-ATF transcription by Rb also will be presented. The above observations suggest that Rb does not function solely by interacting with and inactivating certain transcription factors, but instead plays a more active role in transcriptional initiation.

G 329 IDENTIFICATION OF AN INSERTION IN EXON FOUR OF THE VON RECKLINGHAUSEN NEUROFIBROMATOSIS GENE, A PUTATIVE TUMOR SUPPRESSOR GENE.

Peter T. Rowley, Barbara A. Kosciolek, and Gary R. Skuse. University of Rochester School of Medicine and Dentistry, Rochester, New York.

von Recklinghausen neurofibromatosis (NF1) is an autosomal dominant disease caused by a single gene and involves a diverse set of manifestations including benign and malignant tumors. In order to determine whether the NF1 gene product has a tumor suppressor function we have tested the two-hit hypothesis using markers which detect restriction fragment length polymorphisms (RFLPs) from the region of chromosome 17 surrounding the NF1 gene. We have previously reported a loss of heterozygosity for those markers in malignant tumors but not in benign neurofibromas (Skuse et al., Genes Chromosomes Cancer 1, 36-41, 1989). This finding suggests that inactivation of the NF1 gene leads to malignant tumor formation, a contention supported by others when sequence homology was found between a region of the NF1 gene and the human GTPase activating protein (GAP) and the yeast inhibitor of ras (IRA) genes (Xu et al., Cell 62, 599-608, 1990). Our findings and the sequence homology suggest that the NF1 gene product plays an important role in the regulation of cell growth. In order to delineate the precise mutations leading to tumor formation in NF1, we have been using a combination of the polymerase chain reaction and single strand conformation polymorphism analysis (PCR-SSCP). Using primers flanking the 193 bp fourth exon of the NF1 gene, we have detected an insertion in a patient with the familial form of NF1. An insertion of approximately 200 bps was found approximately 37 bases from the 5' end and 130 bases from the 3' end of the exon. There appears to be a 26 bp deletion of exon 4 accompanying this insertion. It is likely that this interruption of the NF1 gene led to its inactivation and to the disease manifested in this patient.

G 330 Effect of mutated alleles of RSR1, a gene homologous to Krev-1, on the RAS-mediated growth pathway in Saccharomyces cerevisiae. R. Ruggieri, DNAX Res. Inst., Palo Alto, and CETUS, Emeryville, CA 94608, A. Bender, Indiana Univ., Bloomington, Y.Matsui and Y. Takai, Kobe Univ., Japan, S. Powers, UMDNJ-R. Wood Johnson Med. Sch., J. Pringle, Michigan Univ., Ann Arbor, and K. Matsumoto, Nagoya Univ., Japan.

The Saccharomyces cerevisiae RAS-like gene, RSR1, was originally isolated as a multicopy suppressor of cdc24 and found to be highly homologous to the mammalian Krev-1 gene. We showed that this sequence homology extended to a functional homology because expression of Krev-1 in yeast suppressed the growth defect of cdc24 as RSR1 did. Since Krev-1 is known to suppress transformation by ras, we tested the ability of RSR1 to affect the RAS pathway in Saccharomyces cerevisiae. Overexpression of $RSR1^{val12}$, which contains a mutation analogous to that of mammalian ras^{val12} and yeast $RAS2^{val19}$, suppressed the lethality of a strain lacking both RAS genes through activation of adenylyl cyclase. The interaction of RSR1 with the yeast effector of RAS provides in vivo evidence suggesting that in mammalian cells Krev-1 may revert ras-induced transformation by affecting ras interaction with its effector.

G 331 INFLUENCE OF DE NOVO DNA METHYLATION ON C-HA-RAS EXPRESSION AND GROWTH OF NEOPLASTICALLY TRANSFORMED SYRIAN HAMSTER EMBRYO (SHE) CELLS Dietmar Schiffmann, Rolf Ebert and Reinhard Pechan,Institute of Toxicology, University of Würzburg, 87 Würzburg, Germany

The degree of DNA methylation is inversely correlated with gene expression. In vitro methylation of a number of genes causes inhibition of their expression after transfer into cells. - It is known that Concanavalin A (Con A) is able to activate the de novo methylation activity of the cytosine-5-methyltransferase without affecting the maintenance methylation activity of this enzyme. Moreover, Con A inhibits tumor cell growth in vivo and in vitro. - Con A (2 µg/ml, nonagglutinating dose) induces a 33% growth inhibition after 5 days in a SHE cell line neoplastically transformed by diethylstilbestrol (DES T2). With another cell line, transformed by ethionine, growth inhibition and induction of de novo methylation were observed simultaneously. No change in growth and de novo methylation of normal SHE cells was found (same conditions). In DES T2 cells we observed a 6-fold overexpression of the c-Ha-ras gene (compared to normal SHE). Since it is known that expression of c-Ha-ras is influenced by its methylation status, we have investigated whether Con A mediated de novo methylation reduces c-Ha-ras overexpression. In fact we found a reduction to 2-fold expression (48 h Con A treatment). These results suggest that stimulation of de novo memethylation may reduce oncogene expression and most likely, as a consequence, tumor cell growth. Our findings also provide a possible new approach to influence the malignant phenotype of transformed cells.

G 332 IMMUNOFLUORESCENCE DETECTION OF THE RETINOBLASTOMA PROTEIN IN MOUSE FETUSES Laszlo Szekely, Wei-Qin Jiang; Floriana Bulic-Jakus; Anders Rosen; Nils Ringertz; George Klein and Klas G. Wiman, Department of Tumor Biology and Department of Medical Cell Genetics Karolinska Institute, S-104 01 Stockholm, Sweden

The expression pattern of the retinoblastoma (Rb) protein has been studied at the single cell level in frozen sections of 16-18th days old SCID mouse fetuses by immunofluorescence staining with mouse monoclonal and rabbit polyclonal antibodies, using conventional epifluorescence and confocal laser scanning microscopy. The nuclei of megakaryocytes, hemopoetic islands of the fetal liver, osteo- amelo- and odontoblasts, and skeletal muscle were strongly stained. There was no detectable Rb in the basal cell layers of stratified squamous epithelia, but the differentiating, more superficial layers were positive. Intestinal crypts were negative, while the villi were positive. In the retina the Rb protein was detectable in the inner ganglion layer but not in the outer neuroblastic layer. In the central nervous system the Rb was expressed in neurons and glia cells as well. The nuclei in the collecting tubules of the kidney, in the pancreas and the adrenal cortex showed positive staining. Analysis of the differentiation dependent expression of the Rb protein in relation to the prospective life cycle of the cells in which it appears may pave the way towards an understanding of the strictly tissue specific oncogenic effect of Rb loss in families with hereditary retinoblastoma.

G 333 ACTIVATION OF Ki-ras IN RADIATION-INDUCED PRENEOPLASTIC VARIANTS OF RAT TRACHEAL EPITHELIAL CELLS, David G. Thomassen and Gregory Kelly, Inhalation Toxicology Research Institute, Albuquerque, NM 87185

Specific, transforming point mutations of Ki-ras have been described in proliferative lesions and neoplasms in the lungs of rats exposed to ^{239}Pu by inhalation (Molecular Carcinogenesis 4:43-51, 1991). Forty-six percent of the radiation-induced neoplasms and 27% of the proliferative lesions had point mutations in the 12th codon of Ki-ras. Eighty-eight percent of the observed mutations were G to A transitions in the first position of the 12th codon. No mutations were observed in the 13th or 61st codons. These data suggest that Ki-ras activation is an early lesion associated with the multistage development of radiation-induced pulmonary tumors in the rat. To further characterize the time during pulmonary carcinogenesis when Ki-ras activation may be important, we have examined the 12th, 13th, and 61st codons of Ki-ras in a series of X ray (4 variants) and alpha radiation (9 variants) induced preneoplastic variants of rat tracheal epithelial cells using oligonucleotide mismatch hybridization. In contrast to the results described above for radiation-induced proliferative lesions and tumors, none of 13 radiation-induced variants isolated following selection in vitro had mutations in the first position of the 12th codon of Ki-ras. Some variants may have G to T transversions in the second position of the 12th codon, which will be clarified by DNA sequencing. No mutations were observed in the 13th or 61st codons of Ki-ras in any of the variants. These data suggest (1) that alterations in Ki-ras are not critical events at the earliest detectable stage of carcinogenesis of respiratory epithelial cells, (2) that pathways of carcinogenesis for respiratory cells irradiated and selected in vivo may be different from those for cells irradiated in vivo or in vitro and selected in vitro, or (3) that pathways of carcinogenesis for proximal and peripheral airway cells may be different. (Research was supported by U.S. DOE/OHER under contract No. DE-AC04-76EV01013.)

G 334 DIFFERENTIAL EXPRESSION OF TWO TYPES OF THE NEUROFIBROMATOSIS TYPE 1 (*NF1*) GENE TRANSCRIPTS RELATED TO NEURONAL DIFFERENTIATION, Toru Nishi, Hiroshi Mochizuki, Polly Lee, Athanassios Kyritsis, Kenneth K. Tanabe, Janet Bruner, Victor Levin, and Hideyuki Saya, Department of Neuro-Oncology and Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030

The human *NF1* gene which is responsible for the genesis of neurofibromatosis type 1 has been recently identified. A 360-residue region of the *NF1* product shows significant homology to the catalytic domains of both mammalian GTPase-activating proteins (GAP) and yeast IRA proteins. This GAP-related domain of the *NF1* product (*NF1*-GRD), like the GAP and IRA protein, has been reported to mediate hydrolysis of *RAS*-bound GTP to GDP, resulting in inactivation of *RAS* protein. This result suggested that the *NF1* gene plays a role in the signal transduction pathway by interacting with the *RAS* gene product or *RAS*-like protein and is, thereby, heavily involved in growth and differentiation of cells. In these studies, we identified two different types of the *NF1*-GRD transcripts in both normal and cancer cells. One (type I) had the sequence identical to that previously reported, and the other (type II) contained an additional 63 bp insert that encodes for a region of 21 amino acids in the center of the *NF1*-GRD molecule. The alternative splicing appears to be the most likely mechanism by which the type I and type II *NF1*-GRD transcripts arise. We confirmed that the rat *NF1* gene also has two types of *NF1*-GRD transcripts and the 63 bp insert sequence in type II transcript is identical to the human sequence. The type I transcript was predominantly expressed in undifferentiated neuronal cells, whereas the type II transcript predominated in differentiated cells. We also examined the type I/type II ratio of the *NF1* transcript in brain tumor tissue samples by RNA-PCR method. As consistent with the result of cell line analysis, primitive neuroectodermal tumor tissues predominantly expressed type I transcript. The type I/type II ratio is a potential indicator for assessing the differentiation level of neuroectodermal tumors. In addition, the expression pattern of the two types of *NF1*-GRD transcripts immediately changed, from type I to type II, in a neuroblastoma cell when the neuronal differentiation was induced by retinoic acid treatment. We propose that this evolutionarily conserved alternative splicing might regulate the catalytic activity of the *NF1* product and play an important role in the implementation of a neuroblast differentiation program.

G 335 GROWTH SUPPRESSION BY THE RETINOBLASTOMA (RB) GENE IN OSTEOSARCOMA CELLS IS ABROGATED BY CONSTITUTIVE C-MYC EXPRESSION, Klas G. Wiman, Laszlo Szekely, Gabor Dobos, George Klein, and Joseph Segal, Department of Tumor Biology, The Karolinska Institute, Box 60400, S-104 01 Stockholm, SWEDEN

The retinoblastoma (Rb) gene is inactivated or lost in a variety of human tumors. Introduction of an intact Rb gene into tumor cells lacking a functional Rb gene may antagonize the malignant phenotype. Rb interacts with several cellular proteins, including the E2F transcription factor, suggesting that Rb could function by regulating the expression of other genes. Experimental evidence points to a negative effect of Rb on c-myc and c-fos transcription. These findings prompted us to ask whether Rb inhibits growth by downregulating c-myc. We have transfected OHS osteosarcoma cells that do not express any Rb protein, with plasmids containg Rb and c-myc, alone or in combination. pTKRbneo contains Rb driven by the HSV thymidine kinase promoter, and the neomycin resistance marker. pSVmyc carries the two coding c-myc exons driven by the SV40 early promoter, and is thus devoid of the normal c-myc promoter. Transfection of pTKRbneo alone into OHS cells resulted in complete inhibition of growth. However, when pTKRbneo was cotransfected with pSVmyc, numerous G418 resistant cells were obtained that continued to grow. Cells from one colony, that expressed both Rb and exogeneous c-myc, divided with a prolonged doubling time compared to the parental OHS cells, or cells transfected with pSVmyc and a neomycin resistance plasmid. Soft agar clonability and tumorigenicity in SCID mice were also reduced. Cotransfection of Rb and constitutvely expressed c-myc thus rescues the cells from Rb-mediated growth suppression. The effect falls short of complete reversion of suppression, however, since the cells were phenotypically different from the parental OHS cells. This implies that Rb may inhibit the expression of other genes in addition to c-myc. In conclusion, our results suggest that downregulation of c-myc is required for suppression of cell growth by Rb.

DNA Viral Oncoproteins and Growth Control; Other Growth Inhibitory Peptides and Genes

G 400 TRANSCRIPTIONAL ACTIVATION MEDIATED BY SV40 LARGE T ANTIGEN. James C. Alwine, Maryann C. Gruda, Gwen Gilinger, Mariana Nacht, Henry Chiou and James C. Alwine. Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104.

The transcriptional activation function of T antigen (T Ag) has been most studied using the SV40 late promoter. However, T Ag is a promiscuous activator of many heterologous promoters which may account for the dramatic alterations in cellular gene expression seen during transformation and lytic infection. Our analysis of the transcriptional activation mechanisms mediated by T Ag has shown that the promoter structure necessary for activation is relatively simple. A TATA or initiator element with an upstream binding site for a transcription factor is sufficient. A great deal of variability in elements is allowed which may explain promiscuous activation by T Ag. Our data suggest that the late promoter is an overlapping set of these more simple promoters. Through the study of simple promoters we have found that a late promoter element containing overlapping binding sites for octamer and TEF transcription factors (OCT/TEF region), is a specific upstream region through which T antigen mediates transcriptional activation. Activation is mediated through the TEF sites, while octamer factor binding potentially mediates negative regulation. In the simple promoter context, the OCT/TEF region, plus either a TATA or initiator element, can be transcriptionally activated by T Ag. In the context of the whole late promoter the OCT/TEF region functions as in the simple promoters, showing activation mediated through TEF sites and possible negative regulation through the OCT sites. A monkey cell binding factor with properties similar to HeLa TEF-1 has been identified and is being characterized. The late promoter can be transcriptionally activated in Saos-2 cells which are mutant for both the RB protein and for p53; hence interaction with these proteins does not appear to be essential for transcriptional activation of this promoter.

G 401 Purification and analysis of growth regulating proteins, secreted by a human melanoma cell line R.Apfel, U.Bogdahn, F.Lottspeich, J.Hoppe, C.Behl Department of Neurology, University of Wuerzburg, Department of Biochemistry, MPI Martinsried, Department of Physiological Chemistry, University of Wuerzburg

A human melanoma cell line, HTZ-19, was shown to secrete a tumor-specific growth-inhibiting activity (MIA). The crude activity is correlated to a pattern of 7 proteins in the lower molecular weigth (21 kD-6 kD), detectable in SDS-PAGE and 2D-Gel analysis after a BioGel P-10 gelfiltration chromatography. Further purification by rpHPLC, and amino-terminal sequencing, respectivly CNBr or trypsin cleavage and purification of the resulting peptides, followed by sequencing, resulted in the identification of **Tissue Inhibitor of Metalloproteinases 2** (TIMP-2, 21 kD), **Diacepam Binding Inhibitor** (DBI, (8 kD)), **Beta-2 Microglobulin** (10 kD) and **Ubiquitine** (6 kD).

The main inhibitory activity is related to a single 11 kD protein with unique amino acid sequences of the amino terminus, as well as of the tryptic peptides, sequenced so far.

The secretion of **DBI** and the sensitivity of the cell line (HTZ-19) to **Diacepam** in a dose dependent inhibitory manner let us suggest a growth-regulatory activity of **DBI** *in vitro*.

G 402 LIGAND BINDING TO FCγRIIIA INHIBITS IL-2-INDUCED PROLIFERATION IN HUMAN NATURAL KILLER CELLS

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Interaction of IL-2 with the β chain of the IL-2R, constitutively expressed on NK cells, results in ehancement of NK cell cytotoxicity, c-fos and c-myc mRNA accumulation, production of lymphokines and proliferation. FcγRIII (CD16) ligands induce similar effects, except for the proliferation. IL-2 and FcγR ligands synergize to induce lymphokine production and accumulation of c-fos and c-myc mRNA. We analyzed the effects of simultaneous stimulation of NK cells with mitogens and FcγR ligands on their proliferation. The IL-2 induced proliferation is inhibited under these conditions. However, proliferation induced by Natural Killer cell Stimulatory Factor (NKSF, IL-12) is not. In order to study the role of FcγR-dependent signals on cell proliferation in the abscence of IL-2, Jurkat cells expressing FcγRIII have been produced and studied. Stimulation of either FcγRIII A or B induces activation of the transfected Jurkat cells, as detected by the c-fos mRNA accumulation, independently of whether increased $[Ca^{2+}]_i$ is induced (FcγRIIIA) or not (FcγRIIIB). This is analogous to what is observed in peripheral blood granulocytes and NK cells. Both in FcγRIIIA and in FcγRIIIB-transfected Jurkat cells proliferation is not significantly affected upon FcR ligand binding, unlike what we observed in IL-2 stimulated NK cells. Altogether these data support the hypotheses that 1) the proliferative capability of NK cells is not directly affected by FcγRIII ligand binding, and 2) IL-2-induced proliferation is specifically inhibited in NK cells by FcγR ligands, possibly depending on effects on the IL-2R signalling pathway. This hypotheses are now being investigated.

G 403 SYNERGISTIC EFFECTS OF CHEMOTHERAPY AND ANTI-EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MONOCLONAL ANTIBODIES (MAB). J Baselga, W Miller, L Norton, H Masui, C Cordon-Cardo, MG Kris, and J Mendelsohn. Memorial Sloan-Kettering Cancer Center, New York, NY. U.S.A.

Overexpression of EGFr occurs frequently in non-small cell lung cancer. To determine whether chemotherapy effects the EGFr or its ligand, transforming growth factor-α (TGFα), we conducted experiments with squamous carcinoma cell line A431 that expresses high numbers of EGFr. Treatment of cultured A431 cells with Adriamycin (ADR) at a concentration of 40 nM produces a 5 fold increase of TGFα mRNA. This increase is detected during the first 2 hrs, reaches its peak at 6 hrs, and returns to baseline by 72 hrs. Since this could represent an activation of the TGFα/EGFr loop in response to ADR, we then added anti-EGFr MAb 528 to the ADR treated A431 cells. While ADR alone (20nMx2days) caused a 10% inhibition by day five, t s inhibition increased to 67% when anti-EGFr MAbs was added. In nude mice with established A431 xenografts we tested ADR (10mg/kg), anti-EGFr MAb 528 (1 mg twice weekly), or a combination of both, all given intraperitoneally. While ADR or MAb 528 alone produced transient growth inhibition, all animals treated with ADR plus anti-EGFr MAb had complete regression of their tumors, with no regrowth after 70 days. The animal death rate was also significantly lower with the combination. We conclude that in A431 cells: 1) ADR increases TGFα mRNA; 2) The combination of ADR and anti-EGFr MAb is synergistic in cell culture and xenografts; 3) Trials in humans with combination chemotherapy plus anti-EGFr MAbs are warranted.

G 404 DIRECT INHIBITORY EFFECT OF THE TETRAPEPTIDE ACETYL-N-SER-ASP-LYS-PRO (AcSDKP) ON THE PROLIFERATION OF HUMAN PURIFIED BONE MARROW $CD34^+$ CELLS

, Dominique Bonnet, François M. Lemoine, Claude Baillou, Sabine Pontvert-Delucq, Albert Najman and Martine Guigon, Department of Hematology, Faculté de Médecine St Antoine, Paris, France

We have shown that the synthetic tetrapeptide AcSDKP, an inhibitor of murine CFU-S, reversibly reduces the number and the percentage in DNA synthesis of human marrow CFU-GM and BFU-E. Here, we evaluated the effect of AcSDKP on highly purified hematopoietic stem cells (HSC), when these are stimulated by various combinations of recombinant human growth factors (GFs). Therefore, HSC were sorted by flow cytometry from bone marrow mononuclear cells after staining with CD34 and HLA-DR monoclonal antibodies. The $CD34^{++}$ $HLA\text{-}DR^{high}$ cell fraction was seeded in liquid culture in the presence of optimal concentrations of either 3GFs : interleukin-3 (IL3), Tumor Necrosis Factor alpha and Stem Cell Factor (SCF) or 7GFs : IL-3, IL-1 beta, IL-6, granulocyte-macrophage colony stimulating factor, granulocyte-colony stimulating factor, erythropoietin and SCF ; AcSDKP (10^{-10}M) was added daily for 6 days. Then, cell proliferation was measured by ^{3}H-thymidine incorporation. As compared to control (FCS 5% alone), the addition of 3GFs or 7GFs increased the $CD34^{++}HLA\text{-}DR^{high}$ cell proliferation 30 and 95 fold respectively. Interestingly, under these conditions, whatever the combination of GFs used, the addition of AcSDKP decreased this proliferation by more than 50%. These data indicate that AcSDKP has a direct inhibiting effect on HSC.

G 405 CELL CYCLE-DEPENDENT INDUCTION KINETICS OF CYTOKINE-MEDIATED TUMOR CELL DEATH

Jörg Breder, Jürgen van der Bosch, Stephan Rüller and Max Schlaak, Forschungsinstitut Borstel, 2061 Borstel,FRG

During monocyte/tumor cell(TC)-interaction in serum-free medium TC accumulate in G1 and die due to cytokines including TNF, IFN-α, IFN-β and IL-1, which are released into the supernatant (SU). SU exerts basically growth inhibitory effects and TC responding to it by transit into G0 are protected against induction of death. On the other side, growth promoting signals favouring G1/S-transit increase TC-susceptibility to induction of cell death by SU. In order to unravel the complex interrelationships between stimulatory and inhibitory pathways involved, TC-phases sensitive to signal reception have been discriminated from TC-phases during which the lytic pathway is activated. With synchronous TC-populations it is found that TC in G1 can be induced by SU to die within the same G1-phase. In order to induce measurable rates of cell death a minimal SU-pulse of 3h to 6h duration is neccessary. Such an SU-pulse has to be positioned in G1. It is not effective, if applied in the preceeding cell cycle. Minimal lag periods of about 20 h between SU application and initiaton of the lytic pathway and maximum cell death rates are observed if the signal pulse is applied between 9 and 15 hours following stimulation of G1 cells with fresh growth factor supplemented medium. Glucocorticoids prevent the induction of cell death if applied at least 24 h before induction by SU, suggesting not a direct interference with mechanisms of the lytic pathway but rather transcriptional modulation of the components involved. The fact that rather late in G1 (9 to 15 h after stimulation by growth factors) a maximum of sensitivity for induction of cell death exists, suggests that preparations for entry into S-phase have to be rather advanced before a state permissive of cell death is reached.

G 406 CONSERVED REGION 1 OF ADENOVIRUS 5 E1A ONCOGENE, BUT NOT CONSERVED REGION 2, INDUCES TRANSFORMED PHENOTYPE IN A RODENT CELL LINE, Thierry Buchou, Hans van Dam, Alt Zantema, Frederick L. Hall*, and Alex van der Eb, Department of Medical Biochemistry, Sylvius Laboratory, PO Box 9503, 2300 RA Leiden, The Netherlands, and * Division of Orthopaedic Surgery, Childrens Hospital of Los Angeles, PO Box 54700, Los Angeles, California 90054-0700, USA.

Adenovirus E1A proteins carry two conserved regions (CR1 and CR2) which are required for cooperation with other oncogenes in the transformation of primary rodent cells. Sequences in these regions are essential for the association of E1A protein to cellular regulators of the cell division cycle, including p58-cyclinA and p105-retinoblastoma proteins. However, association experiments have been performed in cellular systems other than those used for transformation assays. In normal rat kidney cell lines expressing different mutant Ad5 E1A genes, we show that CR2-, but not CR1-deletion mutants, can induce a transformed phenotype as manifested by morphological transformation, absence of density-arrest, loss of requirement for serum factors, and resistance to butyrate. No strict correlation between p58-cyclinA and p105-RB bindings to E1A protein, and 5E1A-mediated transformation was observed: CR1- and CR2-mutants both bind significantly smaller amounts of p58 and p105 as compared to wild type E1A. However, total p58 levels are higher in cell lines of transformed phenotype. Although 5E1A proteins are sufficient to trigger transformation in NRK cells, a more pronounced effect is obtained when 5E1B proteins are co-expressed. One of the 5E1B effects, is a sequestering of the p53-tumor supressor protein as a p53/5E1B-55kDa complex into a filamentous cytoplasmic cluster. Interestingly, in 5E1B-55kDa expressing cell lines, we observed p58 not only in the nucleus but also a fraction in the cytoplasmic cluster with p53. The functional relevance of the cytoplasmic localization of p58-cyclinA will be discussed.

G 407 RECOMBINANT HUMAN INTERFERON ALFA-2a SUPPRESSES COLONY FORMATION OF HUMAN MARROW FIBROBLASTS (CFU-F). Barrett H. Childs, Cornel Tomelden, Richard J. O'Reilly and Hugo Castro-Malaspina. Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

Alpha interferon has been shown to inhibit colony formation of human multipotential (CFU-GEMM), burst-forming unit-erythroid (BFU-E) and day 7 and day 14 granulocyte-macrophage (CFU-GM) hematopoietic progenitor cells. To assess the influence of this agent on the proliferation of human marrow fibroblast progenitors (CFU-F), we cultured human marrow cells in the presence of varying doses of alpha interferon. Normal human buffy coat marrow cells, 5 x 10^6/flask, were inoculated in 75 cm^2 tissue culture flasks in alpha medium containing 20% fetal bovine serum. The flasks were incubated at 37°C in a humidified chamber with 5% CO_2. After 24 hours the flasks were washed twice with PBS to remove nonadherent cells. Fresh medium along with varying concentrations (0, 1, 10, 100, 1000, and 10,000 units/cc) of recombinant human alfa-2a (Roche Laboratories, Nutley, NJ) were added to the flasks and incubation was resumed. Flasks were scored for CFU-F after 14 days of culture by Wright-Giemsa staining. Colonies were defined as aggregates of >50 cells with fibroblastoid morphology. Alpha interferon at concentrations of 0, 1, 10, 100, 1000, and 10,000 units/ml inhibited day 14 CFU-F production by a mean (six experiments) of 0, 17.1, 23.8, 41.8, 64.0, and 91.8%, respectively. Monocyte depletion by 2 hour incubation of Ficoll-Hypaque separated marrow cells with monoclonal antibody to CD14 and rabbit complement prior to adherence did not affect the results, ruling out an indirect effect of interferon, via monocytes, on fibroblast progenitors. We conclude that recombinant human interferon alfa-2a suppresses in vitro proliferation of human marrow fibroblast progenitors, similar to its previously described effects on human hematopoietic progenitors. Clinical trials incorporating alpha interferon as treatment for disorders associated with abnormal marrow fibroblast proliferation, such as primary myelofibrosis, may be warranted.

G 408 STUDIES OF MUTANT SV40 LARGE T ANTIGENS REVEAL RELATIONSHIPS AMONG IMMORTALIZATION, TRANSFORMATION, AND BINDING TO RB AND p53. Charles N. Cole, Jiyue Zhu, Philip W. Rice, Lisa Gorsch, and Marina Abate. Department of Biochemistry, Dartmouth Medical School, Hanover, NH 03756 .

We have been characterizing the properties of mutants of SV40 which contain small insertions or deletions within the T antigen gene. We have determined their ability to transform two established cell lines, mouse 10T1/2 cells and rat REF-52 cells, to immortalize primary mouse and rat fibroblasts, and to bind p53 and Rb. The only mutants unable to transform 10T1/2 cells at near wildtype efficiency were those with mutations in the first exon of large T (shared with small t) or one affecting the unique region of small t antigen. Mutations in the Rb binding domain had only a small effect on transformation efficiency. Therefore, neither Rb nor p53 binding appear to be required for 10T1/2 cell transformation. Mutants unable to transform REF-52 cells at near wildtype efficiency included those with mutations in the first exon defective for 10T1/2 transformation, mutations in the Rb binding domain (aa 105-114), and mutations at various sites within the C-terminal half of large T. All mutants within the C-terminal half of large T that were unable to transform REF-52 cells also failed to bind p53. Therefore, binding to Rb and to p53 appear necessary, but not sufficient for REF-52 transformation, and binding of T antigen to an additional cellular protein may be required for transformation of REF-52 cells. Immortalization of primary MEFs required only those portions of large T between aa 345 and 626. All mutants with small deletions or linker insertions within the 345-626 region that failed to immortalize were also unable to bind to p53. Thus, immortalization of primary MEFs co-segregated with p53 binding but did not appear to require either Rb binding or the first exon of large T. This is in contrast to immortalization studies in other laboratories using primary rat embryo fibroblasts, where Rb binding appeared to be required. Experiments are currently in progress to compare immortalization in rat and mouse cells using the same set of mutants.

G 409 LEUKEMIC CELLS (HL-60) CONSTITUTIVELY RELEASE A METALLOPROTEASE WHICH MODULATES BONE MARROW FIBROBLAST EXTRACELLULAR MATRIX TURNOVER AND CELL GROWTH. Klaus H. Dittmann [1], Friedrich Lottspeich [2] and Petro E. Petrides [1,3]

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The extracellular matrix of the bone marrow plays an important role for the development of normal hematopoietic cells. Malignant hematopoiesis in leukemia is accompanied by defective interactions of leukemic cells with this stromal cell derived matrix. In a coculture system of human bone marrow fibroblasts and leukemic cells (HL-60) we have identified a leukemic cell derived proteolytic activity which causes loss of spreaded shape of stromal fibroblasts and inhibition of cell growth. The release of this activity is discontinued after induction of terminal differentiation of HL-60 cells to phenotypically normal monocytic cells. Purification to homogeneity shows this activity to be a type IV collagenase distinct from HL-60 derived gelatinase. SDS-gel electrophoresis reveals a molecular mass of 55 kDa for the enzyme. The protease cleaves peptide bonds between alanine and glycine residues. Although it is unable to degrade purified native collagen type I, it causes collagen degradation by fibroblasts in tissue culture. The degradation occurs in the presence of a fibroblast derived collagenase inhibitor (TIMP). This lack of inhibition may be due to a proteolytic degradation of such an inhibitor, since the metalloprotease described by us is able to degrade recombinant tissue inhibitor of metalloproteases.

N-terminal sequence analysis reveals a homology to plastin, a protein present in transformed fibroblasts, which is probably involved in cytoskeleton remodeling.

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G 410 A NATURALLY OCCURRING CELL SURFACE NEGATIVE REGULATORY SIALOGLYCOPROTEIN AND IT'S SIGNAL TRANSDUCTION. D. J. Enebo, P. J. Moos, H. K. Fattaey, and T. C. Johnson, Division of Biology, Kansas State University, Manhattan, KS 66506.

An 18 kDa cell surface sialoglycopeptide (SGP) released by mild proteolysis and purified from intact bovine cerebral cortex cells, has been shown to be a potent and reversible inhibitor of cell proliferation. The SGP binds to a specific cell surface receptor, arrests and synchronizes a wide variety of exponentially growing cell at the G1/S interphase of the cell cycle and antagonizes the mitogenic activity of epidermal growth factor (EGF), platelet-derived growth factor (PDGF), bombesin and the tumor promoter 12-o-tetradecanoylphorbol-13-acetate (TPA). The early SGP signal opposes Ca^{2+} mobilization and cytosol alkalinization although not the expression of several genes associated with cell proliferation (ie., JE, KC, c-myc, c-fos, c-ras, ornithine decarboxylase, and thymidine kinase). The SGP is shown to abrogate the phosphorylation of the retinoblastoma susceptibility gene product (RB), and the SV40 transformed cell line SVT-2, in which the large T antigen binds RB, is refractory to the biological inhibitory activity of the SGP. A highly specific polyclonal antibody, raised to the SGP, was used to visualize the parental sialoglycoprotein (pSGP) on the cell surface by immunofluorescence. The pSGP has been identified on plasma membranes by Western analysis and purified from bovine cerebral cortex cells. The pSGP has been shown to have a molecular weight of 66,000, a pI of 5.1, a relative biological inhibitory specific activity consistent with that of the SGP, and may potentially play the role of a tumor suppressor.

G 411 SUPPRESSION OF B CELL COLONY FORMATION BY IMMUNOGLOBULIN, Louis A. Fernandez, J. Michael MacSween and Donalda A. Robson, Department of Medicine, Camp Hill Medical Center and Dalhousie University, Halifax, Nova Scotia, Canada B3H 3G2

We have previously reported the existance of feedback suppression of B cell colony formation by mature colony cells with the markers of CD5+ B cells. We therefore investigated the nature of the suppression. Supernatants from either CD5+ or CD5- B cells from mature colonies mediated a similar degree of suppression of new colony formation. In trying to identify candidate mediators of suppression we found that transforming growth factor beta and tumor necrosis factor did not suppress B cell colony formation. We therefore explored the possibility that immunoglobulin might be responsible for feedback suppression. Cells were recovered from B cell colonies and their capacity to produce immunoglobulin tested using reverse hemolytic plaque assays. One million colony cells contained 2130 ± 353 IgG, 447 ± 211 IgA, and 509 ± 184 IgM producing cells. Addition of purified gammaglobulin (Cutter Ltd) resulted in suppression of B cell colony formation in a concentration dependent fashion. These results are consistent with the concept that feedback suppression of B cell colony formation is mediated by immunoglobulin produced by mature colony cells.

G 412 THE 13S PRODUCT, BUT NOT THE 12S, OF THE ADENOVIRUS E1A GENE BLOCKS DIFFERENTIATION AND ,IN COOPERATION WITH OTHER ONCOGENES,INDUCES THE NEOPLASTIC PHENOTYPE OF THE RAT THYROID CELL LINE EPITHELIAL CELL LINE PC Cl 3. Alfredo Fusco*, Maria T. Berlingieri**, Caterina Battaglia*, M. Grieco** and Massimo Santoro**.*) Dipartimento di Medicina Sperimentale e Clinica, Facoltà di Medicina e Chirurgia di Catanzaro, CATANZARO **)Dipartimento di Biologia e Patologia Cellulare e Molecolare, II Facoltà di Medicina e Chirurgia di Napoli, NAPOLI

The PC Cl 3 cell line is a well characterized epithelial thyroid cell line of Fischer rat origin. This cell line has the peculiarity to retain in vitro the typical markers of rat thyroid differentiated markers (thyroglobulin synthesis and secretion, iodide uptake and dependence on TSH for the growth) and does not become always fully malignantly transformed after infection with several murine acute retroviruses, even though the thyroid cells become no longer dependent on TSH for the growth after retroviral infection.

The PC Cl 3 cells have been transfected with the E1A gene of Adenovirus 5. The E1A transfected cells, PC E1A, became partially independent from TSH for the growth and lost the capability to trap iodide and synthesize thyroglobulin, however no typical markers of the neoplastic phenotype appeared. A highly malignant phenotype was achieved after infection with retroviruses carrying the v-raf, v-abl and the polyoma virus middle T oncogenes. In contrast the PC E1A transfected with the E1B gene of Adenovirus are not tumorigenic at all, and those infected with retroviruses carrying oncogenes of the ras family showed a very weak tumorigenic phenotype. All these effects induced by the E1A gene are due to the 13 S E1A product, since the same effects were obtained transfecting the thyroid cells with a vector carrying only the 13S gene, whereas no effect on differentiation and on transformation was achieved after transfection of the PC Cl 3 with a construct coding for the 12S E1A product only.

G 413 **TISSUE SPECIFIC EXPRESSION OF HPV-16 E6 AND E7 ONCOGENES LEADS TO DEVELOPMENTAL DEFECTS AND TUMOR FORMATION IN TRANSGENIC MICE.** **Anne E. Griep[1,2], Renee Herber[3], Jan Lohse-Heideman[2], Saewha Jeon[3], and Paul F. Lambert[3], Department of Anatomy[1], Biotechnology Center[2], and McArdle Laboratory for Cancer Research[3], University of Wisconsin, Madison, WI 53706.**

The human papillomavirus type-16 (HPV-16) is one of the papillomavirus genotypes which is frequently associated with cervical carcinoma. In cervical carcinoma tissue and in cell lines derived from cervical carcinoma, two translational open reading frames of the HPV-16 genome, E6 and E7, are found to be structurally intact and actively transcribed, implicating their gene products as agents in the development of epithelial cancer. The E6 and E7 gene products have been characterized as immortalizing agents on the basis of *in vitro* assays and are also known to interact with tumor suppressor gene products, p53 and retinoblastoma, respectively. To study the specific activities of the E6 and E7 gene products in a differentiating epithelial tissue *in vivo*, we created transgenic mice carrying a chimeric DNA fragment in which the E6 and E7 open reading fames are fused to the transcriptional regulatory signals for the αA crystallin gene. This promoter directs expression of E6 and E7 specifically to the developing ocular lens. All transgenic mice in three independent lineages developed bilateral microphthalmia during embryonic development. Histological examination of lenses from transgenic mice indicated an efficient inhibition of lens cell differentiation and hyperproliferation of undifferentiated lens cells. Lens tumors were detected in these mice, but only rarely and after a long latency. Lens epithelial cells placed in tissue culture from transgenic animals are immortalized, in contrast to the limited lifespan of normal lens epithelial cells. After continued passage, these cells acquired a transformed phenotype, as measured by growth in soft agar and tumor formation in syngeneic mice. E6 and E7 mRNAs were detected specifically in the lens tissue of neonatal and adult transgenic mice. Our results suggest that the primary effect of HPV-16 E6 and E7 gene expression on this epithelial cell type is an inhibition of differentiation and an induction of proliferation. Subsequent tumor formation requires additional events and perhaps is dependent upon the level of E6 and E7 expression. These preneoplastic and neoplastic characteristics may relate to the etiologic role of these genes in cervical carcinoma.

G 414 A DOMINANT MUTATION IN THE WILMS' TUMOR GENE WT1 COLLABORATES WITH E1A TO TRANSFORM PRIMARY KIDNEY CELLS.

Daniel A. Haber, H. Th. Marc Timmers, Jerry Pelletier, and David E. Housman , Center for Cancer Research, Massachusett Institute of Technology, Cambridge, MA 02139 and Massachusetts General Hospital Cancer Center, Charlestown, MA 02129.

The WT1 gene maps to the chromosome 11p13 locus associated with Wilms' tumor and encodes a zinc finger protein whose expression is strictly regulated during normal kidney development. A proportion of Wilms' tumors show mutations and deletions within this gene, consistent with its identification as a potential tumor suppressor gene. However, not all WT1 mutations found in Wilms' tumors are homozygous, as would be expected if the inactivation of both alleles were essential for a malignant phenotype. We have previously described a WT1 mutation in which the third zinc finger domain and alternative splice II have been removed by an in-frame deletion. This sporadic Wilms' tumor (AR) expressed equal levels of wild type and mutated WT1 transcripts, leadingus to suggest that the mutant gene product (WT/AR) might act by a dominant suppressor mechanism. We have now investigated the properties of both wild type WT1 constructs and the mutant WT/AR gene in the baby rat kidney (BRK) transformation assay. The BRK system appears particularly well suited for functional analysis of a Wilms' tumor gene, since the 5 day old rat kidney cells used as recipients are derived from the developmental time when WT1 expression peaks. In this assay, the mutant WT/AR gene was found to collaborate with the adenovirus E1A gene, as demonstrated by growth in soft agar and tumorigenicity in nude mice. The wild type WT1 gene, in all of its alternatively spliced forms, neither suppressed E1A-induced focus formation, nor collaborated with E1A. Our results indicate that an alteration in the DNA binding domain of the WT1 tumor suppressor gene can result in a dominant suppressor mutation, which is capable of collaboration with another nuclear oncogene.

G 415 TRANSFORMATION BY ADENOVIRUS E1A INDUCES HYPERPHOSPHORYLATION OF THE C-JUN TRANSACTIVATION DOMAIN.

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In cells that are transformed by the adenovirus E1A oncogene, the genes encoding the metalloproteases stromelysin and collagenase are strongly repressed. This repression seems to be a specific feature of E1A-induced transformation since several other oncogenes, including c-jun, c-fos, ras and src give rise to an elevated expression of these genes. Our previous work showed that repression of the collagenase promoter by E1A was mediated through the TPA-responsive element, which is the binding site for the transcription factor AP-1, a heterodimer of the oncoproteins c-Jun and c-Fos. The inhibition of AP-1 by E1a was not caused by decreased c-Jun and c-Fos levels. Also, the in vitro binding of AP-1 to its target sequence in the collagenase promoter did not seem to be affected. However, immunoprecipitation studies showed that E1A-transformed human embryonic kidney cells (293 cells) and human embryonic retinoblast cells contain multiple forms of Jun protein with different electrophoretic mobilities while in untransformed cells only the form with the highest mobility is found. Tryptic phosphopeptide mapping of these proteins revealed that also the additional bands were c-Jun and that in E1A-transformed cells the c-Jun protein is phosphorylated to a higher extent than c-Jun from untransformed cells. Phosphorylated tryptic peptides are largely from the N-terminal transactivation domain of the protein. This E1A-induced hyperphosphorylation of the c-Jun transactivation domain may hamper its transcriptional activation function.

G 416 BIOCHEMICAL AND BIOLOGICAL DIFFERENCES BETWEEN E7 ONCOPROTEINS OF THE HIGH AND LOW RISK HPV TYPES, Donald V. Heck, Carole L. Yee, Peter M. Howley, and Karl Münger Laboratory of Tumor Virus Biology, National Cancer Institute Bethesda, MD 20982

The E7 proteins of the "high risk" HPVs differ from the E7 proteins of the "low risk" HPVs in a number of biological and biochemical properties. Differences which have been mapped to the amino terminal half of E7 include aberrant migration on SDS-polyacrylamide gels, cooperation with a *ras* oncogene to transform primary baby rat kidney cells (BRK assay), high affinity binding of the retinoblastoma tumor suppressor gene product (pRB), and abrogation of TGFß mediated repression of c-*myc* expression. In addition, the amino terminal half of the E7 proteins is a substrate for phosphorylation by casein kinase (CK) II at serines 31 and 32. In this study, a series of chimeric HPV-16/HPV-6 E7 proteins was synthesized. All of these contain the carboxy terminus of HPV-16 E7. The amino termini contain combinations between HPV-16 E7 and HPV-6 E7 of the following regions: the extreme amino terminus, which shares homology with conserved region I of the adenovirus E1A proteins; the pRB binding domain; and the CKII phosphorylation site. Also presented are two E7 point mutations which decrease or increase the pRB binding affinity of HPV-16 E7 and HPV-6 E7, respectively. Finally, three HPV-16 E7 phosphorylation mutants were synthesized by exchanging for serines 31 and 32 two cysteine, two aspartic acid, or two alanine residues. The biological and biochemical activities of these recombinant E7 proteins are presented.

G 417 A YEAST SYSTEM TO STUDY THE INTERACTION BETWEEN HUMAN PAPILLOMAVIRUS TYPE 16 E7 AND THE RETINOBLASTOMA (Rb) GENE PRODUCTS. D.L. Horner-Nielsen and J.S. Logan. DNX, Inc. 303B College Road East, NJ 08540

Human papillomaviruses (HPV), type 16 and 18 are associated with human cancer, particularly cervical carcinomas. The regions thought to be responsible for the transforming properties of HPV are localized to two open reading frames designated E6 and E7. The HPV E7 and E6 gene products interact with cellular tumor suppressor genes, the retinoblastoma (Rb) and p53 gene products, respectively. It is likely that these interactions render the Rb and p53 gene products inactive in their normal roles of cell cycle regulation. To define the sites of interaction between HPV16 E7 and the human Rb gene, we have a set up a system in yeast which will allow a genetic evaluation of the interactions. The system utilizes a transactivator protein, HPV E2, which regulates the synthesis of the β-galactosidase gene. The HPV E2 gene contains two domains, one for DNA binding and one for transactivation. The first of the two constructs fuses the human Rb gene to the DNA binding domain of HPV E2. The second fuses the E2 transcriptional activation region to the HPV E7 protein. Either construct expressed alone with a reporter plasmid in yeast yields no β-galactosidase. However, when the two constructs are expressed together β-galactosidase is expressed and the yeast are blue. This occurs when the binding of HPV E7 to human Rb brings the transactivator domain of the E2 protein into the vicinity of the promoter to which the DNA binding domain is bound. Therefore, the color of the yeast colonies indicates interactions between HPV E7 and human Rb. In the case of a mutant in HPV E7, which no longer binds human Rb, the yeast will be white. By setting up an assay in the appropriate manner, revertants of mutant HPV E7 proteins will be the result of compensating mutations within human Rb and thus, should define the direct interaction site between HPV E7 and human Rb. By using the advantages of yeast genetics, we can define the interaction site of HPV E7 on human Rb. This site is likely to be important in normal human Rb function.

G 418 CELL CYCLE CONTROL BY THE RETINOBLASTOMA GENE IN QUIESCENT AND SENESCENT HUMAN EMBRYO FIBROBLASTS: DEPENDENCE ON DIFFERENTIATION STATE, Kazuichi Sakamoto, Tazuko H. Howard, Claire T. Ranjan, and Bruce H. Howard, Laboratory of Molecular Growth Regulation, NICHD, NIH, Bethesda, MD 20892

A novel gene transfer approach was used to investigate whether the retinoblastoma (Rb)-binding domain of simian virus 40 (SV40) T antigen is required for optimal T antigen-mediated stimulation of DNA synthesis in quiescent or senescent human embryo fibroblasts (HEF). In senescent cells, comparison between wild type T antigen and a mutant defective for Rb binding (107Glu→Lys) revealed the latter to have ≈4-fold lower mitogenic activity. This finding supports a previous study which reported that Rb hypophoshorylation correlates with senescence in HEF (Stein, et al. (1990) *Science* 249, 666). In contrast, when wild type and Rb^- T antigen mitogenic activities were compared in serum-starved quiescent cells, no reproducible difference (≤2-fold) was observed. Interestingly, quiescent HEF could be rendered sensitive to the activity of Rb by treatment with the differentiating agent sodium butyrate: thus, in HEF maintained for 48 hrs in low serum medium containing this differentiating agent, a large (≈15-fold) difference between wild type and Rb^- T antigen activities was evident. These results indicate that Rb (or another protein that binds T antigen via its Rb-complementary domain) is a major determinant in the growth arrest state characteristic of senescence. The apparent difference between the magnitude of Rb control in quiescence vs. senescence is likely to reside in post-translational modification(s), since the quantity of Rb protein is similar in young and old HEF (Shigeoka and Yang (1991) *Mech Ageing Res* 57, 63-70). These results further imply that the role of Rb in control of the cell cycle is dependent both on differentiation state and the mechanism of G1 phase arrest.

G 419 Modulation of the Intracellular Status and Transcriptional Activity of the human E2F protein by viral oncoproteins P. Jansen-Dürr, Projektgruppe Angewandte Tumorvirologie; Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 506; D-6900 Heidelberg/FRG.

We are interested in the modulation of the transcriptional activity of cellular genes by viral transforming proteins. In particular we study the effects exerted by the E7 gene products of human papillomaviruses on a subset of cellular genes, the expression of which is correlated with increased cell proliferation. Such studies should provide a biochemical basis for the process of immortalization and transformation of mammalian cells by viral oncoproteins.

It has been shown by several laboratories that E2F can form complexes with pRB as well as with cyclin A.We show that binding of E2F to RB is abolished in HPV 16 immortalized keratinocytes and in cell lines derived from HPV-positive cervix carcinomas. In keratinocytes which became spontaneously immortalized in the absence of HPV DNA, the interaction of pRB with E2F is preserved. These findings are consistent with a role for pRB in repressing E2F activity.

It has been published that cyclin A is removed from E2F by viral oncoproteins like E1a. In contrast to these findings, we report that E2F/cyclin A-complexes are stable in cell lines expressing high doses of E7. In vitro binding assays confirm that cyclin A remains attached to E2F under conditions where the E2F/pRB-complex is destroyed. These results point to a possible positive role of cyclin A in the process of oncogene-mediated activation of E2F. This question has been addressed more directly in a separate set of experiments involving cyclin A protein and cDNA.These results will be discussed.

G 420 Schwannoma-derived growth factor (SDGF) promotes the neuronal differentiation of PC12 cells. Hideo Kimura and David Schubert, The Salk Institute, P.O. Box 85800, San Diego, CA 92186-5800

Schwannoma-derived growth factor (SDGF) was initially isolated from schwannoma cells as a mitogen for glial cells and fibroblasts. The present data show that SDGF causes the morphological and molecular differentiation of rat PC12 cells in a manner similar to, but distinguishable from, NGF. SDGF induced changes include neurite outgrowth and the induction of the mRNAs for GAP-43 and transin, proteins which are highly expressed in axons. In addition, both SDGF and NGF induce the transcription factor, NGFI-A. The time course of the response to SDGF is similar to that for NGF. GAP-43 mRNA induction by both SDGF and NGF is inhibited by dexamethasone, but dexamethasone has no effect on NGFI-A mRNA synthesis. These observations show that SDGF has a differentiation promoting effect on PC12 cells in addition to its mitogenic activity on glial cells and fibroblasts.

G 421 ISOLATION OF A GROWTH INHIBITOR FROM HUMAN FIBROBLASTS, A. Macieira-Coelho, A. Söderberg and A. Wasteson, Department of Cell Biology, Faculty of Health Sciences, 581 85 Linköping, Sweden.

We isolated a novel glycopeptide from cultivated human fibroblasts in resting phase. The molecule was purified by its molecular mass, electric charge and affinity chromatography. The molecular weight is around 1,200 Daltons. It was found to be a growth inhibitor. Its synthesis decreases during the early stages of the G1 period after adding growth factors to resting phase cultures. The synthesis remains steady during the S period and increases progressively back to resting phase levels during the G2 period. The molecular weight of this glycopeptide and the kinetics of its synthesis in relation to the division cycle, are different from that of all growth regulators described so far. The reduced synthesis of this inhibitor induced by growth factors, seems to be one of the requirements for the initiation of the division cycle by normal human fibroblasts.

G 422 NEGATIVE GROWTH REGULATION INDUCED THROUGH MODULATION OF CELL SURFACE RECEPTORS, Raphael J. Mannino, Department of Laboratory Medicine and Pathology, UMDNJ-New Jersey Medical School, Newark, N.J. 07103-2714

A wide variety of transformed cells can be inhibited by succinylated concanavalin A, (succinyl-conA), a non-toxic and non-agglutinating derivative of the Jack bean lectin concanavalin A. Cells inhibited by succinyl-conA accumulate in the G1 phase of the cell cycle, analogous to contact-inhibited normal cells. They re-enter the cell cycle upon the addition of α methyl mannoside, a con A hapten sugar. The density at which growth ceases is inversely proportional to the concentration of succinyl-conA in the medium. Cultures inhibited by succinyl-con A become quiescent at sub-confluent densities, accumulating in small patches, leaving large areas of the substratum devoid of cells. This is not due to a decrease in interaction with the substratum, but rather an increase in lateral cell to cell adhesion. Scanning electron microscopy indicates a greater number of "microvilli-like" structures connecting cells in the presence of succinyl-conA than are seen in control cell cultures. This is a specific phenomenon, as other molecules which cause increased cellular adhesion do not cause growth arrest. While many structural and biochemical characteristics of the cell which are not causally related to transformation remain the same upon succinyl-conA treatment, several changes at the molecular level which have potential functional importance have been noted. Succinyl-conA induced growth arrest is characterized by reduced SV40 T antigen and p53 messenger RNA and protein levels. In contrast, mRNA for the GAS-1 gene, which is associated with density-dependent growth arrest of 3T3 cells, is increased. Upon release from succinyl-conA growth arrest by treatment with α methyl-mannoside, levels of SV40 T antigens and P53 rapidly rise, overshooting control levels at 1 hr. Our current working hypothesis is that succinyl-conA interacts with cell surface receptors resulting in increased cell-cell contacts which stimulate an intracellular negative growth regulating pathway.

G 423 **The Role of PDGF in Myelination of the Rat Optic Nerve: Further Evidence for Oligodendrocyte Formation via a Constitutive Developmental Pathway.** **Huseyin Mehmet and Martin Raff, Biology Department, Medawar Building, University College London, Gower Street, London WC1E 6BT, U.K.**

Oligodendrocytes, the major myelin-forming cells in the CNS, arise on a precise developmental schedule from a bipotential precursor, termed the oligodendrocyte-type-2 astrocyte (O-2A) progenitor cell. Current evidence indicates that oligodendrocytes are formed by default when O-2A progenitors are deprived of environmental signals. Under normal conditions each progenitor cell undergoes a limited number of divisions before it differentiates into a non-dividing oligodendrocyte. It has been demonstrated that the mitogen responsible for the proliferation of O-2A progenitor cells is platelet-derived growth factor (PDGF). It appears that an intrinsic 'clock' in the progenitor cell limits the number of times it can divide in response to PDGF. At this point the cell drops out of the division cycle and, consequently, differentiates into an oligodendrocyte.

Since the number and binding capacity of PDGF receptors is initially conserved on newly-formed oligodendrocytes, it is likely that a block in a post-receptor signalling step causes the eventual loss of the mitogenic response to PDGF in O-2A progenitor cells. In order to address this point we have used the growth inhibitor amiloride to block the mitogenic response of O-2A progenitor cells treated with PDGF. We found that amiloride inhibited DNA synthesis in PDGF-treated progenitor cells. Furthermore, in parallel cultures the proportion of oligodendrocytes increased markedly in the presence of amiloride. These results indicate that inhibiting cell division in O-2A progenitors, normally responsive to PDGF, is sufficient to trigger differentiation and also provide further evidence that oligodendrocytes are the product of a constitutive developmental pathway.

G 424 **GROWTH INHIBITION IN HUMAN LEUKEMIC CELL LINES BY A tRNA-MEDIATED MECHANISM.** CJ Morgan, GM Siravo-Sagraves, BT French, RW Trewyn, Departments of Surgery and Medical Biochemistry, The Ohio State University, Columbus OH 43210.

The development of resistance in patients with acute lymphocytic leukemia to the chemotherapeutic agents 6-mercaptopurine (6MP) or 6-thioguanine (6TG) is often associated with the generation of HGPRT-deficient lymphocytes, lacking the purine salvage pathway. In human leukemic cell lines made HGPRT-deficient by selection with 6MP, the cytotoxicity of 6TG is reduced, but it is still able to inhibit cell proliferation and induce differentiation, putatively by a tRNA-mediated mechanism. In normal mammalian cells, guanine in the anticodon wobble position of tRNAs for aspartate, asparagine, histidine and tyrosine is replaced enzymatically with the 7-deazaguanine analog queuine. Exogenous 6-thioguanine can be incorporated into tRNA in place of queuine, and this anticodon alteration has been implicated in the differentiation of HGPRT-deficient HL-60 cells (Kretz etal., Mol. Cell. Biol. 7:3613-3619, 1987; French etal., PNAS 88:370-374, 1991). Treatment with 6TG inhibits growth and induces differentiation of the human HGPRT-deficient leukemic cell lines HL-60, CEM and U937 along the granulocytic, lymphocytic and monocytic pathways, respectively. In HL-60 cells, 6TG induction of differentiation is associated with early down-regulation of c-myc mRNA expression. Addition of the tRNA base queuine prevents growth inhibition, such that differentiation is delayed until cultures reach confluence, but does not affect the early down-regulation of c-myc induced in HL-60 cells. These observations suggest that 6TG-induced growth inhibition is a tRNA-mediated process. In ongoing studies, the role of the cytokines TGFβ, TNF, lymphotoxin and IFNγ in this process is being investigated.

G 425 OPTIMIZATION OF INTERFERON PRODUCTION FROM HUMAN PERIPHERAL BLOOD LEUKOCYTES, Manisha Padhye, Abbas Rashidbaigi, Mei-June Liao, and Douglas Testa, Interferon Sciences, Inc. 783 Jersey Avenue, New Brunswick, N.J. 08901

Human leukocyte interferons (IFN-alpha) are a family of more than 15 proteins with anti-viral, antigrowth and immunoregulatory activities, and have been demonstrated to have important medical applications. Large scale interferon production is required for clinical trials, and for those patients who are under interferon therapy. Alpha interferons are produced by inducing human leukocyte suspensions with Sendai virus. Methods previously reported were not efficient, and therefore large scale production of natural interferons from leukocytes was an expensive multi-step procedure. We have made attempts to optimize the different steps used in the production of natural alpha interferon. This could make the process more efficient and less expensive. We show that the major factors which cause significant variation in the amounts of interferon produced from peripheral blood leukocytes are the source of leukocytes, density of cells used during induction, and the Sendai virus itself. Composition of the media plays a very significant role in affecting the alpha interferon yeilds. We also show that kinetic factors for priming cells, storing leukocytes for various times, and methods for challenging cells with virus, all have important effects on the amount of alpha interferons produced.

G 426 TRANSFORMING GROWTH FACTOR-α FORMS AN AUTOCRINE GROWTH INHIBITORY FEEDBACK LOOP IN PITUITARY CELLS THAT IS UNDER ESTROGEN REGULATION. John S. Ramsdell, Debbi A. Paquette and Eric L. Finley, Department of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425.

Transforming growth factor-α (TGF-α) is a polypeptide factor expressed by prolactin-secreting cells of the anterior pituitary (AP) gland. Using dual label immunofluorescence of enzymatically dispersed rat AP cells, we find that TGF-α and its receptor are expressed in the same cells, suggestive of an autocrine mechanism. To evalulate the use of a clonal cell line for regulatory studies, we next examined whether TGF-α is also expressed GH_4 cells, a rat pituitary tumor cell line that secretes prolactin. Immunostaining of western transfers of extracts of GH_4 tumors, identified TGF-α as a 15-18 kDa form and to a lesser extent, a 24 kDa transmembrane precursor. No evidence of the fully processed 6 kDa form was evident in GH_4 tumor or rat AP gland extracts. A growth regulatory action of TGF-α was assessed by cell cycle distribution using flow cytometry of bromodeoxyuridine- and DNA-labeled cells. TGF-α (10 nM) decreased the percentage of S-phase cells by 74 ± 3 at 18 h and caused a proportional increase in G_0-G_1 phase cells. This indicates that TGF-α has a cytostatic action to block G_1 cells from entering S-phase. We next examined regulation of TGF-α *in vitro* and *in vivo*. Treatment of GH_4 cells for 48 h with 10 nM EGF (which binds the same receptor as TGF-α, yet does not crossreact with TGF-α antisera) caused a 2-3 fold increase in TGF-α expression as determined by ELISA. Another growth inhibitory agent for GH_4 cells, 12-O-tetradecanoylphorbol-13-acetate, caused a smaller increase in TGF-α expression and yet, given together with EGF, caused a marked increase. The effectiveness of these agents (EGF+TPA>EGF>TPA) to enhance TGF-α expression matches their effectiveness to inhibit GH_4 cell proliferation and suggests that TGF-α expression may be a common mediator for growth inhibitory agents in these cells. Rats bearing GH_4 tumors and treated for 10 days with implants containing the synthetic estrogen, diethylstilbestrol, showed a substantial increase in TGF-α immunoreactivity as judged by immunostaining of paraffin sections and by western analysis of tumor extracts. Taken together, these findings demonstrate that TGF-α forms a positive autocrine feedback loop that inhibits proliferation of GH_4 pituitary tumor cells and is under estrogen regulation *in vivo*. These findings are of importance because they have identified an estrogen regulated autocrine pathway; one that may serve to shut off estrogen induced proliferation.

G 427 RESTORATION OF DENSITY DEPENDENT GROWTH CONTROL IN TUMOR CELLS BY MONOCYTES SIGNALS

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It is shown in a serum-free,hormone supplemented system with human tumor cells (TC), that during monocyte (MO)/tumor cell interactions signals are generated, which restore the principles of density dependent inhibition of growth in tumor cell populations. Tumor cells, which fail to comply with these principles are induced to die. These signals can be detected in supernatant (SU) of MO/TC- interaction cultures and consist of several cytokines including TNF, IL-1, IFN-a and IFN-ß each of which by itself is not able to mimic these effects. TC continuously exposed to SU for several days reach a stationary cell density, which is approached from lower cell densities by attenuated growth rates and from higher cell densities by cell loss due to cell death. This cell death is an active process in target cells involving proteinbiosynthesis and tumor cell derived signals. The former is shown by cycloheximide, which can prevent SU induced cell death and the latter is shown by TC-conditioned medium which causes increased TC-death rates. TC-contributions to SU-induced cell death are also demonstrated in co-culture experiments involving SU-pretreated TC and untreated TC. In such co-cultures - depending on the ratio of the two cell types and on overall cell density - growth inhibitory effects and induction of TC-death can be observed. These effects are due to the SU-pretreated TC-population, which causes the overall population to adjust its cell density in a density dependent manner reminiscent of normal cell populations like mouse 3T3 cells, suggesting that SU-pretreatment has led to a restoration of density dependent growth control in previously unrestricted growing TC.

G 428 Does down-regulation of elongation factor 2 kinase mediate the antimitogenic effect of Nerve Growth Factor? Alexey G. Ryazanov[2,4] Victor S. Prisyazhnoy[2], Karine Kindbeiter[3], Jean-Jacques Diaz[3], Jean-Jacques Madjar[3], Haya Abdelmajid[1] and Brian B. Rudkin[1]. [1]Laboratoire de Biologie Moleculaire et Cellulaire, UMR 49 CNRS/Ecole Normale Supérieure de Lyon, 69364 Lyon Cedex 07, France. [2]Institute of Protein Research of the Academy of Sciences of the USSR, Pushchino, Moscow Region, USSR; [3]Faculté de Médicine Alexis Carel, Lyon France, [4]Rutgers University, Campus at Newark, Newark, NJ USA. Elongation factor 2 (eEF-2), a 100 kd protein which catalyses ribosomal translocation, is the substrate for Ca2+/calmodulin-dependent eEF-2 kinase. Phosphorylation of eEF-2 by eEF-2 kinase makes it inactive in translation (reviewed in Ryazanov A.G. and Spirin A.S. (1990) New Biol. **2**, 843-850). During the cell cycle eEF-2 becomes phosphorylated only at mitosis (Celis J.E. et al. (1990) Proc. Natl. Acad. Sci. USA **87**, 4231-4235), i.e. when there is transient increase of Ca2+ concentration and decrease in the rate of protein synthesis. It was suggested that inhibition of protein synthesis during mitosis is important for degradation of short-lived proteins. According to this idea, in situations where cells are induced to exit from the cell cycle (*e;g;* during differentiation) there should be mechanisms for preventing eEF-2 phosphorylation and the resulting inhibition of protein synthesis during mitosis. Treatment of the rat pheochromocytoma cell line PC-12 with nerve growth factor (NGF) has been shown to result in the stimulation of neurite outgrowth, accompanied by the cessation of cell division. Flow cytometric analysis has indicated that the cells so treated accumulate in a G1-like state of the cell cycle. The mechanism by which this antimitogenic effect of NGF occurs remains to be elucidated. An early effect of NGF treatment on PC-12 cells is the down-regulation of the eEF-2 kinase and it was suggested that this down-regulation serves to prevent phosphorylation of eEF-2 and the subsequent inhibition of protein synthesis during mitosis - conditions which could facilitate arrest in a G1-like state (Rudkin B.B. et al. (1989) EMBO J. **8**, 3319-3325). Here we report results supporting this hypothesis. eEF2 kinase activity, the degree of eEF-2 phosphorylation and the rate of protein synthesis were monitored in PC12 cultures enriched in cells at different phases of the cell cycle. We found that : 1) eEF-2 becomes maximally phosphorylated at mitosis, 2) NGF treatment decreases phosphorylation of eEF-2 at mitosis, 3) Protein synthesis rate during mitosis was significantly higher in NGF treated cells than in control cells 4) *in vitro* eEF2 phosphorylating activity of extracts varies during the the cell cycle eliciting a maximum during the M phase. It appears, therefore, that previous results describing the effect of NGF addition on kinase activity in asynchronous cultures reflects the response of only those cells undergoing mitosis. The fact that NGF treatment prevents the normally-occuring phosphorylation of eEF2 during the M phase, suggests that it may be essential in preparing the cells for arrest in G1.

G 429 MECHANISMS OF CELL CYCLE ARREST IN G_1 BY α-INTERFERON. N.S.B. Thomas, A. Bybee and L. C. Burke, Dept. of Haematology, University College and Middlesex School of Medicine, 98 Chenies Mews, London WC1E 6HX, England.

We have shown that cell cycle arrest of a human B-cell line (Daudi) by α-IFN results in an accumulation of cells in early and late G_1 (G_{1A} and G_{1B} respectively) and these cells contain mainly pRB^{110}. In contrast, actively cycling human lymphoid cells contain partially phosphorylated pRB even in G_{1A}. These G_{1A} cells contain intermediate forms of pRB which become further phosphorylated to $pRB^{112\text{-}114}$ as cells traverse G_{1B}. Since pRB^{110} is thought to prevent cell proliferation, our data would suggest that the cytostatic effect of α-IFN may occur by preventing the initial phosphorylation of pRB during or prior to G_{1A}. The cdc2 kinase family may play a role in pRB phosphorylation; we have shown that G_1 arrest caused by α-IFN or phorbol ester leads to dephosphorylation of $p34^{cdc2}$, which may inhibit its kinase activity against pRB. However, others have shown by antisense methods that cdc2 may not be required for the initial, partial phosphorylation of pRB, suggesting that at least one other unidentified kinase is needed. The action of the non-cdc2 kinase(s) which partially phosphorylates pRB in G_{1A} is therefore also inhibited by α-IFN. pRB may function in the G_1 to S-phase transition by interacting with transcription factors and also with cyclin-A. We have found that cyclin-A is not present in α-IFN-arrested cells, however it is present in proliferating cells and in cells arrested in late G_1 by aphidicolin. Taken together our data suggest that α-IFN may cause cell cycle inhibition in G_1 by preventing the phosphorylation of pRB by $p34^{cdc2}$ and by other kinase(s) and by preventing the synthesis of cyclin-A. To investigate the mechanisms of cell cycle arrest in more detail we are currently characterizing proteins which complex with pRB and with $p34^{cdc2}$ and other related kinases in proliferating and in α-IFN-inhibited cells.

Supported by the Kay Kendall Leukaemia Research Fund and The Cancer Research Campaign.

G 430 CYTOKINE-MEDIATED TUMOR CELL DEATH - A CONSEQUENCE OF SIMULTANEOUSLY ACTIVATED GROWTH STIMULATORY AND INHIBITORY SIGNAL TRANSDUCTION PATHWAYS

Jürgen van der Bosch, Stephan Rüller, Jörg Breder and Max Schlaak, Forschungsinstitut Borstel, 2061 Borstel, Germany.

Cytokine-containing supernatants (SU) generated during interaction of elutriated human monocytes (MO) with various clonal human tumor cell (TC) lines in a serum-free medium are shown to cause cell cycle arrest in G1 in subconfluent and cell death in postconfluent TC-populations. Removal of individual cytokines (TNF, IFN-α, IFN-β, IL-1) by affinity chromatography causes *coordinate* reduction of SU-induced cell death and growth inhibition, suggesting partially overlapping signal transduction pathways leading to G1-arrest and cell death. This view is supported by the observation that hydrocortisone (HC) also causes *coordinate* suppression of TC-susceptibility to growth inhibition and to induction of cell death. Furthermore, aurintricarboxylic acid, an endonuclease inhibitor, not only suppresses TC-death but also growth arrest in G1. During SU- or MO-exposure part of the TC-population is driven into the quiescent (Ki67-negative) state G0. This reversibly arrested TC-population is shown to be insusceptible to induction of cell death. Factors promoting G1/S-transit (EGF, insulin) on the other hand increase TC-susceptibility to induction of cell death. *These* data suggest a coupling or overlap of signal transduction pathways leading to G1/S-transit and to TC-death. Based on these observations the hypothesis is put forward that susceptibility to induction of cell death is a consequence of simultaneously activated growth inhibitory and growth stimulatory signal transduction pathways, the stimulatory pathway causing chromatin relaxation and thereby exposing DNA to nucleases activated by the inhibitory pathway.

G 431 A CELLULAR MUTATION MEDIATES RESISTANCE TO E1 BUT NOT TO TRANSFORMATION BY SV40-T AND HPV16-E7, Joseph M. Weber, Sucheta Sircar, Joseph Horvath, Dominique Roberge and Mounir Diouri, Department of Microbiology, Fac. Medicine, University of Sherbrooke, Quebec, Canada, J1H 5N4

An adenovirus-specific transformation resistant cell line (G2) expressing biologically active E1a proteins and originally isolated as a revertant from Ad2-transformed rat cells (F4), was shown to form stable Rb-E1a and 300K-E1a complexes in immunoprecipitation experiments. Consistent with the transformation resistant phenotype, cell hybrids between G2 and F4 were all nontumorigenic. In addition fusion between G2 and normal rat cells (FR3T3) failed to restore the transformed phenotype, as might be expected if G2 contained a defective cellular transformation facilitator gene. Fusion of G2 cells with the E1 transformed human 293 cells also resulted in a less transformed phenotype without any effect on the expression of E1a proteins. This transformation resistant phenotype does not apparently extend to other members of the p105Rb-binding family of DNA virus oncoproteins: Both SV40-T and HPV16-E7 transformed G2 cells readily. Retrovirus insertion mutagenesis resulted in tumorigenic cell lines and identified a common locus responsible for the E1a-specific dominant tumor suppressor phenotype of G2 cells. Studies are underway to determine the nature of the insertion sites.

G 432 INACTIVATION OF THE RETINOBLASTOMA GENE PRODUCT VIA TRANSGENE EXPRESSION OF THE POLYOMAVIRUS LARGE T ANTIGEN IN MOUSE MAMMARY EPITHELIUM. Marc A. Webster and William J. Muller, The Institute for Molecular Biology and Biotechnology, Mc Master University, Hamilton, Ontario, Canada, L8S 4K1.

The inactivation of the retinoblastoma gene product (*Rb*) has been implicated as an obligatory event in either the initiation or progression of many human cancers including those of the eye, bladder, prostate, lung, and breast. To directly assess the role of *Rb* in mammary tumorigenesis, we have taken advantage of the ability of polyomavirus large T (PyLT) antigen to complex and inactivate cellular *Rb* function. Eight transgenic strains carrying a cDNA encoding PyLT antigen linked to the Mouse Mammary Tumor Virus (MMTV) promotor/enhancer were generated. While mammary gland specific expression of PyLT has thus far demonstrated no apparent abnormalities, these mice have recently developed testicular tumors. Offspring from one line exhibited expression of the transgene in the brain lending to a severe central nervous system demyelinating effect similar in phenotype to the genetically inherited neurological disorder '*jimpy*' in mice. Currently we are examining whether PyLT antigen expression (and by extension, inactivation of *Rb*) can cooperate with other oncogene products to transform the mammary epithelium.

G 433 A NOVEL INTRACELLULAR ANTIPROLIFERATIVE PROTEIN, PROHIBITIN, IS THE MAMMALIAN EQUIVALENT OF THE D. MELANOGASTER GENE PRODUCT, Cc, ESSENTIAL FOR DEVELOPMENT. C.M. Wood[1], D.A. Stewart[1], M.J. Nuell[1], J.J. White[1], J.K. McClung[2], C.C. Morton[3], and D.B. Danner[1]. [1]Lab. of Molecular Genetics, NIH, NIA, Baltimore; [2]Biomedical Division, The S.R. Noble Foundation, Ardmore, OK; & [3]Dept. of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston.

Prohibitin has been recently identified by mRNA microinjection as a novel antiproliferative protein of 30 kD; its exact intracellular role is currently under investigation. Prohibitin is expressed in all tissues examined, is of moderate abundance, and is associated with a protein complex of approximately 500 kD. Peptide-generated antibodies against prohibitin suggest that it exists in two different forms, one in the nucleus and one in the cytoplasm. The protein was found to resemble the Cc gene product, a protein which is vital for Drosophila development during the larval to pupal metamorphosis. Several rat cDNAs as well as cDNAs from human and Drosophila have since been isolated and sequenced. Between rat and Drosophila, the amino acid conservation is 76% identity with 84% strong similarity, while rat and human proteins are identical. Moreover, the two mammalian transcript forms, 1.2 and 1.9 kb, encode the same protein and utilize two alternative polyadenylation sites. Drosophila prohibitin (Cc) mRNA blocks DNA synthesis in human fibroblasts nearly as well as rat prohibitin mRNA. Chromosomal mapping of the functional human prohibitin gene shows localization to the region of 17q21-22, a region genetically linked to early-onset human breast cancer.

G 434 CHARACTERIZATION OF THE FUNCTIONAL DOMAINS OF THE ADENO-ASSOCIATED VIRUS REP PROTEIN: AN ANTI-PROLIFERATION GENE PRODUCT, Qicheng Yang and James P. Trempe, Department of Biochemistry and Molecular Biology, Medical College of Ohio, Toledo, OH43699

The Adeno-Associated Virus (AAV) is a defective human parvovirus. In addition to a helper virus, two AAV DNA sequences, the rep gene and the terminal repeats, are required for its replication. Four species of Rep proteins are produced in AAV infections due to alternate initiation from either P_5 or P_{19} promoter and mRNA splicing. The two largest Rep proteins (Rep78/Rep68) are essential for AAV DNA replication and trans-activation of the capsid gene, and can suppress oncogene-mediated cellular transformation. To identify the functional domains of the Rep proteins involved in these processes, we constructed a series of Xho I linker insertion and deletion mutants. Their behavior in nuclear localization, AAV DNA replication, trans-activation of the capsid gene and suppression of oncogene-mediated transformation were compared to the wild type Rep proteins. We have found a basic amino acid-rich region, which is similar in structure to other known nuclear targeting signals, that is required for nuclear localization. Three domains, separated by "hinge" regions, are required for AAV DNA replication. Mutants that cannot support AAV DNA replication are defective for trans-activation of the capsid gene with the exception of one mutant in the amino terminus of the Rep protein. Domains necessary for suppression of E1a/ras oncogene-mediated transformation are found primarily in the amino terminus of the protein.

Regulation of Gene Expression

G 500 RETROVIRAL INSERTIONS DOWNSTREAM OF THE GENE FOR RNA-BINDING A1 IN ERYTHROLEUKEMIA CELLS INDUCED BY FRIEND LEUKEMIA VIRUS. Ben-David[1], Y., Chabot[2], B., De Koven[1], A. and Bernstein[3], A. [1]Division of Cancer Research, Sunnybrook Health Science Centre, Toronto, Ontario, Canada, [2]Department of Microbiology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada and [3]Division of Molecular and Developmental Biology, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada.

The multistage erythroleukemia induced by the various strains of Friend leukemia virus involved inactivation of the *p53* tumor suppressor gene and frequent activation of either the *Spi-1/PU.1* or *Fli*-1 genes. Both *Fli*-1 and *Spi*-1 are members of the *ets* oncogene family of transcription factors and are activated as a result of proviral integration events in a majority of erythroleukemia cell lines induced by either Friend murine leukemia virus (F-MuLV) or Friend spleen focus-forming virus (SFFV), respectively. In this report, we have identified a novel common integration site, designated *Fli*-2 (Friend leukemia integration-2), from an erythroleukemia cell line induced by F-MuLV. Rearrangements at the *Fli*-2 locus were found in 2/11 erythroleukemia cell lines independently induced by F-MuLV and 1/19 erythroid cell clones derived from spleens of mice infected with the polycythemia or anemia strains of Friend virus (FV-P abd FV-A respectively). A cDNA corresponding to a transcript originating from genomic DNA adjacent to *Fli*-2 was isolated using a junction fragment 5' to the site of the proviral integration site. The deduced amino acid sequence of this cDNA is identical to the human *hnRNP* A1 gene, a member of the gene family of RNA binding proteins involved in RNA slicing. In one erythroleukemia cell line, A1 expression is undetectable as a result of F-MuLV integration in one allele and loss of the other allele. These results

G 501 **A CELL-TYPE SPECIFIC AND ENHANCER-DEPENDENT SILENCER IN THE PROMOTER OF THE HUMAN UROKINASE PLASMINOGEN ACTIVATOR GENE** Roberta BENFANTE, Raffaele CANNIO and Francesco BLASI. Institute of Microbiology, University of Copenhagen, Øster Farimagsgade 2A,1353 Copenhagen K, Denmark

A trascriptional silencer in the 5' regulatory region of the human urokinase plasminogen activator (uPA) gene is able to block transcription from the human uPA as well as the rabbit ß-globin promoters in a cell type specific and orientation independent way. The silencer is enhancer dependent and is active in two cell lines (HeLa and CV-1) which produce little if any uPA, but not in the high uPA producer PC3.

Silencing activity and enhancer dependence can be separated, the first can be mapped between -660 and - 536, the enhancer dependence between -536 and -308.

In the silencer- containing fragment, the sequence -549/-538 has a 9/10 identity to the TGF-ß1 responsive element of the transin/stromelysin gene (TIE element). This sequence, that is present in the promoter of c-myc, elastase, collagenase and proliferin genes, has been shown to bind a nuclear protein complex which includes the product of the c-fos oncogene. DNAse I footprinting analysis showed a pattern of protected sequences covering most of the region -660/-537. Gel-shift experiments with both HeLa and CV-1 nuclear extract show the presence of a specific DNA-protein complex which binds TIE containing oligonucleotide. Preliminary results also indicate an inhibition of the formation of this complex by c-fos antibodies.

The low level homology of this sequence with the AP-1 consensus sequence and the absence of TIE element in the c-fos promoter (which is known to be negatively autoregulated by c-fos product itself) suggest that sequences other than AP-1 can be recognized by Fos protein, possibly in a complex with proteins other than those belonging to the Jun family. We are now attempting to characterize the nature of this complex and to understand the role of the silencer and of the TIE sequence in the regulation of the expression of the urokinase gene.

G 502 DIFFERENTIAL TRANSCRIPTION OF TRANSFORMING GROWTH FACTOR BETA FAMILY MEMBERS FOLLOWING RETINOIC ACID (RA) AND HEXAMETHYLENEBIS-ACETAMIDE (HMBA) TREATMENT OF THE NTERA-2cl.D1 (NT2/D1) HUMAN TERATOCARCINOMA CELL, Martina Burchert, Heidi Giordano and Ethan Dmitrovsky, Department of Medicine, Memorial Sloan-Kettering Cancer Center, NY, NY 10021

The NT2/D1 human teratocarcinoma cell is multipotential, with distinct phenotypes induced by RA and HMBA treatment. This provides an opportunity to explore the mechanism of inducer mediated growth inhibition and how these inducers affect the TGF-ß growth inhibitory pathway in an embryonal cancer cell. We report here that transforming growth factors-ß1 and -ß2 are differentially upregulated in the NT2/D1 cell treated with HMBA and RA. Compared to untreated cells, HMBA causes a marked increase in TGF-ß1 mRNA expression, while RA treatment primarily augments TFG-ß2 expression. Neither inducer increases the low level of TGF-ß3 expression observed in untreated NT2/D1 cells. Exogenous TGF-ß1 protein added to the media of NT2/D1 cells inhibits growth comparable to HMBA and RA treatment. Gel retardation assays show that nuclear extracts of untreated, RA treated and HMBA treated cells bind to different elements of the TGF-ß1 promoter. Nuclear extracts from untreated and RA treated cells contain protein(s) which bind to an AP-1 rich region between -453 and -313 of the TGF-ß1 promoter, while nuclear extracts from HMBA treated cells lack these binding proteins. Supershift experiments using polyclonal antibodies indicate these binding proteins belong to the jun family. This suggests that an AP-1 complex is a negative regulatory element in the regulation of TGF-ß1 expression in untreated and RA treated cells, and that HMBA treatment results in deregulation of these binding proteins, thereby allowing transcription of TGF-ß1. Which nuclear proteins are involved in transcriptional regulation of TGF-ß2 following RA treatment is currently under investigation. Taken together, these data indicate that different TGF-ß members cause growth inhibition through distinct pathways in the same cell.

G 503 TGF-B INDUCED TRANSCRIPTION OF JUNB IS BLOCKED BY EXPRESSION OF ADENOVIRUS E1A PROTEINS WHILE TGF-B INDUCTION OF CJUN REMAINS RAPID AND TRANSIENT, Lisa M. Coussens and Robert Chiu, Departments of Biological Chemistry and Surgical Oncology, UCLA School of Medicine, Los Angeles, CA 90024

The multipotent growth factor TGF-B regulates the expression of many important genes critical to cell cycle progression and development. We study the ability of TGF-B to induce the transcription of the AP-1 associated gene product JunB. Our data suggest that the transcriptional induction of junB occurs in a primary response manner in the rat embryonal fibroblast line 3Y1. Additionally, we have found that in an isotypic derivative of 3Y1 cells stably transfected with adenovirus-13 E1A, AE1AY1, the induction of junB mRNA normally observed following cellular exposure to TGF-B is abrogated while induction following cellular exposure to serum or TPA resembles 3Y1 cells. Other genes known to be transcriptionally regulated by TGF-B, specifically PAI-1 and TGF-B are also unresponsive in the AE1AY1 cells while c-jun gene expression is induced in a rapid and transient manner identical to that of the 3Y1 derivative. Three high molecular weight receptors known to bind with various affinities to TGF-B appear to be present on AE1AY1 cells based on affinity labeling experiments. Other 3Y1 isotypic derivative lines stably transfected with SV40 large T, papiloma virus E6 and E7, as well as a mutant adenovirus 13-E1A remain responsive to TGF-B action resulting in normal induction kinetics of junB mRNA. These data suggest a defect in TGF-B signalling distal to ligand-receptor interaction specifically imposed by the transforming proteins of adenovirus 13-E1A. Furthermore, these studies suggest that regulation of the highly homologous AP-1 associated proteins c-jun and junB are not regulated coordinately atleast in rat embryonal fibroblasts exposed to TGF-B.

G 504 FUNCTIONAL INTERACTIONS BETWEEN cJun / cFos AND THE ESTROGEN RECEPTOR IN BREAST CANCER CELLS, **Vassilis Doucas, Giannis Spyrou°, Curt Pfarr and Moshe Yaniv**, Unité des virus oncogènes, UA 1149 du CNRS, Département des Biotechnologies, Institut Pasteur, Paris.

Overexpression of c-Jun or c-Fos proteins in human MCF-7 breast cancer cells inhibits estrogen dependent transcription of an ERE (estrogen responsive element) containing reporter gene, without affecting its basal level of expression. Overexpression of JunB or JunD produced little or no effect on estrogen-dependent trascription, respectively. Contrary to transcriptional activation of promoters containing AP1 sites no synergy is observed between cJun and cFos, suggesting that DNA binding of c-Jun or c-Fos is not necessary for repression of estrogen activity. Overexpression of the estrogen receptor largely relieved the c-Fos but not the c-Jun mediated inhibition, further supporting the idea that both can act independently. Mutagenesis analysis revealed that deletion of either one of two regions of c-Jun, one adjacent to the basic DNA binding domain and the other containing the leucine repeat blocked repression. These results can be explained by a direct protein-protein interaction between either cJun or cFos and the estrogen receptor or an indirect interaction mediated by an additional factor.

It was previously shown that TPA treatement supresses estrogen induced growth of the MCF-7 cells and provoques changes in their morphology. We are currently studing if this inhibition is mediated by the increase in AP1 activity after TPA treatement.

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G 505 A HELIX-LOOP-HELIX PROTEIN APPEARS INVOLVED IN GLUCOCORTICOID REGULATED EXPRESSION OF Mu CLASS GLUTATHIONE S-TRANSFERASE IN A SMOOTH MUSCLE TUMOR CELL LINE, Weimin Fan, Tina Cooper, Yinong Liu and James S. Norris*, Departments of Medicine and *Biochemistry/Molecular Biology, Medical University of South Carolina, Charleston, SC 29425

Glucocorticoid treatment of the Syrian hamster tumor cell line, DDT_1 MF-2, results in Go/G_1 arrest and concomitant induction of a 29 kD protein identified as a mu class glutathione S-transferase (hGSTYBX). The expression of the *hGSTYBX* in DDT_1 MF-2 cells is transcriptionally up-regulated by glucocorticoids. Secondly, *hGSTYBX* expression is dependent upon protein synthesis, suggesting that induction is a secondary hormonal response. To study the mechanism of induction we have cloned and sequenced the *hGSTYBX* gene including its 5' flanking regions. When linked to a *CAT* reporter gene the 5' flanking domain directs glucocorticoid inducible transcription. Through a series of 5' deletion mutants, we have localized the glucocorticoid inducible regulatory element between nucleotides -356 to -236. No classical glucocorticoid response elements are found within this region, however, four potential helix-loop helix (HLH) binding sites (CANNTG) are embedded in two 16 bp repeats. Using electrophoretic mobility shift assays under competition conditions, we identified a specific nuclear protein that binds to the HLH domain. Oligomers representing GRE or AP1 elements failed to interfere with protein binding. These results suggest that a HLH transactivator protein(s) may be involved in glucocorticoid induction of GST.

By immunocytochemistry we localized the *hGSTYBX* gene product to the nuclei of glucocorticoid treated cells. Because of this nuclear localization under conditon of Go/G_1 arrest, we decided to see if *hGSTYBX* was involved in regulation of cell growth. Expression vectors with either sense or antisense *hGSTYBX* cDNA under the control of an MMTV promoter were constructed and stably transfected into DDT_1 MF-2 cells by G418 selection. The results indicated that *hGSTYBX* is unlikely to be the only factor responsible for glucocorticoid induced cell growth cycle arrest since growth of either sense or antisense GST transfectants was still arrested by glucocorticoids. However, transfection of sense GST enhanced overall cell growth while the cells transfected with antisense GST always grew slower. These observations suggest that the *hGSTYBX* gene product may play a role in promoting tumor cell growth or survival by mechanisms not yet revealed. Supported by NIH CA 49949 and 52085.

G 506 C-FOS PROTEIN SYNTHESIS AND GROWTH REGULATION IN BOVINE CORNEAL ENDOTHELIAL CELLS

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We evaluated the effects of stimulation (by serum and three peptide growth factors: fibroblast growth factor, insulin and transforming growth factor ß) on the expression of the protein product of the immediate early gene, c-*fos* in bovine corneal endothelial cells. We compared these results to cells which were made quiescent by serum starvation. We also examined the effect of these same growth factors on DNA synthesis. Quiescent cells expressed low levels of c-*fos* protein. Serum was the most potent stimulator whereas FGF and insulin were modest stimulators. TGFß did not significantly stimulate c-*fos* production. The results from DNA synthesis were different. Serum and FGF were still the most potent stimulators while insulin and TFGß were weak stimulators. These data suggest that growth factors induce c-*fos* protein in BCE and that this may in part regulate the downstream event, cellular proliferation. Further investigation into the regulation of this and other proto-oncogene products may provide insight into the mechanisms which modulate corneal endothelial cell growth in humans.

G 507 A MEMBER OF THE ATF/CREB FAMILY WHICH INHIBITS TRANSCRIPTION, Helen C. Hurst and Ian G. Cowell, ICRF Oncology Group, Hammersmith Hospital, London W12 OHS, U.K.

Most specific promoter and enhancer binding sites are recognised by a family of related nuclear proteins. A major question in understanding the control of cellular growth at the level of transcription is to establish what role all these family members play in determining patterns of gene expression.

We have been examining the family of bZIP transcription factors which bind the CGTCA motif. This includes the cAMP inducible factor CREB and the homologous but distinct factor ATF1 which is not a convincing activator of transcription, but may play a role in modulating CREB activity. We have also isolated a novel member of this family, E4BP4 which is currently being characterised. Transfection assays indicate that E4BP4 is an inhibitory factor whose action is reversed in the presence of adenovirus E1a to the extent that it may become slightly stimulatory. As E4BP4 is a phosphoprotein whose DNA binding activity is dependent on its phosphorylation state, we are currently examining whether external stimuli can affect the binding potential and hence the activity of this factor and thus give some insight as to its role in modulating cellular transcription.

G 508 Transcriptional regulation of human α-actin gene by Ha-*ras* oncogene.

Chandra Kumar, C*, Earl Ruley+, Wen Chang+ Fernando Ramirez*, Jae-Hong Kim*, and Pierre Bushel*, *Dept. of Tumor Biology, Schering-Plough Research, Bloomfield, N.J 07003 and +Center for Cancer Research, Mass. Institute of Technology, Cambridge, Ma 02139.
Neoplastic transformation of mammalian cells leads to dramatic changes in the synthesis of several cytoskeletal proteins resulting in altered morphology. Our studies have shown that smooth muscle specific isoforms of Myosin light chain-2 and α-actin are selectively repressed in *ras* oncogene transformed fibroblast cells. We are using the α-actin gene as the prototype to elucidate molecular mechanisms involved in cytoskeletal alterations in transformed cells. We have recently characterized the 5' flanking sequences of the human α-actin gene. Using plasmids that contain the α-actin promoter fused to various reporter genes such as ß-Galactosidase, Chloramphenicol Acetyl Transferase (CAT) and human Growth Hormone (hGH), we have shown that the α-actin promoter activity is repressed in *ras* transformed fibroblast cells. Transient transfection analysis of plasmids containing progressive deletions of 5' flanking sequences of the α-actin gene fused to CAT reporter gene indicates that the Ras Responsive Element (RRE) is localized within 120 bases from the transcription start site. This region of the promoter has two CArG motifs that are evolutionarily conserved. The CArG motif is also part of the Serum Response Element (SRE) of *c-Fos* gene and plays an important role for both transcriptional activation and repression of c-*Fos*. Our preliminary studies indicate that these CArG boxes also play an important role in down regulating the α-actin transcription in response to transformation by *ras* oncogene.

G 509 MODULATION OF THE TGF-α PROMOTER BY TGF-β1 IN HUMAN COLON CARCINOMA CELLS. Mark J. Lynch, Lenore Pelosi, June Merwin, Department of Molecular and Cellular Biology, Bristol-Myers Squibb, Wallingford CT 06492.
In studying synergy between different growth factors on human colon carcinoma cells we have found that treatment of the cell line FET with transforming growth factor-beta 1 (TGF-β1) leads to a 2 fold increase of transforming growth factor-alpha (TGF-α) produced in the conditioned medium. To study the molecular mechanisms involved in this induction we used a luciferase reporter construct containing 2816 base pairs of TGF-α 5'-flanking region (pTGFα2816Luc) in transient transfection studies. pTGFα2816Luc transfected FET cells treated with TGF-β1 gave a dose dependent increase in luciferase activity with a maximal increase of 7.5 fold at 10 ng/ml TGF-β1 as compared to untreated controls. This increase in activity was specific for the TGF-α promoter as other promoters including the Rous sarcoma and human immunodeficiency virus LTRs and metallothionine promoter showed no modulation by TGF-β1. In addition, TGF-β1 had no effect on the TGF-α promoter in the human colon carcinoma cell line HCT116 which is insensitive to TGF-β1. Using luciferase constructs containing progressively smaller portions of the TGF-α promoter region, we have localized this TGF-β1 responsive element to between -201 and -77 base pairs relative to the AUG codon. This region contains sequences with homology to other known TGF-β1 responsive elements. Gel retardation analysis is being performed in an effort to characterize the transcription factor(s) interacting with this putative TGF-β1 responsive element.

G 510 RETINOIC ACID-INDUCED CHANGES IN THE EXPRESSION OF RETINOBLASTOMA & P53 PROTEINS IN NON-SMALL CELL LUNG CARCINOMA LINES Steve A. Maxwell and Jack A. Roth, Department of Thoracic Surgery, UT M.D. Anderson Cancer Center, Houston, TX 77030

The proliferation of non-small cell lung carcinoma (NSCLC) lines is increased 30 to 90% after 8 h of exposure to 5 uM retinoic acid. However, after 48 to 72 h treatment with retinoic acid, cell growth is reduced by 50% as measured by tritiated thymidine incorporation. We investigated whether the expression of tumor suppressor gene products correlated with the retinoic acid-induced changes in growth of NSCLC lines. The expression of retinoblastoma P105 and p53 was examined by immunoblotting of SDS extracts from cells treated with 5 uM retinoic acid. In the H460 and H226 cell lines, which express endogenous wild-type p53, both Rb-p105 and p53 were induced several-fold over untreated cells after 8 h of incubation with retinoic acid. After 48 h of growth in retinoic acid, Rb-p105 converted from a superphosphorylated form to an underphosphorylated species. Both Rb-p105 and p53 were almost undetectable in H460 and H226 cells after 72 h of retinoic acid treatment. A different retinoic acid-induced pattern of expression of Rb-p105 and p53 was observed in two other cell lines that express endogenous p53 mutated at residue 273 (H322,H820). In contrast to that observed in H460 and H226 cells, no change in p53 was observed after 72 h of retinoic acid treatment. The Rb-p105 was induced several-fold in H820 and H322 cells after 8 h retinoic acid treatment, but in contrast to H460 and H226 cells the expression and phosphorylation of Rb-p105 thereafter remained unchanged even after 72 h of retinoic acid treatment. A cell line containing a homozygous p53 deletion (H358) and a cell line negative for Rb (H596) displayed growth properties in response to retinoic acid that were similar to the other p53/Rb-positive lines. We conclude that the retinoblastoma and p53 gene products do not play a role in the retinoic acid-mediated growth alteration of NSCLC lines. However, a positive correlation was observed between the expression of wild-type p53 and the ability of retinoic acid to induce dephosphorylation of Rb-p105.

G 511 IDENTIFICATION OF A GENE WHOSE EXPRESSION CORRELATES WITH TUMORIGENICITY IN THE WILMS' TUMOR CELL LINE G401. McMaster ML, Dowdy SF, West A, Stanbridge E, and Weissman BE. Lineberger Comprehensive Cancer Center; University of North Carolina at Chapel Hill; Chapel Hill, North Carolina 27599

The development of malignancy is believed to be a multistep process involving several factors including activation of oncogenes and inactivation of genes functioning normally in cellular growth control. Analysis of Wilms' tumor (WT), a pediatric nephroblastoma, has revealed at least two candidate tumor suppressor loci, at 11p13 and 11p15, and suggests the presence of a third. Studies of WT have documented the presence of a gene, designated WinF4, whose expression correlates with the tumorigenic phenotype of the WT cell line G401, and which does not map to any locus on chromosome 11. Preliminary studies suggest that WinF4 is expressed constitutively in the tumorigenic G401 parent cell line but is differentially expressed in response to serum stimulation and transferrin stimulation in the suppressed microcell hybrid, G401.5, indicating that it is a growth response gene, control of which is abrogated in the tumorigenic G401 phenotype. Sequence analysis of WinF4 indicates that it is homologous to the L9 ribosomal protein recently described in rodents. These studies raise questions regarding the role of ribosomal proteins in the development of the tumorigenic phenotype.

G 512 TRANSFORMATION BY V-SIS IS ACCOMPANIED BY PHOSPHORYLATION OF MULTIPLE TRANSCRIPTION FACTORS

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Transformation by v-*sis* has been examined in a model cells bearing v-*sis* under the control of a metallothionine promoter which exhibit rapid, complete and reversible transformation upon induction of v-*sis*[1]. Transformation leads to hyperphosphorylation of cJun at serines 63 and 73 which have previously been associated with increased AP-1 transactivation potential[2]. To study other factors, antibodies have been prepared against Jun-B, Jun-D, Fos-B, Fra-I and Early Growth Response-I transcription factor (Egr-I). These sera precipitate the expected size products from serum-stimulated fibroblasts and exhibit several criteria of specificity. Using these sera, we have observed prominent phosphorylation of Jun-B, Jun-D and, especially, Egr-I. Phosphorylation persists for at least the first 48 hours of *sis*-induced accelerated growth but is nearly undectable during normal log-phase growth. Focus forming assays with NIH-3T3 cells show that v-*sis* DNA causes mostly large foci whereas inclusion of Egr-I DNA leads to a striking >10-fold increase in foci which are characteristic and small. v-*sis* DNA in combination with antisense Egr-I DNA leads to a 3-fold *decrease* in foci and restoration of size. Therefore multiple factors are phosphorylated in v-*sis* transformation and phosphorylated Egr-I may complement *sis*-induced transformation.

[1] Carpenter, P. et al. Antisense Res. Dev. 1991;1:(in press);[2] Boyle, W. et al. Cell 1991;64:573-84.

G 513 MECHANISMS OF TRANSACTIVATION BY HTLV-I p40^{tax}. Masataka Nakamura, Kiyoshi Ohtani, Shigeto Miura and Kazuo Sugamura, Department of Microbiology, Tohoku University School of Medicine, Sendai 980, Japan.

A *trans*-acting transcriptional activator, p40^{tax}, of human T-cell leukemia virus type I (HTLV-I) is thought to be implicated in leukemogenesis by HTLV-I through abnormal activation of enhancers of cellular genes. Most of the cellular genes which are activated by HTLV-I p40^{tax} are expressed or induced in T lymphocytes under normal conditions. In spite of the activity of HTLV-I p40^{tax} to activate enhancers, its activity to bind to enhancer DNA has not been proved, rather it has been shown that at least two cellular transcription factors mediate function of transactivation by HTLV-I p40^{tax} so far. cAMP responsive element binding factor (CREB)-like and NF-κB-like factors are shown to be involved in activation of the HTLV-I 21-bp core enhancer element, and enhancers of interleukin 2 receptor α chain (IL-2αR), simian virus 40 (SV40) and human immunodeficiency virus type I (HIV-1), respectively. We have recently identified that a membrane glycoprotein (gp34) with a molecular weight of 34,000 whose expression is restricted in cells expressing HTLV-I is transcriptionally induced in a T-cell line, Jurkat, by HTLV-I p40^{tax}. gp34 is different from the other cellular genes that are transactivated by HTLV-I p40^{tax} in that gp34 is not expressed in normal, even activated, T lymphocytes. Cloning study revealed that there are two species of gp34 mRNA generated from one copy gene and that gp34 lacks typical signal peptides, and has a hydrophobic stretch near the amino terminal, which possibly acts as a membrane anchore and four possible N-linked glycosylation sites in the carboxy-terminal portion. Also this supports that gp34 belongs to the family of membrane proteins whose carboxy terminal protrude out of cells. We found that gp34 is constitutively expressed in normal lung and testis but not in heart, liver and spleen. In addition, some cell lines originated from the nerve tissues were found to express gp34, suggesting a possible involvement of HTLV-I infection in a nerve disorder known as HAM. Kinetic study showed a relatively delayed induction of gp34, compared to the other genes activated by p40^{tax}, such as IL-2Rα and *c-fos*, indicating that mechanism in HTLV-I p40^{tax}-dependent induction of gp34 may be distinct from those characterized previously. Indeed, a 110-bp fragment upstream of the gp34 gene responsible for HTLV-I p40^{tax}-dependent activation has the ability to bind to nuclear factors, but concensus sequences for CREB and NF-κB are not effective in competition of complex formation with the 110-bp fragment.

G 514ISOLATION OF A GENE SEQUENCE ENCODING LEU-ZIPPER STRUCTURE INDUCED BY TGFβ AND OTHER GROWTH FACTORS. Kiyoshi Nose, Motoko Shibanuma, and Toshio Kuroki, Department of Cancer Cell Res. , Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan.

TGF β modulates cell growth both positively and negatively depending on cell types. To better understand the molecular mechanisms of TGF β actions, we have been isolating a set of gene sequences induced by TGF β_1 from a cDNA library of a mouse osteoblastic cell line. One of the clones, TSC-22, had a novel sequence, and seemed to encode a polypeptide with a molecular weight of 18 kDa with a putative leucine zipper structure. This gene was induced by TGF β_1 with a peak of 2 hr, and was also induced by serum, cholera toxin, dexamethasone or tumor promoting phorbol ester with a similar kinetics. Antiserum was raised against TSC-22 protein synthesized in *E. coli*, and it detected a cellular protein with a molecular weight of 18 kDa both in cytoplasmic and nuclear fractions. TSC-22 protein seemed to bind with several cellular proteins, as detected by probing Western blot of nuclear proteins with biotinyl-TSC-22 protein, even it did not associate with c-FOS, c-JUN or CREBP. Cells transfected with expression vectors of TSC-22 did not show any change in phenotypes, but this gene is expected to have some regulatory roles on cellular functions.

G 515 ANALYSIS OF THE TRANSCRIPTIONAL REPRESSION FUNCTION ENCODED BY THE WT1 WILMS' TUMOR GENE PRODUCT, Frank J. Rauscher, III, Stephen L. Madden, Donna M. Cook, Jennifer F. Morris, and Robin A. Bhaerman. The Wistar Institute of Anatomy and Biology, 3601 Spruce St., Philadephia, PA 19104

We have been analyzing the biochemical properties of the zinc finger protein present at the chromosome 11p13 Wilms' Tumor locus. The *wt1* gene encodes a 52 Kd nuclear protein (WT1) which is rich in glutamine, proline and glycine residues and contains four contiguous zinc fingers of the C_2H_2 class. The WT1 protein binds to the EGR-1 consensus sequence (5'-CGCCCCC GC-3') and mutations within the finger region which have been identified in patients with Wilms' Tumor abolish DNA binding activity. Since the EGR-1 protein also binds to the EGR site and serves as a positive activator of transcription, we sought to determine the transcriptional regulatory potential of WT1. Cotransfection experiments using synthetic and natural promotors containing EGR binding sites demonstrated that the WT1 protein functioned as a potent repressor of transcription. Repression of transcription mediated by WT1 required an intact zinc finger region and the negatively charged N-terminus. Truncated proteins lacking either of these domains failed to repress transcription. To map the domains of WT1 and EGR-1 required for repression and activation respectively, we constructed chimeric EGR-1-WT1 proteins. Transfer of the 307 amino-acid N-terminal segment of WT1 to the EGR-1 zinc finger domain created a potent transcriptional repressor. In addition, fusion of the same 307 amino-acid segment of WT1 to the GAL4(1-147) DNA binding domain also created a repressor of transcription. A high resolution mutagenic analysis of the WT1 repression domain was performed which included deletion of homopolymeric stretches of proline and glycine residues and disruption of a potential leucine zipper (LLQLY); however none of these alterations significantly affected the repression function. In summary, the WT1 Wilms' tumor gene product is a transcriptional repressor which may function by antagonizing the actions of EGR-1, a growth-factor-inducible immediate-early gene product. Supported by the W.W. Smith Charitable Trust & the Hansen Memorial Trust.

G 516 A COMPOSITE TGF-betal REGULATORY ELEMENT IN THE TYPE-1 PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) GENE. Andrea Riccio, Peter A.Andreasen* and Paolo V.Pedone. Centro di Endocrinologia ed Oncologia Sperimentale, CNR, University of Naples, Italy; *Institute of Molecular Biology, University of Aarhus, Denmark.

Transforming growth factor-beta is the name of a group of closely related polypeptides characterized by a multiplicity of effects, including positive or negative control of cell growth and regulation of extracellular matrix turn-over. PAI-1 gene transcription is strongly induced in several cell types, independently of the effects that TGF-betal exerts on cell growth. We have identified a TGF-betal responsive element in the 5'-flanking region of the human PAI-1 gene. This DNA sequence confers TGF-betal responsiveness to a linked unresponsive promoter. In vitro DNA binding assays showed that two different nuclear factors, detected in exracts from both TGF-betal-treated and non-treated cells, bind to adjacent sequences contained in the responsive unit. A palindromic sequence binds trans-acting factor(s) of the CTF/NF-1 family. This site partially overlaps a dyad simmetry containing a CACGTG motif, that interacts with a second protein. Cross-competition for binding of nuclear proteins in gel mobility shift assays was observed between the dyad simmetry and the adenovirus major late promoter upstream element (USE), suggesting that the basic-Helix-Loop-Helix (bHLH) factor USF that binds the USE also interacts in vitro with the TGF-betal responsive element of the PAI-1 gene. Mutations which abolish the binding of either the CTF/NF-1 or the USF-like factor result in reduction of transcriptional activation upon exposure to TGF-betal, showing that both elements of the unit cooperate in the TGF-betal response. This regulatory region, thus, reflects the organization of the composite responsive elements, in which the association of different motifs strengthens the response of the unit. We will discuss the possible relationship of these findings to the complexity of the TGF-betal action.

G 517 Regulation of Pax2 gene expression during development, Uwe W. Rothenpieler, Victoria Steele-Perkins and Gregory R. Dressler, Laboratory of Mammalian Genes and Development, National Institutes of Health, Bethesda, MD 20892

Pax2 is a DNA binding protein expressed in condensing mesenchyme and early epithelial structures of the developing kidney. Pax2 expression is downregulated as mesenchyme derived epithelial cells differentiate into mature tubular epithelium and glomeruli. High levels of Pax2 expression can also be detected in the epithelial components of Wilms' tumor, an early childhood kidney tumor of embryonic origin. Wilms tumor derived cells can differentiate into a variety of kidney cell types, whose differentiated state reflect the developmental potential of the transformed cell. These data suggest that Pax2 functions in the mesenchyme to epithelial transition in the early kidney development and that failure to properly downregulate Pax2 expression may contribute to tumorigenesis. Thus, the correct terminal differentiation of renal epithelial structures may require the specific shut off of Pax2.

The WT1 gene maps to the Wilms' tumor suppressor locus and encodes a zinc-finger containing DNA binding protein expressed in the developing kidney. Peak levels of WT1 expression appear in more developed structures, at later developmental stages, relative to Pax2 expression. Current studies address the potential interaction of WT1 with Pax2 transcription regulatory elements in vitro and in vivo.

G 518 *JUN*-B HAS PROPERTIES OF A TUMOR SUPPRESSOR GENE SIMILAR TO *P53*

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The c-*jun* and *jun*-b genes share high sequence homlogy with the v-*jun* oncogene. They belong to the immediate early gene group. C-jun together with c-fos constitutes the transcription factor AP-1. We inhibited expression of c-*jun* and *jun*-b in different cell lines using phosphorothioate oligodeoxynucleotides as described (1). Western blot analysis confirmed a reduction in c-jun protein level after antisense treatment by more than 90%. This inhibition in c-jun protein expression lead to a reduction in 3H-thymidine incorporation by more than 50% in NIH 3T3 mouse fibroblasts, primary human fribroblasts, SK-Br-3 human carcinoma cells and rat PC12 cells. Inhibition of c-*fos* expression had similar effects on cell proliferation.

In the same cell lines inhibition of *jun*-b expression however, drastically increased 3H-thymidine uptake to more than 3-fold. This was very similar to the effects resulting from suppression of the anti-oncogene *p53* but different from the results of *rb* gene suppression. We conclude that, while c-*jun* is a proto-oncogene, *jun*-b has the characteristics of an anti-oncogene with strong antiproliferative action, similar to *p53*. Our results show *jun*-b and c-*jun* to be functional antagonists with regard to their effect on cell growth. This is in line with findings that c-*jun* and *jun*-b negatively regulate each others expression (2). Transfection experiments with *jun*-b by Minna et al. also support the idea that high *jun*-b expression leads to an arrest in cell growth (Minna, personal communication). Preliminary results from blocking *jun*-d expression suggest that *jun*-d, like *jun*-b displays properties of an anti-oncogene.

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2. Chiu R, Angel P, Karin M: *Jun*-b differs in its biological properties from, and is a negative regulator of, c-*jun*. Cell (1989) 59:979-986.

G 519 ISOLATION OF GENES WHICH ARE INDUCED BY TGFβ1 AND WHOSE EXPRESSIONS ARE SUPPRESSED IN v-RAS TRANSFORMED CELLS.

Motoko Shibanuma, Toshio Kuroki, and Kiyoshi Nose, Department of Cancer Cell Res., Institute of Medical Science, University of Tokyo, Japan.

TGFβ1 has an inhibitory effect on cellular growth of various types of cells, and. we have previously found that it inhibits DNA synthesis of mouse osteoblastic cells (MC3T3-E1) in late G_1 phase. To understand the molecular mechanism underlying the inhibitory effect of TGFβ1 on cellular growth, we attempted to isolate genes induced by TGFβ1 from a λZAP cDNA library constructed from MC3T3 cells treated with TGFβ1 for 4 hr in late G_1 phase, and obtained six different clones. Expression of these genes were induced by TGFβ1 with a peak of 6 - 8 hr after stimulation, and were also induced by serum. These were unknown genes except one, TSC (TGFβ1-inducible clone)-160, which was revealed to be rrg (ras recision gene) from partial sequencing. Endogenous expressions of two other genes (TSC-5, -36) were also remarkably reduced in v-K-ras transformed cells, compared with those of parental cells and their expressions were restored in one of the flat revertant. These results suggest that these genes are involved in the negative regulation of cellular growth, and their expressions are lost in v-ras transformed cells.

G 520 MOLECULAR CLONING OF 2-5A-DEPENDENT RNase: AN ENDORIBONUCLEASE INVOLVED IN INTERFERON ACTION,

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2-5A-dependent RNase (RNase L or F) is the terminal factor in an interferon-regulated, RNA-degradation pathway. The 2-5A-dependent RNase may be involved in the molecular mechanism of interferon action and in the fundamental control of cell growth and differentiation. In addition, 2-5A-dependent RNase is intrinsically interesting as it is the only ribonuclease known which requires an allosteric effector [2-5A or $p_3(A2'p)_nA$] for its activity. To obtain direct evidence for the involvement of 2-5A-dependent RNase in interferon action we undertook the molecular cloning of this enzyme. Using a bromine-substituted, ^{32}P-labeled 2-5A analog as probe, we isolated a cDNA containing the complete coding sequence of 2-5A-dependent RNase from a cDNA expression library we prepared from interferon (alpha + beta)-treated mouse L929 cells. The identity of the recombinant 2-5A-dependent RNase was confirmed by its reactivity with affinity-purified antibody to 2-5A-dependent RNase. The protein produced *in vitro* by first transcribing the cDNA and then translating the mRNA showed an affinity for 2-5A which was comparable to that of natural 2-5A-dependent RNase as determined in reactions containing various 2-5A analogs as competitors. Expression of the 2-5A-dependent RNase cDNA in mammalian cells is being performed to determine the negative growth effect of the 2-5A system.

G 521 INDUCTION OF c-jun EXPRESSION BY TRANSFORMING GROWTH FACTOR-β IS INDEPENDENT OF RETINOBLASTOMA GENE EXPRESSION,

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Transforming growth factor-β (TGF-β) has both stimulatory and inhibitory effects on cellular proliferation depending on the cell type. It is thought to exert some of its growth inhibitory effects through blockade of phosphorylation of the retinoblastoma (Rb) protein. The c-jun oncogene encodes the major component of the AP-1 transcription factor which induces expression of the early response genes required for cellular proliferation. It is of interest to determine whether TGF-β affects expression of c-jun, and, if so, whether Rb protein is required for this effect. Two osteosarcoma cell lines, U2OS, which expresses normal Rb, and SAOS-2, which does not express Rb, were treated with TGF-β. Total cellular RNA was isolated and Northern blot analysis was performed using a c-jun probe. Increased expression of c-jun was seen following treatment with TGF-β in both Rb-positive and Rb-negative cell lines. To confirm these results, two small cell lung cancer cell lines, NCI-H69 which is Rb-positive, and NCI-H82, which is Rb-negative, were treated with TGF-β. Again expression of c-jun was elevated following TGF-β treatment in both cell lines. These findings demonstrate that TGF-β can induce c-jun expression in an Rb-independent manner which suggests that TGF-β may have some modulatory effects on cellular growth and proliferation which do not require the presence of the Rb protein.

G 522 A STRATEGY FOR EMPLOYING ANTISENSE OLIGODEOXY NUCLEOTIDES (ODNs) TO INHIBIT SMOOTH MUSCLE CELL PROLIFERATION. E. Speir, E. Brinkmann, S. Biro, Y.-M. Fu, Z.-X. Yu, S.E. Epstein, Bethesda, MD 20892

Smooth muscle cell (SMC) proliferation and migration play pivotal roles in restenosis following coronary angioplasty. Because proliferating SMCs express multiple growth factors and proto-oncogenes, we reasoned that a strategy employing ODNs complementary to the mRNA of such factors (antisense ODNs) might inhibit SMC proliferation, and perhaps migration. (By hybridizing with a specific mRNA, antisense ODNs interfere with translation of that mRNA.) To evaluate an antisense strategy for preventing restenosis, antisense phosphoroamidate ODNs (15-18 mer) complementary to c-myc and to PCNA mRNA were introduced into the medium of cultured rat aortic SMC.

Both antisense ODNs:

1)inhibited SMC proliferation in a dose-dependent manner ODNs with the same nucleotides but a scrambled sequence had no or less of an effect);

2)decreased SMC expression of c-myc and PCNA proteins.

In addition, antisense to c-myc mRNA inhibited SMC migration (modified Boyden chamber method). Of note, targeting of the mRNAs was highly specific, as shifts in targets of only a few base pairs profoundly altered inhibitory capacity.

In summary, our results indicate that expression of the c-myc and PCNA gene products importantly facilitates SMC proliferation, and that c-myc gene expression appears to be involved in a signal transduction pathway that modulates SMC migration. These results suggest a potential role of antisense strategies in the prevention of coronary restenosis.

G 523 IDENTIFICATION AND CHARACTERIZATION OF CELLULAR FACTORS WHICH INTERACT WITH THE RETINOBLASTOMA CONTROL ELEMENT, Kevin R. Webster and Kevin G. Coleman; Department of Cellular and Molecular Biology, Bristol-Myers Squibb Company, Wallingford, CT. 06492

The retinoblastoma suceptability gene (Rb) encodes a 105 kd protein which is a negative regulator of cell growth. The loss of Rb function has been observed in a number of human carcinomas, thus leading to its clasification as a tumor suppressor gene. Recent findings have suggested that Rb protein functions at the transcriptional level in controlling the expression of immediate-early genes. The transcriptional activity of both the *c-myc* and *c-fos* promoters is down-regulated by Rb protein. Rb-mediated repression of *c-fos* transcription has been shown to be dependent upon a 30 base pair DNA fragment (termed the Rb control element or RCE) within the *c-fos* promoter.

We have examined the factors that interact with the RCE using both south-western analysis and direct cloning methods. South-western analysis of total cell lysates from: Hela, Caski and IMR90 cell lines has identified seven proteins which can specifically interact with the RCE. These proteins are: 150, 115, 88, 76, 46, 34 and 29 kd in size and are present in all three cell lines. A number of these proteins have been partially purified and their identity is being pursued by protein sequence analysis. In addition we have screened a λgt11 cDNA expression library derived from IMR90 cells for proteins that can interact with the RCE. To date 25 clones have been isolated and are currently being characterized.

G 524 POSITIVE AND NEGATIVE REGULATION OF EXPRESSION OF THE MGSA GENES IN Hs294T MELANOMA CELLS. Lauren D. Wood, Rebecca L. Shattuck, and Ann Richmond. Departments of Cell Biology and Medicine, VA Medical Center(Nashville) and Vanderbilt University School of Medicine, Nashville, TN 37232

Melanoma growth stimulatory activity (MGSA) is a cytokine associated with neutrophil chemotaxis and growth regulation. Expression of MGSA in melanoma cells and fibroblasts is controlled at both the transcriptional and post-transcriptional levels. We have isolated genomic clones for α,β and γ forms of the MGSA gene and sequenced over 1Kb of the 5' regulatory regions for these genes. A number of concensus sites for regulatory elements have been identified, including putative $NF_{k\beta}$, SRE, AP3, CEBP, TCE and TIE elements. Based upon Northern analyses, nuclear run on analyses, and CAT assays, the positive modulators of expression include IL-1, serum, TNFα, cycloheximide and phorbol ester. The contribution of these agents to transcriptional activation versus mRNA stabilization has been evaluated using Northern analysis of 5,6-dichloro-1-β-D-ribofuranosyl-benzimidazole (DRB) treated Hs294T melanoma cultures. TGFβ is reported to be a negative modulator of MGSA expression in a number of cell systems. In our studies, using both Northern analysis and CAT assays, TGFβ produced an approximately 50% inhibition of basal and phorbol ester induced mRNA levels and CAT conversion. Serial 5' MGSAα promoter deletion constructs have been made to identify the promoter elements involved in the TGFβ suppression of the MGSAα gene. Finally, a putative fourth MGSA gene has been isolated. Sequence analysis of the 5' regulatory region reveals several of the same consensus sequences found in the α, β and γ genes, including $NF_{k\beta}$ and TCE/TIE-like regions. Investigations are currently underway to determine which cell systems express this form of MGSA and to determine which cytokines regulate expression of this form of MGSA.

G 525 EXPRESSION OF THE RETINOIC ACID RECEPTORS IN HUMAN PRIMARY NEONATAL MELANOCYTES AND METASTATIC MELANOMA CELL STRAINS. Douglas T. Yamanishi, Julie A. Buckmeier, Mark J. Graham, Joel E. Voboril, and Frank L. Meyskens Jr., Clinical Cancer Center and College of Med., Dept. of Hem./Onc., U.C. Irvine, Irvine, CA 92717.

The expression of the retinoic acid receptors (α, β, and γ) in primary neonatal melanocytes and metastatic melanoma cell strains was investigated using Northern blot analysis. The three retinoic acid receptor (RAR) isotypes were detected in melanocytes. The RAR RNA transcript expression levels of the melanoma cells were of two distinct patterns. Melanoma cell strains c81-46a and c81-46c expressed all three RAR isotypes. However, melanoma cell strains c81-61 and c83-2c expressed only the RAR α and γ isotypes. The effect of trans-retinoic acid (tRA) on melanocytes and melanoma cells was investigated using cell survival and Northern blot analysis. Cells expressing the RAR β isotype were sensitive to tRA. tRA treatment of cells induced the RAR β isotype RNA transcripts in melanocytes and melanoma cells c81-46a and c81-46c. RAR β isotype RNA transcripts were not induced in melanoma cell strains c81-61 and c83-2c. Melanocytes are very sensitive to tRA and c-jun RNA transcripts were induced following tRA treatment. Melanoma cell strains c81-46a and c81-46c are less sensitive to tRA and no effect on c-jun RNA transcripts expression levels was detected. Melanoma cell strains c81-61 and c83-2c are insensitive to tRA and c-jun RNA transcripts are repressed by tRA treatment. Thus one of the ways the RARs could effect human melanocytes is through the AP-1 pathway.

G 526 PROTEIN KINASE C-MEDIATED GENE REPRESSION AND CELL DEATH IN A HUMAN PROSTATIC ADENOCARCINOMA CELL LINE. Charles Y.-F. Young, Paul E. Andrews, and Donald J. Tindall, Department of Urology and Biochemistry and Molecular Biology, Mayo Clinic/Foundation, Rochester, MN 55905.

Recent studies have demonstrated that protein kinase C (PKC) activity increases in the rat prostate gland following castration. Since the proliferation of prostate cells is highly dependent upon androgen stimulation, we investigated the role of PKC in the involution of prostate cells in culture. LNCaP cells (a cell line derived from human prostate carcinoma) were incubated in the presence of androgens and the phorbol ester, 12-O-tetradecanoyl phorbol-13-acetate (TPA), a potent PKC activator that exhibits diversified effects on cell growth and differentiation. We observed that TPA exerted a profound cytotoxic effect in a dose-dependent manner. As little as $5x10^{-10}$M TPA-induced cell death within 48 hours. In the first several hours, cells underwent profound morphological changes, followed by cell death. This killing effect appeared to be specific for this androgen-sensitive cell line, since TPA (up to 10^{-6}M) had very little or much less effect on two androgen-independent prostate adenocarcinoma cell lines, PC-3 and DU145. Furthermore, staurosporine (100 nM), a potent PKC inhibitor, effectively prevented the TPA-induced cell death. We next examined the effects of TPA on the androgenic regulation of a marker of prostate epithelial cell differentiation, prostate-specific antigen (PSA). Previously, we have established that PSA is up-regulated by the ligand-activated androgen receptor in LNCaP cells at both the mRNA and protein levels. In this study, we have demonstrated that TPA exerted a time- and dose-dependent repression of the androgenic regulation of PSA mRNA and protein. PSA mRNA decreased rapidly within a few hours following TPA treatment. However, during that time the androgen receptor protein content was not changed by TPA treatment, suggesting that additional factor(s) may be involved in the repression of the androgenic induction. Moreover, staurosporine blocked the TPA-mediated repression of the androgenic stimulation of PSA protein. In summary, the cell death of LNCaP caused by TPA appears to be mediated by the PKC pathway. The repression of androgenic stimulation of PSA, which occurs at an early stage of TPA treatment, is also mediated by the PKC pathway. Thus, PKC-mediated actions in prostatic cells may play an important role in counteracting the androgenic stimulation of cell growth and gene expression.

Late Abstracts

ADULT KERATINOCYTES ARE RESISTANT TO THE DIFFERENTIATION-INDUCED DECREASE IN HA-RAS mRNA LEVELS OBSERVED IN NEWBORN KERATINOCYTES, Natalie A. Betz and Jill C. Pelling, Department of Biochemistry and Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, NE 68198.

During two-stage mouse skin tumorigenesis, the mouse c-Ha-ras oncogene undergoes activation by point mutation following initiation. Furthermore, initiated epidermal cells containing an activated Ha-ras oncogene have been shown to be resistant to calcium-induced terminal differentiation. However, the relationship between Ha-ras expression and the differentiation process is not well understood. In the present studies, Northern blot analysis was used to compare Ha-ras expression in normal newborn and adult epidermal cells undergoing differentiation. Steady state levels of Ha-ras mRNA remained unchanged in primary cultures of normal adult epidermal cells during calcium-induced differentiation, while steady state levels of Ha-ras transcripts decreased 2.0-4.4 fold during calcium-induced differentiation in primary newborn epidermal cells. The decrease in levels of Ha-ras mRNA observed during differentiation in newborn keratinocytes occurred as a relatively late event in the differentiation process, was specific for the Ha-ras gene, and was not due to a general decrease in transcriptional activity during differentiation. A characteristic pattern of keratin gene expression was observed, verifying that induction of the differentiation process had occurred in both the primary adult and newborn epidermal cells. The fact that adult keratinocytes are resistant to the differentiation-induced reduction in Ha-ras mRNA expression observed in newborn keratinocytes may explain the difference in in vivo tumorigenic potentials of newborn and adult skin.

THE t(15;17) TRANSLOCATION OF ACUTE PROMYELOCYTIC LEUKEMIA GENERATES A FUNCTIONALLY ALTERED RETINOIC ACID RECEPTOR. Hugues de Thé, Catherine Lavau, Christine Chomienne*, Agnès Marchio, Laurent Degos* and Anne Dejean, UREG, Institut Pasteur and *Hôpital Saint-Louis, Paris, France.

A specific translocation t(15,17) has been reported in every patient with acute promyelocytic leukemia (APL). We have previously reported that, in an APL-derived cell-line (NB4), this translocation fused the retinoic acid receptor α (RARα) gene to a previously unknown locus, initially called *myl* and now renamed PML (Nature, 347, 558-561, 1990). Moreover, genomic alterations of either RARα or PML loci were demonstrated in most patients, suggesting that the fusion of these two genes might be a general situation in APL.

We now report the molecular cloning of the wild-type PML and hybrid PML-RARα transcripts. The PML gene product displays a C_3HC_4 motif found in several DNA-binding proteins and could encode a transcription factor. Two hybrid cDNAs, that differ by an alternatively spliced coding exon of PML, were isolated from the NB4 cell-line and shown to encode proteins containing most of the PML sequences fused to a large part of RARα, including its DNA- and hormone- binding domains. In transient expression assays, the hybrid protein exhibits altered *trans*-activating properties if compared to the wild-type RARα progenitor. These observations suggest that in APL, the t(15;17) translocation generates a retinoic acid receptor mutant that could contribute to leukaemogenesis through interference with promyelocytic differentiation.

GH4C1 PITUITARY TUMOR CELLS SECRETE A GROWTH INHIBITORY FACTOR IN RESPONSE TO ESTRADIOL, C.A. Gilchrist, and J.D. Shull, Eppley Institute for Research in Cancer and the Department of Biochemistry, University of Nebraska Medical Center, Omaha, NE 68198-6805.

Our laboratory has shown that population density drastically affects the responses of GH4C1 cells to 17ß-estradiol (E2). When cells are plated at low density, E2 stimulates proliferation, induces prolactin (PRL) mRNA, and increases progesterone receptor (PR). In contrast, when cells are plated at a fourfold higher density, E2 inhibits proliferation and does not affect PRL mRNA, yet still increases PR levels. To test the hypothesis that this selective loss of responsiveness to E2 at the high density is due to the release of a paracrine factor, cells were plated at low density, and the ability of E2 to induce cell proliferation in fresh culture medium and in conditioned media collected from control and E2 treated, high density cultures was examined. In both the collection and treatment phases of these experiments, culture media were changed daily to reduce the possibility of nutrient depletion or toxification. In the fresh medium, E2 stimulated cell proliferation, and cells cultured in conditioned medium collected from high density control cultures proliferated at a rate comparable to control cells in fresh medium. However, low density cells cultured in media conditioned by E2 treated, high density cells were unable to proliferate over the five day culture period. Proliferation of the cells was assessed by measuring total DNA per plate and by measuring the ability of isolated nuclei to incorporate [^{32}P]dTTP into acid precipitable material. E2 also failed to induce PRL mRNA in these normally responsive low density cultures. Media were rapidly conditioned with this activity: medium collected from high density cells treated for one day with E2 blocked the induction of DNA synthesis in low density cultures. These data suggest that E2 induces the synthesis or release of a factor that functions as a paracrine regulator of cell proliferation and PRL gene transcription. We are presently attempting to identify and characterize this factor. Supported by grants from the National Institutes of Health, American Cancer Society, and the Nebraska Department of Health.

The Role of PDGF in Myelination of the Rat Optic Nerve: Further Evidence for Oligodendrocyte Formation via a Constitutive Developmental Pathway.
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Oligodendrocytes, the major myelin-forming cells in the CNS, arise on a precise developmental schedule from a bipotential precursor, termed the oligodendrocyte-type-2 astrocyte (O-2A) progenitor cell. Experiments *in vitro* show that oligodendrocytes are formed by default when O-2A progenitors are deprived of environmental signals. Under normal conditions, however, each progenitor cell undergoes a limited number of divisions before it differentiates into a non-dividing oligodendrocyte. It has been demonstrated that one of the mitogens responsible for the proliferation of O-2A progenitor cells is platelet-derived growth factor (PDGF). It appears that an intrinsic 'clock' in the progenitor cell limits the number of times it can divide in response to PDGF. At this point the cell drops out of the division cycle and, consequently, differentiates into an oligodendrocyte.

Since the number and binding capacity of PDGF receptors is initially conserved on newly-formed oligodendrocytes, it is likely that a block in a post-receptor signalling step causes the eventual loss of the mitogenic response to PDGF in O-2A progenitor cells. In order to address this point we have used the growth inhibitor amiloride to block the mitogenic response of O-2A progenitor cells treated with PDGF. We found that amiloride inhibited DNA synthesis in PDGF-treated progenitor cells. Furthermore, in parallel cultures the proportion of oligodendrocytes increased markedly in the presence of amiloride. These results suggest that inhibiting cell division in O-2A progenitors, normally responsive to PDGF, is sufficient to trigger differentiation and also provide further evidence that oligodendrocytes are the product of a constitutive developmental pathway.

INITIAL INCREASE IN PtdInsP and $PtdInsP_2$ INDUCED BY PDGF IN SERUM STARVED HUMAN FIBROBLASTS,
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PDGF induces immediate effects on actin polymerization and motile activities at the cell surface. Contrary to the effect of PDGF-BB stimulation with PDGF-AA does not efficiently provoke these effects. Earlier findings suggest that actin polymerization is driven by the PtdInscycle and we therefore followed the kinetics of initial PtdIns turnover induced by PDGF in serum-starved human fibroblasts labeled for 5 minutes with ^{32}P-orthophosphate. PDGF-AA and PDGF-BB both cause an initial increase in PtdInsP and $PtdInsP_2$ that appears to precede $PtdInsP_2$ hydrolysis. This suggests that the PtdIns4'- and PtdIns(4)P5'-kinases are stimulated by receptor activation prior to the stimulation of $PtdIns(4,5)P_2$ hydrolysis. For comparison we measured the time dependent cellular binding of the two PDGF isoforms under the same conditions, i.e. at 37°C. While very limited binding of ^{125}I-PDGF-AA occurred after 5 min, there was a continued binding to new receptor sites for ^{125}I-PDGF-BB during 1 h of incubation.
Despite a significaltly lower number of binding sites occupied by ^{125}I-PDGF-AA than by ^{125}I-PDGF-BB the initital PtdInsP and $PtdInsP_2$ formation was stimulated to a similar degree by the two isoforms. The subsequent $PtdInsP_2$ hydrolysis, however, appeared to correlate with the rate of binding to new receptor sites, i.e. PDGF-BB continously stimulated PtdIns turnover, whereas the effect of PDGF-AA ceased. These data demonstrate that the maintenance of PtdIns turnover is dependent on a continous ligand binding to cell surface receptors.
The initital relatively higher $PtdInsP_2$ formation than $PtdInsP_2$ hydrolysis induced by PDGF-AA can be discussed in relation to recent findings that components of the microfilament system have a regulatory function in growth factor induced PtdIns cycle turnover.

SUPPRESSION OF PDGF ALPHA AND BETA RECEPTOR mRNA EXPRESSION BY SV40 T/t ANTIGEN,
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The early region of simian virus 40 (SV40) encodes two proteins, large T antigen (94 kilodaltons) and small t antigen (17 kilodaltons). Both of the two tumor antigens are active in the process of virus-induced neoplastic transformation. It is generally believed that the T antigen is necessary for initiation of DNA replication and for cell transformation. Small t antigen seems to act cooperatively with large T antigen to enhance the transforming activity. Small t alone cannot transform established cell lines or immortalize primary cells in culture. During the study of PDGF receptors in human embryonic lung fibroblasts (2264 HEL) by Northern hybridization and ^{125}I-labeled PDGF receptor binding assay, we have found that the SV40 early region can suppress PDGF receptor expression. We found a total loss of the alpha receptor mRNA and protein and partial loss of the beta receptor signal. Microinjection of SV40 wild type DNA into cell nuclei followed by double immunostaining of PDGF beta receptor and SV40 T antigen, showed that the PDGF beta receptor reduction in the SV40 T positive cells could be observed already after 48 h. Large T antigen alone could not induce a total inactivation of PDGF alpha receptor mRNA expression, suggesting that small t antigen is also required for this process.