NEGATIVE CONTROLS ON CELL GROWTH

Organizers: Harold Moses and Robert Weinberg
March 3-9, 1990

Plenary Sessions Page

March 4:
The Genetics of Cancer-Somatic Alteration 250
The Human Genetics of Familial Cancers 252

March 5:
Growth Suppressing Genes-Somatic Cell Genetics 253
Oncogene-Reverting Genes 255

March 6:
Oncogenes and the Rb Gene 257
TGF Beta 259

March 7:
TGF Beta Family 260
Negative Growth Factors 262

March 8:
Growth Suppressing Genes 263
Receptors Negatively Regulating Growth 265

Poster Sessions

March 4:
Genetic Alterations in Cancer Cells; Familial Cancers (I100-131) 267

March 5:
Tumor Suppressor Genes; Growth Suppressing Genes (I200-238) 277

March 6:
TGF Beta; TGF Beta Family (I300-332) 290

March 7:
Growth Inhibitory Peptides (I400-427) 301

Negative Controls on Cell Growth

The Genetics of Cancer-Somatic Alteration

I 001 MOLECULAR BASIS OF TUMOR SUPPRESSION BY THE HUMAN RETINOBLASTOMA GENE,
Wen-Hwa Lee, Robert Bookstein and Eva Lee, Department of Pathology, M-012 and Center for Molecular Genetics, School of Medicine, University of California, San Diego, La Jolla, CA 92093. A class of cellular genes in which loss-of-function mutations are tumorigenic has been proposed. Such genes would normally act to suppress the cancer phenotype at the cellular or organism level. The gene determining susceptibility to hereditary retinoblastoma (RB) appears to operate in exactly this fashion, and is the first cancer suppressor gene to be molecularly cloned. 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 106 kd. Several antibodies, prepared according to the sequence, recognized the putative RB protein (apparent m.w. 110 kd) in normal cells, but do not detect this protein in retinoblastoma cells. The RB protein is a nuclear phosphoprotein capable of binding to DNA and to form a complex with oncoproteins of several DNA tumor viruses.

Consistent with its ubiquitous expression pattern, RB gene inactivation is not only limited to retinoblastomas. Many other cancers such as osteosarcoma, breast carcinoma, small cell lung carcinoma and prostate carcinom also contain inactivated RB genes, suggesting that RB may act as a general cancer suppressor gene. We have introduced, via retroviral-mediated gene transfer, a cloned RB gene into retinoblastoma, osteosarcoma, prostate carcinoma and breast carcinoma cells that have inactivated endogenous RB genes. Expression of the exogenous RB gene consistently suppressed their tumorigenecity in nude mice. Each individual clones of these tumor cells with exogenous RB protein expression were all non-tumorigenic in nude mice. However, after prolonged culturing some of the clones lost the expression of the RB protein and regained tumorigenic ability. This tight correlation strongly indicated that the RB gene is essential to tumorigenesis in these tumor cells.

In an attempt to address the potential cellular function of this gene, we have observed that Rb protein phosphorylation oscillates with cell-cycle and the unphosphorylated form is present predominantly in the G_0/G_1 phase. Furthermore, when cells were induced to differentiate only the unphosphorylated form of Rb could be detected, suggesting that RB protein was modulated through phosphorylation, may play an important role in these cellular functions. A working hypothesis is proposed to interpret how Rb participates cell proliferation and differentiation in relation to its role in tumorigenesis.

1. Lee, W-H *et al.* (1987) The retinoblastoma susceptibility gene enclodes a nuclear phosphoprotein associated with DNA binding activity. *Nature (London)* **329**: 642-645.
2. Huang, H-JS *et. al* (1988) Suppression of the neoplastic phenotype by replacement of the retinoblastoma gene product in human cancer cells. *Science* **242**: 1563-1566.
3. Chen, P-L *et al.* (1989) Phosphorylation of the Retinoblastoma Gene Product Is Modulated during the Cell Cycle and Cellular Differentiation. *Cell* **58**: 1193-1198
4. Bookstein, R et al. (1989) Replacement of Mutated Retinoblastoma Gene Suppresses the Tumorigenic Potential of Human Prostate Carcinoma Cells. (unpublished)

I 002 REDUCTION OR MUTATION OF THE Nm23/awd PROTEIN IN CANCER METASTASIS AND ABERRANT *DROSOPHILA* DEVELOPMENT
Patricia S. Steeg, Ariella Rosengard, Alvaro Leone, Allen Shearn+, C. Richter King*, and Lance A. Liotta, Laboratory of Pathology, National Cancer Institute, NIH, Bethesda Md. 20892,+Department of Biology, The Johns Hopkins University, Baltimore Md., *Molecular Oncology Inc., Gaithersburg, Maryland.
Invasion and Metastasis, the major cause of death for cancer patients, may be determined by loss of genes such as the Nm23/awd which regulate morphogenesis and differentiation in normal cells. The *nm*23 gene was identified by differential hybridization using RNA from K-1735 melanoma lines of varying metastatic potential. *nm*23 RNA levels were uniformly reduced in high metastatic potential rodent cell lines and human infiltrating breast duct carcinomas with lymph node metastasis. The murine and human cDNA clones contain an open reading frame which predicts a protein of 17.18 K. Affinity purified anti-Nm23 peptide antibodies were used to detect the 17 K nuclear and cytoplasmic protein.The human Nm23 protein has remarkable sequence homology, over the entire translated region, with a recently described developmentally regulated protein in *Drosophila*, encoded by the abnormal wing discs (*awd*) gene. Mutations in *awd* cause abnormal tissue morphology and widespread aberrant differentiation in *Drosophila*, analogous to changes in malignant progression. Negative regulation of Nm23/awd by mechanisms including allelic loss or mutation may lead to a disordered state favoring malignant progression.

I 003 THE MOLECULAR PATHOGENESIS OF LUNG CANCER INVOLVES THE ACCUMULATION OF A LARGE NUMBER OF MUTATIONS IN DOMINANT ONCOGENES AND MULTIPLE TUMOR SUPPRESSOR GENES (RECESSIVE ONCOGENES), John D. Minna, Takashi Takahashi, Marion M. Nau, Itsuo Chiba, Jean Viallet, Jochen Schütte, Rhoda Maneckjee, Frederick Kaye, Jacqueline Whang-Peng, and Adi F. Gazdar, NCI-Navy Medical Oncology Branch, National Cancer Institute, and Uniformed Services University of the Health Sciences, Bethesda, MD 20814

We have found that lung cancer cells produce autocrine growth factors, *jun* family transcription factors, nicotine and opioid receptors which could aid in tumor promotion allowing the accumulation of a large number of genetic lesions. These lesions documented in both fresh tumor specimens and tumor cells lines involve the activation of the dominantly acting cellular proto-oncogenes (particularly those of the *myc* family) and the inactivation of the recessive or "tumor suppressor" genes particularly for the retinoblastoma (*rb*) and *p53* genes as well as putative suppressor genes in chromosome regions 3p and 11p. Lung cancer cells have a large number of clonal structural and numerical cytogenetic abnormalities. These include chromosomal deletions with a prominent deletion occuring in chromosome region 3p(14-23) as well as changes on chromosomes 1, 4, 5, 11, 13, 16, and 17. RFLP analysis shows 3p allele loss in nearly all small cell lung cancers and 50% or more of non-small cell lung cancers as well as allele loss on chromosome regions 13q and 17p and other chromosomes which pointed to involvement of the *rb* and *p53* genes respectively. Similarly, the allele loss in chromosome regions 3p and 11p have prompted a search for new anti-oncogene(s) in these areas. Studies of the *rb* gene suggest it may be altered in nearly all cases of small cell lung cancer (SCLC) and at least some non-small cell lung cancers (Non-SCLC), while *p53* appears mutant in 40% or more of all lung cancers. In comparing mutations in *rb* and *p53* we find occasional examples of homozygous deletions in both. More frequently we find absent expression of *rb* mRNA and/or protein, while for *p53* the commonest lesions are point or small mutations leading to the production of a mutant p53 protein. The latter findings are consistent with a working model of mutant *p53* functioning in a dominant negative fashion. The number of lesions required for both the dominant and recessive oncogenes raise the question of Mendelian inheritence and acquistion of lesions during embryonic development as well as from carcinogen exposure in adult life. While lung cancer is not generally considered to be an inherited disease, there are actually several pieces of evidence indicating potential inherited predisposition including patients with familial retinoblastoma cured of their retinoblastoma who have an increased risk of developing lung cancer, and the 2-4 fold increased risk of several types of cancer in family members of lung cancer patients. In conclusion, our studies showed that lung cancer cells exhibit a large number (10-20 per tumor) of lesions involving both dominant oncogenes and the tumor suppressor genes. These findings suggest that detection of molecular genetic abnormaliaties in these genes may be applied in studies of early diagnosis, prognosis, and familial inheritence of cancer.

I 004 GENETIC ALTERATIONS ACCUMULATE DURING COLORECTAL TUMORIGENESIS, Bert Vogelstein, Janice M. Nigro, Suzanne J. Baker, The Johns Hopkins Oncology Center, 424 N. Bond Street, Baltimore, MD 21231

Colorectal tumors evolve through a series of progressive stages defined clinically and histopathologically. We have attempted to define the genetic alterations that accompany, and are presumably responsible for, this progression. These studies have revealed that genes on several different chromosomes (prominently including chromosomes 5, 12, 17 and 18) are involved in this process; alterations of specific oncogenes and tumor suppressor genes on these chromosomes gradually accumulate as tumors progress from small benign adenomas to large, invasive carcinomas. Although some of the alterations usually precede others, the total accumulation of changes is more important in determining biologic behavior than the order of changes with respect to one another.

The study of mutations within two genes, *RAS* and p53, has revealed some interesting similarities and differences. For example, *RAS* gene mutations occur in only a few types of cancers besides those of the colon, while p53 gene mutations occur commonly in most human tumor types. Both *RAS* and p53 are usually altered through point mutations, but there are many more target codons for p53 than for *RAS*. The targets for p53 gene mutations include more than 20 codons dispersed over four hotspots, whereas only three codons are mutated in *RAS* genes. This difference in target size is consistent with the fact that p53 gene mutations probably exert a dominant negative effect, while *RAS* gene mutations are selected by virtue of a positive effect. Mutations in p53 genes initially occur in the presence of a wild-type p53 gene on the homologous chromosome 17. At a later stage of tumorigenesis, the wild-type p53 gene is usually lost, leading to further tumor progression. In contrast, *RAS* gene mutations usually are not associated with the later loss of the wild-type *RAS* gene from the homologous chromosome 12. These mutations provide some insight into the nature of the processes underlying the development of cancer in humans. They will be discussed with respect to a genetic model for colorectal tumorigenesis.

Negative Controls on Cell Growth

The Human Genetics of Familial Cancers

I 005 MOLECULAR GENETICS OF GLIOMA PROGRESSION, Webster K. Cavenee[1], Tom Mikkelsen[1], C. David James[2], and V. Peter Collins[3], [1]Ludwig Institute for Cancer Research, Montreal H3A 1A1 Canada; [2]Department of Neurosurgery, Henry Ford Hospital, Detroit, MI 48202; Ludwig Institute for Cancer Research, Stockholm S105 01 Sweden.

The development of human cancer is generally thought to entail a series of events that cause a progressively more malignant phenotype. Such a hypothesis predicts that tumor cells of the ultimate stage will carry each of the events, cells of the penultimate stage will carry each of the events less the last one and so on. That is to say a dissection of the pathway form a normal cell to a fully malignant tumor may be viewed as the unraveling of a nested set of aberrations. In experiments designed to elucidate these events we have compared genotypic combinations at genomic loci defined by restriction endonuclease recognition site variation in normal and tumor tissues from patients with various forms and stages of cancer. The first step, inherited predisposition, is best described for retinoblastoma in which a recessive mutation of a locus residing in the 13q14 region of the genome is unmasked by aberrant, but specific, mitotic chromosomal segregation. A similar mechanism involving the distal short arm of chromosome 17 is apparent in astrocytic tumors and the event is shared by cells in each malignancy stage. This is distinct from a loss of heterozygosity for loci on chromosome 10 which is restricted to the ultimate stage, glioblastoma multiforme. These results suggest a genetic approach to defining degrees of tumor progression and means for determining the genomic locations of genes involved in the pathway as a prelude to their molecular isolation and characterization.

I 006 EXPRESSION OF GENETIC PREDISPOSITION TO BREAST CANCER,

Mark H. Skolnick, John H. Ward, C. Jay Marshall, G. Berry Schumann, Lisa A. Cannon-Albright, David E. Goldgar, William P. McWhorter, Harmon J. Eyre, Univeristy of Utah Medical Center, Salt Lake City, UT, 84132.
Women with proliferative breast lesions have an increased risk of developing breast cancer. We developed a sensitive method for detecting proliferative breast disease (PBD) in the absence of a detectable lesion. This technique, systematic fine needle breast aspiration, was used to screen asymptomatic women in high risk families. Proliferative breast disease was frequently detected in relatives of pairs of both premenopausal and postmenopausal breast cancer probands. We hypothesize a common major gene for proliferative breast disease which in its most extreme form is expressed as breast cancer. Preliminary analysis of proliferative breast disease indicates that it may be a commonly inherited precursor which is responsible for a sizeable fraction of breast cancer cases. Attempts to map a gene for breast cancer are thwarted by late age onset of disease, deceased family members, and sex specific expression. Furthermore, rare families segregating for early onset and/or bilateral breast cancer may represent a different genetic predisposition than the more common susceptibility suggested by this study. Analysis of genetic markers in breast cancer families typed for proliferative breast disease offers a different avenue to mapping a breast cancer susceptibility gene and may lead to an understanding of the proliferation of breast cells in these women.

Negative Controls on Cell Growth

Growth Suppressing Genes-Somatic Cell Genetics

I 007 TUMOR SUPPRESSOR GENES AS NEGATIVE REGULATORS OF GROWTH FACTOR RESPONSES,
J. Carl Barrett, Jeffery Boyd, Cynthia Afshari, Lois Annab, Junichi Hosoi, Jeffry Montgomery and Roger Wiseman, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709
Tumor suppressor genes control the neoplastic phenotype of tumor cells, but the function of these genes in normal cells is unknown. We have shown that the loss of a tumor suppressor gene function releases negative controls on the growth of cells in agar. Clonal derivatives of nontumorigenic, immortal Syrian hamster cell lines were isolated that either retained (termed $supB^+$) or had lost (termed $supB^-$) the ability to suppress tumorigenicity of chemically transformed tumor cells in cell hybrids. Neither $supB^+$ nor $supB^-$ variants grew in agar under conditions that allowed efficient growth of the tumor cells. However, $supB^-$ cells, but not $supB^+$ cells, were reversibly induced to grow in agar in the presence of tumor cell-conditioned media or by a combination of growth factors. These results indicate the tumor suppressor gene function in $supB^+$ cells negatively regulates the growth response of cells in agar to mitogenic stimuli. This growth regulation depends on cell shape or adhesion because $supB^+$ and $supB^-$ cells grown attached to plastic responded similarly to growth factors. After growth factor stimulation in suspension, quiescent $supB^-$ cells, but not $supB^+$ cells, exhibit a characteristic elevation in steady-state levels of several immediate-early mRNAs. Nuclear run-on analyses indicated that this is due to greater post-transcriptional mRNA degradation in the $supB^+$ cells. We propose that loss of a tumor suppressor gene function allows the anchorage-independent growth of $supB^-$ cells through the differential post-transcriptional regulation of immediate-early gene expression, and that a disruption of the normal actin microfilament system seen in $supB^-$ cells may be related to this process. In an attempt to identify the $supB^+$ gene product, we performed differential screening of a $supB^+$ cDNA library with cDNA probes prepared from $supB^+$ cells and $supB^-$ cells. We identified three $supB^+$ specific mRNAs (type II collagen, type IX collagen, and H19). The steady-state levels for these mRNAs are considerably higher in $supB^+$ cells than in $supB^-$ cells. We performed nuclear run-on assays to test the hypothesis that these genes are transcriptionally regulated by the supB tumor suppressor gene function, and higher transcription rates for type II collagen and H19 were observed in $supB^+$ cells. DNA sequence comparisons revealed that a 10-base pair element previously identified in the mouse H19 enhancer was also present in the 5' flanking region of the rat $\alpha 1$ type II collagen gene. By gel shift assays, a factor that could bind this sequence motif was found in nuclear extracts from $supB^+$ cells. Further characterization of this binding factor and its possible role in the $supB^+$ function will be discussed.

I 008 TUMOR SUPPRESSOR GENE CONTROL OF AN INHIBITOR OF ANGIOGENESIS,
Noel P. Bouck, Deborah J. Good, Peter J. Polverini, and F. Rastinejad, Departments of Microbiology-Immunology and Pathology and Cancer Center, Northwestern University Medical and Dental Schools, Chicago, IL 60611
As a normal cell develops into a solid tumor it undergoes a series of changes. At the genetic level, oncogenes are activated and multiple tumor suppressor genes are inactivated. At the physiological level, growth is enhanced, immunity evaded, and neovascularization induced. Neovascularization appears to be a prerequisite, for experimental solid tumors are unable to grow beyond a few mm in thickness without a blood supply. Using the immortal, non-tumorigenic hamster cell line, BHK21/cl13, we have linked the genetic loss of a tumor suppressor gene with the physiological acquisition of angiogenic activity. These cells produce both angiogenic factors and a 140 kD inhibitor that blocks the action of these factors. The production of the inhibitor, and thus the inability of the cells and their conditioned media to induce neovascularization, is strictly dependent upon the presence in the cells of an active suppressor gene.

This 140 kD suppressor-dependent inhibitor of angiogenesis has been identified as a portion of the well characterized adhesive glycoprotein thrombospondin. In addition native human thrombospondin has been found to inhibit angiogenesis efficiently in vivo and to suppress endothelial cell migration in vitro. Thrombospondin is abundant in the platelet alpha granule and is produced and incorporated into matrix by a variety of other cell types. These results suggest the possibility that its presence may play an inhibitory role important in the resolution of the neovascularization accompanying wound repair and that its absence may contribute to the pathological angiogenesis on which the growth of solid tumors depends.

I 009 STRUCTURE AND FUNCTION OF THE *gas* GENES, V. Sorrentino, C. Schneider and L. Philipson, European Molecular Biology Laboratory, 6900 Heidelberg, F.R.Germany
A set of growth arrest specific (*gas*) genes whose expression is negatively regulated by serum has recently been identified (Cell 54, 787-793, 1988). The sequence of full-length cDNA has been determined for 3 of these genes. Although accumulation of *gas* gene mRNA is down-regulated by serum, nuclear transcription of the *gas* 2, 3 and 5 genes is observed in serum stimulated cells indicating that posttranscriptional events may regulate the mRNA levels. In contrast, the *gas* 1 gene seems to be transcriptionally regulated during the growth cycle. Cell cycle regulation and the serum response of *gas* 1 is lost in *ras* transformed cells. Sequence similarities to both membrane and secreted proteins have been identified among the *gas* cDNAs. Since the *gas* gene expression shows strong tissue specificity and developmental control they may not be the primary mediators for inducing growth arrest. In accordance, the expression of *gas* genes is down-regulated during the final steps of terminal differentiation in hematopoetic and muscle cells

I 010 MONOCHROMOSOME TRANSFER AND TUMOR SUPPRESSION, Scott A. Bader, Steven F. Dowdy Eric J. Stanbridge, Department of Microbiology and Molecular Genetics, University of California, Irvine, California College of Medicine, Irvine, CA 92717
Evidence has been accumulating for loss of genetic information associated with various human malignancies. This evidence has been based upon cytogenetic analyses and restriction fragment length polymorphism (RFLP) analyses. Using these techniques deletion of discrete regions of specific chromosomes have been detected. Human cancers have been associated with specific deletions on single chromosomes (e.g.retinoblastoma) or multiple chromosomes (e.g.colorectal carcinomas). These deletions have been interpreted as inactivation of tumor suppressor genes. With few exceptions (rb-1 and p53) the genes have not been cloned and linkage analysis data are not available. In order to provide functional evidence for unknown and putative tumor suppressor genes we have developed techniques for single chromosome transfer. Data will be presented indicating tumor suppression via monochromosome transfer. In most cases the predicted chromosome had a tumor-suppressing effect. In others e.g.neuroblastoma, this was not the case. Furthermore, we have been able to dissociate the induction of differentiation from tumor suppression in neuroblastoma. Recent refinements of the microcell transfer technique have allowed us to transfer portions of single chromosomes, thereby, more finely mapping the region responsible for tumor suppression.

Oncogene-Reverting Genes

I 011 MOLECULAR ANALYSIS OF SUPPRESSION OF TRANSFORMATION Benjamin G. Neel,[1] Daniel L. Simmons,[2] Christine A. Jost,[1] Jonathan Chernoff,[3] Raymond L. Erikson[3] and Andrea Schivella[3], [1]Molecular Medicine Unit, Beth Israel Hospital, Boston, Mass. 02215, [2]Department of Chemistry, Brigham Young University, Provo, Utah 84602, [3] Department of Cellular and Developmental Biology, Harvard University, Cambridge, Mass. 02138. We have taken two approaches towards identifying genes that suppress transformation. First, using retroviral insertional mutagenesis of a dominant revertant (F2) of a KiSV-transformed NIH3T3 cell line (DT), we have identified and cloned the cDNA for a new, highly conserved gene (skr-1) which may play a role in suppression of v-ras^{Ki}-transformation. There is a rare Bam H1 polymorphism in skr-1 present in DT and its derivative lines. Integration in one retransformant of F2 occurs into the polymorphic allele. Four other retransformants (out of 11) have lost one copy of this allele as have two cell lines established from a tumor arising after a long latent period in nude mice injected with large numbers of F2 cells. The gene encodes a 5.7 kb mRNA which is expressed at low levels in all mouse tissues examined. The largest cDNA obtained is 5.4 kb; it lacks the extreme 3' end but contains a long open reading frame the predicts a 130kD protein. The coding sequence shows no significant similarity to any known sequence. There is a potential leuicine zipper domain near the N-terminus, but no other features suggestive of a DNA binding protein. We are currently attempting direct tests of the biologic function of skr-1 using expression vectors.

Second, we reasoned that since many proto-oncogenes encode tyrosine kinases, some suppressor genes might encode tyrosine phosphatases. We therefore cloned the major tyrosine phosphatase of human placenta (PTPase 1B) using RNA-based PCR. Sequence analysis and in vitro transcription/translation indicate that the full length protein is approximately 10 kD longer than reported from direct protein sequencing experiments. Current experiments involving expression of the cloned gene and isolation of related genes will be discussed.

I 012 ISOLATION OF POSSIBLE TUMOR SUPPRESSOR GENES BY FUNCTIONAL ASSAYS *IN VITRO*, Makoto Noda, Hitoshi Kitayama, Susumu Kanazawa, Satoru Murata, Tomoko Matsuzaki and Yoji Ikawa, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), 3-1-1 Koyadai, Tsukuba, Ibaraki 305, Japan

To understand the molecular mechanism of malignant cell transformation, we have been isolating and characterizing suppressor mutants (flat revertants) from a v-Ki-*ras*-transformed N1H3T3 cell line, named DT. Through this method, we have isolated and molecularly characterized four biologically active cDNA species. The *Krev*-1 cDNA encodes a member of the *ras* protein super-family. *Krev*-2 encodes a small and, possibly, secretory polypeptide(s). *Krev*-3 encodes a trancated form of an extracellular matrix protein, tenascin (also known as cytotactin, hexabrachion, myotendinus antigen, etc.). The *Krev*-4 cDNA has no obvious open reading frames, and the possibility that the gene acts at the RNA level is currently being tested. We have extensively examined the biological activities of the *Krev*-1 cDNA and its point mutants, and found that this gene suppresses not only the transformed morphology of DT cells but also other important phenotypes such as anchorage-indepenendent growth and *in vivo* tumorigenicity of DT cells as well as of HT1080 human fibrosarcoma cells. We have also found, through the experiments using a series of *Krev*-1/H-*ras* chimeric cDNA molecules, that the determinant of the unique transformation suppressor activity of *Krev*-1 resides in the first 60 amino acid portion of the protein . Thus, this approach is allowing us to isolate a series of biologically interesting genes, and further studies on these genes and their products may yield important insights into the regulatory mechanisms of cell growth and, possibly, into the etiology of some naturally occuring tumors.

I 013 MOLECULAR CLONING AND FUNCTIONAL ANALYSIS OF TWO CANDIDATE TUMOR SUPPRESSOR GENES, Reinhold Schäfer, Arto C. Nirkko, Johannes Balmer, Colin Wickenden, Patrice M. Ambühl and Roman Klemenz, Division of Cancer Research, Institute of Pathology, University of Zürich, CH-8091 Zürich, Switzerland

The neoplastic phenotype of H-ras transformed rat FE-8 cells and of spontaneously transformed Chinese hamster Wg3-h-o cells is suppressed upon fusion with normal diploid cells. We have now isolated phenotypic revertants from these tumorigenic rodent cell lines following transfection of normal human DNA in two consecutive cycles. We have molecularly cloned the human DNA associated with the reversion of the tumorigenic phenotype of FE-8 and Wg3-h-o cells.

A candidate suppressor gene, designated NTS-1, has been cloned from secondary FE-8 transfectant DNA in the EMBL3 phage vector. Transfer of recombinant phage DNA into FE-8 cells resulted in the suppression of anchorage- and serum-independent growth, and a reduction in tumorigenicity. The transfected FE-8 cells exhibited the suppressed phenotype in spite of the continued expression of the ras oncogene. We have analyzed NTS-1 expression in FE-8 transfectant RNA, have determined its genomic nucleotide sequence, and its location in the human genome. The suppressor gene is highly expressed in FE-8 revertants. The NTS-1 locus has been tentatively assigned to human chromsome 20. The analysis of the nucleotide sequence has not revealed any homology to known genes.

The human DNA sequence associated with the reversion of the malignant hamster Wg3-h-o cells has been molecularly cloned in a cosmid vector. Transfer of recombinant cosmid DNA into hamster Wg3-h-o cells induced the suppressed phenotype in only a subset of transfectants. At present, we do not know whether the reversion of Wg3-h-o cells is due to the down-regulation of oncogene expression, since a transforming gene has not yet been identified in the hamster cell line. However, both Wg3-h-o DNA and revertant DNA exhibit transforming activity in a NIH/3T3 transfection assay. The locus of the candidate suppressor gene, designated NTS-2, has been assigned to human chromsome 8.

I 014 ISOLATION OF TRANSFORMATION EFFECTOR AND SUPPRESSOR GENES USING IN VITRO ASSAYS, J.R. Van Amsterdam, C. Hoemann, C.-J. Kho, M.O. Boylan, V.L. Afshani, R.C. Sullivan and H. Zarbl. Division of Toxicology, Whitaker College of Health Sciences and Technology. Massachusetts Institute of Technology. Cambridge, MA 02139.

Three in vitro assays are being used to identify transformation effector and suppressor genes. One approach involves the isolation of revertants from mutagenized populations of transformed cells using a fluorescence-activated cell sorter. A previous study showed that revertants of v-fos-transformed fibroblasts (Cell 51:357, 1987) are resistant to transformation by a wide variety of oncogenes, implying that they had sustained causal mutations in genes which comprise transformation pathways common to several oncogenes. These revertants were transfected with human cDNA expression libraries and retransformants which acquired a functional effector gene were isolated. Polymerase chain reaction using vector primers was utilized to amplify the cDNA inserts present in these retransformed cells. Sequence analysis of these amplified inserts encoding the putative effector genes is in progress. Results from preliminary experiments have indicated that the phenotype of at least one revertant resulted from a mutation in the c-jun proto-oncogene, implying that c-jun is necessary for v-fos mediated cell transformation. This notion was corroborated by the ability of anti-sense c-jun RNA to revert the phenotype of v-fos transformants. Isolation and characterization of additional revertants from tumor cell lines by chemical and retroviral insertional mutagenesis is in progress. In the second approach, revertants are isolated from tumor cell lines that were transfected with genomic DNA or cDNA expression libraries prepared from normal human cells. Transfectants which acquire a functional suppressor genes should display a non-transformed phenotype. Using this tact, several putative revertant cell lines have been isolated by cell sorting. These clones exhibit reduced cloning efficiencies in agar medium, and when inoculated into syngeneic rats and/or athymic (nude) mice showed prolonged latency periods and reduced tumor size, consistant with the presence of a functional tumor suppressor gene. The suppressor genes expressed in these revertants are currently being analyzed. The final approach involves the identification of genes that are differentially expressed between transformed cells and revertants. We have found that $\alpha 1$(I) and $\alpha 2$(I) procollagen genes are expressed in Rat-1 fibroblasts, but not in v-fos transformants. The genes are again expressed in revertants, indicating that their regulation resulted from v-fos transformation rather than v-fos expression. By studying the transcriptional regulatory regions of These genes, We hope to identify transcriptional factors which function as effector genes in v-fos cell transformation.

Oncogenes and the Rb Gene

I 015 FROM DISEASE TO GENE AND BACK AGAIN: LESSONS LEARNED FROM RETINOBLASTOMA, Brenda L. Gallie, The Hospital for Sick Children Research Institute and the University of Toronto, Toronto, Ontario, Canada M5G 1X8.
Unique clinical features of retinoblastoma (RB) have made possible the discovery of mechanisms fundamental to cancer in general. The intraocular location of tumors allowed early diagnosis and curative treatment; survivors demonstrated dominant transmission of predisposition in 40% of cases; and patients with germline deletions indicated the genetic locus of the gene, RB1. Molecular markers distinguishing alleles revealed that inactivation of both alleles of this tumor suppressor gene during normal retinal development initiates RB tumors, usually by loss of heterozygosity (LOH), a previously unrecognized mechanism of human cancer. The gene, RB1, was identified by homozygous deletion in a few RB tumors, and successfully cloned. The recessive nature of the RB1 gene implicated it in control of the cell cycle. Isolation and characterization of the ubiquitously expressed, nuclear phosphoprotein has supported this model. In addition, the RB1 gene turns out to be frequently mutated as a progressive event in malignancies associated with RB. We have identified mutations in RB1 in 13/21 RB tumors by RNase protection of polymerase chain reaction (PCR) amplified cDNA from tumors and identified the germline RB1 mutation in 8 families, allowing accurate diagnosis of the risk for RB in 2 infants. Thus the research made possible by the children with RB, that has uncovered basic mechanisms of human cancer, has returned to benefit the RB children and their families.

I 016 CELLULAR TARGETS FOR TRANSFORMATION BY DNA TUMOR VIRUSES

Nicholas Dyson, Karen Buchkovich, Qianjin Hu, and Ed Harlow
Cold Spring Harbor Laboratory, P.O. Box 100, Cold Spring Harbor, NY 11724

Tumor suppressor genes are genetic loci whose loss has been associated with tumor development. Because the inactivation of both alleles of these genes is a key feature in the genesis of certain tumors, it has been postulated that the protein products of tumor suppressor genes function in the negative regulation of cell proliferation. Tumor suppressor genes have been identified by genetic analysis either as loci associated with an inherited predisposition to certain tumors or by karyotypic studies that have localized putative tumor suppressor genes to loci that show reduction to homozygosity or loss of heterozygosity during tumor development. Second, recent work from a number of laboratories has shown that the protein products of tumor suppressor genes often form protein/protein complexes with the transforming proteins of small DNA tumor viruses. The retinoblastoma gene, RB-1, is one of the best studied examples of the tumor suppressor genes. It was originally identified and cloned through its association with childhood retinoblastoma. However, it is also a key target for transformation by the oncogenes of several small DNA tumor viruses. The E1A proteins of adenovirus, the large T antigens of polyomaviruses, and E7 protein of papillomaviruses all bind to p105-RB. Genetic studies of all three viruses have shown that any mutation that destroys binding to p105-RB also destroys the ability of these proteins to transform cells, suggesting that interaction with the RB gene product is a key event in viral transformation. In addition to interacting with p105-RB, the adenovirus E1A proteins and the polyomavirus large T antigens also bind to other cellular proteins. One of these, a protein with a molecular weight of 107,000 daltons, 107K, binds to E1A and large T at the same amino acid region as p105-RB, suggesting that the 107K and p105-RB proteins may have structural similarities. These observations and other comparisons raise the question whether 107K may be functionally related to p105-RB. If so, the 107K protein may be another example of a product of a tumor suppressor gene interacting with the transforming proteins of small DNA tumor viruses.

I 017 PAPILLOMAVIRUSES AND TUMOR SUPPRESSOR GENES, Peter M. Howley, Bruce A. Werness, and
Karl Münger, Laboratory of Tumor Virus Biology, NCI Bethesda, MD 20892
Compelling evidence now associates certain specific human papillomaviruses (HPVs) with specific types of human cancer. HPV-16 and HPV-18 DNA sequences can be found in a large percentage of biopsies of cervical carcinoma tissues and in cell lines established from human cervical carcinomas. These DNAs are generally transcriptionally active in the tissues and in the cervical carcinoma cell lines, and a variety of studies have mapped the transcripts to the E6 and E7 open reading frames. E7 is an oncoprotein which is sufficient for transformation of NIH 3T3 cells and can cooperate with an activated ras oncogene to transform primary baby rat kidney cells. The E7 proteins of the genital tract papillomaviruses have functional and amino sequence similarities to the adenovirus E1A proteins (1). These regions correspond to the regions in adeno E1A involved in complex formation with pRB. The E7 proteins of the human genital papillomaviruses, complex with pRB (2). Efficient transformation of human keratinocytes requires E6 as well as E7 for HPV-16 and HPV-18 (3). The E6 protein is a cysteine rich protein which has been studied with respect to cellular proteins with which it may interact. In collaboration with Arnold Levine, we have demonstrated that E6 can complex with p53 in vitro. The consequence of this interaction in papillomavirus infected cells is currently under investigation. Thus, it appears that the adenoviruses, the polyomaviruses, and the genital papillomaviruses may target similar cellular pathways in transformation through the proteins with which their oncoproteins associate. The HPV E6 and E7 oncoproteins are not sufficient for full oncogenic transformation. It is clear that additional cellular events are necessary for progression to invasive cervical cancer.

(1) Phelps, W.C., Yee, C.L., Münger, K., and Howley, P.M. Cell 53:539, 1988.
(2) Dyson, N., Howley, P.M., Münger, K., and Harlow, E. Science 243:934, 1989.
(3) Münger, K., Phelps, W.C., Bubb, V., Howley, P.M., and Schlegel, R. J. Virology 63:4417, 1989.

I 018 FUNCTIONAL ANALYSIS OF COMPLEX FORMATION BETWEEN THE RETINOBLASTOMA SUSCEPTIBILITY GENE PRODUCT AND SV40 LARGE T ANTIGEN, David M. Livingston, James A. DeCaprio, Mark E. Ewen, William G. Kaelin, Jr., and John W. Ludlow. The Dana-Farber Cancer Institute and The Harvard Medical School, Boston, MA 02115.

The product of the RB-1 locus is a 928 residue nuclear, DNA binding polypeptide believed to exert some measure of suppression of neoplastic growth in multiple cell types. It exists as a complex family of unphosphorylated (pRB) and differentially phosphorylated (pRBphos) species. A series of DNA tumor viral transforming proteins, including the adenovirus E1A products, SV40 large T antigen (T), and HPV E7, all form stable complexes with the RB-1 product, and complex formation likely participates in the mechanisms underlying the transforming functions of these proteins. Specifically, the data suggest that each of these elements can modulate (? inhibit) the growth suppressing function of the RB-1 product. In this presentation, we will review evidence from our laboratory pointing to a possible cell cycle regulatory role for pRB which is conditioned by its cyclical phosphorylation and dephosphorylation. At least part of the effect of T-pRB complex formation may be to by-pass checkpoint(s) in the cycle at specific point(s) which depend, in some measure, upon the maintenance of pRB cell cycle regulatory function. Moreover, evidence describing the existence of discrete T and pRB domains which, in part, control T-pRB complex formation will be described, along with data pointing to a role for the quarternary structure of T in this process.

This abstract has been submitted to the UCLA Symposium "Molecular Pathways of Cytokine Action," Park City, Utah, January 1990.

Negative Controls on Cell Growth

TGF Beta

I 019 TRANSFORMING GROWTH FACTOR β1 SIGNALLING PATHWAYS, Philip H. Howe and Edward B. Leof, Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232.

Transforming growth factor β1 (TGFβ1) is a 25 kDa homodimeric polypeptide which is prototypic of a larger family of related polypeptides which regulate cell growth and differentiation. The response of cultured cells to TGFβ1 is divergent; mesenchymal cells are routinely growth stimulated by TGFβ1 while the majority of other cell types are inhibited. Addition of TGFβ1 to plasma membranes from AKR-2B cells, a mesenchymal cell line growth stimulated by TGFβ1, increased the binding of the non-hydrolyzable GTP analog GTPγ[^{35}S] as well as increased GTPase activity. Pretreatment of the membranes with pertussis or cholera toxin inhibited both TGFβ1-stimulated GTPγ[^{35}S] binding and GTPase activity. Furthermore, the stimulatory effects of TGFβ1 on c-sis and c-myc transcription were shown to be toxin sensitive while extracellular matrix/cytoskeletal gene expression was toxin insensitive. The data indicate that in AKR-2B cells TGFβ1 uses both G protein-dependent and independent signalling pathways. Similar G protein-dependent coupling was observed in CCL64 epithelial cells inhibited by TGFβ1. Isolation of variants resistant to TGFβ1 growth inhibition was associated with a defect(s) in this G protein coupling as determined by GTPγ[^{35}S] binding, GTPase activity, and pertussis toxin ribosylation. Lastly, our recent data indicate that the effects of TGFβ1 in early G0/G1 are insufficient for growth inhibition. Addition of TGFβ1 at any time during the prereplicative G1 period inhibited DNA synthesis in CCL64 cells to a similar extent as when added at the time of restimulation. Moreover, cultures can progress to the G1/S phase boundary in the continual presence of TGFβ1, but not enter DNA synthesis. Associated with this block at G1/S is a modulation in the phosphorylation of the cdc 2 protein kinase.

I 020 DIFFERENTIAL REGULATION OF EXPRESSION OF THE TGF-ß'S IN GROWTH AND DEVELOPMENT. Anita B. Roberts, Sonia B. Jakowlew, Paturu Kondaiah, Kathleen C. Flanders, Adam B. Glick, Lalage Wakefield, Seong-Jin Kim and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD 20892.

Transforming growth factors-ß (TGF-ß) are 25 kDA homodimeric peptides with multifunctional actions in control of the growth, differentiation, and function of a broad range of target cells of both epithelial and mesenchymal derivation. They are expressed early in embryogenesis and their tissue-specific and developmentally dependent expression is strongly suggestive of an essential role in particular morphogenetic and histogenetic events. Five distinct TGF-ß's have been characterized thus far, each approximately 65-80% homologous to the others. Mammalian species appear to express predominantly TGF-ß's 1-3, while chickens express TGF-ß4 in addition. So far, expression of only TGF-ß's 2 and 5 has been detected in the frog. Using both molecular biological and immunohistochemical techniques, we are currently attempting to define specific sites of expression of the different TGF-ß's and to determine whether TGF-ß's 1-5 might have unique functions developmentally and in the mature organism. Preliminary results suggest selective expression of TGF-ß3 in the mammalian nervous system, and possible substitution, in avian species, of TGF-ß3 for certain functions of TGF-ß1 in mammals. Agents such as TGF-ß itself, Ca^{2+}, and retinoids have been shown to affect expression of TGF-ß's 1-3 selectively. To understand the molecular mechanisms responsible for such selective expression, we have begun a comparative study of the promoter regions for the different TGF-ß's and for any particular TGF-ß in different species is also underway. In terms of their biological activities, TGF-ß's act to control gene expression of their target cells, many of their actions converging on a complex, multifaceted scheme of control of matrix proteins and their interactions with cells; these effects on matrix are thought to mediate many of the effects of TGF-ß on development.

TGF Beta Family

I 021 EXPRESSION OF MULLERIAN INHIBITING SUBSTANCE GENES IN TRANSGENIC MICE

R.R. Behringer[1,4], R.L. Cate[2], G.J. Froelick[3], R.D. Palmiter[3], and R.L. Brinster[1].
[1]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104. [2]Biogen, 14 Cambridge Center, Cambridge, MA 02142. [3]Department of Biochemistry and Howard Hughes Medical Institute, University of Washington, Seattle, WA 98195. [4]Present address: Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

Mullerian inhibiting substance (MIS), also known as anti-Mullerian hormone is a glycoprotein expressed specifically in Sertoli cells of the fetal and adult testis and granulosa cells of the postnatal ovary. During fetal development both males and females possess Mullerian ducts, the anlagen of the uterus, oviducts, and upper vagina. In the male fetus, MIS production brings about the regression of the Mullerian ducts. MIS has also been implicated in testicular differentiation, as suggested by in vitro studies of fetal ovaries exposed to purified MIS. These ovaries lose germ cells and develop seminiferous cord-like structures.

We have generated transgenic mice chronically expressing human MIS under the influence of the mouse metallothionein-1 promoter to investigate its role in sexual development. Nine founder mice were generated (2 females and 7 males). Seven of these mice including both females had circulating levels of human MIS ranging from ~50-600 ng/ml. In comparison, MIS is undetectable in plasma from human adults. Both founder females were sterile and possessed a blind vagina, and no uterus or oviducts. Surprisingly, ovaries were also not detected. All of the expressing founder males were fertile and transmitted the transgene to progeny. Female progeny that inherited the transgene from these males exhibited the same phenotype as the founder females. A developmental study revealed that transgenic females did possess ovaries at birth; however, germ cells were not as prevalent as in controls. Subsequently, the ovaries became devoid of germ cells and this was followed by the organization of the somatic components of the ovary into structures bearing a striking resemblance to the seminiferous tubules of the male gonad. A proportion of males from the 2 highest expressing lines exhibited feminization of the external genitalia, impairment of Wolffian duct development, and undescended testes.

These results demonstrate in vivo that MIS negatively influences the differentiation of the Mullerian ducts. In addition, our findings support the notion that this factor may be actively involved in testicular morphogenesis. MIS may also affect androgen or androgen receptor biosynthesis perhaps by influencing Leydig cell differentiation. Finally, MIS appears to be involved in testicular descent.

I 022 REGULATION OF MULLERIAN INHIBITING SUBSTANCE, Richard L. Cate, Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142

Mullerian inhibiting substance (MIS), also known as Anti-Mullerian Hormone (AMH), is a member of the TGF-β family of growth and differentiation factors. In males, MIS is produced in Sertoli cells of the fetal and postnatal testis, and causes regression of the Mullerian duct, the anlagen of the uterus, oviducts, and upper vagina. Recent experiments suggest that it also may be involved in testicular differentiation. In females, expression of MIS occurs after birth in granulosa cells of the ovary, but its function is not known. Inappropriate expression of MIS during fetal development of the female can lead to obliteration of the Mullerian ducts and sterility. Hence, the regulation of the MIS gene and MIS activity is of central importance during the reproductive development of both sexes.

MIS mRNA has not been detected in cells other than Sertoli or granulosa cells, indicating that the cell-specific expression of MIS is achieved at the level of transcription. Since MIS expression is a very early event in testicular differentiation, transcriptional regulation of the MIS gene in Sertoli cells may be mediated by the testis determining factor encoded by the Y chromosome. A different mechanism must be responsible for the expression of MIS in granulosa cells. We are currently trying to identify tissue specific factors that regulate transcription of the MIS gene and the DNA sequences that interact with these factors.

Regulation of MIS also occurs at the post-translational level. Expression of the human MIS gene in Chinese hamster ovary (CHO) cells has revealed that MIS is synthesized as a latent molecule that undergoes proteolytic processing. Human MIS is synthesized as a 140 kd dimer composed of two identical subunits. Concurrent cleavages on both chains of the MIS dimer produce a 25 kd TGF-β-like dimer from the C-terminus and a 110 kd dimer from the N-terminus of the protein. A mutant in which the cleavage site has been altered is resistant to proteolytic cleavage and is inactive in the Mullerian duct regression assay, indicating that processing is obligatory for biological activity. Fragments of MIS are being generated in order to characterize the active form of MIS.

I 023 THE *decapentaplegic* GENE AND PATTERN FORMATION IN *DROSOPHILA*, William M. Gelbart, Kristi A. Wharton, Richard W. Padgett, Laurel A. Raftery, Robert Ray, Ronald K. Blackman, Michele Sanicola, Seth Findley, Deborah A. Hursh, Donald W. Nelson and Vernon Twombly, Department of Cellular and Developmental Biology, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138-2097. The *decapentaplegic* (*dpp*) gene in the fruit fly, *Drosophila melanogaster*, is a complex genetic locus encoding a member of the TGF-β family of secreted proteins. It is required for determination of dorsal ectoderm in the early embryo, for specification of the proximal-distal axis of developing adult appendages, and for other, less well-characterized developmental events. We have been employing a variety of genetic, molecular and biochemical approaches to gain an understanding of the contribution of the *dpp* gene to spatial patterning. By means of reporter gene constructs and germ-line transformation techniques, we have been dissecting the large cis-regulatory apparatus of the *dpp* gene. We have identified several regions of the gene containing elements which drive specific subsets of the complex *dpp* expression pattern. We have been defining important sequences in the activity of the *dpp* polypeptide by interspecific sequence comparisons, by sequencing of available mutations and by the construction and reintroduction of chimeric genes. We have also been engaged in searches for other members of the TGF-β family in *Drosophila*. We have begun extensive mutational screens for loci which interact with the *dpp* gene. From such screens, we hope to identify functions involved in the binding of *dpp* protein to target cells, in the subsequent signal transduction process, as well as molecules engaged in regulating the pattern of *dpp* gene expression. Progress on these several research directions will be discussed.

I 024 TRANSFORMING GROWTH FACTOR-BETA-RELATED GENES AND MAMMALIAN DEVELOPMENT, Karen Lyons, C. Michael Jones, Ron W. Pelton, Riet Van der Meer-de Jong, Victor Fet, Mary E. Dickinson and Brigid L.M. Hogan, Department of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232-2175. There is strong biochemical and genetic evidence that molecules related to polypeptide growth factors and their receptors play important roles in embryonic development in organisms such as Drosophila, Xenopus and Caenorhabditis. Examples include the decapentaplegic (dpp) gene product in Drosophila and the Vg-1 gene product in Xenopus, both of which are related to TGF-β and form part of a distinct dpp-like subfamily. Other members of this subfamily include the Bone morphogenetic proteins -2a, -2b and -3 and the recently described murine VgR-1 (Lyons et. al. (1989) *Proc. Natl. Acad. Sci.* **86**: 4554-4558). As part of a program to study cell differentiation and pattern formation in the mouse embryo we have been following the expression of various members of the TGF-β gene superfamily, including TGF-β1, -β2, -β3, bone morphogenetic protein -2a and Vgr-1, by *in situ* hybridization and Northern analysis. Maternal mRNA for VgR-1 is found at high levels in primary oocytes but the level of RNA declines after fertilization and is not detectable at the 4-8 cell stage. RNA levels subsequently increase above background in the blastocyst and early post-implantation embryo, but do not appear to be localized to any specific region. In contrast, high levels of Vgr-1 RNA are present in differentiating epidermis. Indeed all of the members of the TGF-β gene family so far studied, are expressed in distinct temporal and spatial patterns in the suprabasal cells of the embryonic and post-natal epidermis and hair and whisker follicles. Distinct patterns of expression are also seen in developing teeth and in cartilage and bone. The results suggest that TGF-β related genes may be playing a role in epithelial-mesenchymal interactions at several stages during mammalian development, and in the progression of embryonic stem cells through specific differentiation pathways. More recent experiments suggest that new member(s) of the dpp-subfamily may be present in the mouse genome and may be expressed at the egg cylinder/primitive streak stage when mesodermal induction is taking place as well as at later stages of development.

Negative Controls on Cell Growth

Negative Growth Factors

I 025 THE PLEIOTROPIC ACTIONS OF LEUKEMIA INHIBITORY FACTOR (LIF), Nicholas Gough, Walter and Eliza Hall Institute of Medical Research, Post Office Royal Melbourne Hospital, Parkville Victoria 3050, Australia.

We have recently described a novel factor, leukemia inhibitory factor (LIF), which is able to enforce the differentiation and suppress the clonogenicity in vitro of certain myeloid leukemic cells[1]. We have cloned the genes for LIF from several species (mouse, human, ovine and porcine), and all encode highly homologous proteins. LIF is encoded by a unique gene, located at 11A1-A2 in the mouse and 22q12 in man. Recombinant LIF has been expressed in a number of host cell systems. Within the hemopoietic system, receptors for LIF are present on cells of the monocyte/macrophage series suggesting some, yet to be determined function, on these cells.

Over the past year a diverse array of functions for LIF, as both a positive and negative regulator, have been described[1]. We recently noted several similarities between LIF and a factor (Differentiation Inhibitory Activity, DIA) able to inhibit the differentiation and maintain the pluripotential state of embryonic stem (ES) cells. Subsequent work has demonstrated that LIF is likely to be equivalent to DIA: all ES and EC cells examined display specific high affinity LIF receptors; all sources of DIA examined contain LIF; and most convincingly, LIF can substitute for DIA in the maintenance of ES cells in vitro and such cells can give rise to germline chimaeric mice[1,2].

A role for LIF in bone metabolism has been determined using a model animal system[3]. Cells of the murine hemopoietic cell line, FDC-P1, were multiply infected with a LIF- expressing retrovirus and a clone expressing high levels of LIF isolated. Injections of these cells into syngeneic DBA/2 mice resulted in animals engrafted with LIF-producing cells in the hemopoietic organs. These mice developed within 12-70 days a novel syndrome characterized by irritability, cachexia, bone overgrowth and calcification in muscle tissue. These observations suggest that LIF may be a potent cachexia-inducing agent and have marked effects on bone and calcium metabolism. The effects on bone are in accordance with our demonstration of LIF receptors on osteoblasts and that LIF can induce calcium release from bone in vitro. The cachexia induction is similarly in accordance with the recent observation that melanoma-derived lipoprotein lipase inhibitor is equivalent to LIF[4].

Finally, LIF has also been shown to be equivalent to hepatocyte stimulating factor III, a factor which induces acute phase protein synthesis in hepatocyte[5].

1. Gough, N.M. and Williams, R.L. (1989) Cancer Cells (in press).
2. Williams, R.L. et al (1988) Nature 336: 684-687.
3. Metcalf, D. and Gearing, D.P. (1989) Proc. Natl. Acad. Sci. USA 86: 5948-5952.
4. Mori, M.R. et al (1989) Biochem. Biophys. Res. Commun. 160: 1085-1092.
5. Baumann, H. and Wong, G.G. (1989) J. Immunol. 143: 1163-1167.

I 026 MOLECULAR MECHANISMS OF GROWTH SUPPRESSION BY INTERFERONS. Kimchi, A., Resnitzky, D, Tiefenbrun, N., Yarden, A. and Berissi, H. Department of Molecular Genetics and Virology, The Weizmann Institute of Science, Rehovot 76100 - Israel.

The mouse M1 myeloleukemia cell line was used to study the post-receptor elements that mediate the growth suppressive effects of IFN and TGF-β. Nuclear run-on experiments revealed that IFN (α or β) selectively reduced the initiation rate of c-myc transcription while TGF-β reduced mRNA expression at the post-transcriptional level. Transfection of M1 cells with constructs of c-myc in which the two coding exons are linked to SV40 early promoter led to constitutive expression of exogenous c-myc mRNA after IFN treatment while it did not interfere with the negative responses to TGF-β. This genetic manipulation abrogated one of the main antiproliferative responses to IFN that involves prolongation of the G1 phase of the cell cycle but did not rescue all the growth responses to the factor. Depletion of protein kinase C (PKC) from M1 or Daudi cells by chronic exposure to phorbol esters also generated the same partial resistant phenotype that failed to change the cell cycle distribution in response to IFN although reduction of c-myc mRNA and protein levels continued to take place in the depleted cells. Taken together, these data suggest the existence of at least two independent molecular pathways in the negative signalling system of IFN, one of which requires active PKC while the other is PKC independent and leads to c-myc transcriptional inhibition. Each of these pathways is necessary but not sufficient by itself to transduce the cell cycle inhibitory effects of IFN. Along this line, the molecular characterization of 20 independently isolated IFN resistant M1 variant clones that express functional cell surface receptors revealed that some failed to reduce the c-myc mRNA levels while others displayed genetic deregulations along the other pathway(s) or downstream to the c-myc protein. TGF-β uses different post-receptor elements to reduce cell growth as documented by the findings that unlike IFN the myc transfected clones as well as the PKC depleted M1 cells continued to respond to TGF-β in a normal G0/G1 type of arrest and no cross resistance was so far detected between IFN and TGF-β when both groups of resistant M1 mutants (IFN^r and $TGF\text{-}\beta^r$) were analyzed. Finally the process of terminal division that takes place during the IL-6 induced differentiation of M1 cells towards monocytes was analyzed. By using specific tools such as anti IFN-β antibodies, the distinct groups of IFN resistant mutants and the myc transfected clones, the precise contribution of an endogenously produced IFN-β to the more complicated process of terminal cell division has been determined and will be described in details.

Negative Controls on Cell Growth

I 027 INTERLEUKIN-6: A MODULATOR OF CELL PROLIFERATION,
Pravinkumar B. Sehgal, The Rockefeller University, New York, NY 10021
Human interleukin-6 (IL-6) consists of differentially modified phosphoglycoproteins in the size range 19-45 kDa derived from a single polymorphic gene located at 7p21 in the human genome. In addition to its major effects on hepatic and non-hepatic acute phase plasma protein synthesis and on the proliferation and differentiation of cells of different hematopoietic lineages, IL-6 modulates the proliferation of a variety of epithelial tissues. IL-6, which is itself induced by growth factors like serum, IL-1, TNF, EGF and PDGF, inhibits the proliferation of the human breast carcinoma cell lines T47D, ZR-75-1 and MCF-7 but can enhance that of normal human keratinocytes. Despite an inhibition of proliferation of T47D and ZR-75-1 cells, IL-6 enhances the motility of these cells. There is a decrease in adherens junctions (desmosomes, focal adhesions) in IL-6-treated cells; under these experimental conditions, IL-6 is a regulator of cell-cell interactions. The cytokine- and second messenger-inducible IL-6 enhancer contains sequence elements similar to the SRE and adjacent AP-1-like site in the *c-fos* enhancer; promoter cross-competition experiments in HeLa cells suggest that many of the same transcription factors regulate both IL-6 and *c-fos* promoters. A single copy of a 23-bp DNA segment located within the 115-bp IL-6 enhancer confers serum, IL-1, TNF, forskolin and phorbol ester inducibility on to a chimeric herpesvirus thymidine kinase/CAT gene. This 23-bp element, the multiple response element or MRE, binds nuclear factors which also bind *c-fos* SRE and AP-1-like site oligonucleotides even though the MRE does not contain any apparent dyad symmetry. Mutations in the CGTCA motif in the IL-6 MRE inhibit inducibility by phorbol ester and forskolin but not by serum, IL-1 and TNF. The ability of the IL-6 promoter to respond to other growth modulating cytokines ensures the participation of IL-6 in the regulation of cell proliferation.

Growth Suppressing Genes

I 028 MOLECULAR GENETICS OF STEROID AND THYROID HORMONE RECEPTORS, Evans, R. M., Howard Hughes Medical Institute, The Salk Institute, La Jolla, California 92037
We have identified receptors for steroid and thyroid hormones and for the vitamin A-derived morphogen retinoic acid. The homology of these receptors and the cDNAs which encode them define the existence of a superfamily of related regulatory proteins. We demonstrate that these molecules contain homologous structures that include domains required for DNA binding, ligand binding and trans-activation. Site-directed mutagenesis was used to identify the DNA-binding specificity of the glucocorticoid receptor. We have demonstrated that a single amino acid change can redirect the GR so that it now recognizes an estrogen response element (ERE). Interestingly, this mutant receptor retains GRE specificity. These results indicate that it is possible to generate a receptor that can recognize and activate through two different sequences. A change in the second zinc finger transforms the specificity to that of the thyroid hormone receptor.

Recent studies indicate that the thyroid hormone receptor can, in the absence of its ligand, suppress activity of a responsive promoter. Addition of thyroid hormone, however, results in the stimulation of expression. The oncogenic derivative of the thyroid hormone receptor, v-erbA, acts as a constitutive repressor and when co-expressed with the receptor, blocks activation by thyroid hormones. Thus, v-erbA is an example of a dominant negative oncogene.

Recent studies from our lab have shown that the retinoic acid receptor is capable of binding to and activating thyroid hormone responsive promoters. This suggests a potential relationship between retinoid and thyroid hormone receptors. To pursue this hypothesis we have examined the action of the erbA oncogene on retinoic acid receptor activity. As with the TR, v-erbA functions as a retinoic acid receptor antagonist and in principle, may be used as a molecular tool to dissect the inductive events of retinoids in cell culture and in transgenic animals. Studies on the relationship of thyroid and retinoid receptors will be presented.

I 029 THE P53 GROWTH SUPPRESSOR GENE, A.J. Levine, P.W. Hinds, C.A. Finlay, R. Quartin, J. Sherley, S. Baker, J. Nigro and B. Vogelstein, Department of Biology, Princeton University, Princeton, NJ 08544-1014, and Department of Oncology, Johns Hopkins School of Medicine, Baltimore, MD 21231.

About 70-80% of the human colorectal carcinomas examined contain a loss of one allele of the p53 gene and a point mutation in the other allele of this gene. Several cDNAs containing these point mutations have been cloned from these tumors or cells derived from these tumors. These missense mutations cluster between amino acid residues 118-307 (out of 393 amino acids). When these mutant p53 cDNAs are transfected along with the activated *ras* oncogene into primary rat embryo fibroblasts in culture, transformed cell foci arise that produce permanent cell lines. These cells express high levels of the mutated human p53 protein that is bound to the cellular heat shock protein, hsc70, in the transformed cells. When the wild-type human p53 protein was expressed in REF cells it did not bind to the hsc70 protein and was detected at only very low levels. The half-life of the wild-type human p53 protein in REF cells was about 20 minutes while the half-life of the mutant p53 protein was extended some 5- to 7-fold leading to increased levels of this mutant protein.

The role of the p53 wild-type protein in cell growth suppression has been explored by introducing the wild-type mouse p53 cDNA into a murine permanent cell line under a temperature sensitive inducible vector. At the permissive temperature where 3-fold more wild-type p53 protein is produced, the cells enter a long lag period blocked in the G_0/G_1 phase of the cell cycle and then grow more slowly. The available evidence suggests that p53 may be involved in regulating events in the cell cycle.

I 030 p53 - A POTENTIAL SUPPRESSOR GENE? Daniel Eliyahu, Dan Michalovitz, Orit Pinhasi-Kimhi, Siona Eliyahu and Moshe Oren, Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Mutant forms of the p53 cellular tumor antigen elicit neoplastic transformation in vitro. Recent evidence indicates that loss of normal p53 expression is a frequent event in certain types of tumors, raising the possibility that such loss provides transformed cells with a selective growth advantage. Thus, it was conceivable that the mutants may contribute to transformation by abrogating normal p53 function. We therefore studied the effect of plasmids encoding wt p53 on the ability of primary rat embryo fibroblasts to be transformed by a combination of mutant p53 and ras. It was found that wt p53 plasmids indeed caused a marked reduction in the number of transformed foci. Furthermore, wt p53 plasmids also suppressed the induction of transformed foci by combinations of bona fide oncogenes, such as myc plus ras or adenovirus E1A plus ras. On the other hand, plasmids carrying mutations in the p53 coding region totally failed to inhibit oncogene-mediated focus induction, and often even slightly stimulated it. Hence, such mutations completely abolished that activity of wt p53 which is responsible for the "suppressor" effect. The latter fact is of special interest, since similar mutations in p53 are often observed in human and rodent tumors. The data support the idea that wt p53 expression may be restrictive to neoplastic progression. Hence, p53 possesses at least several attributes of a "tumor suppressor". It should be noted that the observed effects depended on the use of plasmids capable of substantially overexpressing wt p53. Results suggesting that overexpressed wt p53 may arrest cell proliferation will also be discussed.

1031 MYOGENIN AND THE HLH FAMILY OF MUSCLE REGULATORY FACTORS, Woodring E. Wright, Karen Farmer and Victor Lin, Department of Cell Biology and Neuroscience, U.T. Southwestern Medical School, Dallas, TX 75235. A variety of skeletal muscle specific regulatory factors have recently been identified, including myogenin, myoD, myf-5 and myf-6. Transfection of one member of this family into non-muscle cells frequently results in the autoactivation of the endogenous gene, transactivation of other members of the family, and the conversion of the target cell into a myoblast capable of apparently normal myogenic differentiation. All of these proteins share a region of homology to c-myc that includes a basic region thought to be responsibile for the specificity of DNA binding and a helix-loop-helix (HLH) region involved in dimerization. In gel shift experiments, bacterially expressed factors bind to a 14 bp sequence present in the muscle creatine kinase enhancer and a large number of other muscle-specific genes. Antibodies against these proteins cause double-shifts of several muscle-specific bands produced using nuclear extracts, indicating that the native complexes can recognize the same sequences. Myogenin and myoD can form low affinity homomers and heteromers as well as high affinity complexes with E12/E47. Myogenin is expressed at high levels in the myotomal region of the somites in the mouse as early as 8.5 days of gestation, almost two days before myosin heavy chain and myoD transcripts can be detected, whereas there is no significant temporal discordance in the appearance of myogenin and myoD transcripts in the developing limb bud at 11.5 days. Growing evidence suggests that myoD and myf-5 are alternative factors that must cooperate with myogenin in regulating muscle cell differentiation.

Receptors Negatively Regulating Growth

1032 TGF-ß RECEPTORS AND TGF-ß BINDING PROTEOGLYCANS. Joan Massagué, Janet L. Andres, Frederick T. Boyd, Sela Cheifetz, and Marikki Laiho. Cell Biology and Genetics Program, Sloan-Kettering Institute, New York, NY 10021

TGF-ßs are pluripotent diffusible polypeptides that can act as growth inhibitors, regulators of cell phenotype and regulators of the cell adhesion apparatus. These activities may be interrelated in part, and may be mediated by mechanisms distinct from known growth factor signalling pathways. Studies addressing the mechanism of action of TGF-ß have identified three widely distributed cell surface components that bind this factor. Two of these components are glycoproteins of 53 kDa (type I receptor) and 73-83 kDa (type II receptor), both with high affinity for TGF-ß (Kd 5-50pM). These two receptor species are present in all known TGF-ß-sensitive cell lines. The selective and highly frequent loss of these membrane proteins in cell mutants resistant to growth suppression by TGF-ß identifies them as components of the TGF-ß receptor complex. Their loss of function correlates with loss of all measurable responses to TGF-ß. Genetic cell mutant complementation analysis is in progress to determine if receptor types I and II interact with each other. A third TGF-ß-binding species is betaglycan (type III receptor), a membrane proteoglycan whose 110 kDa core protein binds TGF-ß (Kd 30-300pM) even in the absence of glycosaminoglycan chains. Betaglycan is not detectable in hematopoietic, myogenic or endothelial cells that respond to TGF-ß; its participation in TGF-ß signal transduction is uncertain. Betaglycan is released by cells into the medium, and is found in extracellular matrices and in serum. Membrane-bound and released forms of betaglycan might be involved in controlling the availability of active TGF-ß to cells. Two interesting aspects of TGF-ß action are modulation of the cell adhesion apparatus and control of cell cycle regulatory proteins. TGF-ß regulates expression of genes that encode components of the cell adhesion apparatus and this response may in turn mediate effects of TGF-ß on cell migration, growth and phenotype. However, the growth inhibitory action of TGF-ß correlates with its ability to rapidly block phosphorylation of the Rb protein, an event that might mediate the growth inhibitory response to this factor.

I 033 RECEPTOR-LINKED PROTEIN TYROSINE PHOSPHATASES

Haruo Saito, Michel Streuli, Neil X. Krueger and Alex Y.M. Tsai, Division of Tumor Immunology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.

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). As was the case with PTKases, there are two types of PTPases: the Type 1 PTPases are soluble cytoplasmic enzymes that have single catalytic domain, whereas the Type 2 PTPases are transmembrane molecules that have two cytoplasmic PTPase domains and an extracellular receptor domain. By using consensus oligonucleotide probes or cross-hybridization to previously isolated PTPase cDNAs, we have obtained cDNA clones encoding several Type 1 and Type 2 PTPases. The structures of these PTPases suggest an evolution by gene reshuffling. The human LAR PTPase and *Drosophila* DLAR PTPase are about 77% identical within the enzyme domain, indicating that the function of these molecules are conserved throughout the evolution of metazoa. The extracellular receptor domains of the Type 2 PTPases are not homologous to each other. However, the extracellular receptors of human LAR and *Drosophila* DLAR and DPTP are all composed of several immunoglobulin-like domains and several Fibronectin type-III domains. This organization is similar to that of cell adhesion molecules such as N-CAM. Thus, LAR, DLAR, and DPTP might be themselves cell adhesion molecules. This raises a possibility that the function of LAR, DLAR, and DPTP, is to suppress cell growth upon cell-cell contact. Both Type 1 and Type 2 PTPases share several common properties including a requirement for the reduced form of a cysteine residue, presumably located at the catalytic center. The cDNA segments encoding the cytoplasmic enzyme domains of the prototype Type 2 PTPases, LCA and LAR, were separately inserted into a prokaryotic expression vector, pKKUC12, and transformed into *E. coli*. The overproduced LCA or LAR cytoplasmic domain was enzymatically active. Deletion analysis as well as site-directed mutagenesis analysis of the conserved cysteines indicated that only the first PTPase domain is enzymatically active, even though the second PTPase domain is highly homologous to other PTPases including the Type 1 enzymes. However, the second PTPase domain has profound effect on the specificity of the first domain. By site-directed mutagenesis, chemical mutagenesis, and cassette mutagenesis, a series of mutations were generated in the first PTPase domain of LAR. The effects of these mutations on the activity and specificity of enzyme will be discussed.

Negative Controls on Cell Growth

Genetic Alterations in Cancer Cells; Familial Cancers

I 100 SURVEY OF PROTO-ONCOGENE STRUCTURE AND EXPRESSION IN CORE MEMBERS OF CANCER-PRONE KINDREDS, Rémy A. Aubin, Ron M. Fourney*, Razmik Mirzayans**, Kevin D. Dietrich** and Malcolm C. Paterson**, Health and Welfare Canada, DID/Biotechnology, Sir F.G. Banting Res. Ctr., Tunney's Pasture, Ottawa Ont. K1A OL2, *Central Forensic Lab, R.C.M.P., DNA/Serology, 1200 Alta Vista Dr., Ottawa Ont. K1G 3M8, **Molecular Genetics and Carcinogenesis, W.W. Cross Cancer Institute, 11560 University Ave, Edmonton, Alt T6G 1Z2, Canada.

We have tested the hypothesis that the aggregation of tumours occurring in cancer-prone families might stem from an inherited propensity to activate specific proto-oncogenes by searching for evidence of proto-oncogene amplification, allelic polymorphism and aberrant expression in the somatic cells of cancer-prone subjects. Genomic DNA samples from over 90 non-cancerous fibroblast strains derived from 82 cancer-prone individuals (representing 40 distinct cancer families) and 8 healthy volunteers were submitted to RFLP and Northern blot analyses with a wide panel of oncogene probes. While amplification events were not detected in any of the strains, evidence that rare oncogene alleles might signify increased susceptibility to familial cancer was uncovered in one pedigree. A rare 5.0 kbp EcoR1 *c-mos* allele was detected in the normal and tumour-derived cell lines from 2 of 11 members of a family afflicted with the dysplastic nevus syndrome combined with hereditary cutaneous malignant melanoma. We also detected the loss of a metallothionein gene allele and observed~2.5-fold higher levels of *c-myc* transcription in skin fibroblasts from members of a family prone to diverse cancers (e.g. osteosarcoma, soft tissue sarcomas and breast cancer) and whose cells demonstrate enhanced radioresistance and marked disturbances in *de novo* DNA synthesis following gamma-ray exposure. In contrast, no evidence for alterations in the physical structure or the expression of the *L-myc, N-myc, c-sis, c-raf, c-Ha-ras, c-mos, c-fms, c-abl, c-met* or *c-erb2* proto-oncogenes were detected in any of the 24 members of this particular family. All genomic DNA samples assayed scored negative in the NIH 3T3 focus formation assay.

I 101 EFFECTS OF CHROMOSOME TRANSFER ON HUMAN NEUROBLASTOMA CELLS, Scott A. Bader, Garrett M. Brodeur* and Eric J. Stanbridge, Department of Microbiology and Molecular Genetics, University of California at Irvine, CA 92717, *Departments of Pediatrics and Genetics, Washington University, Saint Louis, MO 63110.

Partial monosomy of chromosome 1p is the most common cytogenetic abnormality associated with human neuroblastoma. The smallest visible region of cytogenetic deletion and the region that shows loss of heterozygosity most consistently is 1p36.1 - 1pter. The location of a tumor suppressor gene is thus postulated to reside on the distal short arm of chromosome 1. We have introduced chromosomes 1p and 1q independently into a neuroblastoma cell line, NGP.1A.TR1, using the microcell-mediated chromosome transfer technique to determine the effects of each portion of this chromosome. Using the same method we have introduced chromosomes 11 and 17 as "irrelevant" chromosomes in separate experiments. Only chromosome 1p was predicted to suppress tumorigenicity of the neuroblastoma cells. If suppression is due to the reinstatement of differentiation then there might also be some differentiation in vitro. Transfer of chromosomes 1p and 11 dramatically affected the in vitro morphology of the neuroblastoma cells indicative of differentiation, while chromosomes 1q and 17 did not. These results and those of tumorigenicity assays are presented.

I 102 FAMILIAL MULTIPLE ENDOCRINE NEOPLASIA TYPE I (FMEN 1): CELLULAR BIOLOGY AND MOLECULAR GENETICS, Maria Luisa Brandi, Stephen J. Marx*, Mark B. Zimering*, Eitan Friedman**, Kazushige Sakaguchi*, Alberto Falchetti, Elizabeth A. Streeten* and Gerald D. Aurbach*, Endocrine Unit, Florence University School of Medicine, 50139 Florence, Italy and *Metabolic Diseases and **Molecular Pathophysiology Branches, Bldg. 10, Room 9C101, NIH, Bethesda, MD 20892, USA

FMEN 1 is a familial form of hyperparathyroidism, usually referred to as hyperplasia, with tumors of pituitary and pancreatic islets as well. Using a long-term culture system of parathyroid cells we showed that plasma from subjects with FMEN 1 contains a unique mitogenic activity (Brandi et al. NEJM 1986, 314:1287). The mitogen shares many features with basic Fibroblast Growth Factor (bFGF), eventhough substantial biological differences exist between the two mitogens. Using a bFGF antiserum which cross-reacts with the FMEN 1 mitogen we found recognizable entities in the conditioned medium of FMEN 1 pituitary cells in primary culture. No immunoreactivity was released from human sporadic pituitary tumor cells in primary culture. These observations prompted linkage studies in a single large kindred using int-2, a bFGF-related oncogene known to be localized to 11q13, were the FMEN 1 gene has recently been mapped. int-2 locus seems to be closely linked to the FMEN 1 gene. More recently the monoclonal development of the FMEN 1 parathyroid tissue has been demonstrated. The origin of the FMEN 1 mitogen, its nature and the relation between extrinsic and intrinsic factors in FMEN 1 tumor predisposition and progression will be discussed.

I 103 GENETIC ALTERATIONS IN PROSTATE CANCER, Bob S. Carter and William B. Isaacs
James Buchanan Brady Urological Institute Research Laboratories,
The Johns Hopkins Hospital, Baltimore, MD 21205
Allelic loss at specific chromosomes and point mutations in ras-oncogenes have been noted to occur frequently in many human tumors. Vogelstein et al. noted that allelic loss at chromosomes 17 and 18 can be documented in at least 75% of all human colorectal tumors and that point mutations of the K-ras gene occur in 40-50% of these tumors. A tumor suppressor function has been postulated for the p53 gene product which is encoded at chromosome 17. We are currently studying the genetic alterations which occur in benign and malignant neoplasms of the prostate. Using Southern blot analysis of normal and tumor prostatic tissue we have examined allelic loss at chromosome 17 in 18 patients (14 informative: 12 localized cancers, 4 metastatic cancers). Only 1 tumor of the 14 informative cases was found to have allelic loss at this chromosome. In addition, we have examined 12 of these tumors (9 localized cancers, 3 metastatic cancers) and 10 specimens of benign prostatic hyperplasia for mutations at codons 12/13 of the K-ras gene using differential hybridization to detect wild type and mutant alleles in genomic DNA amplified using the polymerase chain reaction. To date, we have not detected any mutations at codons 12/13 of the K-ras gene in these specimens. These results imply that allelic loss at chromosome 17 and point mutations of the K-ras gene codons 12/13 are not frequent events in prostate cancer tumorigenesis.

I 104 TRANSFORMING GROWTH FACTOR-α GENE EXPRESSION IN REGENERATING ENDOTHELIUM AFTER BALLOON DENUDATION, Bojan Cercek, Mehran Khorsandi, James S. Forrester, Richard H. Helfant, James A. Fagin. Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, California 90048.
The rapidity and extent of reendothelialization after balloon denudation has been implicated in modulating the degree of neointimal proliferation. Transforming growth factor-α (TGF-α) has a mitogenic affect on cultured endothelial cells and is believed to be capable of autocrine growth regulation. This prompted us to investigate the expression of TGF-α gene after arterial wall injury in vivo.
Rats were killed 1, 3, 7 and 14 days after aortic balloon denudation. Regeneration of the endothelium was determined with injection of Evans blue prior to the kill and the reendothelialized area expressed as percent of total area. TGF-α mRNA was detected by solution hybridization/ RNase protection assay using the .4 kilobases long fragment of the 5' end of the cDNA clone of rat TGF-α .
The denudation was complete (stained blue) 1 day and 3 days after intervention. Rendothelialization (stained white) was 48±10% and 78±17% at 7 and 14 days after denudation, respectively. TGF-α mRNA was not detected until 7 days after denudation. Protected TGF-α transcripts were no longer present when the endothelium was mechanically removed prior to RNA extraction.
Conclusion: In rats, aortic TGF-α mRNA is expressed during reendothelialization after balloon denudation, probably in the regenerating endothelial cells. This finding suggests that TGF-α may be involved in the autocrine regulation of endothelial regeneration after arterial wall injury.

I 105 A COMMON GENETIC DEFECT IN THE CONTROL OF TUMORIGENIC EXPRESSION IN FOUR ADULT SARCOMA CELL LINES, Pengchin Chen, Scott Bader[1] and Bernard E. Weissman,
Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27599 and [1]Dept of Microbiology and Molecular Genetics, University of California School of Medicine, Irvine, CA 92715
The development and progression of tumors involves genetic alterations which result in the abnormal behavior of individual cells. One type of change involves activation of oncogenes which function in a dominant genetic fashion. In contrast, a second group of genes designated tumor suppressor are lost or inactivated upon tumor formation. Associations between specific chromosome deletions and distinct types of cancer as well as the existence of different complementation groups for tumorigenic expression in human tumor cells suggest that tumors from the same origin may share a common defective tumor suppressor gene. Initial studies showed that four human carcinoma cell lines belonged to the same complementation group. We have extended these studies to four human adult sarcoma cell lines and a neuroblastoma cell line. Four sets of hybrid cell lines were isolated using by fusion of HPRT-deficient sarcoma cells to a common TK-deficient cell line. Hybrid identity was confirmed by chromosome and RFLP analyses. Tumorigenic potential was tested by inoculation of $1x10^7$ cells subcutaneously into athymic nude mice. All hybrids remained tumorigenic suggesting a common genetic defect in the control of tumorigenic potential. Expression of two known tumor suppressor genes, rb-1 and p53, was examined in the.hybrid cells and the tumors and was not correlated with tumorigenicity. We are currently using the technique of microcell hybridization to determine the chromosomal location of this common tumor suppressor gene.

I 106 DOES 17p DELETIONS AIM INACTIVATION OF THE p53 GENE? P.Chumakov,[1] E.Fleischman[2], B.Kopnin[2] and J.Jenkins[3], Institutes of Molecular Biology[1] and Cancerogenesis[2], Moscow, USSR, Marie Curie Cancer Research, Oxted, Surrey, U.K.

Previous studies indicate that mutated p53 gene can act as a dominant oncogene, immortalizing primary cell cultures and cooperating with activated *c-Ha-ras* gene for complete transformation. Recently it has been suggested that wild-type p53 possesses a tumor suppressor activity that can be inactivated either by chromosomal deletions, or by oligomer complexing with mutated p53. Human p53 gene is localized in chromosome 17, band 17p13.1, within the region of frequent chromosomal deletions in a number of malignancies. If p53 is the critical target of 17p deletions required for tumor progression, it seems reasonable to expect that the remaining copy of the gene had been previously inactivated either by rearrangements, or by point mutations. To test this idea we have analysed the structure of the p53 gene in primary human tumors with defined karyotypic abnormalities involving 17p. mRNA-coding exons of the p53 gene were amplified by PCR and sequenced. Sequence poymorphism within two sites of amplified regions served as an internal control for the presence or absence of p53 gene alleles in DNA samples. We observed frequent point mutations within evolutionary conserved domaines of the p53 protein-coding regions. However, at least in colorectal carcinomas, we found no correlation between the deletions in one and point mutations in the other allele of the p53 gene. Wild-type p53 gene was frequently found as a single allele and was able to specify wild-type p53 mRNA as revealed by cDNA sequencing. The data, while leaving open the question of tumor suppressor properties of wild-type p53, suggestthat 17p deletions might have no relation to p53 gene.

I 107 A NOVEL GENETIC SYSTEM FOR CLONING GROWTH RESTRICTION GENES, Louis Deiss, and Adi Kimchi, Department of Molecular Genetics and Virology, The Weizmann Institute of Science, Rehovot 76100 - Israel

Cells in vivo and in vitro are under some proliferation constraints which are manifested through cell-to-cell and cell to environment signals. Most of the pathways in which these signals are transduced have not yet been rigorously defined. Some of these restrictions are abrogated as cells become more "Tumorigenic". The final phenotype of a tumor is probably the result of a combination of recessive and dominant genetic changes. We have devised a new genetic approach which should facilitate the isolation of genes which function in growth suppressing pathways and are good candidates for "Recessive" or "anti" oncogenes. The system works as follows: cells which are sensitive to a specific proliferation restriction are converted to resistant by the inactivation of gene(s) specific to the restriction pathway. This inactivation is accomplished via an anti-sense expression vector. An entire cDNA library is screened and elements of the proliferation restriction pathway are isolated. In a test system, the inactivation of a specific gene converted HeLa cells to partially resistant to growth inhibition by IFN-γ Details of the genetic system and some characterization of the specific gene will be presented at the meeting.

I 108 CORRELATION OF THE INABILITY TO GROW IN SERUM-FREE MEDIum WITH SUPPRESSION OF TUMORIGENICITY IN WILMS' TUMOR.

Steven F. Dowdy, Kin-Man Lai, Clare L. Fasching, Bernard E. Weissman* and Eric J. Stanbridge, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717, and *Lineberger Cancer Research Center, University of North Carolina, Chapel Hill, NC 27514.

Deletion of the 11p13 region has been associated with Wilms' tumor, a pediatric nephroblastoma, and therefore has been postulated to contain tumor suppressive genetic information. In a previous report (Weissman *et al.*, (1987) Science 236:175), this laboratory transferred a single, selectable human t(X;11) chromosome into a Wilms' tumor cell line, G401.6, resulting in microcell hybrids, 110/G401, that were nontumorigenic when injected subcutaneously into athymic nude mice. Segregation of the t(X;11) resulted in reexpression of the tumorigenic phenotype. The reversioqn of the tumorigenic phenotype in the microcell hybrids suggests that a tumor suppressor gene(s) present on the t(X;11) was introduced into G401.6. Comparative analyses between the nontumorigenic microcell hybrids and tumorigenic cell lines have failed to demonstrate any significant differences in their *in vitro* properties (namely: growth rate in fetal calf serum, morphological alterations, soft agar clonability, fibronectin production, and alteration of proto-oncogene expression). However, when the cell lines are grown in serum-free (SF) medium the microcell hybrids failed to divide and demonstrated gross morphological alterations. The parental and segregant cell lines continued to divide and did not alter morphologically. Currently, the SF medium is being supplemented with grow factors in an attempt to 'override' the negative growth regulation present in the microcell hybrids. In addition, we are examining the cell lines grown under SF conditions for transcriptional alterations.

I 109 MUTATIONS IN THE *RB1* GENE WHICH INITIATE TUMORIGENESIS, James M. Dunn, Karen Johnston, Xiaoping Zhu, Andrew J. Becker, Robert A. Phillips and Brenda L. Gallie, Division of Heamatology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8. Inactivation of both alleles of the *RB1* gene during normal retinal development initiates the formation of a retinoblastoma (RB) tumor. In order to identify the mutations which inactivate *RB1*, 21 RB tumors were analyzed with the polymerase chain reaction (PCR) and/or an RNase protection assay. Mutations were identified in 13/21 RB tumors. Sequence analysis of the PCR amplified cDNA from a tumor with undetectable alterations demonstrated a point mutation which created a new termination codon. In 8 tumors the precise errors in nucleotide sequence were characterized: in 4/5 germline mutations a small deletion or duplication led to premature termination, while 3 somatic mutations were point mutations leading to splice alterations and loss of an exon from the mature *RB1* mRNA. We were unable to detect expression of the mutant allele in lymphoblasts and/or fibroblasts of 3/4 bilaterally affected patients, although the mutation was present in the genomic DNA and transcripts containing the mutations were obvious in the RB tumors in the absence of a normal *RB1* allele. We are investigating these results through the study of the wild type and mutant mRNA half-lives and transcription rates in normal and RB cells.

I 110 A NOVEL cDNA FROM WERNER SYNDROME (WS) FIBROBLASTS IS RELATED TO CELLULAR SENESCENCE AND QUIESCENCE, S. Goldstein, R. Thweatt, R. Fleischmann*, E.J. Moerman, R.A. Jones, R.J. Shmookler Reis, S. Murano, Departments of Medicine and Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, National Cancer Institute, Bethesda, MD*, and VA Medical Center, Little Rock, AR 72205.

We have attempted to clone the gene(s) inducing senescent replicative arrest of human diploid fibroblasts (HDF). Based on evidence that such genes might be overexpressed in WS, an inherited disorder featuring premature aging and a curtailed HDF replicative life-span, we inserted WS mRNA as cDNA into a eukaryotic expression vector. By plus/minus screening of this WS library with cDNA probes derived from RNA of WS vs. normal, early-passage (young) HDF we have identified several differentially expressed clones, most of which were homologous to known genes including procollagen $\alpha1(I)$ and $\alpha2(I)$, osteonectin, and fibronectin. However, one clone was unique and hybridized strongly to a 2.5 Kb band on Northern analysis of RNA from WS and late-passage (old) HDF, either at subconfluent density or rendered quiescent by high density, but gave much less signal with RNA of young cells during logarithmic growth or high-density arrest. In contrast, RNA from young cells rendered quiescent by low serum gave a strong signal at 2.5 Kb. DNA sequencing of the entire cDNA and computer analysis indicates that this cDNA is novel and has an open reading frame of 1023 nucleotides. Current studies should reveal if this cDNA inhibits growth during senescence and low-serum quiescence alone, or requires the concerted action of other cDNA species, and if alterations of this gene are involved in the degenerative and malignant disease, respectively, in WS and aging normal persons.

I 111 TUMOR SUPPRESSION INVOLVES DOWN-REGULATION OF INTERLEUKIN-3 (IL-3) EXPRESSION IN MASTOCYTE/MASTOCYTOMA CELL HYBRIDS
Hans H. Hirsch, Ioannis D. Diamantis, Asha P.K. Nair, and Christoph Moroni, Institut für Medizinische Mikrobiologie, Petersplatz 10, CH-4053 Basel, Switzerland.
Transfection of *v-H-ras* oncogene into the transformation competent IL-3 dependent mastocyte line PB-3c results in the generation of two classes of autocrine IL-3 secreting mastocytomas. Class I mastocytoma lines (prototype V2D1) show no detectable rearrangement 5' to the IL-3 gene by Southern blot analysis, whereas a 5' IL-3 gene rearrangement is found in class II mastocytoma lines (prototype V4D6). Class I cell hybrids between the parental IL-3 dependent PB-3c and the non-rearranged autocrine V2D1 show dramatic down-regulation of IL-3 mRNA and dependence on exogenous IL-3 for proliferation *in vitro*, whereas the $p21^{v\text{-}H\text{-}ras}$ levels appeared unaffected. The class I hybrid cell lines generated tumors *in vivo* with drastically prolonged latency times when compared to the tumor parent (10 *vs.* 2 weeks). We hypothesize that a negative regulator of IL-3 expression present in PB-3c acts as a tumor suppressor in class I cell hybrids (*Diamantis et al.,1989, PNAS, in press)*. Data on the analysis of class II cell hybrids PB-3c/V4D6 will be presented.

I 112 POSSIBLE ROLE FOR ALU INTERDISPERSED REPETITIVE SEQUENCES IN NEGATIVE REGULATION OF CELL GROWTH, Bruce H. Howard, Kazuichi Sakamoto, C. Michael Fordis, Christopher D. Corsico, Wolfgang Holter and Tazuko H. Howard, National Cancer Institute, Bethesda, MD 20892

Several lines of evidence suggest a role for the 7SL RNA/Alu family of RNA polymerase III-transcribed interspersed repetitive sequences in mammalian cell growth regulation: i) transfection of either Alu or 7SL RNA gene sequences into HeLa cells can mediate inhibition of recipient cell DNA synthesis and proliferation; ii) such inhibition is dependent on at least two potential DNA-binding protein recognition motifs that occur within these genes - the B block element of the RNA polymerase III promoter, and a GAGGCNGAGGY element; iii) expression of Alu elements is strongly perturbed by a variety of agents which have in common the alteration of cell growth. Taken together, these findings suggest that 7SL RNA/Alu gene sequences (and/or their transcripts) interact with *trans*-acting factor(s) that are involved in the control of cell proliferation. Of note in this regard is a highly significant "homology" between the Alu consensus sequence and the SV40 core DNA replication origin at the level of short (5-7 bp) sequence motifs, especially the GAGGC pentamer that serves as the recognition site for SV40 T antigen. Our results suggest that a HeLa cell fraction previously reported to bind both the SV40 origin and Alu sequences (Podgornaya et al. FEBS Lett. 232: 99) exhibits a complex pattern of single and double strand binding activities for both the GAGGY motif and a second shared motif, CTG(G/T)AAT.

I 113 TUMORIGENICITY AND RFLP ANALYSES OF MONOCHROMOSOME-CELL HYBRIDS AND SOMATIC CELL HYBRIDS, Deborah Iman, Mary McMenamin, Anne Van der Meeren, Elisa Spillare, Edward Kaighn, Eric Stanbridge[1], Thomas Shows[2], William Benedict[3], and Curtis C. Harris. NCI, Bethesda, MD 20892; [1]UCI, Irvine, CA 92717; [2]Roswell Park Memorial Institute, Buffalo, NY 14262; and [3]Baylor College of Medicine, The Woodlands, TX 77381.

Two sets of somatic cell hybrids between immortalized human bronchial epithelial cells and the mucoepidermoid carcinoma line HUT292DM were made to determine whether normal epithelial cells could suppress the tumorigenic HUT292DM cell line. Tumorigenicity was found to be suppressed (77% in one set and 52% in the other) and tumor latency was 3 times that of HUT292DM. Analysis of c-Ha-ras (11p15) alleles in the hybrids showed that 1)the more tumorigenic hybrids were more likely to have lost a ras allele and 2)the tumors that formed had fewer ras alleles than the hybrid clones injected.

In other experiments, individual human chromosomes have been introduced into HUT292DM by microcell transfer. Microcell hybrids containing regions of chromosome 11 were isolated and tested for tumorigenicity. Most colonies displayed a decreased probability of tumor formation and an increased latency. RFLP analysis was performed and, in both sets of microcell hybrids, the informative donor loci were present in every colony examined. Tumor DNA samples were also examined and the banding pattern was always identical to that of the colony injected. A normal size retinoblastoma (Rb) message was seen in HUT292DM and in two of the hybrids, and Rb protein was seen as well. Since the donated chromosome 11/X is not lost in the tumors, we conclude that this chromosome alone is not able to completely suppress tumorigenicity.

I 114 DELETION MAPPING OF CHROMOSOME 13 IN RETINOBLASTOMAS, Ishizaki K., Kato M., Sasaki S. M., Radiation Biology Center, Kyoto University, Yoshida-konoecho, Sakyo-ku, Kyoto 606, Japan

In development of retinoblastoma, both alleles of retinoblastoma susceptibility gene (RB gene) should be inactivated, which was a consequence of two mutational events. The first event is supposed as germinal or somatic mutation and most of the second steps are deletions of whole or part of chromosome 13. This chromosomal deletion was observed as loss of heterozygosities of polymorphic loci on chromosome 13. In the present study, we have analyzed 34 retinoblastomas by using 8 polymorphic DNA markers which are located on various regions of chromosome 13 to know the mode of chromosome loss. Out of 34 retinoblastomas, 25 showed loss of heterozygosity at least at one locus. In only one case interstitial deletion was observed. Recombinational loss was observed in two cases. In the other 22 cases, total loss of a chromosome 13 was supposed. These results suggests that in retinoblastomas segregation of chromosome 13 is the major pathway in the second steps for inactivation of the RB gene.

I 115 DETECTION OF GENETIC ALTERATIONS IN SOLID HUMAN TUMOURS AND CELL LINES BY SYNTHETIC AND GENOME-DERIVED HYPERVARIABLE MULTILOCUS PROBES,

Olaf-Georg Issinger, Pierre Lagoda, Özlem Türeci and Hans Fischer+, Institute for Human Genetics, University of the Saar, D-6650 Homburg/Saar and +DKFZ, D-6900 Heidelberg, FRG.

1. Human cell lines were treated with carcinogens (ENU, NMU, MNNG) for various times and concentrations. DNA from treated cells and controls was digested with Hae III and probed with VNTR-specific probes (GTG)5 and 33.15. The observed differences in the DNA fingerprint pattern suggest a possible application of this method for routine screening of cells after exposure to mutagens.
2. Using a human glioblastoma cell line we have investigated the influence of passage number, virus transformation and heterotransplantion into nude mice on the DNA fingerprint pattern. It turns out that all these events lead to noticeable changes in the DNA fingerprint pattern. Especially the results, where we show that passage number leads to observable changes in the DNA fingerprint pattern strongly suggest to establish a "DNA fingerprint passport" for each cell line used. This will help to establish identity of cell lines whenever circumstantial results are obtained.

I 116 THE HUMAN GENOME CONTAINS TWO DISTINCT GENES RELATED TO THE PUTATIVE ANTI-METASTATIC MURINE GENE NM23, King, C.R., Porter, L., Liotta, L.A., and Steeg, P.S. Molecular Oncology Incorporated, Gaithersburg, MD. and National Cancer Institute, Bethesda, MD.

Previous experiments have indicated that the murine nm23 gene is expressed in amounts inversely related with the metastatic potential of a variety of experimental tumor cell lines. Recently, human breast tumors have been reported to express little nm23 mRNA when spread to the lymph node has occurred. In order to investigate the human nm23 system we have isolated two distinct cDNAs showing substantial sequence similarity to the murine nm23 mRNA and to the drosophila developmental mutant abnormal wing discs (awd). One human nm23 cDNA has been expressed in E.Coli and specific antibodies prepared. Investigations of human tumor cells indicate significant differences in the level of expression of nm23 protein.

I 117 IDENTIFICATION OF GENES CAPABLE OF SUPPRESSING THE INVASIVE PHENOTYPE Shunichiro Kubota and Yoshihiko Yamada
Laboratory of Developmental Biology and Anomaly, NIDR, NIH, Bethesda, MD20892

Tumorigenecity is often suppressed in somatic cell hybrids between tumor cells and normal cells. From this finding, the hypothesis has evolved that rearrangements, mutation, or deletion of tumor suppressor genes contribute to the conversion of normal cells to malignant cells. In this study, to identify and isolate the genes capable of reverting the invasive and metastatic phenotype, B16-F10 mouse melanoma cells which are highly invasive and metastatic were transfected with cDNA expression library from human fibroblasts. Transfectants were selected in medium containing G418. The population of transfected G418 resistant cells were then treated with colchicine to eliminate rapidly proliferating cells. Invasiveness of each surviving colony was measured by Boyden chamber invasion assay. Fifteen less invasive cell lines were selected and plasmids were recovered from these cells. The sizes of their inserts were around 1.0 kb. After second transfection of genes, various clones with different levels of invasiveness were obtained. We are currently studying the correlation between the expression of the genes and invasiveness.

I 118 THE HUMAN CBL PROTO-ONCOGENE AND ITS RELATIONSHIP TO BREAKPOINTS ON THE 11q23 REGION OF THE HUMAN GENOME. W.Y. LANGDON[1], P. Savage[2], M. Shapiro[3], C. Croce[4], J. Kersey[2] & H.C. Morse III[3]. [1]Div. of Human Immunology, Inst. of Medical & Veterinary Science, Adelaide, South Australia 5000; [2]Uni of Minnesota, Minneapolis, MN55455, [3]Lab Immunopathology, NIAID, Bethesda, MD 20892, [4]The Wistar Inst, Philadelphia, PA 19104.
v-cbl, the oncogene carried by the murine Cas NS-1 retrovirus, induces fibroblast transformation in vitro and pre-B and myeloid tumors following innoculation into newborn mice. Cellular sequences incorporated in v-cbl have no homology with previously described oncogenes, but the deduced amino acid sequence shows some similarity to the yeast transcriptional factor GCN4. Probes prepared from v-cbl detect the human cellular homolog at high stringency and the human CBL proto-oncogene was mapped to the 11q23 region of the human genome. Panels of rodent-human somatic cell hybrids were used to study the relationship of CBL to translocations occurring in acute leukemias and Ewing's sarcoma. CBL was found to translocate from chromosome 11 to 4 in acute B cell leukemias characterized by t(4;11) (q21;q23) and t(11;14) (q23;q32). In an Ewing's sarcoma cell line with a t(11;22) (q23;q12), CBL remained on chromosome 11. Studies of other genes in the region demonstrate the gene and breakpoint (BP) order: NCAM--CD3--t(4;11)BP--THY-1--t(11;14)BP--CBL--ETS1--t(11;22)BP. The gross structure of the CBL gene was not altered in these tumors, however the v-cbl probes used in this study only represent 10% of the c-cbl transcript.

I 119 ROLE OF INTESTINAL ALKALINE PHOSPHATASE IN THE TUMORIGENIC EXPRESSION OF HELA X FIBROBLAST CELL HYBRIDS, Kathryn M. Latham and Eric J. Stanbridge, Department of Microbiology and Molecular Genetics, University of California-Irvine, Irvine, CA 92717. The aberrant expression of alkaline phosphatases in human cancer has been widely observed and yet the significance is not known to be functional or one of misregulated gene expression. We recently have identified p75/150, a cell surface tumor antigen, as the HeLa form of intestinal alkaline phosphatase (IAP). Previously, HeLa x fibroblast (H/F) cell hybrids were generated that were stably suppressed for tumorigenicity yet exhibited properties of a transformed phenotype and from these, rare tumorigenic segregants arose. IAP was found to be abundantly expressed on the surface of the HeLa parents and tumorigenic segregants, yet was not detected in the nontumorigenic H/F cell hybrids. To test whether IAP may be playing a direct functional role in the tumorigenic behavior of H/F cell hybrids, the nontumorigenic H/F cell line, CGL1, was transfected with a *B*-actin promoter/HeLa IAP cDNA expression vector, pHIAP. It was determined by various analyses (Northern, Western, immunodetection, and alkaline phosphatase enzymatic assays) that high levels of IAP were appropriately expressed on the surface of the pHIAP transfectants. Tumorigenicity assays were performed by subcutaneous injection into athymic nude mice. To date, no tumors have arisen from injection of the pHIAP transfectants. Therefore, it seems that expression of IAP in this suppressed H/F cell hybrid is not *sufficient* to cause tumorigenicity. The results of ongoing experiments to determine whether IAP is *necessary* for expression of tumorigenicity will also be discussed and these involve transfection of an antisense IAP expression vector into the tumorigenic segregant, CGL4. Furthermore, we are currently studying the transcriptional regulation of IAP as a marker for tumor suppression.

I 120 IMMUNOPRECIPITATION OF P53 AND HEAT SHOCK PROTEIN COMPLEXES IN HUMAN BRONCHIAL EPITHELIAL CELLS AND HUMAN CARCINOMA CELL LINES, Teresa A. Lehman[1], Rama V. Modali[1], Stephen Ullrich[2], Ettore Appella[2], Brenda I. Gerwin[1] and Curtis C. Harris[1], [1]Laboratory of Human Carcinogenesis; [2]Laboratory of Cell Biology, NCI, NIH, Bethesda, MD 20892.
Previously reported data show that in many murine and a few human systems, wild type p53 binds to SV40 T antigen, while mutated p53 frequently binds to heat shock proteins. We investigated the status of p53 in various human cell lines using immunoprecipitation with antibodies to p53, heat shock protein (hsp) and T antigen. In a human bronchial epithelial cell line immortalized with T antigen (BEAS-2B), both p53 and T antigen were detected by immunoprecipitation with anti-p53 antibodies. Similarly, antiserum to T antigen coimmunoprecipitated p53 and T antigen. This result implies that the p53 in the BEAS-2B cell line is wild type, and that in vitro immortalization of normal human bronchial epithelial cells is not dependent upon mutation in the p53 gene. Further immunoprecipitation analyses were performed on a variety of lung cancer cell lines including adenocarcinomas, mucoepidermoid carcinomas, and a squamous cell carcinoma. Each cell line was examined using p53 antibodies and anti-hsp peptide serum. In 6/7 lung carcinoma cell lines examined, p53 and hsp proteins were coimmunoprecipitated using both antibodies. This indicates that mutations are present in the p53 gene in many human lung carcinoma cell lines.

I 121 DNA SEQUENCE DELETIONS FROM CHROMOSOME 11 AND 3 IN HUMAN NON-SMALL CELL LUNG CARCINOMA: Ludwig Ch.U., Dalquen P., Stulz P., Bertschmann W. and Obrecht J.P., Division of Oncology, Department of Research of the University, Kantonsspital, CH-4031 Basel, Switzerland.
So far most reports on DNA sequence deletions in human lung cancer are confined to studies of small-cell lung cancer and have described an associated 3p deletion. However the importance of loss of heterozygosity at the 3p locus in non-small cell lung cancer (NSCLC) remains controversial concerning frequency and correlation with histological subtypes.- We are analyzing NSCLC for allelic deletions on chromosome 11p, 3p and more recently also for 17p. For this purpose chromosome 11p, 3p and 17p specific DNA probes are used and their RFLP's exploited. In 50 patients DNA of lung cancer and of normal lung tissue of the same patients (matched pairs) was extracted. So far the DNA of about 20 patients has been analyzed on southern blots, using 5 different chromosome 11p specific DNA probes as well as one chromosome 3p specific probe. Our preliminary results demonstrate deletions with the following probes (informative cases only): IGF-II 2/9, PTH 1/26, CGRP-I 2/7, beta-FSH 1/6, Catalase 4/11 (chromosome 11p probes) and pH2H3 3/7 (chromosome 3p) . An update of these results and correlation with histological subtype will be presented.
Supported by grants No. 3100-009140 and 3231-025149 from the Swiss National Foundation.

I 122 AN EXPRESSION CLONING SYSTEM TO ISOLATE TRANSFORMING AND TUMOR SUPPRESSOR GENE cDNAs, Toru Miki[1], Timothy P. Fleming[1], Christopher J. Molloy[1], Marco Crescenzi[1], Shelly B. Blam[1], Steven H. Reynolds[2] and Stuart A. Aaronson[1], [1]*Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892,* [2]*Laboratory of Biochemical Risk Assessment, National Institute of Environmental Health Services, Research Triangle Park, NC 27709*

A new expression cloning system using a λ-plasmid composite vector, λpCEV27, was developed to clone cDNAs of transforming and tumor suppressor genes. This vector contains (1) a mechanism for the efficient construction of unidirectional cDNA libraries, (2) the M-MLV LTR promoter for high-level expression of cDNA inserts, (3) the SV40 promoter-driven *neo* gene as a selectable marker, (4) a mechanism that allows rescue of plasmids from transfected eukaryotic cells, and (5) some sequences useful to construct subtraction libraries.

To examine the performance of this system, a λpCEV27 cDNA library was constructed from a transformant induced by mouse hepatocellular carcinoma DNA, and the library DNA was used to transfect NIH/3T3 cells. From one of the foci obtained, four different plasmids were rescued. Transfection assay by the individual plasmids showed that one had transforming activity. Molecular characterization of the cDNA clone will be described. Our results indicate that this cloning system may be well suited for isolation of dominant and as well as recessive oncogenes.

I 123 PCR ANALYSIS SHOWS LACK OF SEQUENCE HOMOLOGY IN THE 5'FLANKING REGION OF THE HUMAN C-H-RAS PROTO-ONCOGENE. Antonio Milici, Jean-Numa Lapeyre, Frederick F. Becker, Department of Molecular Pathology, The University of Texas M. D. Anderson Cancer Center, Houston TX 77030. A series of Southern blot and PCR analyses were performed to determine the structure of the 5' flanking region of the c-H-ras oncogene. DNA derived from normal peripheral lymphocytes and lung tumors was first digested with BAM HI and hybridized with a 6.6 kb probe containing the complete c-h-ras gene to determine the total length of the gene. The same samples were also digested with BamHI/XhoI and BamHI/KpI. If the 5' flanking region of the c-H-ras gene conformed to its previously reported structure, a fragment of approximately 200 bp should have been detected in the BamHI/XhoI digestion when hybridized with a 1.9 kb H-ras probe containing the 5' flanking region and the first exon of the c-h-ras gene. None of the DNAs examined showed the expected 200 bp fragment. To examine the possibility of restriction enzymatic polymorphism in the 3' end of the gene namely VNTR, genomic DNA was digested with MspI and HpaII, and hybridized with a 1 kb probe homologous to the VNTR region. All of the samples yielded the predicted 1 kb fragment. To further examine the promoter region of c-H-ras, PCR analysis of the first 200 bp of the 5' flanking region of the gene spanning from the BamHI site to the XhoI site was performed. None of the genomic amplified samples demonstrated the 200 bp fragment which is clearly amplified in the PucEJ plasmid control containing the cloned H-ras gene. These data suggest that the 5' end of the c-H-ras proto-oncogene shows a lack of homology with its previously reported structure.

I 124 PARENTAL ORIGIN OF ALLELES LOST IN EMBRYONAL TUMORS, Paolo Radice, Marco A. Pierotti, Savino Lacerenza, Patrizia Mondini, Maria G. Borrello, Monica Miozzo, Maria T. Radice, Silvana Pilotti, Giuseppe Della Porta, Istituto Nazionale Tumori, Milano, Italy.
The preferential loss of maternal alleles observed in sporadic cases of Wilms' tumor and osteosarcoma, but not in retinoblastomas, suggests that in some types of childhood cancers there is a bias towards initial mutation on the paternal chromosome. Therefore, it has been speculated that in certain tissues the paternal and maternal alleles of onco-suppressor genes may differ in their behaviour because of epigenetic modification (i.e. methylation) resulting from genomic imprinting. In our laboratory, we have detected loss of heterozygosity (LOH) on the short arm of chromosome 11 (11p) in seven Wilms' tumors and two rhabdomyosarcomas. In all the four Wilms' tumors cases whose parental genotypes could be analysed, it has been found that the lost alleles were of maternal origin. On the contrary, in one rhadbomyosarcoma the allele loss occurred on the paternal chromosome.

I 125 CHROMOSOME MUTATION PRECEDES NEOPLASTIC TRANSFORMATION AND IMMORTALIZATION OF HUMAN DIPLOID FIBROBLASTS EXPRESSING THE SV40 LARGE T ANTIGEN F. Andrew Ray, David S. Peabody, L.Scott Cram and Paul M. Kraemer, Los Alamos National Laboratory, Los Alamos, NM 87545
To determine if the SV40 T antigen caused neoplastic transformation of human diploid fibroblasts, by an indirect mutational mechanism or by a direct effect on the entire population of cells, we have constructed a minimal plasmid containing large T antigen sequences but, with no origin of replication or small t antigen. We co-electroporated this plasmid with the neo gene and selected colonies of cells on the basis of G418 resistance. Newly expanded, pre-crisis, T antigen positive clones were assayed immediately for anchorage independence, colony formation in 1% serum, immortalization, tumorigenicity and chromosome damage. In cell populations which were 100% T antigen positive, the karyotypes of the cells were virtually 100% aberrant, either structurally or numerically. In contrast, the cells were neither anchorage independent nor could form colonies in low serum. None of the cells were immortal or tumorigenic when first assayed. Later, after crisis, several immortal cell lines arose from the T+ clones and these cell lines usually had increased percentages of cells capable of colony formation in 1% serum . Large numbers of dead cells were generated in these cultures through crisis. These results indicate that the primary role of SV40 T antigen in the transformation and immortalization of human fibroblasts is to act as a sublethal mutagen analogous to continual carcinogen or radiation treatment.

I 126 THE NEUROFIBROMATOSIS-1 GENE MAY ENCODE A TUMOR SUPPRESSOR
Peter T. Rowley, Barbara A. Kosciolek, and Gary R. Skuse, Department of Medicine and Division of Genetics, University of Rochester School of Medicine, Rochester, NY 14642.
The commonest inherited syndrome in man predisposing to neoplasia is neurofibromatosis-1 (von Recklinghausen disease)(NF1). NF1 is an autosomal dominant syndrome characterized by cafe au lait spots, neurofibromas, and a variety of inconstant features. NF1 individuals have a four-fold increased risk of malignancy, an increased risk of types of malignancy uncommon in the general population, an earlier mean age of tumor presentation, and an increased risk of multiple primary malignancies. We have recently reported a loss of heterozygosity in malignant tumors in NF1 subjects in the region of the NF1 locus (Genes, Chromosomes, Cancer 1, 36-41, 1989). We have obtained further evidence by analyzing additional tumors and using additional probes, permitting the following overall summary. DNA from tumor and control tissue from 31 unrelated NF1 patients was analyzed by Southern blotting using restriction enzyme polymorphisms and using DNA probes recognizing sequences linked to the NF1 locus on chromosome 17. A loss of heterozygosity was observed in 10/31 or 32% of all tumors, in 10/18 or 56% of gliomas and malignant peripheral nerve tumors, but never in neurofibromas. In the two cases in which NF1 was inherited and the parents were both available and informative, the allele remaining in the tumor was inherited from the affected parent, confirming its abnormality. Taken together, these data support the hypothesis that individuals with NF1 carry a single inactive allele at the NF1 locus and that a malignant tumor arises from a cell in a susceptible tissue in which the remaining normal allele is lost or inactivated.

I 127 TUMOR SUPPRESSOR GENE p53 ALLELE LOSS IN HEPATITIS B VIRUS-POSITIVE HEPATOCELLULAR CARCINOMAS FROM CHINA, Betty L. Slagle[1], Yi-Zhong Zhou[2] and Janet S. Butel[1], [1]Division of Molecular Virology, Baylor College of Medicine, Houston, TX 77030, and [2]Shanghai Institute of Biochemistry, Shanghai, China.

Hepatitis B virus (HBV) is clearly a factor in the development of hepatocellular carcinoma (HCC) in certain parts of the world. Although the exact mechanism by which the virus contributes to tumorigenesis remains unknown, the HCCs that arise in HBV-endemic areas (such as China) frequently contain HBV sequences integrated into the tumor DNA. Analysis of the viral and flanking cellular DNAs cloned from HCCs has revealed that the site of HBV integration is very frequently associated with gross chromosomal abnormalities (deletions, duplications, and translocations). It is possible that the loss of specific domains of chromosomes is an important feature of hepatoma development.

Of the cloned HBV inserts mapped to chromosomes, 50% have been assigned to either chromosome 11 or 17, suggesting that genes on these chromosomes may be particularly important in the development of liver cancer. We have analyzed 19 matched normal and tumor samples from China using restriction fragment length polymorphism (RFLP) probes to determine the frequency of specific chromosome loss in these tumors. A series of chromosome 17-specific RFLP probes revealed that 44% of the samples had a tumor-specific loss of markers from the short arm of the chromosome. The 17p deletions frequently included the p53 gene; of 9 patients with constitutional heterozygosity at the p53 locus, 5 (55%) showed a tumor-specific loss of one allele of p53. We are currently attempting to determine if the remaining p53 alleles contain mutations. Analyses of other chromosomes for allele loss are also in progress. These studies demonstrate that tumor-specific loss of chromosome 17p occurred in nearly half of the informative patients. It remains to be established if these chromosome 17p losses are related to HBV integration events and/or to the genesis of HCC.

I 128 CLONING THE HUMAN GENE FOR THE M1 SUBUNIT OF RIBONUCLEOTIDE REDUCTASE, P.J. Smith, J.A. Byrne and M.H. Little, Queensland Cancer Fund Research Unit, Department of Pathology, University of Queensland Medical School, Herston, Queensland, Australia, 4006.

Ribonucleotide reductase catalyses the reduction of ribonucleoside diphosphates to their corresponding deoxyribonucleoside diphosphates and is therefore essential for DNA synthesis. The mammalian holoenzyme is composed of two non-identical subunits known as M1 and M2. While M2 is necessary for catalysis, allosteric binding of effectors to M1 determines substrate specificity and overall enzyme activity, and therefore ensures a balanced supply of deoxynucleotides for DNA synthesis.

Recently a cDNA for a mutant mouse M1 subunit with altered allosteric control has been shown to produce a dominant mutator phenotype in mammalian cells (Caras and Martin, 1988). The increased mutation rate was proposed to result from aberrant deoxynucleotide levels. It has also been shown that the M1 gene forms part of a syntetic group of genes mapping to chromosome 7 in mouse and to chromosome band 11p15 in the human. Rearrangements of this chromosome band have been found in several embryonal tumours including Wilms' tumour. The M1 gene therefore appears to be of interest with regards to tumourigenesis.

We are currently cloning the human M1 genomic sequence with a view to subsequently examining whether embryonal tumours contain M1 gene rearrangements. A chromosome 11 specific large insert library (LL11NL01, Lawrence Livermore National Laboratory) was screened with a cDNA for wild-type mouse M1. Four positive clones were identified and purified through second and third round screenings. Characterization of clone inserts with respect to size, restriction sites and their hybridization with different regions of the murine M1 cDNA revealed two clone types which do not show significant overlap. Restriction fragments from both clones are currently being subcloned in preparation for sequencing.

I 129 ONCOGENE AND TUMOR SUPPRESSOR GENE INTERACTION IN RAT HEREDITARY RENAL CARCINOMA. C. Walker[1], J.J. Freed[2] and A.G. Knudson, Jr.[2], [1]CIIT, Research Triangle Park, NC and [2]Fox Chase Cancer Research Center, Philadelphia, PA.

Cellular oncogene activation and loss of tumor suppressor gene function are critical events in tumor development. Each can occur frequently in specific tumors, but how these two events interact during carcinogenesis is largely unknown. To identify such interactions, we have analyzed gene alterations in a rat tumor suppressor gene model. Long-Evans rats that carry a single gene mutation in a putative tumor suppressor gene develop kidney tumors at 10 mos. of age (Eker, Nature 1961). This gene mutation has 100% penetrance resulting in multiple bi-lateral tumors in all animals that carry the mutation; non-carriers have an incidence of renal carcinomas < 0.1%. We have isolated cell lines from these hereditary renal carcinomas and asked which oncogene and/or growth factor/receptor alternations occur in the transformed cells that lack tumor suppressor gene function. TGFα is expressed only by the transformed cells whereas TGFβ transcripts, which are abundant in normal kidney, are decreased in the tumor derived cell lines. Of the other growth factors examined, EGF and IGF-I were only expressed by normal kidney; PDGF and IGFII were expressed by both normal and transformed cells. Insulin receptors were expressed at detectable levels only in normal kidney; EGF and IGF-I receptors were expressed by both normal kidney and the cell lines. The expression of several oncogenes including c-abl, c-yes and H-ras was altered in the transformed cells relative to normal cells. Transcripts for genes with tumor suppressor function, R_b and p53, were expressed in all lines examined. In addition, the transformed cell lines contained an activated cellular oncogene detectable in the NIH3T3 nude mouse assay. Taken together, these results suggest that in hereditary cancers tumor formation may require alterations in cellular oncogenes in addition to loss of tumor suppressor gene function.

I 130 SUPPRESSION OF ADENOVIRUS MEDIATED ONCOGENIC TRANSFORMATION BY A CELLULAR MUTATION, Joseph M. Weber, Mabel Rodrigues and Sucheta Sircar, Department of Microbiology, Faculty of Medicine, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4.
Using an indirect selection method based on drug-resistance, we have previously reported the isolation of a flat revertant (G2) from adenovirus-2 transformed rat cells. The G2 cells contain 16 copies of the E1 transformation genes, elaborate E1a and E1b proteins indistinguishable from those of the transformed parent (F4), are resistant to re-transformation by E1 genes and complement the replication of an E1a deletion mutant. These properties suggest that the viral transformation genes retained their functional integrity and point to a possible cellular mutation. This hypothesis is supported by the results of cell-fusion experiments. Hybrids between F4 and G2 cells and between G2 and normal FR3T3 cells were nontumorigenic. Immunoprecipitation with anti-E1a serum revealed the presence of Rb105-E1a complexes in F4, G2 and the hybrids. These results suggest that the G2 cells express a dominant E1a-specific tumor suppressor gene, and shows that binding of the retinoblastoma protein is not sufficient for transformation.

I 131 GENE REGULATORY EFFECTS ASSOCIATED WITH SUPPRESSION OF TUMORIGENCITY OF A WILMS' TUMOR CELL LINE FOLLOWING INTRODUCTION OF A PORTION OF CHROMOSOME 11. Thomas T. Wheeler*, Steven F. Dowdy°, Eric J. Stanbridge°, and Donald A. Young*. *Departments of Medicine and Biochemistry, University of Rochester, Rochester NY 14642, and °Department of Molecular Genetics and Microbiology, University of California, Irvine CA 92717. Wilms' tumor has been associated with deletions in chromosome 11. Introduction of human chromosome t(X;11) into a Wilms' tumor cell line suppresses tumor formation in nude mice (Weissman et al., 1987.Science 236, 175). We have used ultra-high resolution "giant" two-dimensional electrophoresis (Young et al., 1983 Methods Enzymol. 91,190) to identify changes in gene expression in three cell lines: a tumorigenic cell line (G401.6) from a patient with Wilms' tumor, a derived non-tumorigenic microcell hybrid line (110.1/G401.1) into which the t(X;11) has been introduced, and a segregant of this in which the introduced chromosome has been lost and the tumorigenic phenotype restored (G401.2/6TG.1). Our initial experiments reveal an apparant influence of chromosome 11 on relative rates of synthesis of of at least 26 proteins analysed in whole cells (15 increases and 11 decreases). On subcellular fractionation two of these appear enriched in nuclei. This procedure also reveals at least four consistant changes in nuclear proteins not observed in the analysis of whole cells. Analysis of in vitro translation products of mRNA isolated from the cell lines reveals that at least five of the changes occur at the level of message. Interestingly, these are all repressions, suggesting a suppressive influence of a chromosome 11 gene product on the expression of other cellular genes. A goal of this work is to identify the Wilm's tumor suppressor gene product. To date we have identified tentatively one protein apparantly expressed exclusively in cells in which the tumorigenic phenotype has been suppressed but not seen in either of the tumorigenic cell lines. Further progress will be presented. Supported by NIH grants CA19401-14, CA47650, DK16177, and the Council for Tobacco Research, U.S.A., 1774.

Tumor Suppressor Genes; Growth Suppressing Genes

I 200 FREQUENT INACTIVATION OF THE P53 ONCOGENE AS RESULT OF SFFV INTEGRATION IN FRIEND VIRUS-INDUCED ERYTHROLEUKEMIAS. Yaacov Ben-David, Alain Lavigueur and Alan Bernstein. Division of Molecular and Developmental Biology, Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Ontario, M5G 1X5, Canada. Rearrangements within the p53 proto-oncogene is observed in a high proportion of erythroleukemic cell lines derived from the spleens of mice infected with Friend leukemia virus. The majority of these cells which show at least one rearranged allele of p53 do not express detectable levels of p53 protein. Here, we shown that in 6 out of 30 erythroleukemic cell lines induced by FV-P or FV-A strain of Friend virus, the p53 gene is rearranged as a result of integration of SFFV (spleen focus forming virus). Integration of SFFV within the p53 gene results in inactivation of the gene expression as determined by Western blot analysis. Using Southern blot analysis we have located the integration sites to a 1 kbp region between intron 7 and 9 of the p53 gene. In addition, loss of the normal allele of p53 was observed in 3 of the erythroleukemic cell lines which carried a rearranged p53 gene. The lack of p53 expression in at least 30% of erythroleukemic cell lines as a result of retrovirus integration provides further evidence that inactivation of p53 confers a selective growth advantage to transformed cells during progression of Friend disease. These results also agree with earlier suggestions from our lab (Mowat et al, Nature 314, 633-636, 1985) that p53 is an anti-oncogene whose expression acts in a negative way to control cell growth. (Supported by the Leukemia Research Fund, the MRC and NCI of Canada).

Negative Controls on Cell Growth

I 201 POSSIBLE MECHANISM FOR REGULATION OF GENE EXPRESSION BY A TUMOR SUPPRESSOR GENE, Jeff A. Boyd, Cynthia A. Afshari, Carlton J. Zdanski, and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, NIH/NIEHS, Research Triangle Park, NC 27709. Carcinogen immortalized, preneoplastic SHE cells were subcloned based on their ability to suppress (sup+) or not suppress (sup-) the tumorigenic phenotype of tumor cells in cell hybrids. The sup- cells do not exhibit autonomous anchorage-independent growth, but will grow in agar or suspension in response to a combination of growth factors. Sup+ cells in agar or suspension are growth inhibited even in the presence of growth factors, although growth factor receptor numbers and affinities on sup+ and sup- cells are identical. After growth factor stimulation in suspension, quiescent sup- cells, but not sup+ cells, exhibit a characteristic elevation in steady-state levels of several immediate-early mRNAs. Nuclear run-on analyses indicate that this difference is due to greater post-transcriptional mRNA degradation in the sup+ cells than in the sup- cells. Furthermore, these studies indicate that control of immediate-early gene expression in both cell types in suspension is through a post-transcriptional mechanism. The sup- cells also exhibit a subtle structural alteration, specific for the actin cytoskeleton. We propose that loss of a tumor suppressor gene function allows the anchorage-independent growth of sup- cells through the differential post-transcriptional regulation of immediate-early gene expression, and that disruption of the normal actin microfilament system may be related to this process.

I 202 FAR1: A GENE NECESSARY FOR CELL CYCLE ARREST BY NEGATIVE GROWTH FACTORS IN S. CEREVISIAE. Fred Chang and Ira Herskowitz, UCSF, San Francisco, CA 94143-0448.
In response to mating factors, cells exhibit many changes, including cell cycle arrest in G1, induction of genes involved in mating, and formation of the shmoo morphology. We are interested in determining how mating factors cause cell cycle arrest. Many components in the response pathway have been identified genetically and include the receptor, G protein, kinases, and a transcription factor. To identify components in the mating factor response pathway which might interact directly with general cell cycle controls, we have sought mutants which affect arrest but not other aspects of the mating factor response. The far1 (factor arrest) mutants were isolated as *mutants defective in cell cycle arrest but not defective for transcriptional responses or morphological changes.* Cells carrying a multicopy FAR1 plasmid are supersensitive to α-factor in arrest but are not supersensitive in transcriptional or morphological responses. Thus FAR1 functions specifically in cell cycle arrest and not in transcriptional response, and may be an effector of arrest. far1 mutants do not appear to have phenotypes outside of mating factor response, and thus may act solely in mating factor response and not in more general cell cycle controls. The sequence of FAR1 reveals no signicant homologies or motifs. The FAR1 transcript is induced by α-factor and is dependent on components of the response pathway for expression.

The target of FAR1 may be the S. cerevisiae homologs of cyclins. Three cyclins, CLN1,2,3, appear to have roles in G1 regulation and in α-factor response (S. Reed and F. Cross, personal communication). A deletion of all three cyclins causes cells to arrest in G1, suggesting that α-factor might arrest cells by inactivating these cyclins. Recently, we have found that cln1cln2 suppresses far1, suggesting that FAR1 may act to inactivate CLN1 and CLN2 in response to α-factor.

I 203 CHARACTERIZATION OF GAS 5 GENES AND ITS EXPRESSION DURING THE CELL CYCLE AND DIFFERENTIATION. Eliana M. Coccia', Claudia Cicala, Giovanni B.Rossi°, Lennart Philipson and Vincenzo Sorrentino. 'Lab. of Virology, Istituto Superiore di Sanità, Rome, Italy; European Molecular Biology Laboratory, Heidelberg, F.G.R.
Few studies have so far concentrated on gene expression at growth arrest. A group of cDNA has been recently cloned from mRNA preferentially expressed in quiescent cells). Expression of these genes is negatively regulated by serum and growth factors. To further clarify the expression of GAS gene clones we analized their expression during differentiation, a particular stage of the cell cycle where growth arrest is specifically induced. As a model system we have used Friend Leukemia cells (FLC) that undergo to erytroid differentiation in vitro after treatment by several agents. GAS 5 is the only growth arrest specific gene expressed in these cells, its expression is cell cycle regulated at growth arrest but it is suppressed when the cells are induced to terminally differentiate. However, if differentiation is not accompanied by irreversibile exit from the cell cycle the GAS 5 gene can still be expressed. Both transcriptional and post-transcriptional mechanisms appear to control the expression of GAS 5 in growing and differentiating cells. These data suggest that genes expressed at growth arrest of the cell cycle are not expressed at the terminal divisions during differentiation of FLC.

I 204 HUMAN PROVIRAL mRNAs DOWN-REGULATED IN CHORIOCARCINOMA CONTAIN A KRUPPEL-RELATED OPEN READING FRAME, Maurice Cohen[1], Nobuyuki Kato[2], Kunitada Shimotohno[2] and Donald VanLeeuwen[1]; [1]Abbott Laboratories, D-93D, AP20/5, Abbott Park, IL 60064; [2]Virology Division, National Cancer Center Research Institute, Tsukiji 5-Chome Chuo-ku, Tokyo, Japan

We previously described the HERV-R (ERV3) human endogenous retroviral genome, a single-copy provirus on chromosome 7, that is highly expressed as mRNA throughout gestation. HERV-R mRNAs of 3.5, 7.3 and 9 kb are most abundant in the chorionic villi of first trimester and term placenta. These mRNAs are all spliced forms lacking most of the gag and pol genes, and all contain a non-defective env glycoprotein gene and 3'-LTR. The 9 and 7.3 kb mRNAs extend into the human flanking region where, as as result of splicing, they are linked to human sequences not contiguous with HERV-R. Because HERV-R mRNAs are specifically down-regulated in malignant cell lines derived from the placenta, termed choriocarcinoma, it was postulated that another cistron would be found in these transcripts that might encode a negative regulator of a gene or genes involved in growth. We now report the isolation and characterization of HERV-R-initiated cDNA clones encoding a zinc-finger protein, H-plk, closely related to the Drosophila developmental gene, Kruppel. Because Kruppel can act as a positive or negative transcriptional modulator in Drosophila, we are investigating the possibility that the putative H-plk protein can act similarly in humans.

I 205 THE EXPRESSION OF CELLULAR GENE rrg IS ASSOCIATED WITH THE REVERSION OF ras-TRANSFORMED NIH 3T3 BY INTERFERON, Sara Contente, Kaylene Kenyon and Robert M. Friedman, Department of Pathology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814. NIH 3T3 cells that have been transformed by LTR-c-Ha-ras can be reverted by prolonged treatment with IFN α/β. When IFN is removed, the revertants persist as morphologically normal and remain non-tumorigenic in nude mice, yet they express pre-reversion levels of ras mRNA and p21. Our working hypothesis is that the action of interferon in the reversion process involved either the inactivation of a gene(s) required to co-operate with ras, or the reactivation of a gene(s) downregulated by ras. In screening cDNA libraries of the transformed and persistent revertant lines for differentially expressed genes, we isolated a partial cDNA of a gene (rrg, ras regulated gene) whose message is strongly expressed in the persistent revertant and in NIH 3T3, but very weakly expressed in the transformed line. When DNA expression vectors carrying a partial cDNA of rrg in the antisense orientation are transfected into persistent revertants, the clonal lines generated take on a transformed morphology and are tumorigenic in nude mice. Transfection with vectors carrying rrg cDNA in the sense orientation results in clonal lines that are morphologically normal and non-tumorigenic in nude mice. Analysis of the antisense- and sense-transfected cell lines shows that the level of rrg mRNA in the antisense lines is markedly decreased, while mRNA levels in the sense lines are comparable to those in the persistent revertant. Further studies are in progress on this gene, which appears to be involved in the pathway of cellular transformation by ras and can be regulated by IFN.

I 206 BALANCE BETWEEN NORMAL AND MUTATED Ha-ras GENES IN CHEMICAL CARCINOGENESIS, C.J. Conti, A.B. Bianchi, and C.M. Aldaz, Department of Carcinogenesis, University of Texas M.D. Anderson Cancer Center, Science Park, Smithville, TX 78957. We have recently identified non-random chromosomal abnormalities during mouse skin chemical carcinogenesis. Trisomies of chromosomes 6 and 7 were shown to be characteristic of the progression from premalignant papillomas to the fully invasive squamous cell (SCC) carcinoma. Trisomy 7 (Ts7) was found in more than 90% of SCC induced by initiation with dimethylbenz(a)anthracene (DMBA) and promotion with 12-O-tetradecanoylphorbol-13-acetate (TPA). Since initiation in this model was previously postulated to be a point mutation at codon 61 of the Ha-ras gene, which has been mapped to mouse chromosome 7, we have speculated about a possible link between the dosage of the mutated gene and the generation of Ts7 by non-disjunction. For that purpose, we have determined, by Southern analysis, Ha-ras allelotypes of skin tumor cells free of stromal and inflammatory contribution. Our results indicate that Ts7 occurs by non-random duplication of the chromosome 7 carrying the mutated c-Ha-ras allele. Most tumors (75%) were well differentiated SCC and presented a 1:2 (normal:mutated) allelotype, whereas a small percentage (20%) was poorly differentiated presenting a 0:3 allelic composition. These results suggest that the normal Ha-ras gene may be acting as a suppressor gene whose inactivation, loss, and/or overbalancing by its mutated counterpart may be a necessary step for the development of the fully malignant phenotype. (Supported by CA 42157).

I 207 CHINESE HAMSTER X CHROMOSOME INDUCES SENESCENCE OF A NICKEL-TRANSFORMED CELL LINE WITH Xq DELETION, Kathleen Conway, Lois Annab*, Xin-Wei Wang, Xin Hua Lin, Catherine B. Klein, Rupinder K. Bhamra, J. Carl Barrett* and Max Costa, New York University, Institute of Environmental Medicine, New York, NY, and National Institute of Environmental Health Sciences, Research Triangle Park, NC*.

Male Chinese hamster (CH) embryo cells transformed by nickel compounds exhibited partial or complete deletions of the heterochromatic long arm of the X chromosome. The relevance of the X chromosomal deletions to the CH embryo cell transformation process was addressed using the microcell-mediated chromosome transfer technique. Microcells of two different clones of the mouse A9 line, each carrying a normal CH X chromosome, were fused with the nickel-transformed, hprt-deficient Ni-2/TG^R. This recipient line is immortal, anchorage-independent, tumorigenic and exhibits a complete Xq deletion. All of 42 HAT-resistant hybrid colonies derived from these fusions senesced after reaching 25-200 cells in size. Human chromosome 11, tagged with the neo resistance gene, was also transferred to Ni-2/TG^R. Two microcell hybrid clones were obtained and both were immortal, morphologically-transformed and anchorage-independent, but were no longer tumorigenic in nude mice. The senescence-inducing effect was therefore specific for the X chromosome, since even a chromosome such as human 11 which is known to carry tumor suppressor genes did not induce senescence of the Ni-2/TG^R line. Further studies are currently in progress to localize the senescence-inducing activity along the CH X chromosome.

I 208 ISOLATION AND CHARACTERIZATION OF HUMAN cDNA CLONES PREFERENTIALLY EXPRESSED IN QUIESCENT WI38 FIBROBLASTS. Donald L. Coppock and Suzanne Scandalis, Oncology Research Laboratory, Winthrop University Hospital, Mineola, NY and Dept. of Medicine SUNY, Stony Brook, NY. Normal fibroblasts can leave the proliferative cell cycle and enter a reversible quiescent state (G_0). Entry into G_0 is regulated by positive and negative growth factors, nutrients and space. In contrast, most cancer cells do not enter a viable quiescent state when presented the normal environmental signals. To investigate the molecular mechanisms which regulate the entrance into G_0, we have made a cDNA library from mRNA isolated from quiescent human fibroblasts (WI38). A differential screen of 30,000 recombinants yielded 500 clones with increased expression in G_0. Partial characterization has yielded 4 groups of unrelated clones. We propose the name "quiescins" for these genes. Quiescin 1 (clone SS3), appears to have a 5.0 kb mRNA and is induced about 5 fold in quiescent as compared to cycling WI38 cells. We have sequenced several partial cDNAs from the 3' end and have found a signal for poly A addition but no open reading frame in over 1000 bases. Quiescin 1 (clone SS3) is not homologous to any sequences in Genbank (Release 59). Quiescins 2,3 and 4 are induced more highly than quiescin 1. These clones are of medium to rare abundance (0.02-0.05% of the recombinants). We will use these molecular probes to analyze the mechanism which controls whether a cell will enter a new proliferative cycle or G_0 and how this mechanism is altered in cancer cells. Supported in part by a grant from the Elsa U. Pardee Foundation.

I 209 Analysis of the Leucine Repeat region of the human Retinoblastoma gene product Sean E. Egan, Dennis J. Templeton, and Robert A. Weinberg. The Whitehead Institute for Biomedical Research. 9 Cambridge Center, Cambridge MA.,02142.
Sequence analysis of the retinoblastoma gene product reveals that a heptad repeat of leucine residues is conserved between humans and rodents. This type of structure is responsible for protein-protein interactions such as homodimerization of *myc* and heterodimerization of *jun* and *fos*. In order to assess the functional significance of this motif in the Rb protein we have mutated the second or third leucines to prolines. In addition, these residues were altered to valines either independently or together. These mutant Rb genes as well as the wild type allele have been linked in an expression vector also coding for hygromycin resistance allowing for selection in cultured cells. This series of Rb genes will be tested for tumor suppressor activity, toxicity and anti-proliferative effects on various cell types. In addition, cells expressing these genes either transiently or stably will be analyzed for alterations in Rb protein physical characteristics such as covalent modifications and protein-protein interactions.

I 210 EXPRESSION OF THE MURINE RETINOBLASTOMA GENE PRODUCT IN INSECT CELLS, Barton W. Giddings and Robert A. Weinberg, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139 and Whitehead Institute for Biomedical Research, Cambridge, MA 02142.

The role of the retinoblastoma gene product (RB) in mammalian cells is unknown but has been the focus of intense investigation. In an attempt to facilitate the biochemical study of this protein, we have overproduced the mouse retinoblastoma gene product in a recombinant baculovirus expression system. A cDNA coding for the murine retinoblastoma gene (kindly provided by Dr. Rene Bernards) was inserted into the genome of the baculovirus *Autographa californica* Nuclear Polyhedrosis Virus (AcNPV) immediately downstream from the strong polyhedrin promoter. *Spodoptera frugiperda* (fall armyworm) cells infected with the recombinant virus produce significant amounts of mouse RB, which co-migrates with RB from mouse cells (104kD). Like RB from mouse cells, the insect cell-produced protein is readily precipitated by specific antibodies, binds to the adenovirus E1A protein, and binds DNA. The retinoblastoma protein produced should be useful for a variety of studies, including affinity chromatography for proteins which interact with RB and three-dimensional structural studies of the protein using X-ray crystallography.

I 211 TWO NOVEL GENES INDUCED AS A PRIMARY RESPONSE TO DIFFERENTIATION AND GROWTH ARREST Of MYELOID CELLS. Barbara Hoffman-Liebermann, Dan A. Liebermann and Kenneth A. Lord, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Phil., PA 19104-6059. The sequence of the complete cDNAs for two novel genes induced as a primary response to differentiation and growth arrest of myeloid cells has been accomplished. The deduced coding region for one gene is 243 amino acids in length. This gene is stably induced within thirty minutes following stimulation of myeloid cells with physiological inducers. The deduced coding region for the other gene is about 400 amino acids and within this region there are 4 and 1/2 repeats of 38 amino acids. This gene is also induced within thirty minutes; however, its expression is cyclic. After a time it drops and then increases and continues to be expressed. In addition, for this latter gene IL6 and γ-interferon synergize the induced response.

I 212 **Characterization of a Human Placental Phosphotyrosine Phosphatase Gene**

David E. Hill, Sheryl Brown-Shimer, Karen A. Johnson, and Arthur Bruskin.
Applied bioTechnology, Inc. 80 Rogers St. Cambridge, MA 02142. 617-492-7289.

Using degenerate oligonucleotides deduced from the amino acid sequence of a 35000 dalton placental phosphotyrosine phosphatase (PTPase)[1], we have obtained cDNA clones encoding a full length PTPase. The single open reading frame deduced from sequencing the 3.2 kb cDNA predicts a protein of 435 amino acids having a molecular mass of 50000. The placental PTPase shares approximately 65% amino acid similarity with the 415 amino acid open reading frame encoded by a T cell-specific PTPase cDNA[2]. In vitro transcription and translation of the placental PTPase cDNA clone produces a protein of approximately 50000 daltons as determined by SDS/PAGE analysis. The structural similarity to the T cell-specific PTPase cDNA and the existence of an open reading frame of 435 amino acids for placental PTPase cDNA suggests that the purified 35000 dalton protein is a processed form of an otherwise 50000 dalton primary translation product. Using the cDNA as a probe, a genomic placental PTPase clone has been obtained. Partial sequence analysis of the genomic clone suggests that the exon/intron structure of placental PTPase is similar to the corresponding exon/intron structure determined for the PTPase domains of CD45[3]. In a co-transfection with selection for hygromycin resistance, the PTPase gene has been introduced into 3T3 cells, however, fewer than 20% of the hygromycin resistant transfectants appeared to express the PTPase cDNA as determined by RNA dot blot analysis. Experiments are currently underway to characterize the role(s) of PTPase in signal transduction.

1. Charbonneau, et al (1988) Proc. Natl. Acad. Sci. USA 85, 7182-7186.
2. Cool, et al (1989) Proc. Natl. Acad. Sci. USA 86, 5257-5261.
3. Hall, et al (1988) J. Immunol. 141, 2781-2787.

I 213 Inactivation of Retinoblatoma Gene Expression Through a Mutation in its Promoter,
Frank Hong, Pascale Rio and Wen-Hwa Lee, Department of Pathology, University of California at San Diego, La Jolla, California 92093
Retinoblastoma (RB) gene is the prototype for a class of tumor suppressing genes. Its expression occurs ubiquitously and the primary structure of its promoter seems to concur with this observed pattern of expression. The inactivation of the RB gene is considered a principal determinant of the retinoblastoma formation and its inactivation could arise through a mutation in its promoter. Here we report on a case where a single base deletion has been detected in RB promoter region. A DNA segment spanning the previously determined 70 bps of transcriptionally critical region was isolated from the genomic DNA of a retinoblastoma through the PCR technique. The fragment was then subcloned and sequenced. Interestingly, the deletion occurred at a base at which majority of RB transcripts appear initiated. A chimeric construct containing the bacterial CAT gene controlled by the mutant promoter has been prepared and tested for its activity. It is anticipated that the study of this type of promoter mutants may yield insights regarding the mechanisms underlying transcriptional regulation of G+C rich promoters that lack the typical TATA box.

I 214 TRANSCRIPTIONAL REGULATION BY A TUMOR SUPPRESSOR GENE FUNCTION,
Junichi Hosoi, Jeffry Montgomery, Roger Wiseman and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709
We have previously shown that loss of tumor suppressor gene function is essential in the multistep process of neoplastic progression of Syrian hamster embryo cells. A panel of clonal variants have been isolated from two chemically transformed preneoplastic Syrian hamster cell lines that either retained ($supB^+$) the ability to suppress tumorigenicity of a benzo(a)pyrene-induced tumor cell line in cell hybrids or had lost ($supB^-$) this ability. By differential screening of a $supB^+$ library with cDNA probes prepared from $polyA^+$ RNA of $supB^+$ cells and $supB^-$ cells, we identified $supB^+$ specific mRNAs derived from the α1(II) collagen, α1(IX) collagen, and H19 genes. Northern analyses demonstrated that the steady state levels of these mRNAs are considerably higher in $supB^+$ cells than in $supB^-$ cells. To test the hypothesis that these genes were transcriptionally regulated by the supB tumor suppressor gene function, we performed nuclear run-on assays. Higher transcription rates for the type II collagen and H19 genes were observed in $supB^+$ cells relative to $supB^-$ cells by this analysis. DNA sequence comparisons showed that a 10 bp element that was previously identified in the mouse H19 enhancer was also present in the 5' flanking reigon of the rat α1 type II collagen gene. Gel shift assays revealed the presence of a factor in nuclear extracts from $supB^+$ cells that bound to an oligonucleotide containing this sequence motif. Further characterization of this potential transcriptional regulator will be reported.

I 215 REPRESSION OF THE TRANSFORMING ACTIVITY OF *NEU* BY DMSO IS CORRELATED TO THE LOSS OF PHOSPHOTYROSINE CONTENT IN THE SUBSTRATE PROTEIN(S) OF *NEU*.
Mien-Chie Hung & Angabin Matin, Dept. of Tumor Biology, University of Texas, M.D. Anderson Cancer Center, Houston, Texas 77030.
The proto-oncogene *neu* (*erb*B-2) encodes a cell surface transmembrane protein closely related to the EGF receptor. When the *neu* oncogene, which has a point mutation in the transmembrane domain, is transfected into NIH 3T3 cells, the mouse fibroblasts are transformed (B104-1-1 cell line). We found that treatment of B104-1-1 cells with the non-polar solvent, dimethyl sulfoxide (DMSO), changes their usual transformed morphology, decreases their growth rate and the cells lose their ability to grow in soft agar. DMSO treatment did not affect the level of *neu* encoded protein or its mRNA. Recently, two tyrosine phosphoproteins of molecular weights greater than 110 kd have been implicated as substrates of the *neu* protein kinase. We found that treatment of B104-1-1 cells with DMSO caused an increase in the phosphotyrosine level of *neu* and a concomitant decrease in the phosphotyrosine levels of the substrates. The increased phosphotyrosine of *neu* and the reciprocal decreased phophotyrosine levels of the substrates suggests that DMSO interferes with the ability of *neu* tyrosine kinase to transfer phosphate groups to its substrates which in turn may be responsible for the observed loss of the transformed phenotype of B104-1-1 cells.

I 216 ANALYSIS OF SV40 T ANTIGEN MUTANTS IN THE RB BINDING DOMAIN, Michael J. Imperiale, Joan B. Christensen, David Kohrman, and Gary Silverstein, Department of Microbiology and Immunology, U. of Michigan Medical School, Ann Arbor, MI 48109-0620.
We have been analyzing the properties of two SV40 T antigen point mutants which map to the binding domain for the retinoblastoma protein and are defective for transformation of rat cells. We have performed genetic complementation assays with various adenovirus E1A genes to show that the defect is in binding of the anti-oncoprotein. For example, co-transfection of a wild type E1A gene with either mutant results in restoration of transformation. Interestingly, if we co-transfect an E1A-T antigen hybrid gene in which domain 2 of E1A is replaced by the homologous region of SV40 (Moran, Nature 334, 168), it complements one of the mutants, 6b, but not the other, 6a. In addition, 6b will transform murine cells even though it cannot transform rat cells. We have isolated a cDNA copy of the rat Rb gene and are using this and a mouse cDNA to produce reagents to study the interaction between the various T antigens and the two rodent Rb proteins.

I 217 SUPPRESSION OF TUMORIGENICITY OF ADENO-TRANSFORMED BRK CELLS BY HUMAN DNA, Aart G. Jochemsen, Ineke de Wit, Eric J. Stanbridge* and Alex J. van der Eb, Sylvius Laboratories, Lab. for Molecular Carcinogenesis, P.O. Box 9503, 2300 RA Leiden, The Netherlands, *Dept. of Microbiology and Molecular Genetics, U.C. Irvine, CA 92717. The transforming E1 region of human adenoviruses (Ad) can not or hardly transform human fibroblasts, in contrast to the reproducible transformation observed with rodent cells. These results could indicate that human cells contain sequences which prevent transformation by Ad E1. Two approaches are being employed to identify these sequences: 1) Microcell mediated chromosome transfer. Applying this technique some human chromosomes have been introduced into Ad-transformed BRK cells. Preliminary results indicate that both chrom. #11 and #17 do (partially) suppress the tumorigenicity of these Ad-transformed cells (1 and 3 microcell hybrids, resp.). Neither chrom. #3 nor #13 appears to influence the oncogenicity (3 and 2 microcell hybrids, resp.). 2) Transfection of human chromosomal DNA. Human DNA was transfected into Ad-BRK cells in the presence of a dominant selectable marker (RSVneo). The transfected cells were selected both for reverted phenotype by methionine- and serum-free selection and for the uptake of DNA (G418 selection). Phenotypically altered cell lines were isolated and tested for tumorigenicity in nude mice. At least one line appears to be (partially) suppressed. Other revertant lines are being tested. The results will be presented.

I 218 MUTATION OF A SINGLE AMINO ACID OF THE RETINOBLASTOMA PROTEIN BLOCKS PHOSPHORYLATION AND ONCOPROTEIN BINDING, Frederic J. Kaye[1,2], Robert Kratzke[1], Jean Gerster[1], and Jonathan Horowitz[3], [1]NCI-Navy Oncology Branch, NIH, and [2]the Uniformed Services University of the Health Sciences, Bethesda, MD 20814, and [3]the Section of Cell Growth, Regulation and Oncogenesis and the Department of Microbiology and Immunology, Duke University Medical Center, Durham, NC 27710.
Previous studies from our laboratories (Harbour et al., Science 241:353-357, 1988; Horowitz et al., PNAS, in press) and others (Yokota et al., Oncogene 3:471-475, 1988) have implicated inactivation of the retinoblastoma (Rb) gene in the etiology of small cell lung carcinomas. Greater than 95% (31/32) of such tumors have lost expression of the product of the Rb gene, p105-Rb, or carry mutant genes encoding aberrant Rb proteins. Immunoprecipitates from one of these tumor cultures (NCI-H209) showed the synthesis of an Rb protein of apparently wild type size that did not become phosphorylated post-translation. RNase protection analysis of Rb mRNA and nucleotide sequencing of PCR-amplified Rb cDNA from this tumor revealed a point mutation resulting in an amino acid substitution (cysteine to phenylalanine) within exon 21. No other amino acid substitution were detected within Rb coding sequences and this single amino acid substitution was not present in mRNA isolated from a lymphoblastoid cell line derived from the same patient. To test whether this mutation affected the ability of Rb to complex with viral oncoproteins, wild-type and mutant Rb cDNAs were transcribed and translated *in vitro* and incubated with cell lysates containing adenovirus E1A or SV40 large-T antigen. Whereas wild type Rb protein could be immunoprecipitated with antisera directed against it or viral oncoproteins, the mutant NCI-H209 Rb protein was immunoprecipitated only by anti-Rb antiserum. The possible mechanisms by which this novel mutation could effect the structural and functional properties of the Rb protein will be discussed.

I 219 HIGH INCIDENCE OF LUNG, BONE AND LYMPHOID TUMORS IN TRANSGENIC MICE OVER-EXPRESSING MUTANT ALLELES OF THE p53 ONCOGENE. Alain Lavigueur[1,2], Victor Maltby[1], David Mock[3], Janet Rossant[1,2], Tony Pawson[1,2] and Alan Bernstein[1,2], [1]Division of Molecular and Developmental Biology,Mount Sinai Hospital Research Institute, 600 University Avenue, Toronto, Canada, M5G 1X5,[2]Department of Medical Genetics, Faculty of Medicine, University of Toronto, [3]Department of Oral Medicine and Pathology, Faculty of Dentistry, University of Toronto, Canada, M5G 1G6
We have investigated the role of the p53 gene in oncogenesis in vivo by generating transgenic mice carrying mutated murine p53 genomic fragments. Each of the two mutated alleles gave transgenic lines which express elevated levels of p53 mRNA in several tissues. Increased levels of p53 protein were also detected in most of the tissues analysed by Western blotting. Because both transgenes encode p53 proteins which are antigenically distinct from wild type p53, it was possible to demonstrate that over-expression of the p53 protein was mostly, if not entirely, due to transgene expression. Neoplasms developed in 20% of the transgenic mice, with a high incidence of lung adenocarcinomas, osteosarcomas and lymphomas. The long latent period and low penetrance suggests that over-expression of p53 alone is not sufficient to induce malignancies and that additional events are required. These observations provide direct evidence that mutant alleles of the p53 oncogene have oncogenic potential in vivo, and that different cell types show intrinsic differences in susceptibility to malignant transformation by p53. Since recent data suggest that p53 may be a recessive oncogene, it is possible that the elevated tumor incidence results from functional inactivation of endogenous p53 by over-expression of the mutant protein. p53 transgenic mice may provide a useful model to investigate the molecular events that underlie lung and bone tumors in humans.

I 220 COMPLEXITY OF THE IMMEDIATE EARLY RESPONSE OF MYELOID CELLS TO TERMINAL DIFFERENTIATION AND GROWTH ARREST INCLUDES ICAM-1, JUN-B, AND HISTONE VARIANTS. Dan A. Liebermann, Barbara Hoffman-Liebermann, and Kenneth A. Lord, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104.

Differentiation inducible leukemic as well as normal myeloid precursors treated with physiological myeloid differentiation inducer have been used to explore the immediate early genetic response of cells to terminal differentiation and growth arrest stimuli. cDNA clones of 12 distinct genes, referred to as MyD genes, which are activated in the absence of protein synthesis following induction of myeloid differentiation and growth arrest have been isolated. Sequence analysis of both ends of MyD cDNA clones, and analysis of MyD gene expression following induced differentiation of M1D+ and normal myeloid precursors , has shown that the immediate early genetic response of myeloid cells to the induction of terminal differentiation is complex. This complex response involves a variety of genes, some of which are known and others unknown, including: transient induction of ICAM-1, a gene encoding for a ligand to a cell surface adhesion receptor; stable induction of Jun-B, a gene encoding for a nuclear transcription factor; and increased expression of histone genes which encode for terminal differentiation histone variants. These findings demonstrate that terminal differentiation and growth arrest immediate early response genes encode for at least three distinct types of gene products, which may play a role to reprogram the transcriptional activity of proliferating and nondifferentiated cells towards their conversion into terminally differentiated nonproliferating cells.

I 221 RETINOBLASTOMA CANCER SUPPRESSOR GENE PRODUCT IS PHOSPHORYLATED BY CELL CYCLE REGULATOR CDC2 KINASE, Bryan T.-Y. Lin, Alex O. Morla, Stefan Gruenwald, Wen-Hwa Lee* and Jean Y. J. Wang, Department of Biology, Pathology*, and Center for Molecular Genetics, University of California at San Diego, La Jolla, CA 92093. Phosphorylation of RB protein has been found to be regulated in the cell cycle and during cellular differentiation. RB is found to be a substrate of the human homolog of the fission yeast cdc2 kinase. Nine out of seventeen RB phosphopeptides are phosphorylated by cdc2 in vitro whereas two other broad substrate specificity protein kinases, Casein kinase II and cAMP dependent protein kinase, fail to phosphorylate RB on its in vivo sites. The G1/S activation of RB phosphorylation observed in vivo can be recapitulated in vitro in a cdc2/RB co-immunoprecipitation kinase reaction. Alternations in the sites of phosphorylation found between S and M phase RB can also be reproduced in vitro by using cdc2 from S or M phase cells. The study of a mutant RB protein indicates that the phosphorylation of RB by cdc2 in intact cells may require a specific interaction between these two proteins. These results strongly suggest that RB protein plays an important role in cell cycle regulation.

I 222 EXPRESSION CLONING OF cDNA SEQUENCES ASSOCIATED WITH REVERSION OF K-ras TRANSFORMATION, M. L. McGeady, N. Talbot, R. Bassin, LTIB, NCI, Bethesda, MD 20892. A morphologically non-transformed revertant cell line, 9CJ (Yanagihara et al., Oncogene, in press), derived from a Kirsten sarcoma virus transformed NIH/3T3 cell line (DT), was used as a source of polyadenylated RNA for the construction of a cDNA expression library. The cDNA expression vector contains the SV 40 origin and early region promoter controlling transcription of the neomycin/G-418 resistance gene and an RSV promoter to drive transcription of cDNA sequences. The cDNA library was transfected into transformed DT cells which were then selected initially in G-418, and, subsequently, in ouabain containing media. Following phenotypic screening of approximately 18,000 G-418 resistant colonies to date, 8 "flat" colonies have been identified, isolated and single-cell cloned. The resulting flat cell lines were fused with COS cells, extrachromosomal DNA was isolated, highly competent bacteria were transformed and neomycin resistant colonies were recovered. Subsequent to isolation and mapping, the cDNA containing plasmids were assayed for the ability to revert the transformed phenotype following transfection into DT cells. Results of these studies and the characterization of the cDNA sequences will be presented.

I 223 **REGULATED EXPRESSION OF THE RB1 GENE IN THE RB1 NEGATIVE BREAST CELL LINE MDA-468-S4.** Michelle M. Menard, Robert A. Phillips and Brenda L. Gallie, The Hospital for Sick Children Research Institute, University of Toronto, Toronto, Ontario, Canada M5G 1X8.
Mutation of the RB1 gene is known to be the initiating event in retinoblastoma, and perhaps osteosarcoma. Deletion of the RB1 gene has been noted in several non-retinoblastoma tumor cell lines, including the well characterized, EGFR-amplified breast cancer line MDA-468. The unamplified subclone S4 was transfected with a plasmid construct containing the entire RB1 coding region under the control of the murine metallothionein promoter. In the absense of induction, a low basal level of RB1 was detected by immunoprecipitation and western blot. Increased RB1 expression was induced by zinc ions. However, no changes in growth or morphology were observed in the induced or uninduced $RB1^+$ S4 cells, despite the reconstitution of these cells with RB1. The discrepancy in the phenotypic effect of RB1 in the breast cancer cell line, in comparison with the published results of RB1 in a retinoblastoma and an osteosarcoma cell line (Huang, et al., Science 1988;242:1563), may reflect the non-initiating role of loss of RB1 in non-retinoblastoma cells.

I 224 **ANTIPROLIFERATIVE EFFECTS OF WILD TYPE HUMAN P53,** W. Edward Mercer*, Michelle T. Shields*, Mamta Amin*, Gordon J. Sauve*, Ettore Appella, Stephen J. Ullrich and Joseph W. Romano, Department of Pathology and Fels Research Institute Temple University School of Medicine, Philadelphia, Pennsylvania, 19140 USA* Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, 20892 USA.
The transformation related p53 protein has been implicated in the processes of normal cell proliferation and neoplastic transformation. In this study the biological activity of human wild-type p53, a deletion mutant derived *in vitro*, and a mutant p53 cloned from a human osteosarcoma cell line which contains a single point mutation were examined. Plasmid constructs in which these p53 cDNAs driven by the mouse mammary tumor virus (MMTV) promoter linked to a dominant biochemical selection marker gpt were used in a colony forming assay employing SV40-transformed hamster HR8 cells. Plasmids encoding wt-p53 but not those encoding a deletion or point mutation significantly reduced the number of gpt^+ colonies obtained after transfection. Stable clonal hamster cell lines that constitutively express wt-p53 were isolated and found to have altered growth characteristics. In addition, wt-p53 plasmid was introduced into human T98G glioblastoma tumor cells and stable cell lines that conditionally-express wt-p53 were isolated. We show that induction of wt-p53 expression by dexamethasone alters the cell cycle and growth properties of these cells. Taken together, the data presented in this communication supports the hypothesis that human wt-p53 expression plays an important role in regulating cell proliferation.

I 226 Interaction of the human papillomavirus E7 proteins with pRB,

Karl Münger[1], Bruce A. Werness[1], Nicholas Dyson[2], Wiliam C. Phelps[3], Ed Harlow[2] & Peter M. Howley[1]. [1]Laboratory of Tumor Virus Biology, National Cancer Institute Bethesda MD 20852, [2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, [3]Burroughs Wellcome Co., Research Triangle Park, NC 27709.

The E7 proteins encoded by the human papillomaviruses (HPVs) associated with anogenital lesions share significant amino acid sequence homology with the Ad E1A proteins and the large T antigens of polyomaviruses. The E7 proteins of these different HPVs were assessed for their ability to form complexes with the retinoblastoma tumor suppressor gene product (pRB). Similar to the E7 protein of HPV-16 (Dyson et al., *Science* 243, 934, 1989) the E7 proteins of HPV-18, HPV-6b and HPV-11 were found to associate with p105-RB *in vitro*. The E7 proteins of HPV types associated with a high risk of malignant progression (HPV-16 & HPV-18) formed complexes with pRB with higher affinities than the E7 proteins encoded by HPV types associated with a lower risk for progression (HPV-11 & HPV-6b). The amino acid sequences of the HPV-16 E7 protein involved in complex formation with pRB *in vitro* have been mapped. Only a portion of the sequences which are conserved between the HPV E7 proteins and AdE1A and the large T antigens of polyomaviruses were necessary for association with pRB. This complex is further studied with respect to the state of phosphorylation of both the E7 protein and pRB.

I 227 TRANS-DOMINANT SUPPRESSOR MUTATIONS OF THE H-*ras* ONCOGENE,

Yoshifumi Ogiso, Lourdes Gutierrez, Linda S. Wrathall, Yu-Wen Hwang¶ and Thomas Y. Shih, Laboratory of Molecular Oncology, National Cancer Institute, Frederick, MD. 21701-1013, and ¶Department of Molecular Biology, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, N. Y. 10314.

Site-directed mutagenesis of the invariant amino acid residues of the highly conserved sequence motifs of the v-H-*ras* oncogene generated a group of mutant p21s defective in GTP-binding. Paradoxically, *some* of these mutants were highly transforming, such as N116I/G12R (mutations designated by proto-oncogene residue at indicated position followed by mutant residue), while most lost their transforming activity. Among this group of transformation defective mutants, we found two mutants derived from the v-H-*ras*, i.e., N116I/G12R/A59T and N116Y/G12R/A59T, that demonstrated a *trans*-dominant *activity* of suppressing the transformed phenotype of NIH3T3 cells induced *by a* LTR-linked c-H-*ras* proto-oncogene. About 8 to 30% of cell colonies selected by the G418-resistant marker *of* the plasmid DNA exhibited the revertant flat cell morphology. The doubling time of cell growth of 6 flat clones averaged 24.9 hr as compared to 14.2 hr for the parental transformed cells. The revertant cell morphology was accompanied by loss of its ability to grow in agar suspension. These suppressed cells expressed a great excess of mutant p21 relative to the c-*ras* p21 present in parental transformed cells. These dominant negative mutants provide a *research* tool to explore the normal cellular function of proto-oncogenes by blocking the resident wild type c-*ras* function. Experiments are in progress to determine the requirement of *ras* function *for* transformation induced by a variety of oncogenes.

I 228 INACTIVATION OF THE RETINOBLASTOMA GENE IN NON-SMALL CELL LUNG CANCER,

Peter T. Reissmann, Hironobu Koga, Rei Takahashi, William F.Benedict, Robert Figlin, E.Carmack Holmes, Dennis J. Slamon, and the Lung Cancer Study Group, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024, and Center for Biotechnology, Baylor University, The Woodlands,TX 77381

The retinoblastoma susceptibility gene (RB) is a tumor suppressor gene which has been implicated in the pathogenesis of a number of human malignancies. Loss or inactivation of the gene appears to be central to the pathogenesis of retinoblastoma, and deletions of the gene as well as abnormalites of the RNA transcript and protein product have been described in osteosarcomas, soft-tissue sarcomas, small cell lung cancers, and other tumors. We studied the RB gene and its expression in 204 cases of non-small cell lung cancer (NSCLC) resected from patients enrolled in Lung Cancer Study Group protocols. DNA and RNA were extracted from specimens of tumor and normal lung tissue collected at the time of surgery. Two of 160 interpretable cases were found to have deletions of the RB gene by Southern analysis. In both cases the deletion was internal to the 5' and 3' ends of the gene, and involved the loss of exons 12-17. DNA from the adjacent lung tissue was normal. The RNA from these two cases demonstrated the presence of truncated RB transcripts of approximately 4.0 kb. Sixteen additional cases were found to lack RB message in the tumor tissue. Normal RB transcripts were found in the normal tissue from these cases, indicating that inactivation of the gene took place in the course of tumor development. Absence or alteration of RB mRNA was found in 7/64 epidermoid carcinomas, 7/68 adenocarcinomas, and 4/21 large cell carcinomas. In total 18/160 cases (11%) of NSCLC were found to have inactivation of the RB gene. The clinical data collected from these cases showed no clear correlation of RB gene inactivation with histology, stage or epidemiologic factors. As the follow-up of these patients matures, the relapse rate, disease-free survival and overall survival of these cases will be studied to assess any posssible impact of RB gene abnormalities on clinical outcome.

I 229 TUMOR SUPPRESSIVE EFFECT OF NORMAL HUMAN CHROMOSOMES INTRODUCED VIA MICROCELL TRANSFER INTO A549 LUNG ADENOCARCINOMA CELL LINE, Hitoshi Satoh, Mitsuo Oshimura, and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC 27709 and Kanagawa Cancer Center, Yokohama, Japan
There is increasing evidence for a family of tumor genes that have to be lost, inactivated, or mutated in a variety of tumors. Mapping of tumor suppressor genes for a specific tumor can be achieved by introduction of normal chromosomes into tumor cells by microcell-mediated chromosome transfer. Using microcell-mediated transfer, chromosomes 3 and 11 were introduced into an A549 lung adenocarcinoma cell line to test which human chromosomes retain the ability to suppress tumorigenicity. These normal human chromosomes, which contain the neo gene as a selectable marker, were transferred into the tumor cells at a frequency of ~ 2×10^{-6}. Two chromosome 3- and four chromosome 11-introduced microcell hybrid clones were isolated and examined for their growth properties and for the tumorigenicity in nude mice. Cytogenetic analysis was performed at the same passage. Both chromosome 3-introduced clones and three of four chromosome 11-introduced clones failed to form tumors 150 to 180 days after injection of 2×10^6 cells per site, whereas A549 cells formed tumors in a period of 32 to 96 days. One chromosome 11-introduced hybrid clone was tumorigenic but did not contain any extra copies of an intact chromosome 11. These results indicate that chromosomes 3 and 11 can suppress the tumorigenicity of A549 lung adenocarcinoma cells and that the putative suppressor gene(s) on chromosome 11 is effective in suppressing several different types of tumors. Experiments with other chromosomes are in progress.

I 230 INTERFERON INHIBITS THE EXPRESSION OF MITOCHONDRIAL GENES, Bei Shan and John A. Lewis, Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, N.Y. 11203
It is well known that interferons exert inhibitory effects on cell growth. These effects are presumed to be mediated by alterations in gene expression, but little is known about the nature of the target genes. To identify genes which may play roles in interferon-mediated growth inhibition, a lambda gt 10 cDNA library from murine L-929 cells was differentially screened and a clone, BS-5, was selected based on reduced expression in interferon-treated cells. It hybridizes to a 1.2kb mRNA and its expression is strongly down-regulated by interferon-ß. Clone BS-5 also recognizes a similar mRNA in human Daudi lymphoblastoid cells that is down-regulated by interferon-α. The regulation of expression requires protein synthesis as it is blocked by cycloheximide. Reduction of the mRNA levels is detected within 4 h of addition of interferon and expression remains at a reduced level for at least 48 h. Removal of interferon is accompanied by a rapid restoration of the normal level. The 1099 bp insert of BS-5 and a 3.6 kb cDNA corresponding to the putative precursor (obtained by rescreening the cDNA library) were sequenced and compared with the Genebank database. The 3.6 kb sequence corresponds to a region of the mouse mitochondrial genome encoding cytochrome b and unidentified reading frames 5 and 6. The 1099 bp clone is identical to the cytochrome b gene. This is the first report that interferons affect the expression of mitochondrial genes. Since cytochrome b is an important member of the electron-transport chain it is possible that mitochondrial function is impaired as a result of interferon treatment. Alterations in mitochondrial gene expression could influence the rate of cell growth.

I 231 THE *DROSOPHILA awd* POLYPEPTIDE IS HOMOLOGOUS TO THE HUMAN *NM23* POLYPEPTIDE WHICH IS DOWN-REGULATED IN TUMORS OF HIGH METASTATIC POTENTIAL. Allen Shearn,[1] Evelyn Hersperger,[1] Joseph Biggs,[1] Nicholas Tripoulas,[1] Charles Dearolf[1], Ariella Rosengard[2], Lance Liotta[2], and Patricia Steeg[2]. [1] Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218. [2] Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892. The *abnormal wing discs* (*awd*) gene was identified in a hybrid-dysgenic screen for third-chromosome, late larval lethal mutations which cause imaginal disc defects. Homozygous larvae, in addition to their imaginal disc defects, have abnormal brains and ovaries. The product of the *awd* gene is a single 0.8 kb poly A+ mRNA It is expressed abundantly in imaginal discs, brains and ovaries of wild-type individuals. Based on the nucleotide sequence of a full length cDNA, the polypeptide product of the *awd* gene would have 153 amino acids and have a molecular weight of 17,158. Antiserum from a rabbit immunized with a *trpE/awd* fusion protein reacts with a polypeptide that is present in normal embryos, larvae, and ovaries but which is absent in homozygous *awd* larvae. We therefore believe that this polypeptide is the product of the *awd* gene. The *Killer of prune* (*K-pn*) mutation described by Sturtevant in 1956 is the result of a single amino acid substitution of a serine for a proline in residue 97 of the *awd* polypeptide. In the absence of *prune* gene function, the *K-pn* mutation acts as a dominant lethal. It causes a failure of *awd* polypeptide accumulation. The entire amino acid sequence of the *awd* polypeptide is 78% identical both to the human and murine *NM23* polypeptide In human breast carcinomas and murine melanomas reduced levels of *NM23* is highly correlated with increased metastatic potential. The extreme degree of conservation between *awd* and *NM23* amino acid sequences implies that they are functionally homologous. Antibodies raised against a peptide of murine *NM23* cross react with the *Drosophila awd* polypeptide. Indirect immunofluorescence of sectioned *Drosophila* ovaries indicates a predominantly nuclear localization of the *awd* polypeptide in that tissue.

I 232 NUCLEAR RETINOIC ACID RECEPTORS: TRANSCRIPTIONAL REGULATION OF RARβ EXPRESSION BY THE RARα GENE PRODUCT. Paul T. van der Saag, Christina van den Brink, Bas H.K. Defize, Frank A.E. Kruyt and Wiebe Kruijer. Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands.

Retinoic acid (RA) is a negative growth regulator for a variety of normal and transformed cells. Moreover, in a more restricted number of cases RA can induce cellular differentiation in vitro, concomitant with growth retardation. Pluripotent embryocarcinoma (EC) cells are efficiently induced by RA to differentiate in derivatives of all three germ layers. The recently identified nuclear receptors for retinoic acid (RARs) belong to a supergene family of steroid and thyroid hormone receptors. So far three different RARs have been identified by others, RARα, β and γ. We have studied RAR expression in mouse P19 EC cells using human cDNA probes for RARα and β. Undifferentiated EC cells express two RARα transcripts constitutively and no RARβ can be detected; upon addition of RA, expression of RARβ is induced within 2 hrs reaching a maximum after 24 hours. In contrast, in a P19-derived cell line (RAC65) resistant to both the growth-inhibitory and differentiation-inducing activity of RA, expression of RARβ cannot be induced, while a smaller RARα transcript is highly expressed. These findings have led us to the hypothesis that RARα protein is involved in the induction of the RARβ gene. In order to test this hypothesis upstream sequences of the human RARβ gene have been isolated to study the regulation of the RARβ gene directly. A 1.5 Kb PstI-BamHl fragment, containing the putative promoter region was cloned from a genomic library of K562 cells. This fragment, when inserted into a promoterless Tk-CAT vector conferred RA-sensitive CAT expression in transient assays in P19 EC cells. However, this fragment was inactive in RAC65 cells, unless expression vector containing hRARα was co-trans-fected. These results confirm our hypothesis and indicate the presence of one or more RA-responsive elements (RARE) in the upstream RARβ gene, which are recognized by RARα.

I 233 IN VITRO ASSOCIATION OF HPV-16 AND HPV-18 E6 PROTEINS WITH p53 Bruce A. Werness[1], Arnold J. Levine[2] and Peter M. Howley[1]. Laboratory of Tumor Virus Biology, National Cancer Institute, Bethesda MD 20892[1], and Department of Biology, Princeton University, Princeton NJ 08540[2]

HPV-16 and HPV-18 contain two transforming genes, E6 and E7, and both together are required for transforming primary human cells. E7, like SV40 large T and adenovirus E1a, has been shown to bind the product of the tumor suppressor gene, pRB. We have investigated the possibility that E6 or E7 proteins encoded by these papillomaviruses may bind p53. Labelled E6 or E7 proteins translated in a rabbit reticulocyte lysate system were mixed with cell lysates containing normal murine p53. HPV-16 E6, but not E7, could be co-immunoprecipitated by two different anti-p53 antibodies in the presence of murine p53. We then tested the E6 proteins from several different genital type HPVs and from bovine papillomavirus (BPV) type 1 to see if these E6 proteins differed in their ability to bind p53, and if so, whether these differences correlated with transforming ability. In vitro translated human p53 and E6 proteins were mixed and immunoprecipitated with anti-p53 antibodies. E6 proteins of HPV types 16 and 18, two viruses associated with a relatively high risk of malignant progression, could be co-immunoprecipitated with anti-p53 antibody, whereas the E6 proteins of HPV type 6b, a benign associated virus, and BPV-1, could not be. The E6 of HPV-11, which is also benign associated, gave equivocal results. Further evidence for complex formation was obtained by mixing p53 with E6 proteins engineered to contain an additional epitope at their amino terminus, and co-immunoprecipitating p53 using antibody to the epitope.

I 234 SUPPRESSION OF TUMORIGENICITY IN HYBRIDS OF V-FGR OR V-FMS TRANSFORMED RAT-1 CELLS AND NORMAL RAT FIBROBLASTS IS DUE TO TRANSCRIPTIONAL EXTINCTION OF THE VIRAL ONCOGENES, Klaus Willecke, Wolfgang Martin, Beate Grohé, and Ekkart Lenz, Institut für Genetik, Abt. Molekulargenetik, Universität Bonn, 5300 Bonn 1, Fed. Rep. of Germany

Rat-1 (F208) cells transformed by proviral DNA containing v-fgr or v-fms grew in soft agar medium and were highly tumorigenic after injection into nude mice. After fusion with embryonic rat fibroblasts the resulting hybrid cells are dependent on anchorage for proliferation and show a drastic reduction of tumorigenicity. The corresponding viral oncogenes are present in the parental and in the hybrid cells. In contrast to the transformed parental cells no transcripts of v-fgr and v-fms were detected in hybrid cells suggesting that suppression of the transformed phenotype is due to transcriptional extinction of the viral oncogenes.

The growth of v-fgr, v-fms, v-raf, or c-Ha-ras transformed Rat-1 cells as measured by colony number and size is strongly inhibited by surrounding non-transformed Rat-1 cells or surrounding rat embryonic fibroblasts. The same extent of strong inhibition was measured under conditions where intercellular coupling through gap junctions was completely blocked. We conclude that growth inhibition of these transformed cells by surrounding normal cells is independent of intercellular communication through gap junctions.

I 235 TRANSFORMATION OF NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS BY HUMAN PAPILLOMAVIRUS TYPES 16 AND 18 DNA, James C. Willey, Ambereen Sleemi and Curtis C. Harris, Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892.
Human papillomavirus types 16 and 18 have been reported recently to immortalize normal human cervical epithelial cells, keratinocytes and mammary gland epithelial cells. In addition, another transforming DNA virus, the SV40 virus immortalizes several types of human epithelial cells _in vitro_, including normal human bronchial epithelial (NHBE) cells. Thus, there is reason to believe that transfection of NHBE cells with HPV16 or 18 may result in immortalization. We obtained HPV16 and 18 DNA cloned into PUC18 and PBR322 respectively and using the lipofection technique, transfected each into NHBE cells. Control cultures ceased dividing in the third passage, acquired a squamous morphology, and after several weeks, sloughed off the surface. In contrast, many colonies of rapidly dividing cells appeared in the HPV16 or 18 transfectant cultures. Both HPV16 and 18 transfected cultures have now achieved greater than 50 population doublings. They stain positive for keratin, are mycoplasma negative and are human by isozymes and cytogenetic analyses. Southern analysis is consistent with integration of HPV sequences into chromosomal DNA. We are presently evaluating expression of E6/E7 genes and tumorigenicity in nude mice. These cell lines should provide useful models for evaluating the role of HPV as a co-factor in bronchial epithelial cell carcinogenesis, alone and in conjunction with substances in cigarette smoke.

I 236 RETINOBLASTOMA IN TRANSGENIC MICE, Jolene J. Windle, Daniel M. Albert*, Joan M. O'Brien*, Dennis M. Marcus*, Christine M. Disteche§, Rene Bernards†, and Pamela L. Mellon, Regulatory Biology Laboratory, The Salk Institute, La Jolla, CA 92037, *Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA 02114, §Dept. of Pathology, University of Washington, Seattle, WA 98195, †Cancer Center, Harvard Medical School, Boston, MA 02114.
Retinoblastoma, a malignancy of the eye occurring in young children, has been widely studied as a model for genetic predisposition to cancer. This disease is caused by mutations in both alleles of an anti-oncogene (the retinoblastoma gene, Rb) which inactivate or eliminate the Rb protein, $p105^{Rb}$. The tumor does not arise spontaneously in animals and the absence of an animal model has limited understanding of the pathogenesis of this disease. Fifteen lines of transgenic mice were obtained with a hybrid transgene containing the protein coding region of SV40 T-antigen driven by the promoter of the luteinizing hormone β-subunit gene. Expression of the transgene was pituitary specific in most lines of mice, but a single male founder developed bilateral, multifocal ocular neoplasms at 5 months of age, and the disease shows nearly complete penetrance in transgenic offspring. The ocular tumors have histologic, ultrastructural, immunohistochemical, and invasive features identical to human retinoblastoma. Integration of the transgene has not disrupted the Rb allele, and cell lines derived from the tumors express an apparently normal Rb mRNA and protein. These observations support a mechanism for the development of the murine retinoblastoma in which normal function of $p105^{Rb}$ is impaired through binding to T-antigen, thus mimicking the situation in human retinoblastoma in which $p105^{Rb}$ is absent, and provide an explanation for the remarkable identity in morphology and malignant characteristics of the murine and human retinoblastomas.

I 237 **TRANSCRIPTIONAL REPRESSION OF THE HER-2/*neu* PROTO-ONCOGENE BY THE ADENOVIRUS-5 E1A GENE PRODUCTS,** Dihua Yu, Ting-Chung Suen, Duen-Hwa Yan, Mien-Chie Hung, Department of Tumor Biology, The University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030 The adenovirus E1A can regulate gene expression at the transcriptional level and has been identified as a viral suppressor gene for metastasis. Here we demonstrate that transcription of HER-2/*neu* proto-oncogene can be strongly repressed by E1A in Rat-1 cells. The transcriptional repression can be attributed to the conserved region 2 of the E1A proteins. The target for E1A repression was localized within a 139-bp DNA fragment in the upstream region of HER-2/*neu* promoter. These results indicate that E1A negatively regulates HER-2/*neu* gene expression at the transcriptional level via a specific DNA element. The product of human retinoblastoma (Rb) susceptibility gene has been shown to complex with E1A products, the role of E1A-Rb complex in the transcriptional repression of *neu* is currently under investigation.

I 238 LOCALISATION OF THE TUMOR SUPPRESSOR GENE INVOLVED IN THE ETIOLOGY OF MENINGIOMA, E.C. Zwarthoff, R.H. Lekanne Deprez, N.A. van Biezen, N.A. Groen, A. Hagemeyer, E. van Drunen, J.W. Koper, C.J.J. Avezaat and D. Bootsma, Department of Pathology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam. The Netherlands.
Meningioma presumably develops when both alleles of a suppressor gene on chromosome 22 are inactivated. In tumor cells from one patient we found that both chromosomes 22 were affected. Cytogenetic analysis shows that one chromosome 22 carries a translocation, resulting in deletion of most of the q arm and replacing this with the q arm of chromosome 1 (22q+). The other chromosome 22 appears to have lost sequences distal from band q11 (22q-). In addition one chromosome 4 had obtained extra sequences (4p+). We have fused these tumor cells with hamster cells, resulting in hybrid cell lines in which chromosomes 4p+, 22q- and 22q+ segregate separately. DNA from hybrid cell lines carrying the 22q- chromosome hybridizes to a probe originating from chromosome 4p. This suggests that in fact this chromosome is one of the products of a reciprocal translocation, resulting in $4p^+$, carrying 22q sequences and 22q-, which carries a cytogenetically invisible piece of the p arm of chromosome 4. Thus, in this particular tumor both chromosomes 22 are involved in translocations. It is very likely that at least one of these has inactivated the suppressor gene. The positions of the translocation breakpoints are being determined.

TGF Beta; TGF Beta Family

I 300 COMPETITIVE INTERACTIONS BETWEEN TGFβ1 AND TGFα IN THE REGULATION OF MURINE KERATINOCYTE PROLIFERATION. C.C. Bascom and H.L. Moses, Department of Cell Biology, Vanderbilt University Medical School, Nashville, TN 37232. BALB/c mouse keratinocytes (BALB/MK), a highly EGF-dependent cell line, are reversibly growth arrested by picomolar concentrations of TGFβ1. Within 48 hrs of removal of EGF from the medium, these cells become quiescent and can be restimulated to proliferate by the re-addition of either EGF/TGFα. The relationship between TGFβ1 and TGFα expression and biological activity has been examined in both proliferating and quiescent BALB/MK cells. In quiescent (EGF-deprived) BALB/MK cells, the re-addition of either EGF or TGFα maximally down-regulates TGFβ1 expression at 2 hrs; in addition, the effect of removal of EGF from the medium on TGFβ1 expression is also being examined. Further experiments have demonstrated that the addition of TGFα, in a dose-dependent manner, to quiescent BALB/MK cells can overcome inhibition of TGFβ1 at low doses (12 pM) but not at higher doses (400 pM). In proliferating BALB/MK cells, TGFβ1 (and TGFβ2) expression increases, and is maximal, when the cells reach confluence and DNA synthesis ceases. In contrast, the expression of *c-myc*, a TGFβ1-repressed gene, is highest in proliferating cells and begins to diminish when TGFβ1 expression is enhanced. The comparison of TGFα expression and the growth of the BALB/MK cells will also be examined. These data suggest that there is a reciprocal relationship between TGFβ1 and TGFα expression (both potential autocrine regulators of BALB/MK proliferation) and, that if this balance between the growth stimulator and growth inhibitor is altered, cell growth is responsive to these changes.

I 301 TRANSFORMING GROWTH FACTOR TYPE ß MODULATES AN INHIBITORY EFFECT OF NORMAL CELLS ON TRANSFORMED CELLS, Georg Bauer and Petra Höfler, Abteilung Virologie, Institut für Med. Mikrobiologie, Universität Freiburg, D-7800 Freiburg, FRG
We have recently shown that TGF-ß is involved in the establishment and maintenance of the transformed state of certain murine fibroblasts. TGF-ß is responsible for the induction of qualitative changes of the cells that lead to the transformed state and is involved in the maintenance of the transformed state due to an autocrinous loop.
Here we report on strong negative effects of TGF-ß on these transformed cells in combination with normal fibroblasts. When single transformed cells are grown together with a surplus of normal cells, exogenously added TGF-ß leads to a net loss of cells with the transformed phenotype, compared to the control. This inhibitory effect requires cell to cell contact. It is dependent both on the dose of TGF-ß and the number of normal cells present. Quantitative and qualitative analysis of this phenomenon show that the inhibitory effect of normal cells, modulated by TGF-ß can be explained by cytotoxic action of the normal cells against the transformed cells.
This elimination step is postulated to be a so far unknown controlling step in carcinogenesis. We presently search for substances that might counteract with that control point.

I 302 GROWTH INHIBITORY RESPONSE TO EPIDERMAL GROWTH FACTOR (EGF), ANTI-EGF RECEPTOR ANTIBODIES, AND TRANSFORMING GROWTH FACTOR (TGF)-BETA IN DIFI COLORECTAL CARCINOMA CELLS. Bruce M. Boman, Robert J. Coffey, Jr., John Mendelsohn, Lynn C. Yeoman, and Gary E. Gallick, Creighton University Cancer Center, Omaha, NE 68178, Vanderbilt University School of Medicine, Nashville, TN 37232, Memorial Sloan-Kettering Cancer Center, New York, NY 10021, Baylor College of Medicine, Houston, TX 77030, and University of Texas M.D. Anderson Cancer Center, Houston, Tx 77030.
The DiFi colorectal carcinoma cell line established from a familial adenomatous polyposis patient has amplification/increased expression of the EGF receptor gene and produces TGF-alpha. We report here that EGF inhibits colony formation in soft agarose (50% at 0.1 nM) and monolayer growth (>0.1 nM) of DiFi cells. EGF stimulates the EGF receptor kinase activity at 0.1 nM, whereas higher concentrations inhibit this kinase activity in DiFi cells. Exogenous EGF (10 ng/ml) and TGF-alpha (10 ng/ml) induces an up-regulation of TGF-alpha gene expression in DiFi cells. Anti-EGF receptor antibodies (528 IgG2a and 222 IgG1) inhibit (>65%) DiFi cell monolayer growth. TGF-beta also inhibits DiFi cell growth in soft agarose (50% at 1.0 ng/ml). Preliminary data indicate that TGF-beta down-regulates c-*myc* gene expression in DiFi cells. These results suggest that TGF-beta receptor and overexpressed EGF receptor pathways have a net growth inhibitory role in DiFi cells.

I 303 A DYE DILUTION TECHNIQUE FOR ISOLATING GROWTH INHIBITED CELLS FROM ACTIVELY GROWING CELL POPULATIONS. Frederick Boyd and Joan Massagué. Cell Biology and Genetics Program, Sloan-Kettering Institute, New York, NY 10021.
In the context of characterizing and attempting to clone the TGF-ß receptor we have developed an assay which may be widely applicable to the isolation of growth inhibitory genes. We have made a panel of mutants from Mv1Lu cells (which are normally growth inhibited by TGF-ß) lacking functional TGF-ß receptors and are in the process of complementing them by cDNA and genomic transfection. The problems in isolating the complemented transfectants are similar to the problems of isolating genes which are growth inhibitory. We have employed two methods for enriching for TGF-ß responsive cells. One is a suicide selection protocol in which cells are growth inhibited with TGF-ß and treated with a cytotoxic agent, such as colchicine or cytosine arabinoside. Cells which are growth inhibited are protected against the drug and have a selective advantage. However, this protocol takes a long time and is an inherently negative selection. Alternatively, we have used a lipophilic, fluorescent dye to label the plasma membranes of transfected cells. Cells which are actively growing dilute the dye by approximately half with each cell division. Cells which are growth inhibited by TGF-ß retain a high concentration of the dye and are visually brighter than nonresponsive cells. Mv1Lu cells were stained and allowed to grow for four days in the presence or absence of 50 pM TGF-ß. The cells were then harvested, counted and analyzed with a flourescence activated cell sorter. Cells treated with TGF-ß were potently growth inhibited (cell number 15% of control) and significantly brighter (9.7 fold brighter) than control. This method provides a positive selection for cells which are growth inhibited in a population of growing cells.

I 304 PROCESSING OF rTGF-ß1 PRECURSOR: EFFECTS OF SITE-DIRECTED MUTAGENESIS OF CYS RESIDUES IN THE MATURE REGION, A.M. Brunner and A.F. Purchio Oncogen, 3005 1st Ave, Seattle, WA 98121.
Mature, bioactive TGF-ß1 is a 24 kDa homodimer, containing a complex array of inter- and intra-molecular disulfide bonds. Nine cysteines are located in mature TGF-ß1 and these residues are strictly conserved among the TGF-ßs, types 1 thru 4. To investigate the importance of these CYS residues in processing, dimerization and for biological activity, we used site-directed mutagenesis to change CYS codons to SER codons and transfected COS cells with the mutant constructs. Supernatants were assayed for biological activity. Of the eight single mutations thus far analyzed, all have resulted in a decrease in biological activity. Analysis of mutant proteins by immunoblotting indicated that some mutations altered protein stability and/or impaired secretion; this is most likely due to confirmational changes. Other mutant proteins were readily secreted, but did not undergo correct proteolytic cleavage and/or were unable to assume a bioactive confirmation. To further study the processing and secretion of TGF-ß1, we are conducting pulse-chase experiments using a CHO cell line expressing high levels of rTGF-ß1. These studies indicate that dimerization of the TGF-ß1 precursor occurs co-translationally or very soon after synthesis and that a 90-110 kDa protein complex and mature, cleaved TGF-ß1 appear intracellularly at approximately the same time.

I 305 EMT6/RO SERUM-FREE SUPERNATANT CONTAINS AN ACTIVE FORM OF TGF-ß A NOVEL ANTI-PROLIFERATIVE FACTOR, Zhong Chen and Edith M. Lord,
Department of Microbiology and Immunology, University of Rochester, Rochester, NY 14642
EMT6/Ro, a spontaneous BALB/c mouse mammary tumor cell line, produces potent immunosuppressive activity in its serum-free culture medium. The tumor induced immunosuppression is antigen nonspecific and severely affects both B and T lymphocyte proliferation. By use of a transforming growth factor-ß sensitive cell line and two neutralizing antibodies, we have identified transforming growth factor-ß like activity in the original EMT6/Ro cell culture supernatant. The EMT6/Ro supernatant induced inhibition of splenocyte mitogenesis was abolished by both polyclonal and monoclonal anti-TGF-ß antibodies. The results indicate that an active form of TGF-ß was produced. However, EMT6/Ro cells were not sensitive to pure TGF-ßs in normal concentrations when grown in serum-free culture media. Functional analysis has also indicated the presence of a novel anti-proliferative factor distinguishable from TGF-ß. Inhibitory activity of the novel factor appeared in 45kd-60kd molecular weight fractions after gel filtration chromatography. This factor inhibited proliferation of a myeloma cell line, P3x63-AG8.653, which was not sensitive to TGF-ß from 0.01ng/ml to 100ng/ml. Our work provides evidence that the negative growth factors produced by tumor cells strongly influence host immunity but fail to regulate tumor cell growth. This may be an important mechanism allowing unlimited proliferation of tumor cells *in vivo* and contributing to the ineffectiveness of host anti-tumor immunity.

I 306 DIFFERENTIAL REGULATION OF TGF-ß1 AND TGF-ß2 EXPRESSION BY RETINOIC ACID (RA) AND EPIDERMAL GROWTH FACTOR (EGF) IN NRK-49F AND A549 CELLS, David Danielpour, Kyung Young Kim, Thomas S. Winokur and Michael B. Sporn,
Laboratory of Chemoprevention, National Cancer Institute, Bethesda, Maryland 20892
Although most biological activities of TGF-ß1 and TGF-ß2 examined *in vitro* are similar or identical, recent studies suggest that each of these factors may be involved in certain unique biological processes *in vivo*. In this study we have used highly sensitive and specific sandwich enzyme-linked immunosorbent assays for TGF-ß1 and TGF-ß2 and show that EGF induces secretion of TGF-ß1 and not TGF-ß2, whereas RA induces secretion of TGF-ß2 and not TGF-ß1 in NRK-49F normal rat kidney fibroblasts and A549 human lung carcinoma cells, after 24 to 48 of treatment under serum-free and growth factor-free conditions; these inductions were 2 to 5-fold. Moreover, EGF diminished the levels of TGF-ß2, while RA decreased the levels of TGF-ß1 in both cell lines. Thus, treatment with EGF alone and RA alone altered the relative proportions of TGF-ß1 and TGF-ß2 by as much as 30-fold. Studies on the transcriptional activation of TGF-ß1 and TGF-ß2 will be presented. In conclusion, EGF and RA are important and opposing differential regulators of the expression of TGF-ß1 and TGF-ß2 in A549 and NRK-49F cells.

I 307 **IDENTIFICATION OF TRANSFORMING GROWTH FACTOR β-MODULATED GROWTH-RELATED cDNA SEQUENCES,** J. Albert Fernandez-Pol, Dennis J. Klos, Paul D. Hamilton, and Vera M. Schuette,
Laboratory of Molecular Oncology, VA Medical Center and Department of Medicine, St. Louis University, St. Louis, MO 63106.

To identify genes mediating the antiproliferative action of transforming growth factor beta(TGFβ), two cDNA libraries were constructed with mRNA from TGFβ-treated and untreated human mammary carcinoma MDA-468 cells which have been previously shown to be sensitive to the antiproliferative effects of TGFβ. Differential screening of these two libraries identified cloned sequences whose expression was either induced or repressed by TGFβ treatment. Rescreening of these sequences with probes constructed from proliferating or quiescent cells led to the identification of TGFβ-induced sequences whose expressions also appeared to be modulated by cell proliferation. Northern blot-hybridization analyses indicated that RNA levels corresponding to these induced genes increased when MDA-468 cells were treated with TGFβ. Expression of these genes was unaffected by TGFβ treatment in variant cells resistant to the antiproliferative action of TGFβ. Taken together, these results suggest that the identified cDNAs correspond to genes that are involved in the antiproliferative action of TGFβ.

I 308 THE ROLE OF TRANSFORMING GROWTH FACTOR BETA IN MURINE EPITHELIAL CARCINOGENESIS
Fowlis, Deborah J., Lehnert, Sigrid A., Balmain, Allan, and Akhurst, Rosemary J.
TGF beta-1 is a polypeptide involved in wound healing, tumour promotion and tissue remodelling. We have shown that TGF beta-1 mRNA is induced in differentiating keratinocytes in vivo after treatment with the tumour promotor 12-tetradecanoyl-phorbol -13-acetate . Since TGF beta-1 is a negative regulator of keratinocyte growth and can induce differentiation in some epithelial cells , any loss of responsiveness to TGF beta-1 may be an important factor in tumour development. We are currently producing transgenic mice that will express the gene for TGF beta-1 and other members of the TGF beta family from various bovine keratin promotors. Thus, expression should be directed to specific cell types of the skin and embryonic epithelia . The use of these mice in tumour induction/promotion studies should provide further information regarding both normal skin development and carcinogenesis.

I 309 GROWTH SUPPRESSION CORRELATES WITH c-jun INDUCTION BY TGF-β1, Andrew G. Geiser, Seong-Jin Kim, Robert Lafyatis, Shinichi Watanabe, Michael B. Sporn, and Anita B. Roberts, Laboratory of Chemoprevention, NCI, Bethesda, MD, 20892.
An important property of TGF-β1 is its ability to inhibit the growth of certain cell types. Recently, we characterized the promoter region of the human TGF-β1 gene and found that induction of promoter activity by TPA and by TGF-β1 itself was mediated by the AP-1 transcription factor complex. TGF-β1 has also been found to increase the transcription of c-jun mRNA. This induction, as well as c-jun autoinduction, may amplify the actions of TGF-β. We have found that induction of c-jun expression by TGF-β1 is cell specific. Induction of c-jun was found in primary cells and cell lines which were growth inhibited by TGF-β1, while expression of c-jun was unaffected in cells which were not growth inhibited by TGF-β1 (even though the cells retained functional TGF-β receptors). These results indicate that c-jun (a component of the AP-1 complex) may play an important role in growth control by TGF-β1. In order to investigate this correlation further, we have generated mutant cells which are not growth regulated by TGF-β1, from the TGF-β1-sensitive cell line CCL-64. While the growth of CCL-64 cells is strongly repressed and c-jun expression is induced by TGF-β1, the mutant cells are nonresponsive and c-jun is unaffected. This correlation between growth suppression and induction of c-jun suggests that growth inhibition by TGF-β1 may be mediated, at least in part, by the AP-1 complex.

I 310 Retinoic Acid Induces the expression of Transforming Growth Factor-ß2 in Cultured Primary Mouse Keratinocytes and Mouse Epidermis, Adam B. Glick, Kathy C. Flanders, David Danielpour, Stuart H. Yuspa* and Michael B. Sporn, Laboratory of Chemoprevention, and *Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health , Bethesda, MD 20892 D.D. is supported by a grant from Johnson & Johnson. Retinoic acid has great clinical value in treatment of hyperproliferative diseases of the skin. We have investigated whether the effects of retinoic acid on the epidermis might be mediated through the expression of TGF-ß. Treatment of cultured primary mouse keratinocytes with retinoic acid resulted in a major induction of TGF-ß2 mRNA's and peptide, of at least 20-fold. Very little effect on the expression of TGF-ß1 was observed. At least 25% of the secreted TGF-ß2 was in the active form, and antibodies specific for TGF-ß2 partially reversed the inhibition of keratinocyte DNA synthesis caused by retinoic acid. These data suggest that part of the biological response to retinoic acid in the epidermis is mediated through the induction of TGF-ß2. Immunohistochemical analysis of mouse skin treated with retinoic acid showed that the specific induction of TGF-ß2 also occurs in vivo.

I 311 INDUCTION OF TGFß-REACTIVITY IS ONE STEP TOWARDS TRANSFORMATION, Michaela Götschl & Georg Bauer, Institute of Virology, D-7800 Freiburg (FRG)

We have been able to show that TGFß1 acts as a potent complete tumor promotor in vitro. At the same time we have defined a strong inhibitory mechanism against transformed cells in cell culture.

Initiation by UV was not sufficient to transform cells in our system. Thus promotion by TGFß1 cannot be simply explained by introducing optimal growth conditions for cells transformed by the initiator. Having found that treatment with the tumor promotor TGFß seems to induce qualitative alterations in the cells, we were interested to define those alterations on the level of single cells. To exclude cellular communication via gap junctions, single C3H10T1/2 cells were seeded into soft agar.The treatment of isolated C3H10T1/2 cells with TGFß led to cells reactive to TGFß. Reactive cells can not grow autonomously in a semi solid medium, but need exogenously added TGFß in a very low dose to show the transformed phenotype whereas the parental cell shows no reaction. On the primise that the ability to respond to TGFß is one step towards transformation, it should be easier to transform TGFß reactive cells than their normal counterpart. Indeed, reactive cells can be transformed by UV-light. These transformed cells are then capable of growing autonomously in a semi solid medium, possibly maintaining their transformed status via an autocrinous loop.

Concerning inhibitory mechanisms, experiments in our laboratory have shown that inhibition of transformed cells depends on the presence of normal cells and can be modulated by TGFß1 (see abstract by G. Bauer). We wanted to know,whether TGFß-reactive cells, similar to the transformed cells, can be recognized by this inhibitory system. We found that the more reactive the cells are the more they are inhibited. Thus, the expression of the transformed phenotype seems to be the signal for inhibitory mechanism. The implication of this phenomenon in multistage carcinogenesis will be discussed.

I 312 TGF-B SELECTIVELY INDUCES NEUTROPHIL CHEMOTAXIS. Leslie I. Gold, Theodore C. Lee, Joan Reibman, Bruce Cronstein, and Gerald Weissmann, Depts. of Pathology and Medicine, New York University Medical School, New York, New York 10016.

TGF-B regulates a cascade of events involved in inflammation and tissue repair such as angiogenesis, granulation tissue formation, and fibrosis. Our studies demonstrate that TGF-B is a potent chemoattractant for human neutrophils (PMN) and therefore may be significant in the recruitment of PMNs to sites of tissue injury. TGF-B induced dose-dependent directed PMN migration when studied under agarose, with a maximal response at 1.0 pg/ml (40fM) and a declining response at 5.0pg/ml; an identical dose-response curve was obtained for peripheral blood monocytes. The PMN chemotactic response to TGF-B was 89% of the response elicited by the potent chemoattractant n-formyl-methyl-leucyl-phenylalanine (fMLP @ 10^{-8}M) and represented a stastically significantly increase over controls receiving no TGF-B (n=5,$p \leq 0.009$). In response to a variety of factors and ligands, PMNS undergo degranulation releasing lysosomal enzymes and toxic oxygen metabolites. Since fMLP and immune complexes activate PMNs via different mechanisms and receptors, we examined the effect of TGF-ß on fMLP and immune complex (IC) induced degranulation and superoxide anion generation (O_2^-). PMNs($5x10^6$) were incubated with 0.001-10 ng of TGF-B for 60 min. at 37°C and subsequently stimulated with fMLP (0.1um,5 min.) or IC (200ug/ml). In contrast to the chemotactic response, TGF-B did not alter PMN degranulation, as measured by per cent of total ß-glucoronidase or lysozyme release, and did not effect O_2^- generation. Similarly, TGF-ß alone did not stimulate these responses. While TGF-ß appears to effect the phagocytic and chemotactic response of monocytes, our studies showed that TGF-ß has a selective effect on neutrophil responses since it has potent chemoattractant activity but does not affect degranulation or O_2^- generation.

I 313 TGF-β-ALTERATION IN GROWTH OF A431 CELLS AND PHOSPHORYLATION OF EGF RECEPTOR. Tzipora Goldkorn and John Mendelsohn, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Transforming growth factor-β (TGF-β) is a disulfide bond crosslinked polypeptide homodimer of mol wt 12,500. TGF-β binds to a specific cell surface receptor of mol wt 180,000 that shows no protein tyrosine activity. Although TGF-β was originally shown to support anchorage-independent growth of various types of cells, it also inhibits the growth of some cultured cells.

Long-term biological effects of human platelet derived TGF-β and epidermal growth factor (EGF) were examined with human epidermoid carcinoma A431 cells. EGF inhibited the growth of A431 cells in both serum containing and serum free media by reducing the growth rate and by lowering the saturation density. TGF-β treatment of A431 cells under the same conditions also resulted in the inhibition of cell growth in a dose and time dependent manner. Using an optimal concentration of 30 pM TGF-β, maximum inhibition was obtained after 72 hours. This TGF-β growth inhibition of A431 was synergistic with the inhibiting effects of EGF. Prolonged incubation of A431 cells with 5 nM EGF increased the total specific phosphorylation of the EGF receptor (EGFR) by 50-60%, as measured by in Vivo ^{32}P-labeling for 6-12 hours. Interestingly the same increase in EGFR phosphorylation was observed when the cells were incubated for 72 hours with 30 pM TGF-β. After these prolonged incubations with either EGR or TGF-β, short 10-30 min pulses of 20 nM EGF further doubled the stimulation of the phosphorylation of EGFR.

The TGF-β induced elevation in the phosphorylation of EGFR may have a role in the augmented growth inhibition of A431 cells.

I 314 **ABERRANT TGF-β GENE REGULATION IN METASTATIC MALIGNANCY,**
Arnold H. Greenberg[#+] Lois C. Schwarz[+], Marie-Claude Gingras[+], Paturu Kondaiah[*], David Danielpour[*], Michael B. Sporn[*] and Jim A. Wright[+]. [+]Manitoba Institute of Cell Biology, 100 Olivia Street, Winnipeg, Manitoba, Canada, R3E 0V9 and *Laboratory of Chemoprevention, National Cancer Institute, NIH, Bethesda, Maryland 20892.

We have examined the possible role of transforming growth factor-β (TGF-β) in metastatic malignancy by analyzing the production and activation of TGF-β_1 and β_2 and the regulation of TGF-β-responsive genes in oncogene-transformed metastatic fibrosarcomas. All transformed lines derived from either NIH 3T3 or 10T½ by either H-ras or protein-kinase encoding oncogenes produced more TGF-β than parental cells and this was primarily TGF-β_1. However, only highly metastatic fibrosarcomas secreted activated TGF-β at rates that were greater than parental fibroblasts. Localization of TGF-β by immunohistochemical methods identified intracellular and extracellular staining in metastatic lung tumor nodules. Cells isolated from tumors successfully metastasizing to the lung expressed TGF-β_1 mRNA levels as much as 19-fold over in vitro controls, and expression increased progressively with time.

Despite the greatly enhanced rate of secretion of activated TGF-β, metastatic cells exhibited markedly altered responses to TGF-β_1 and TGF-β_2, being unable to either increase collagen secretion or enhance collagen α2(I) or TGF-β_1 mRNA levels. This lack of response was not due to either altered TGF-β receptor affinity or numbers. Thus, metastatic progression was associated with an increase in the secretion of activated TGF-β_1 and a loss of the ability to deregulate TGF-β-responsive genes. (Supported by the NCIC)

I 315 ELEVATED EXPRESSION OF TRANSFORMING GROWTH FACTOR BETA GENES IN PAPILLOMAS AND CARCINOMAS PRODUCED BY TWO STAGE CARCINOGENESIS IN MOUSE SKIN, Yu Hashimoto, Hiroki Hashiba, Kiyoshi Nose and Toshio Kuroki, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan.

TGFß is an important regulator of growth and differentiation for a wide variety of cells including transformed cells. We found that TGFß-mRNA was highly expressed in papillomas and carcinomas produced by two stage carcinogenesis protocol, although only slight expression was noted in normal skin. Northern blotting showed two bands with 3.5 Kb and 2.5 Kb, corresponding TGFß3 and ß1, respectively. In addition, we found that mRNA of metallothionein, urokinase, osteopontin and novel TPA-inducible pOTS genes were also constitutively expressed in papillomas and carcinomas, whereas mRNAs of fos, myc and jun were not expressed in these tumors. Expression of TGFß genes in tumors may have some regulating functions in growth and differentiation of tumor cells. Furthermore, the present observation suggests that transcriptional control of gene expression is altered during the process of mouse skin carcinogenesis, probably due to the loss of some regulatory mechanisms.

I 316 SEXUALLY DIMORPHIC EXPRESSION OF HUMAN MULLERIAN INHIBITING SUBSTANCE, PL Hudson, I.Dugas,[‡]PK Donahoe,RL Cate*,J.Epstein,B.Pepinsky*,DT Maclaughlin,Pediatric Research Laboratory,Massachusetts General Hospital,Boston,MA 02114,*Biogen Research Corporation,Cambridge,MA 02142. Mullerian Inhibiting Substance (MIS) is a 140 kDa glycoprotein that causes the regression of Mullerian duct cells in male embryos at the sexually undifferentiated stage, and inhibits oocyte meiosis in adult females. The mechanisms of negative growth modulation (Mullerian duct cells) in males and meiotic arrest (rat,frog oocytes) in females most likely converge. The bovine and human MIS genes have been cloned. A specific enzyme-linked immunosorbent assay (ELISA) has been developed for the detection of human MIS in biological fluids. The ELISA uses a monoclonal primary antibody and a rabbit polyclonal as secondary antibody. The MIS antibody sandwich is detected by an anti-rabbit immunoglobulin horseradish peroxidase reporter. The mono and polyclonal antibodies were raised against intact mono and dimeric MIS respectively. No cross reactivity is seen with gonadotropins,(LH or FSH) or TGFB, the latter factor being a member of the same supergene family as MIS. Male values for MIS rise from 28 ng/ml at birth to 46 ng/ml at 23 months. From 2 years to the pubertal stage of development, MIS values steadily decreasein males. Female values at birth are at the limit of assay sensitivity and show a slow, small increase at puberty. The ELISA is also used to monitor MIS production of CHO transfected cells grown in bioreators. This assay has been used to study the pharmacology of MIS delivery and clearance in mice. We forsee its use in experiments designed to assess MIS anti-proliferative activity in vitro and in vivo. The Elisa has detected MIS in the serum of 4 female patients with granulosa cell tumors and will be essential in understanding the sexually dimorphic physiology of this negative regulator of growth in normal and neoplastic cells.

I 317 ALTERED RESPONSIVENESS OF v-raf TRANSFORMED RAT LIVER EPITHELIAL CELLS TO THE GROWTH INHIBITORY EFFECTS OF TGF-β1, Anthony C. Huggett, Lori L. Hampton, Peter J. Wirth and Snorri S. Thorgeirsson, Laboratory of Experimental Carcinogenesis, NCI, NIH, Bethesda MD 20892. A series of cell lines (R3611T-1,2,3,4,5,7) were isolated from a tumor produced following the injection of a nude mouse with v-raf transformed rat liver epithelial (RLE) cells. Clones were also isolated from the parental v-raf infected cells (R3611-3) and from RLE cells infected with a v-raf/v-myc construct (RJ2-14). The *in vitro* morphologies ranged from near-normal epithelioid (R3611-3, R3611T-4 and 5) to adherent (R3611T-1,2,3 and 7) and nonadherent (RJ2-14) spindle-shaped. All of the oncogene infected cells had increased growth rates and those with a spindle-shaped morphology expressed the neoplastic marker γ-glutamyltranspeptidase and were the most tumorigenic in nude mice. Additionally, the more tumorigenic cells had increased mRNA expression for the growth modulators TGF-α and TGF-β and decreased expression of the extracellular matrix proteins fibronectin and collagen type I. All of the oncogene infected cells were resistant to the growth inhibitory effects of TGF-β1 compared to RLE or control cells. Scatchard analysis of ^{125}I-TGF-β1 binding data showed that RLE and RLEC-2 cells had about 10,000 receptors/cell and an affinity of approximately 30 pM. R3611-3, R3611T-1,4 and 5 had approximately normal TGF-β1 binding in contrast to R3611T-2,3,7 and RJ2-14 which bound negligible amounts of TGF-β1. An analysis of proteins synthesized following treatment of the cell lines with TGF-β1 showed significant differences. These findings suggest that post-receptor mediated events in the TGF-β1 signalling pathway are altered in RLE cells following their transformation with v-raf and also indicate that the phenotypic heterogeneity displayed by these transformed cells may be related to TGF-β1 mediated events.

I 318 DOWN-MODULATION OF COLONY STIMULATING FACTOR RECEPTORS ON FACTOR-DEPENDENT AND INDEPENDENT MURINE MYELOID LEUKEMIC CELL LINES BY TRANSFORMING GROWTH FACTOR BETA. [1]Sten E.W. Jacobsen,[2]Claire M. Dubois [3]Francis W. Ruscetti and [4]Jonathan R. Keller, [1,2,3]LMI-BRMP, [4]BCDP-PRI,NCI-FCRF, Frederick, MD,21701. Our laboratory has previously demonstrated that Transforming Growth Factor-Beta (TGF-ß) is a potent selective inhibitor of both normal and leukemic hematopoietic cell growth. A potential mechanism for this could be by down-modulation of growth factor receptors. To investigate this, equilibrium binding experiments and subsequent Scatchard analysis were performed on factor-dependent myeloid leukemic cell lines, using radioiodinated biologically active recombinant IL-3, G-CSF and GM-CSF. GM-CSF- specific binding on these cell lines was maximally inhibited at 1 pmole/ml of TGF-ß. Scatchard analysis showed an 80 % reduction in GM-CSF receptor number on DA-3, an IL-3-dependent myeloid cell line. The kinetics of inhibition showed no effect of TGF-ß after 2 hours, while a significant down-modulation was observed after 6-12 hours. In addition, TGF-ß had no effect on the Kd for the GM-CSF receptor. Other results show that TGF-ß in a similar fashion down-modulates the receptors for G-CSF and IL-3 on murine myeloid cell lines. The decreased CSF binding following TGF-ß treatment is caused by a reduction in binding sites without change in receptor affinity. Thus, we propose that one mechanism of TGF-ß's antiproliferative effect on hematopoetic cells is through down-regulation of growth factor receptors.

I 319 TGFß1 INHIBITION OF TRANSIN GENE REQUIRES C-*FOS* EXPRESSION AND IS MEDIATED THROUGH A NOVEL *FOS* BINDING SITE Lawrence D. Kerr, Donna B. Miller, Bruce E. Magun*, and Lynn M. Matrisian. Dept. of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232. *Dept. of Anatomy and Cell Biology, Oregon Health Science Center, Portland, OR 97201

Transin is a secreted metalloprotease stimulated by a variety of growth factors and oncogenes and inhibited transcriptionally by TGFß1. Deletion mutagenesis of the transin promoter and in vivo oligonucleotide competetion experiments have revealed the presence of a repressible element at position -709 which is required for the TGFß1 inhibitory effect. The repressible element corresponds to a 10 bp conserved sequence that is shared by several transforming growth factor ß1 (TGFß1) inhibited genes (transin, collagenase, urokinase, c-*myc* , MEP/ cathepsin L, and elastase). Oligonucleotides to the consensus sequence specifically bound a protein complex from TGFß1-treated Rat-2 fibroblast nuclear extracts but not from control extracts. Surprisingly, binding of these protein complexes was inhibited by competetion with unlabelled oligonucleotides to the GCN4/AP-1 binding site sequence recognized to bind the c-*fos*/ c-*jun* protein complex. In vitro translated c-*fos*/ c-*jun* protein products were able to alter the mobility of the labelled TGFß1 inhibitory element (TIE) oligonucleotide and antibodies to c-*fos* were able to abolish the binding of HeLa cell nuclear extracts to labelled TIE oligonucleotide. In addition, a cell line bearing a stablely transfected antisense c-*fos* construct demonstrated the necessity of c-*fos* induction in the TGFß1 inhibition of EGF-induced transin gene expression. The data suggest that TGFß1 inhibition of transin and possibly other TGFß1-inhibited genes is mediated through c-*fos* expression and the binding of a c-*fos* containing protein complex to the TIE promoter sequence.

I 320 PHOSPORYLATION OF THE RETINOBLASTOMA GENE PRODUCT IS REGULATED BY TRANSFORMING GROWTH FACTOR-ß. Marikki Laiho*, James A. DeCaprio#, John W. Ludlow#, David M. Livingston#, and Joan Massagué*. *Cell Biology and Genetics Program, Sloan-Kettering Institute, New York, NY 10021 and #Division of Neoplastic Disease Mechanisms, Dana-Faber Cancer Institute, Boston, MA 02115.

Deletion or mutation of both alleles of the gene coding for the retinoblastoma protein, $p105^{Rb}$, has been linked to the development of tumors of the retina and other tissues. It is thought that this protein has a growth suppressive function that is essential for cell cycle control. $p105^{Rb}$ is a nuclear phosphoprotein whose phosphorylation state oscillates in the cell cycle. $p105^{Rb}$ is underphosphorylated during late M and G1 and becomes rapidly phosphorylated at entry into S phase. Underphosphorylated $p105^{Rb}$ is the target for binding by the transforming proteins of DNA tumor viruses, and is suggested to be the growth inhibitory form of $p105^{Rb}$. We have studied whether regulation of $p105^{Rb}$ phosphorylation is connected with the growth inhibitory properties of TGF-ß. Mv1Lu mink lung epithelial cells which are potently growth arrested at late G1 by TGF-ß, respond to this factor with relatively rapid accumulation of underphospho-$p105^{Rb}$. This response precedes inhibition of DNA replication by TGFß. TGF-ß blocks the progressive accumulation of phospho-$p105^{Rb}$ that occurs upon release of Mv1Lu cells from contact inhibition. The kinetics of this effect indicate that the immediate effect of TGF-ß on Mv1Lu cells is prevention of the activation of $p105^{Rb}$ phosphorylation. The extent of accumulation of underphospho-$p105^{Rb}$ induced by TGF-ß in various cell lines surveyed is proportional to the extent of growth inhibition caused by this factor, and is not observed in cell mutants that lack TGF-ß receptor. These results suggest that prevention of $p105^{Rb}$ phosphorylation might be an event that mediates growth inhibition by TGF-ß.

I 321 CHARACTERIZATION OF THE PROMOTER OF THE TRANSFORMING GROWTH FACTOR β3 GENE, Robert Lechleider, Robert Lafyatis, Sonia Jakowlew, Anita Roberts and Michael Sporn, Laboratory of Chemoprevention, National Institutes of Health, Bethesda, MD, 20892. Expression of the TGF-β3 gene has been shown to vary greatly in the different tissues and species studied to date. In order to study transcriptional regulation of this gene, a portion of the 5' flanking region of the TGF-β3 gene was cloned from a human genomic library. The entire first exon and approximately 2 Kbp of the 5' flanking region were sequenced. S1 nuclease analysis showed a single major transcriptional start site approximately 1 Kbp upstream of the translational start site. Sequence analysis revealed a consensus TATA box 35 base pairs upstream from the site of transcriptional initiation. Further analysis revealed several putative transcription factor binding sequences in the 5' region. Chimeric expression vectors composed of 5' sequences of the gene constructed by the polymerase chain reaction fused to a chloramphenicol acetyl transferase (CAT) coding sequence were made. These CAT constructs showed high activity when transfected into A375 human melanoma cells. This and other cell lines were used to determine the major elements of TGF-β3 transcriptional regulation.

I 322 CHARACTERIZATION OF LATENT FORMS OF RECOMBINANT TGF-ß1 AND TGF-ß2. Mario N. Lioubin and A.F. Purchio. ONCOGEN, Seattle WA 98121.

Recombinant Transforming Growth Factors ß1 and ß2 are secreted from CHO transfected cells in a latent form. Biochemical examination of the latent TGF-ß forms reveal a non-covalent complex of the mature TGF-ßs with their respective pro regions. Upon acidification the complex is dissociated, resulting in a 30 to 50 fold increase in biological activity. Furthermore, TGF-ßs and their respective pro regions which have been dissociated and purified under acid conditions, reassociate when incubated together under physiologic conditions. Both TGF-ßs' pro regions have mannose-6-phosphate residues and bind to the mannose-6-phosphate receptor, which may be involved in transfering the pro-TGF-ß complexes to acidic vescicles, where dissociation and activation may occur.

I 323 THE EFFECT OF TGFβ1 ON GROWTH ASSOCIATED GENES IN MURINE KERATINOCYTES, Duncan A. Miller*, Lester Lau# and Harold L. Moses*, *Department of Cell Biology, Vanderbilt University School of Medicine, Nashville, TN 37232 and #Department of Genetics, University of Illinois-Chicago, Chicago, IL 60612
Transforming Growth Factor β1 (TGFβ1) is known to be a growth stimulator in murine fibroblasts and a growth inhibitor in epithelial cells. Previously the mitogenic activity of TGFβ1 in AKR-2B cells has been attributed to the induction of c-sis and the autoinduction of platelet derived growth factor. The inhibitory mechanism TGFβ1 elicits in keratinocytes is as yet not known. TGFβ1 may inhibit BALB/MK cell growth via either the induction of new genes that inhibit cell growth or by the inhibition of genes required for cell growth. To test the later mechanism we have studied the effect TGFβ1 elicits on several genes that are known to be induced in NIH 3T3 cells in response to a mitogen. The induction of these immediate early genes has been shown to be protein synthesis independent under those conditions. The effect TGFβ1 has on these genes was examined in both AKR-2B and BALB/MK cells. We also studied the alterations in gene expression in response to TGFβ1 treatment on both rapidly growing cells and quiescent restimulated cells to determine the affect of growth state on gene expression. To date some of the genes studied (Jun-B, c-myc, KC, 3CH61, 3CH96) show both TGFβ responsiveness and cell cycle dependent differences. In rapidly growing BALB/MK cells treated with 10 ng/ml TGFβ1 we have observed an initial (15-45 min) increase in gene expression followed by a significant decrease in gene expression (1-8 hrs).

I 324 DENSITY-DEPENDENT REGULATION OF PLATELET-DERIVED GROWTH FACTOR α-RECEPTOR EXPRESSION BY TRANSFORMING GROWTH FACTOR-β_1 IN HUMAN FIBROBLASTS, Ylva Paulsson, Christina Karlsson, Carl-Henrik Heldin and Bengt Westermark, Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden.
We have previously found that transforming growth factor-β_1 (TGF-β_1) inhibits the mitogenic activity of platelet-derived growth factor (PDGF) in human fibroblasts in a density dependent fashion; an inhibitory effect is only seen in confluent or dense cultures. In that study, we used a PDGF preparation from human platelets, that contains two of the PDGF isoforms (PDGF-AB and PDGF-BB). These isoforms are known to interact with both types of PDGF receptors in human fibroblasts (α- and β-receptor). In the present investigation we used PDGF-AA that is specific for the α-receptor. We found that the inhibitory effect of TGF-β_1 on PDGF-AA induced mitogenesis also is density-dependent. No inhibition was seen in sparse cultures, whereas, in confluent cultures, DNA synthesis was completely abolished by the addition of TGF-β_1. We also found that the binding of ^{125}I-PDGF-AA was significantly reduced in confluent, but not in sparse cultures. We therefore conclude that the growth inhibitory effect of TGF-β_1 on PDGF-AA induced mitogenesis is density-dependent and that this effect is partly caused by a downregulation of PDGFα-receptors.

I 325 TGFβ1 BLOCKS *C-MYC* TRANSCRIPTION IN KERATINOCYTES: A MECHANISM OF INHIBITION OF EPITHELIAL CELL PROLIFERATION, J.A. Pietenpol, R.M. Lyons, J.T. Holt, R.W. Stein, and H.L. Moses. Departments of Cell Biology and Molecular Physiology, Vanderbilt University School of Medicine, Nashville, TN 37232. Transforming growth factor β1 (TGFβ1) and other closely-related molecules (TGFβ2 and TGFβ3) are highly potent growth inhibitors for most cell types. Previously, we have shown that TGFβ1 inhibits the growth of rapidly-growing and EGF-restimulated quiescent mouse skin keratinocytes (BALB/MK) and other cell types. TGFβ1 does not appear to interfere with most of the early signal transduction events induced by EGF. However, TGFβ1 does rapidly reduce c-*myc* expression; protein synthesis is required for this reduction of c-*myc* expression in BALB/MK cells. Evidence obtained from c-*myc* antisense oligonucleotide experiments indicates that *myc* expression is necessary for proliferation of the BALB/MK cells. In addition, it was determined that the c-*myc* gene is expressed throughout G1 and that TGFβ1 addition at any point during G1 is able to both reduce c-*myc* expression and inhibit cell entry into S phase. TGFβ1 reduction of c-*myc* expression was shown to occur at the level of transcriptional initiation. Studies with a series of 5'-deletion c-*myc*/CAT constructs indicated that a cis-regulatory element(s), which resides between -100 to +71 relative to P1 transcription start site, is responsible for TGFβ1 responsiveness. Based on this data, it is postulated that the mechanism of TGFβ1 inhibition of keratinocyte proliferation involves stimulation of synthesis of a protein that may interact with a specific element(s) in the 5'-regulatory region of the c-*myc* gene causing inhibition of transcriptional initiation.

I 326 ACTIONS OF TGFβ IN EPIDERMAL HYPERPROLIFERATION AND TUMORIGENESIS Ruben Schnapke, Gerhard Fürstenberger, Peter Krieg[1] and Friedrich Marks, Departments of Biochemistry and Virology[1], German Cancer Research Center, 6900 Heidelberg, FRG.
TGFβ has been shown to be a negative growth regulator for epithelial cells in vivo and in vitro including epidermal cells. In contrast, some carcinoma cell lines failed to respond to the inhibitory effects of TGFβ indicating that this lack of reponse may lead to uncontrolled growth (1). An appropriate model system to study effects of TGFβ on both hyperplastic (transient) and neoplastic (permanent) development is the multistage approach of mouse skin carcinogenesis using DMBA as an initiator and phorbol esters as tumor promoters. Actually, TPA has been found to induce the expression of TGFβ mRNA in mouse skin in vivo (2). To this end, we have investigated the expression of TGFβ mRNA in different hyperproliferative states of epidermis and in cells derived from hyperproliferative lesions. Irritant and hyperplasiogenic stimuli induce the transient overexpression of TGFβ mRNA, whereas non-irritant and non-hyperplasiogenic mitogens are inactive in this respect. An overexpression of TGFβ mRNA could not be observed in papillomas and carcinomas. Furthermore, effects of TGFβ on normal and hyperproliferative epidermis in vivo and in vitro were investigated. While in vivo no distinct effect of TGFβ on epidermal cell proliferation was observed, the factor exhibited a dose-dependent inhibition of growth in cell cultures derived from all hyperproliferative lesions. This inhibition was shown to be reversible in accordance with published data.
1. Shipley, G.B., Pittelkow, M.R., Willie, J.J., Scott, R.E., Moses, H.L. (1986) Cenacer Res. 46:2068.
2. Akhurst, R.J., Fee, F., Balmain, A. (1988) Nature 331:363.

I 327 POSSIBLE INVOLVEMENT OF H_2O_2 IN THE GROWTH INHIBITION OF MOUSE OSTEOBLASTIC CELLS BY TGFß, Motoko Shibanuma, Toshio Kuroki and Kiyoshi Nose, Department of Cancer Cell Research, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108, Japan.
TGFß is known to stimulate or inhibit growth of cells, depending on cell types or growth state; in mouse osteoblastic cell line (MC3T3 E1) TGFß inhibited DNA synthesis when added at log phase. We examined effect of TGFß on DNA synthesis during progression through G1 phase, and possible involvement of H_2O_2 in TGFß-mediated growth regulation. Cells in quiescent state were stimulated by serum to progress through cell cycle, and DNA synthesis was determined by ^{3}H-Thd incorporation during 24-36 hr after stimulation. We found that DNA synthesis was significantly inhibited when TGFß was added at the late G1 phase, but not at the early G1 phase. Catalase was found to decrease the inhibitory effect of TGFß on DNA synthesis. Like TGFß, exogenously added H_2O_2 showed the differential effect on DNA synthesis depending on stage of progression through G1 phase. Indeed, TGFß induced production of H_2O_2 from cells in late G1 phase, but did not from cells in early G1 phase. TGFß and H_2O_2 both increased phosphorylation of the protein with molecular weight of 78KDa. These results indicate that inhibitory effect of TGFß on DNA synthesis is at least partly due to the production of H_2O_2.

I 328 EFFECTS OF TGF-β AND EGF ON GROWTH AND DIFFERENTIATION OF HUMAN MAMMARY EPITHELIAL CELLS IN CULTURE, Martha Stampfer, Paul Yaswen, Myriam Alhadeff, and Junko Hosoda, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.
Normal human mammary epithelial cells (HMEC) show long-term active growth (~45-80 population doublings) in the serum-free medium MCDB 170. We have characterized this cell population for its relationship to cell lineages *in vivo*, its differentiation potential, and its behavior after transformation *in vitro*. Based on the pattern of keratin and milk fat globule expression, the cell population exhibiting long-term growth in MCDB 170 has properties consistent with that of a stem cell population derived from the basal cell layer *in vivo*. Growth of these cells is acutely dependent upon EGF/TGF-α; addition of antibodies to the EGF-R rapidly, and reversibly, leads to cessation of growth. Growth inhibition is also induced by exposure to TGF-β. Normal cultured HMEC express mRNA and protein for several extracellular matrix (ECM) associated products (fibronectin, collagen IV, laminin, plasminogen activator inhibitor-1), and the intermediate filaments keratins 5/14, 8/18, and vimentin. We have also identified a gene product, NB-1, expressed in the normal HMEC but downregulated in transformed HMEC. TGF-β markedly increases the synthesis of the ECM associated products, as well as stimulates the mRNA levels for vimentin and NB-1. The levels of some of these gene products can also be modulated by placement of the HMEC on reconstituted basement membrane material. In general, TGF-β appears to reinforce migratory or wound healing characteristics while the basement membrane substrate appears to induce characteristics of a developmentally more mature phenotype. The response to different growth factors and substrate material has been compared in normal HMEC vs. HMEC cell lines derived from normal cells transformed to immortality after *in vitro* exposure to benzo(a)pyrene, and further transformed to malignancy using oncogenes/oncongenic viruses. Numerous differences in growth factor responses and gene expression suggest that the transformed cells may vary from the normal HMEC in their differentiated state and/or their cell-cell and cell-matrix interactions.

I 329 MULTIPLE LESIONS LEAD TO THE DISRUPTION OF THE TGF-β REGULATORY SYSTEM IN TRANSFORMED RAT TRACHEAL EPITHELIAL CELLS. R. Steigerwalt, J. Rundhaug, T. Gray, P. Nettesheim, Laboratory of Pulmonary Pathology, NIEHS, Research Triangle Park, NC 27709
Positive and negative growth factors play an important role in regulating the proliferation of normal cells by autocrine, paracrine or endocrine mechanisms. Aberrations in the regulatory systems have been found in transformed cells and may be responsible for the deregulated growth exhibited by these cells. Transforming Growth Factor Beta (TGF-β) inhibits the proliferation of normal primary rat tracheal epithelial (RTE) cells. We and others have found that the sensitivity to TGF-β is often lost or diminished in transformed RTE cells. In the present study, we have compared the TGF-β responsiveness, RNA expression, secretion and activation of several transformed cell lines derived from primary RTE cells. All cell lines expressed TGF-β RNA and secreted significant amounts of latent TGF-β into the medium. Two preneoplastic cell lines were as TGF-β sensitive as primary cells, secreted latent TGF-β into the medium, but failed to activate TGF-β in measurable quantities. A neoplastic cell line which secreted TGF-β was unresponsive to TGF-β, yet possessed TGF-β receptors suggesting that the lack of responsiveness was due to post receptor alterations. We conclude that transformed RTE cells can differ in their responsiveness to TGF-β and that multiple lesions can occur which lead to disruption of the TGF-β regulatory systems.

I 330 PHARMACOKINETICS OF THE TGFß-1 LATENT COMPLEX, Thomas S. Winokur, Lalage M. Wakefield, Anita Roberts and Michael B. Sporn, Laboratory of Chemoprevention, NCI, Bethesda, Md.
TGFß-1 is produced and secreted as a latent complex consisting of the latency associated protein(LAP) and the mature TGFß-1 molecule. The biological properties of the complex are poorly understood. We have purified the LAP from two different expression systems and reconstituted the latent complex with iodinated TGFß-1. The resulting complexes were injected into the femoral vein of rats and a time course of disappearance was generated by collecting blood from the contralateral iliac artery. Mature TGFß-1 is cleared with a half life of approximately two minutes. U-LAP, an undersilylated complex, was cleared with a half life of one and a half minutes. S-LAP a fully silylated complex was cleared with a half life of approximately 90 minutes. In addition the volume of distribution and the organ distribution were different for the various forms. These characteristics will be important in the use of TGFß as a pharmacologic agent.

I 331 TGF-β REDUCES c-myc EXPRESSION AND COOPERATES WITH TYPE I MOUSE IFN IN M1 MOUSE MYELOID CELLS. Yarden A., Tiefenbrun N., Berissi H. & Kimchi A. Department of Virology, The Weizmann Institute of Science, Rehovot, Israel. The suppression of the *c-myc* nuclear oncogene is associated with growth arrest and may therefore be directly controlled by naturally occuring growth inhibitors. The effect of transforming-growth-factor-β(TGF-β) and of type I mouse interferon (IFN) on *c-myc* expression was investigated in M1 mouse myeloid cells, which responded to these cytokines by a specific arrest in the G0/G1 phase of the cell cycle. Northern blot analysis indicated that the steady-state levels of *c-myc* mRNA were reduced as early as 5 hours following exposure to TGF-β, while the IFN effect was maximal after 24 hours exposure to the cytokine. Single stranded DNA probes corresponding to the *c-myc* first exon were used for nuclear run-on transcription analysis which showed marked reduction in the transcription rate of the *c-myc* gene following exposure to IFN. Thus, a specific inhibition of the *c-myc* transcription initiation occurs following exposure to IFN. TGF-β treatment did not show any preferential inhibition of the *c-myc* gene transcription. Therefore, TGF-β acts to reduce the *c-myc* mRNA steady-state levels at a post-transcriptional mechanism. Adding the two cytokines together at saturating levels resulted in enhanced inhibition of the *c-myc* mRNA steady-state levels as well as caused a greater accumulation of cells in the G0/G1 phase of the cell cycle than adding each cytokine alone. Inhibition of *c-myc* by the combined amendment of the two cytokines could be the cause of the enhanced increase in the percentage of cells that accumulated in the G0/G1 resting phase of the cell cycle. Our observation that each of the cytokines operates to repress the *c-myc* gene through different intracellular mechanisms may explain our findings that the combination of the two cytokines is more effective in reducing *c-myc* mRNA. Nevertheless, another level of regulation may participate in the cooperativity process.

I 332 SEX SPECIFIC PROTEINS BIND PROMOTER DNA OF THE MULLERIAN INHIBITING SUBSTANCE GENE, Christopher Haqq, Richard Cate*, Patricia Donahoe, Ped. Surg. Res. Lab., Mass. General Hospital, Boston, MA 02114, *Biogen Res. Corp., Cambridge, MA 02142. Mullerian Inhibiting Substance (MIS) is a testicular glycoprotein, related to the TGF-beta family of genes, that causes regression of Mullerian ducts in embryonic males. We have identified putative regulatory sequences in the MIS promoter conserved between the promoter DNA sequences of human, bovine, and newly isolated rat genomic clones. We have demonstrated by electrophoretic bandshift that several DNA motifs specfically bind proteins from testicular nuclear extracts prepared at the same time in development that the MIS gene is transcribed. Two motifs, the M2A and palindromic M2B, exhibit particularly strong binding to a complex of proteins whose ontogeny parallels that of MIS gene expression: M2 proteins are present at highest level in 21 day fetal and neonatal rat testes, with intermediate levels in 13 day postnatal testes, and very low levels in adult testes. In addition to testicular expression, MIS is transcribed by a population of ovarian granulosa cells in females. In ovarian nuclear extracts prepared 3 days postpartum, the M2 region binds a complex of proteins in electrophoretic bandshift assays. We have demonstrated by UV-crosslinking, followed by reducing SDS-PAGE, that one of these proteins is identical in molecular weight to the male M2 binding factor, and that there are two additional DNA-binding factors specific to females. These same complexes are evident using nuclear extract prepared from tumor of a human patient with a MIS producing granulosa cell malignancy. A final indication of cross-species conservation is that the male M2 complex is evident in nuclear extracts from 3 week old bovine testes. We hope to correlate deletion of the M2 region from the MIS promoter with effects on MIS transcription in future studies using transgenic animals.

Growth Inhibitory Peptides

I 400 GROWTH INHIBITION OF PROMONOCYTIC TUMOR CELL LINES BY A NOVEL 7kD T CELL INHIBITORY MONOKINE (TCIM). Monique A. Berman, Christy I. Sandborg, Frank Zaldivar Jr., and Karen L. Imfeld. Department of Medicine, California College of Medicine, University of California, Irvine, CA 92717.

Activated monocytes/macrophages (Mϕ) secrete several growth and immunoregulatory factors. We have purified a novel Mϕ derived protein from culture supernatants of endotoxin and latex bead activated human Mϕ. This factor was purified based on its inhibitory activity on interleukin 1 plus PHA stimulated murine thymocyte proliferation. It differs from other IL-1 inhibitors and known cytokines based on its biochemical characteristics and functional spectrum. TCIM does not inhibit mitogen stimulated T and B lymphocyte proliferation, but does inhibit specific antigen stimulated secondary responses. The growth of B lymphocytes, hybridoma cell lines, a cytotoxic T cell line (CTLL), fibroblasts, epithelial cell lines, and an epithelial tumor cell line is not inhibited. In contrast, proliferation of the promonocytic tumor cell line U937 is strongly inhibited. This factor is not produced by human promonocytic cell lines (THP-1, HL-60, U937), but normal human and murine adherent Mu produce this factor when activated by endotoxin or retovirus (e.g. HIV-1). TCIM may be an endogenous factor that prevents outgrowth of certain tumors.

I 401 IDF45: A BIFUNCTIONAL PROTEIN AS CELL GROWTH INHIBITOR AND AS IGF-I BINDING PROTEIN.[+] C. Blat, [+]J. Delbé, J. Villaudy, [+]G. Chatelain, A. Goldé and [+]L. Harel. [+]IRSC 94802 Villejuif France, Institut Curie 75231 Paris France.

From medium conditioned by 3T3 cells, we had previously purified to apparent homogeneity a novel inhibitory diffusible factor of 45 Kd (IDF45), and then determined the amino terminal sequence. IDF45 prevented reversibly the growth of chick embryo fibroblast (CEF). In these cells, DNA synthesis stimulated by 1 % serum was 50 % inhibited in the presence of 45 ng|ml (1 nM) IDF45.

We show now that, in CEF, DNA synthesis stimulated by IGF-I was 100 % inhibited in the presence of purified IDF45. Furthermore, IDF45 was, after Western blotting, able to bind IGF-I. The inhibitory effect of IDF45 upon serum stimulation did not seem to be the result of its inhibitory activity upon IGF-I stimulation, since stimulation by IGF-I and serum were additive. Moreover, it was possible to dissociate the two inhibitory effects : when added to v-src transformed CEF, IDF45 was able to 100 % inhibit stimulation induced by IGF-I and was unable to significantly decrease stimulation induced by serum, as was previously observed. Taken together, our results strongly suggest that IDF45 has two distinct functions, one of which was to bind IGF-I and the other to inhibit serum stimulation. Indeed, it was impossible to separate the two functions when IDF45 was purified by cation exchange FPLC, a method very different from reverse phase FPLC previously used for purification of IDF45. On the other hand, if the IGF binding activity and inhibitory activity effect upon serum stimulation were carried by two different proteins, the presence of IGF-I (in conditions where most of the 45 Kd proteins were bound to IGF-I) should not have affected the activity of the molecule inhibitoring serum stimulation. However, we observed the contrary : when IDF45 was bound to IGF-I, it lost its inhibitory effect upon stimulation induced by serum. This suggests that the two activities occurred on the same protein and that IDF45 is a bifunctional protein.

I 402 REGULATION OF A NOVEL MAMMALIAN STRESS RESPONSE GENE, *gadd153*, BY GROWTH ARREST AND DNA DAMAGE, Sara G. Carlson, Jennifer D. Luethy, Jong S. Park, Joseph Fargnoli, Albert J. Fornace, Jr.,* and Nikki J. Holbrook, Natl. Inst. Aging, Baltimore, MD 21224, and *NCI, NIH, Bethesda, MD 20892

In most cells, DNA damage is accompanied by the transient inhibition of DNA synthesis and cell growth; such delays can have a protective effect since mutants lacking growth arrest responses are hypersensitive to certain DNA-damaging agents. We have been studying a gene, *gadd153*, whose expression in HeLa cells is highly induced by DNA damage as well as by other treatments which result in growth arrest. In order to study the regulation of *gadd153* by DNA damage and growth arrest, we have isolated a genomic fragment from -767 to +21 relative to the transcription start site and have linked it to the CAT reporter gene. Cells stably transfected with the *gadd153*-CAT constructs were examined for the expression of CAT following treatment with the DNA alkylating agent methyl methanesulfonate (MMS), as well as by treatments which induce growth arrest. These experiments have shown that *gadd153* promoter activity is enhanced more than 20-fold by treatment of cells with MMS. Further deletion analysis indicated that an MMS responsive element lies within the region of the promoter from -232 to -36. In contrast to what is seen with MMS treatment, CAT activity in the stably transfected cells was only minimally enhanced (less than 4-fold) by conditions which resulted in growth arrest. However, the expression of endogenous *gadd153* mRNA was markedly elevated in these same cells. These results indicate that different mechanisms are involved in the regulation of *gadd153* by DNA damage and growth arrest. It is possible that the major regulatory elements responsible for induction by growth arrest lie elsewhere in the gene or gene flanking regions, or alternatively, that the major regulation by growth arrest occurs posttranscriptionally. We are currently exploring these possibilities.

I 403 ARACHIDONIC ACID AS A NEGATIVE CONTROLLER OF CELL GROWTH

U.N.Das

Department of Medicine, The Nizam's Institute of Medical Sciences, Punjagutta, Hyderabad India-500482.

Tumor cells are known to be deficient in delta-6-desaturase activity, an enzyme needed to metabolise dietary cis-linoleic acid to gamma-linolenic acid and arachidonic acid. Tumor cells have low amounts of arachidonic acid (AA), a substantial decrease in the P_{450} system, and an increase in total anti-oxidant capacity compared to normal cells. Lipid peroxidation is decreased in preneoplastic foci and rapidly proliferating fetal and neoplastic cells where as mature cells readily show lipid peroxidation. Tumor cells are known to be highly susceptible to free radical-induced toxicity. In an earlier study I have shown that AA can trigger free radical generation in human neutrophils and tumor cells and that it is a calmodulin-dependent process (1). It was also observed that AA can selectively kill tumor cells in a dose dependent manner. AA-induced tumoricidal action was accompanied by an increase in free radical generation and lipid peroxidation process. AA initially showed a cytostatic action on both normal and tumor cells followed by selective tumoricidal action. The cytotoxic action of AA could be blocked by anti-oxidants. These results suggest that AA may function as an endogenous negative controller of cell growth.

Reference: Sangeetha P, Das UN, Koratkar R. Prostaglandins Leukotrienes Essential Fatty Acids, in press (1989).

I 404 NATURAL INHIBITORS OF ENDOTHELIAL CELL PROLIFERATION, Hannes Drexler and Werner Risau, MPI für Psychiatrie, 8033 Martinsried, W.- Germany.

Most of the potent angiogenic proteins of the heparin binding growth factor family are expressed during embryonic development as well as in the adult. However, angiogenesis can only be observed during development. This paradox led us to the hypothesis that some kind of inhibitor of angiogenesis might be active in the adult and also in certain regions of the developing embryo, where blood vessels regress. We have purified two inhibitors of endothelial cell proliferation from chick embryo extracts: one of low molecular weight (3000 Da) and one of higher molecular weight (65-100 kDa, as determined by gelfiltration chromatography). Minute amounts of the higher molecular weight protein so far prevented a further characterization. Therefore we screened the conditioned medium of several cell lines for the presence of this inhibitor. We found that certain cell lines produce inhibitors with a similar molecular weight. Surprisingly, the same cell lines also secreted a heparin binding protein into the medium which specifically stimulates endothelial cell proliferation. Such cell systems might represent an excellent in vitro model to study the regulation of endothelial cell proliferation and angiogenesis.

I 405 **THE CHICKEN pp59c-*myc* PROTO-ONCOGENE POSSESSES WEAK TRANSFORMING ACTIVITY THAT IS MODULATED BY ADJACENT NORMAL CELL NEIGHBORS.**
Edward J. Filardo and Eric H. Humphries, Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75235
Deregulated expression of the c-*myc* proto-oncogene has been implicated in the development of a variety of avian and mammalian tumors. The relative roles that qualitative and quantitative alterations in c-*myc* expression play in tumor development is unknown. We have constructed an avian retrovirus, EF168, that expresses the chicken pp59c-*myc* proto-oncogene. Compared with the transformation capabilities of MC29, a naturally-occurring oncogenic avian retrovirus that expresses pp110*gag*v-*myc*, EF168 transformed quail embryo fibroblasts and macrophage cultures poorly or not at all. As measured by focus formation or colony growth in soft agar, EF168 initiated transformation 100 to 1000-fold less efficiently. In contrast to MC29-induced foci, EF168 foci were smaller, diffuse and less prominent. While control experiments demonstrated that different embryos were equally sensitive to MC29-induced transformation, transformation by EF168 varied 200-fold with less or no transformation in more rapidly dividing cultures. Several experiments demonstrated EF168 expression in the absence of transformation. These observations indicate that relative to pp110*gag*v-*myc*, the transforming activity of EF168 is weak and that adjacent, normal cell neighbors are able to suppress transformation by structurally unaltered pp59c-*myc*.

I 406 A 33-kDa POLYPEPTIDE IS ASSOCIATED WITH GROWTH SUPPRESSION AND TERMINAL DIFFERENTIATION OF HUMAN FIBROBLASTS, Pal I. Francz, Hans-G. Meinrath, H. Peter Rodemann* and Klaus Bayreuther, Institut für Genetik, Universität Hohenheim, D7000 Stuttgart 70 and *Entwicklungsbiologie, Universität Bielefeld, D4800 Bielefeld 1, F.R.G.
Primary and secondary fibroblasts of chicken, mouse, rat, and man differentiate along a unidirectional multistage sequence in three compartments of the fibroblast stem cell system. The mitotic fibroblasts MF I - MF II - MF III develop in the progenitor compartment, the postmitotic fibroblasts PMF IV - PMF V - PMF VI differentiate in the maturing compartment, and the postmitotic and degenerating fibroblast PMF VII progresses in the degenerating compartment. Differentiation-stage-specific marker polypeptides have been demonstrated by two-dimensional gel electrophoresis of membrane bound, cytoplasmic and nuclear, and secreted proteins. In the human skin fibroblast cell line HH-8 24 cell-type-specific marker proteins have been found in the membrane-bound protein fraction, also 14 cell-type-specific marker proteins in the cytoplasmic and nuclear fraction, and 11 cell-type-specific marker proteins in the secreted protein fraction. A 33-kDa polypeptide (named PIVa) is associated with the shift of the mitotic fibroblast MF III to the postmitotic fibroblast PMF IV. This polypeptide also appears to be a regulatory protein for the maintenance of the postmitotic differentiation stages. As demonstrated by pulse-chase experiments, the PIVa polypeptide has a half-life of 1.5 hr. This polypeptide is expressed in 35 additional human skin and lung fibroblast cell lines (e.g., WI38, CRL1221, GM38, GM1717). Work is in progress on the isolation and characterization of the PIVa polypeptide, and the study of the regulatory role of this polypeptide in the growth control of fibroblasts in vitro and in vivo.

I 407 **CHARACTERIZATION OF A NECROSIS-ASSOCIATED GROWTH INHIBITOR ISOLATED FROM TUMOR SPHEROIDS**, James P. Freyer and Patricia L. Schor, Cellular and Molecular Biology Group, Mail Stop M888, Los Alamos National Laboratory, Los Alamos, NM 87545.

We have isolated a novel growth inhibitor from multicellular tumor spheroids, an *in vitro* culture system used to model the regulation of tumor cell proliferation *in vivo*. Our initial characterization of this inhibitor has shown it to be: 1) 80-90 kD; 2) very heat labile; 3) sensitive to trypsin; 4) cytostatic in all cell cycle phases; 5) active on a variety of cell types; and 6) produced by two distinct cell types (1,2). Interestingly, inhibitory activity was only obtained from spheroids with an extensive necrotic center, not from monolayer cultures or from spheroids without necrosis. This link between growth inhibition and necrosis explains the mechanism of growth saturation in spheroid cultures (1), and may have important implications for the development and regrowth of quiescent cells in tumors. A similar inhibitory activity has recently been isolated from tumors with extensive necrosis. We will report on experiments designed to further characterize this growth inhibitor, and on attempts to establish the mechanism behind the link between necrosis and production/release of the inhibitor.

This work was supported by NCI grants CA-36535 and CA 42314 and by the US DOE.

1. Freyer JP, *Cancer Res.* 48: 2432-2439, 1988.
2. Freyer JP, Schor PL, Saponara AG, *Biochem. Biophys. Res. Commun.* 152: 463-468, 1988.

I 408 TNF α, LIF/HILDA AND TGFß GENES ARE CONSTITUTIVELY EXPRESSED IN HUMAN LEUKEMIC CELLS. J. Gabert, C. Velley, F. Kerangueven, N. Maroc, M. Bellâis, P. Bertault-Perez, F. Birg and P. Mannoni.
NSERM U. 119 and CANCER CENTER- 232 BD Ste MARGUERITE 13009 MARSEILLE FRANCE
Leukemic cells are characterized by the fact that they escape normal cell growth controls leading to the differentiation of normal hemopoietic cells. Some growth inhibitors such as TNF α, LIF/HILDA and TGFβ have been described in some models as inhibitors of normal and leukemic cell proliferation. Therefore expression of the genes coding for these cytokines was studied in human leukemic cells. We analysed mRNA expression in leukemic cells isolated from patients with Acute Lymphoid Leukemias or Acute Myeloid Leukemias. TNFα was expressed in about 70% of the samples whatever their lineage origin. LIF/HILDA mRNA had a restricted expression patern, present mainly in the undifferentiated myeloid leukemias (M1). TGF ß was found expressed, sometimes at very high levels, in all specimens. The expression of these growth inhibitors at the protein level is under investigation. We will discuss the significance of these unexpected results in terms of growth regulation and leukemogenesis.

I 409 RETINOIC ACID INDUCES THE ARREST OF THE CELL GROWTH IN G1 PHASE REGULATING CELL CYCLE CONTROL GENES IN HUMAN NEUROBLASTOMAS (NB) Carlo Gaetano, Kazue Matzumoto and Carol J. Thiele. Molecular Genetics Section, Pediatric Branch, NIH-NCI.
NB is a tumor, like retinoblastoma, in which loss of genetic material may be important in the development of malignancy. The observation that RA treatment of some NB cell lines can arrest cell growth and induce differentiation suggests the genes necessary to control these processes are not lost but that their regulation is altered. To identify the specific genes which regulate these processes in NB cells, we have assessed the ability of RA to control cell growth in NB cell lines; KCNR (NMYC amplified), AS (1 copy NMYC) and 2 subclones of SKNSH (1-copy NMYC), SHEP and SY5Y. Previously, we have shown that RA arrests KCNR cells in the G_1 phase of the cell cycle, causes a transcriptionally regulated decrease in NMYC mRNA and induces differentiation. 5uM RA induces a 90% inhibition in ^{3}H-Thym. uptake in KCNR and SY5Y yet only a 50% inhibition in AS and SHEP. Since withdrawal from cell-cycle is a fundamental event for induction of terminal differentiation and the cyclin gene may be an important component of this process, we analyzed cyclin mRNA levels in NB cells treated for 60 hours with RA. Cyclin mRNA levels are decreased in the growth arrested KCNR and SY5Y cell lines but not in the growth inhibited cell lines AS and SHEP. Associated with the decrease in cyclin levels, the expression of a differentiation-associated gene, GAP43, increases KCNR and SY5Y but not in AS and SHEP. Cyclin mRNA decreases in RA-growth arrested and differentiated NB cells which suggests that the inability of RA to arrest the growth of AS and SHEP cells may be due to regulation of genes proximal to the control of cyclin gene expression. Further studies are in progress to identify these genes.

I 410 PROTEINS WHICH MAY CONTRIBUTE TO MAINTAINING THE ENDOTHELIUM IN AN UNACTIVED STATE. Susan E. Goelz and Brian Lu, Department of Cell Biology, Biogen, Inc., 14 Cambridge Center, Cambridge, MA 02142.
The adhesion of leukocytes to the endothelium is an early event in the inflammatory response. In a resting system, leukocytes do not adhere to the endothelium. Endothelial cells can become activated by various agents including cytokines such as IL-1 and TNF, resulting in both the upregulation and de novo expression of a number of cell surface proteins (including ICAM-1, ELAM-1 and VCAM-1) which can contribute to leukocyte binding. There is some indirect evidence indicating that there might be some molecules on the endothelium which inhibit leukocyte binding. In an attempt to identify candidates for these homeostatic proteins, we ran 2-D gels of membrane proteins from resting and cytokine treated human endothelial cells. We have identified a number of proteins, that appear to be down regulated at various times after cytokine treatment. We are raising antibodies to some of these proteins and have developed a sytem in which these antibodies can be tested.

I 411 CELLULAR GROWTH AND ENCYSTATION OF AXENIC ENTAMOEBA HISTOLYTICA AMOEBAE: A MODEL OF DIFFERENTIATION, Syed A. Imam, Microbiology Dn., Central Drug Res. Inst., Lucknow, India. Entamoeba histolytica grows in axenic medium at 37°C[1], but addition of RNA, DNA, or NAD DPN to the medium and doubling of serum content increases the growth of amoebae to yield 10 million amoebae/10ml.medium[2]. This can be thought of as uncontrolled growth rate under the influence of RNA, DNA, NAD simulating cancerous growth in some aspects. At temperatures below 33°C, at 28°C, 24°C, 18°C the factors causing growth mentioned above encyst Entamoeba histolytica in a medium comprising of RNA/DNA/NAD without cysteine and serum[3]. This could mean that differentiation is affected by the same agents which cause faster growth at higher temperature. In terms of concept of supporting pressure[4] it would mean changes in viscosity and surface tension make up differentiation and dedifferentiation. Hence changes in viscosity and surface tension may act as negative factor for an uncontrolled growth rate in cancerous tissues.

1. Diamond, L.S., J. Parasitol., 1968, 54, 1047.
2. Imam, S.A., Curr. Sci. 1986, 55, 1129.
3. Imam, S.A., Curr. Sci. 1988, 57, 496.
4. Imam, S.A., Jour. Cell. Biochem. 1986 supplement.

I 412 **INHIBITION OF PHOSPHORYLATION OF THE c-erbB-2/HER2 GENE PRODUCT BY A MONOCLONAL ANTIBODY: IMPLICATION IN GROWTH REGULATION.**
Rakesh Kumar[1], Mark J. van de Vijver[2], H. Michael Shepard[3], and John Mendelsohn[1], [1]Memorial Sloan-Kettering Cancer Center, New York, NY 10021, [2]Netherlands Cancer Institute, Amsterdam, The Netherlands, and [3]Genentech Inc., South San Francisco, CA 94080.

c-erbB2/HER2, the human homolog of the rat protooncogene neu, encodes a 185 kDa cell surface glycoprotein with intrinsic tyrosine kinase activity, which is the putative receptor for an unidentified ligand. Overexpression of the c-erbB-2 gene product has been shown to cause transformation of mouse fibroblasts, and has also been associated with poor prognosis in human malignancies.

Monoclonal antibody (mAb) 4D5 against the extracellular domain of $p185^{HER2}$ was used to analyze the phosphorylation of this c-erbB-2 gene product in SK-BR-3 human mammary carcinoma cells. It has been shown that this mAb can partially block proliferation of these cells. The observed steady state level of $p185^{HER2}$ phosphorylation was reduced 50% when SK-BR-3 cells were cultured overnight in the presence of 5µg/ml 4D5. A comparable reduction of phosphorylation was observed when cells were grown in serum free culture. This suggests that a mechanism of mAb-induced reduction of phosphorylation may involve inhibition of binding of a serum growth factor to the $p185^{HER2}$.

I 413 SERUM ALBUMIN AS AN INHIBITOR OF CELL PROLIFERATION OF THE HUMAN BREAST CANCER CELL LINE, MCF-7. Inga Laursen, Per Briand and Anne E. Lykkesfeldt. Department of Tumor Endocrinology, The Fibiger Institute, The Danish Cancer Society, Copenhagen, Denmark.

The estrogen receptor positive human breast cancer cell lines MCF-7 and T47D can be significantly growth inhibited by high concentrations of serum. This growth inhibition can be reversed by simultaneous addition of estradiol. We have identified the inhibitory factor in newborn calf serum as serum albumin. Several proteins in serum associate with serum albumin, and experiments have been conducted to elucidate whether serum albumin itself, or a contaminating protein in the serum albumin preparation is responsible for the growth inhibition. A candidate for an albumin associated protein with growth inhibitory activity is TFG-β; however, the results of our experiments exclude that TFG-β can be responsible for the growth inhibition. We have found no indications that serum factors besides albumin are responsible for the growth inhibition. Albumin influences the pattern of secreted proteins from MCF-7 cells, and we suggest that albumin may affect the cell proliferation by modulating the activities of autocrine growth regulatory factors.

I 414 DETECTION OF ACTIVATED RAS p21 IN MURINE TUMORS AND PLASMA USING A PANEL OF MONOCLONAL ANTIBODIES SPECIFIC TO THE RAS MUTATIONS

Joyce A. LaVecchio, Peter J. Hamer, Jean M. McKenna and Walter P. Carney,
Medical Products Dept., DuPont, N. Billerica, MA 01862

Activated ras proteins have been detected in a variety of human cancers and their preneoplastic lesions. 50-60% of the mutations found in colorectal cancer and leukemia are either arginine, aspartic acid or valine at position 12 or aspartic acid at position 13. We have developed a panel of monoclonal antibodies specific to these substitutions of the ras p21 protein.

The monoclonal antibodies were raised against synthetic peptides varying from 12-15 amino acids in length. The initial screening process of the monoclonal antibodies began with selectivity to the appropriate peptide. Once peptide specificity was determined the monoclonal antibodies were evaluated for capability of detecting the activated ras p21 protein in mouse cell and tumor lysates by Western blot, capture ELISA, and immunoprecipitation. These monoclonal antibodies have been successful in detecting the activated ras p21 protein in both tumors and plasma of tumor bearing nude mice.

Since these mutations are seen in over 50% of colorectal cancer and leukemias and their preneoplastic lesions, detecting these mutations may play a significant role in determining the prognosis of the cancer and following the progression of the disease. We feel that these monoclonal antibodies will be a valuable research and clinical tool in the study of cancer.

I 415 DIFFERENTIAL GENE EXPRESSION DURING HEPATOCYTE PROLIFERATION, Thomas M. Laz, Kenneth L. Mohn, Anna E. Melby, Jui-Chou Hsu, Hung Q. Nguyen and Rececca Taub, Department of Human Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

We are interested in investigating the regulation of growth control. Hepatocytes were chosen as a representative epithelial cell type, in which a large number of human cancers occur. In response to cell loss, liver cells will proliferate until the original liver mass is restored. Two systems are being used to study this tightly regulated growth pattern. Regenerating rat liver is the *in vivo* model, with 70% partial hepatectomy as the stimulus for cell division. The *in vitro* model uses serum-starved Rueber H35 hepatoma cells which respond strongly to insulin as a growth factor. cDNA libraries have been constructed from regenerating liver and H35 cells three hours post mitogenic stimulation in the presence of cycloheximide. Through a combination of differential screening and subtracted cDNA probes, approximately 55,000 recombinant phage from the induced cDNA library have been screened. So far, 310 clones showing induced expression have been identified. After screening for known genes and ruling out duplicate clones, 60 of the induced genes may be novel. Differential screening of a normal rat liver cDNA library to find genes turned off during regeneration resulted in 40 clones not expressed during regeneration, out of 20,000 phage screened. We are continuing to screen these libraries and analyzing specific induced genes in an attempt to characterize the proliferative response and identify liver specific genes involved in its growth regulation.

I 416 REGULATION OF CELL PROLIFERATION AND GROWTH ARREST BY AN AUTOCRINE SOLUBLE VERTEBRATE LECTIN, Livio Mallucci* and Valerie Wells, *Laboratory of Cellular and Molecular Biology, Department of Microbiology, UMDS, Guy's Campus, London Bridge. SE1 9RT UK

We have cloned and expressed in recombinant form a cell growth regulatory molecule which can cause growth arrest at nanogram concentrations. The factor, which belongs to a class of proteins classified as soluble vertebrate lectins, plays a part in the autocrine system of cell growth control as a negative regulator. Both as a regulatory molecule and as a growth arrest factor it controls exit of cells from G0 and traverse from S phase through G2 with a mode of action attributable to that of a cytokine rather than that of a lectin.

I 417 Molecular cloning and characterization of the promoter of the transforming growth factor beta-2 gene. Takafumi Noma, Andrew Geiser, Adam B. Glick, Jeanne E. Miller, and Michael B. Sporn. Laboratory of Chemoprevention, National Cancer Institute, Bethesda, MD, 20892. TGF-β2 is the second member of the TGF-β family. In order to study transcriptional regulation of this gene, the 5' flanking region of the TGF-β gene was isolated from a human genomic library using the 5' region of the monkey TGF-β2 cDNA as a probe. 5.6kb of this upstream region was sequenced. By S1 protection analysis, at least three major transcription start sites were identified. Recombinant constructs between serial deletions of the TGF-β2 promoter and the chloramphenicol acetyl transferase (CAT) coding sequence were generated. Promoter activity was measured following transfection into BSC-1 cells.

I 418 MOLECULAR GENETIC ANALYSIS OF PROHIBITIN, AN ANTIPROLIFERATIVE ACTIVITY IN RAT LIVER, Mark J. Nuell[1], David A. Stewart[1], Varda Friedman[1], Carla M. Wood[1], David B. Danner[1], Robert Dell'Orco[2] and J. Keith McClung[2], Laboratory of Molecular Genetics, National Institute on Aging, Baltimore, MD 21224 (1) and Department of Biochemistry, The Noble Foundation, Ardmore, OK 73401 (2). We have isolated a cDNA clone corresponding to an mRNA preferentially expressed in non-regenerating rat liver. Microinjection into serum-stimulated fibroblasts of a synthetic mRNA transcribed from this cDNA results in a 75% decrease in the number of nuclei incorporating tritiated thymidine, compared to uninjected controls. Microinjection of an antisense oligonucleotide results in a 25% increase. Two mRNAs, 1.2 kb and 2 kb in size, are transcribed from the gene in eight of eight adult organs examined. An open reading frame predicts a protein of approximately 30,000 molecular weight, and this is confirmed by in vitro translation. Hybridization studies reveal that the gene is highly conserved in evolution and expressed in a cell-cycle dependent manner, lowest in S phase.

I 419 PARTIAL PURIFICATION AND CHARACTERIZATION OF A NOVEL PROSTAGLANDIN E2 INDUCED MYELOPOIETIC INHIBITORY ACTIVITY. Louis M. Pelus, Ester Levi and Joachin Buck, Sloan Kettering Institute, 430 E 67 St., N.Y., N.Y 10021.
Injection of PGE2 in B6D2F1 mice induces GMA1.2+, MAC1+, F4/80+ bone marrow (BM) and splenic monocytic cells capable of suppressing normal mouse granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) progenitor cells. This inhibition is mediated by a soluble activity. The PGE2 induced inhibitory cytokine has been purified from serum-free BM cell conditioned medium from PGE2 injected mice by sequential ammonium sulfate precipitation, ion exchange chromatograpy, gel filtration and reverse phase HPLC. Biological activity was determined by its ability to inhibit CFU-GM proliferation in 5-6 day cultures of 75,000 normal mouse BM cells stimulated by 10% v/v pokeweed mitogen spleen conditioned medium (PWMSCM). Fifty Units of activity was assigned to the ID50, calculated by linear regression analysis of titration curves, and multiplied by the reciprocal of the calculated ID50 dilution and total sample volume to obtain total fraction activity. Biological activity of HPLC material ($1.1x10^8$ U/mg protein) was associated with Mr 6,000 and Mr 30,000 activities eluted from preparative acrylamide gels. The inhibitor may exist as a lipid protein conjugate with the active moiety residing in the lipid soluble fraction. Delipidation of active material without protein denaturization results in loss of activity of the protein fraction. Reconstitution of natural protein or albumin with the lipid soluble fraction restores full biological activity. The generation and action of this cytokine may explain the long lasting myelopoietic inhibitory activity of PGE2 despite its short in vivo half-life, and may define a novel regulatory lipoprotein modulating the differentiation of myeloid and erythroid progenitor cells.

I 420 REDUCTION OF c-*myc* EXPRESSION BY INTERFERON AND INTERLEUKIN-6 MEDIATES THE G0/G1 ARREST BUT NOT OTHER GROWTH RESPONSES AND DIFFERENTIATION IN M1 MYELOID CELL LINE, Dalia Resnitzky and Adi Kimchi, Department of Molecular Genetics and Virology, The Weizmann Institute of Science, Rehovot 76100 - Israel.
Growth inhibition by type I interferon, in mouse myeloblastic cell line M1, is characterized by G0/G1 arrest of cells and is preceded by selective reduction of c-*myc* oncogene expression. In order to investigate whether one or more of the growth suppressive effects of interferon are mediated by c-*myc* reduction, the M1 cells were transfected with SV40 driven *myc* plasmid whose mRNA and protein expression is resistant to inhibition by interferon. All *myc* transfected clones have lost the ability to be arrested by interferon in the G0/G1 resting phase of the cell cycle, but were still growth arrested in a non cell cycle specific manner. While interferon functions solely as growth inhibitor, another cytokine, interleukin-6, induces differentiation of M1 cells towards resting non dividing monocytes. Unlike the parental cells that turn off the c-*myc* protein in response to interleukin-6 the *myc* transfected clones displayed constitutive expression of c-*myc* which led to a transient expansion in the limited growth capacity of the cells in the presence of the differentiation inducer and to a complete loss of the G0/G1 arrest. However the transfected cells did develop the full differentiated phenotype. Taken together, it is suggested that the precise role of c-*myc* reduction in responses to interferon and interleukin-6 is in controlling entry into the resting phase of the cell cycle. The latter is not necessarily linked to the development of various differentiation parameters in the monocyte cell lineage.

I 421 PHORBOL ESTER TRANSIENTLY STIMULATES DNA TOPOISOMERASE I IN ARRESTED 3T3 CELLS Erasmus Schneider and Leroy F. Liu, Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205
In order to study the influence of cell stimulation on DNA topoisomerase expression, log phase or confluent Balb/c-3T3 cells were serum starved for three days. After three days the cells were stimulated with either 10% serum or 100 nM TPA and total cell lysates were prepared at different times after stimulation and the amounts of topoisomerase I and II analyzed by western blotting. Treatment of confluent cells with serum or TPA produced a rapid transient stimulation of topoisomerase I levels to a maximum of approx. twice basal levels within four hours, followed by a decrease back to basal levels after ten hours. This transient stimulation was more pronounced with TPA. A second, broader peak was observed after 24 hours. On the other hand, the same treatment in cells with a log phase cell density had no effect on topoisomerase I levels. In contrast, no topoisomerase II was detected in arrested starved cells and stimulation only occurred after 16 -24 hours, in parallel with DNA synthesis. Thus it appears that, while topoisomerase II is regulated concommittant with DNA synthesis, regulation of topoisomerase I involves the protein kinase C mediated signal transduction pathway.

I 422 STABLE REVERSION OF RAS-MEDIATED TRANSFORMATION BY IFN-GAMMA, Barbara Seliger[1,2], Klaus Pfizenmaier[2] and Reinhold Schäfer[3], Ludwig Institute for Cancer Research, Stockholm Branch, Box 60202, 104 01 Stockholm, Sweden[1], Clinical Research Group of the Max-Planck-Society, University of Göttingen, D-3400 Göttingen, F.R.G.[2] and Institute of Pathology, University of Zürich, Zürich, Switzerland.
Morphologically nontransformed (flat) revertants have been isolated from NIH3T3 cells transformed by an activated human ras gene after IFN-gamma treatment in the presence of ouabain. The stable, phenotypically reverted clones had lost both their ability to grow in soft agar and their transformed morphology. However, the revertants still express unaltered steady state levels of ras mRNA, p21 protein and MHC class I antigens when compared to parental cells. IFN-gamma treatment of these cell variants did not induce MHC class I expression in contrast to the situation in the parental transformed NIH3T3 cells. This was not due to loss of specific IFN-gamma receptors, suggesting that ras expresssion dominates H-2 expression. Treatment of revertants with 5' azacytidine causes partial retransformation. Furthermore, the revertants resist the transforming activities of superinfected viruses carrying the v-Ha-ras and v-mos gene, respectively. Thus, the IFN-gamma mediated suppression of oncogene-induced transformation may be due to both hypermethylation of genes, the expression of which is required for the maintenance of the neoplastic state and due to activation of a gene which could suppress the malignant characteristics associated with neoplastic transformation.

I 423 ISOLATION AND CHARACTERIZATION OF MITOTIC INHIBITOR SECRETED BY TISSUE CULTURE CELLS, Chandrashekhar N.Shenoy, Shirin M.Marfatia and Kamlakar A.Chaubal, Biophysics Unit, Cancer Research Institute, Parel, Bombay 400012, INDIA

The growth of cells, passing through different stages of cell cycle, is a well regulated phenomenon. It depends on several factors which play important roles in controlling the overall growth of normal cells. However, no such mechanism of growth control is evident in malignant cells. Though the precise mechanism of growth regulation is not known, several substances affecting growth have been isolated, few theories have been postulated and evidence is accumulating for the definite role played by endogenous inhibitors.
It has been our observation that when the used up medium of Human Amnion cells (HA) in tissue culture was fractionated, using sephadex gel filtration, a fraction having absorption at 260 mu consistently occurred. The addition of this fraction to HA cells caused inhibition of mitosis without any toxicity and the action was reversible. However, the fraction was found ineffective on other cell types. Hence, the inhibitory action was specific.
Further investigations using microplanimetry, showed that the mitotic inhibitory fraction caused enlargement of cells. The effect of the fraction on macromolecular synthesis is being studied using autoradiography and cytophotometry. The chemical analysis of the fraction showed that it consists of protein and DNA. The molecular weight of the fraction was determined by sephadex gel filtration using molecular weight markers.

I 424 **REDUCED SECRETION OF A 45 KDa DNA SYNTHESIS INHIBITOR PROTEIN IN ONCOGENE TRANSFECTED CELLS** S.Srinivas, M.V.V.S. Vara Prasad, T.Nagashunmugam and G.Shanmugam Cancer Biology Division,School of Biological Sciences,Madurai Kamaraj University, Madurai - 625 021, India.
We have recently identified a 45 KDa DNA synthesis inhibitor protein which showed variable expression during S-phase. The secretion of this protein decreased when the cells were synthesizing DNA at peak levels. The level of this protein was monitored in mouse embryo fibroblasts across two cell cycles and a reduction in its level was observed in both S-phases. This protein was purified from the conditioned medium of cycloheximide treated quiescent cells by size exclusion HPLC and shown to have DNA synthesis inhibitory activity. The medium and matrix bound 45 KDa protein was found to be immunologically related to a chicken DNA binding protein which also was shown to be present in lower levels in S-phase cells. Treatment of rat embryo fibroblasts with FCS and growth factors resulted in the induction of synthesis of several proteins including the 45 KDa protein. Transfection of these cells with *myc* and *ras* oncogenes resulted in the specific suppression of the synthesis of the 45 KDa protein. These results suggest that the 45 KDa protein may have an important role in the control of cell proliferation.

I 425 CHANGES IN QUEUINE MODIFICATION OF tRNA AFFECTING GENE EXPRESSION AND CELL GROWTH, R.W. Trewyn, C.J. Morgan, and B.T. French, Department of Physiological Chemistry, Ohio State University, Columbus, OH 43210
In mammalian cells, the 7-deazaguanine analog queuine is exchanged enzymatically for guanine in the anticodon wobble position of tRNAs for aspartic acid, asparagine, histidine and tyrosine. Queuine in the anticodon appears to exert negative control over anchorage-independent growth (AIG) of chemically transformed hamster cells (Muralidhar et al., Cancer Res., in press); cells expressing enhanced H-ras. Mouse C3H10t½ cells transformed with activated H-ras exhibit some degree of hypomodification for queuine compared to control cells and this diminished modification can be accentuated by adding 7-methylguanine to the culture medium for as little as 24 hours. Concurrent changes in protein synthesis are observed, along with enhanced AIG. These results further implicate the dietary factor queuine as a negative regulator of AIG in cells expressing elevated H-ras.
Induction of terminal cell differentiation also offers a means for negative control of cell growth. While queuine per se has not been assigned a role in such induction with human HL-60 cells, changes in queuine modification of tRNA have. 6-Thioguanine (TG) can be incorporated into tRNA in place of queuine, and this structural alteration in the anticodon has been implicated in the differentiation of HGPRT-deficient HL-60 cells (Kretz et al., Mol. Cell. Biol. 7: 3613-3619, 1987). Rapid down-regulation of c-myc mRNA is observed in the TG treated HL-60 cells; the time course preceding growth inhibition. Although queuine does not alter the early c-myc response, it does block the TG-induced differentiation. Therefore, queuine can affect cell growth in a positive or negative fashion depending on the system employed.

1426 GROWTH INHIBITORY EFFECTS OF TGF-β1, IL-6 AND A LIVER-DERIVED GROWTH INHIBITOR ON NORMAL AND TRANSFORMED LIVER EPITHELIAL CELLS. Peter J. Wirth, Lori L. Hampton, Snorri S. Thorgeirsson and Anthony C. Huggett, Laboratory of Experimental Carcinogenesis, NCI, NIH, Bethesda MD 20892.

We have recently developed a reverse phase HPLC procedure for the purification of a liver-derived growth inhibitor (HPI/LDGI) isolated from normal adult rat liver. The growth inhibitory effects of this inhibitor together with those of the well characterized growth modulators TGF-β1 and IL-6 were compared in freshly isolated primary rat hepatocytes and in a liver epithelial (RLE) cell line. All three inhibitors produced an almost complete inhibition of EGF-mediated DNA synthesis in the primary hepatocyte cultures but had contrasting effects on the proliferation of RLE cells. HPI/LDGI produced a 75% decrease and IL-6 a 40% decrease in DNA synthesis which was reversible on removal of the growth inhibitors. In contrast TGF-β1 completely inhibited the proliferation of these cells and this inhibition was irreversible and resulted in a dramatic change in cell morphology. An analysis of the effects of TGF-β1 and HPI/LDGI on RLE protein synthesis by 2D-PAGE revealed significant differences. The effects of these inhibitors on a number of transformed RLE cell lines was investigated. While all of the lines investigated were completely resistant to the growth inhibitory effects of IL-6, the transformed cell lines showed a reduced but variable response to the effects of TGF-β1 and HPI/LDGI. Further analysis of these cell lines demonstrated that they exhibit different numbers of receptors for TGF-β1. These studies indicate that these inhibitors modulate DNA synthesis via different pathways. In addition the observed resistance of transformed liver cells to these inhibitors suggests that the synthesis of these factors during the course of hepatocarcinogenesis may confer a growth advantage to the preneoplastic and neoplastic cells resulting in a tumor promoting effect.

1427 A MATHEMATICAL MODEL OF PROLIFERATION AND AGING OF CELLS IN CULTURE: LAW OF MORTALITY AND IMMORTALITY ?, Tan Zheng, Department of Cell Biology, Institute of Zoology, Beijing 100080, P.R.China

A mathematical model is made in an attempt to explain the diference in duplicative potential between normal and transformed cells in culture based on the DNA damage hypothesis of cellular aging. It is assumed that in a cell population each cell has a total number of gene of N. DNA damage occurs randomly in time and location. The probability that a gene becomes inactivated as a result of DNA damage within an unit time interval is r during cell cycle and q when cell is ouiescent. When the number of defective gene in a cell exceeds L, the cell lose the ability to divide. When it exceeds M, the cell dies. The dividable cells proliferate at the rate of P. The model is able to account for either the limited lifespan of normal cells or the unlimited growth of transformed cells. It is predicted that the destiny of a cell population is determined by two counteracting factors: the proliferation rate of the dividable cells and the gene damage accumulation rate. The establishment of an immortal cell line requires high rate of proliferation and/or low rate of gene damage accumulation. The computer simulations in (1) population growth kinetics; (2) percentage of dividable cells; (3) variation in clonal doubling potential among cells within a bulk culture, though with small N due to technical limits, produced results in agreement in the general properties with experimental observations.