GENES AND CANCER

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The Genetics of Cancer

0052 CLINICAL ECOGENETICS OF CANCER, John J. Mulvihill, Clinical Epidemiology Branch, National Cancer Institute, Bethesda MD 20205.

Ecogenetics, the study of heritable variations in response to environmental agents, may be a useful concept in understanding carcinogenesis, to avoid considering exogenous factors to the exclusion of genetic determinants and vice versa. A paradox in the cancer problem arises because, at the level of the cell, cancer is a genetic disease, whereas, in populations, cancer has minor familial aggregation and is largely attributed to environmental influences. Ecogenetics implies the need for joint studies by epidemiologists (to deal with population characteristics), clinicians (to provide information and specimens on patients), and laboratory scientists (to dissect mechanisms within tissues, cells, and genes). Clinical geneticists recognize 200 single gene traits that have neoplasia as feature or complication; 13 of these, as well as at least one inborn cytogenetic disorder, clearly represent inborn susceptibility to environmental carcinogens. Examples include radiation sensitivity in xeroderma pigmentosum, ataxia-telangiectasia, and four other traits; viruses in the X-linked hyperproliferative syndrome; and diet in the polyposes coli, hemochromatosis, and tyrosinemia. The mapping of single gene traits in man is merging with the chromosomal abnormalities in cancers of both environmental and inherited origins. Among the leads for further studies the c-onc genes draw current attention. Other loci deserve exploration, such as nucleoside phosphorylase on chromosome 14q, neurofibromatosis possibly of 4p, and the Y chromosome in gonadal dysgenesis and the Klinefelter syndrome. Specimens from well characterized patients and families, stored at the National Cancer Institute and elsewhere, could be a source of DNA and cell lines for further studies of cancer genes.

0053 GENE MAPPING IN CANCER STUDIES, Robert S. Sparkes, Department of Medicine, University of California, Los Angeles, CA 90024

A number of recent developments have contributed to our understanding of the role of genetic factors in cancer. Among these has been the expansion of capabilities to map genes. This will be illustrated through the use of gene mapping studies in human retinoblastoma, which is a developmental eye tumor and occurs with a frequency of 1/20,000 in the first few years of life. In slightly less than half the cases, there is a positive family history following an autosomal pattern of inheritance. The remaining cases are sporadic, but among these, there is a small group which has a partial deletion of the long arm of a chromosome 13. A gene for the enzyme esterase D had been mapped previously to chromosome 13 by using interspecific somatic cell hybrids. This enzyme was evaluated in the patients with the small chromosome deletion of 13 and was found to be half normal; based upon a gene dose relationship, this indicated that the genetic locus for esterase D is located in the deleted segment. Through the study of several patients, it was found that the common region of deletion was band 13q14 and that patients having this deletion had half normal enzyme activity. These studies suggested a genetic factor located in this band related to retinoblastoma. It was possible in one patient to identify a small chromosome deletion which was too small to be seen by cytogenetic techniques, but was inferred by the patient having half normal esterase D activity. The patient's tumor tissue was examined and was found to have no esterase D activity; chromosome studies on this tumor demonstrated only a single chromosome 13, which although on cytogenetic evaluation appeared to be normal, was interpreted to be the chromosome 13 containing the submicroscopic deletion. These studies suggested that at the cellular level, the manifestation of the retinoblastoma follows a recessive mechanism. These studies are being followed up by recombinant DNA analysis to isolate probes close to the retinoblastoma gene with the ultimate objective being to isolate the retinoblastoma gene and identify its normal function. Another question addressed was whether the gene for the hereditary form of retinoblastoma is located in the same chromosomal region. Because esterase D shows a genetic polymorphism on electrophoresis, it was possible to carry out these enzyme studies in families with the hereditary form of retinoblastoma and to demonstrate close genetic linkage between the gene for the hereditary retinoblastoma and esterase D.

0054 THE GENETICS OF SUSCEPTIBILITY TO THYMIC LYMPHOMA IN MICE. Frank Lilly and Maria L. Duran-Reynals, Albert Einstein College of Medicine, Bronx, NY 10461.

During the last twenty years, a number of mouse genes that influence the occurrence of thymic lymphoma (resistance genes) have been identified. The mechanism of each of these genes appears to be different from that of any other gene of the set. Thymic lymphoma in mice can either be MuLV-associated (i.e., induced by administration of exogenous MuLV or associated with endogenous MuLV sequences, as in spontaneous AKR lymphoma) or it can result from treatment of mice with physical or chemical agents (e.g., x-rays or methylcholanthrene). These two types of thymic lymphoma are difficult or impossible to distinguish from each other clinically and phenotypically. Nevertheless, it appears that the resistance genes that can interfere with the emergence of one of the two types of the disease have little or no effect on the occurrence of the other type. These genes will be briefly described and their mechanisms summarized. It is suggested that, if endogenous MuLV sequences are involved in the second category of lymphoma (i.e., induced by x-rays or methylcholanthrene), as has seemed possible on certain experimental grounds, then the molecular mechanisms of their involvement are likely to fundamentally different from those in classical MuLV-induced thymic lymphoma.

DNA Damage and Tumorigenesis

0055 THE ROLE OF THE CELLULAR GENOME IN THE STAGES OF CARCINOGENESIS, Henry C. Pitot, McArdle Laboratory, The Medical School, University of Wisconsin, Madison, Wisconsin 53706

The process of carcinogenesis in most instances can be dissected into three distinct stages, initiation, promotion and progression. Initiation is an irreversible, hereditary process which appears to involve single hit kinetics but no readily measurable threshold in response to known physical, chemical and biologic carcinogenic agents (1). On the basis of such findings initiation is presumably the result of an alteration in the DNA of the cellular genome. Tumor promotion, which follows the process of initiation is readily modulated by environmental factors such as nutrition, hormonal status and age, and does exhibit a threshold or no-effect level as well as a maximal effect following a single dose of an initiating agent (2). Some promoting agents exhibit tissue specificity through their interaction with tissue-specific receptors. The direct action of promoting agents appears to result from their ability to alter gene expression. Indirect actions of some promoting agents include toxic effects on the cell resulting from the formation of reactive radicals of oxygen (3). This latter process may effect the transition from the stage of promotion to that of progression. Although promoting agents cannot initiate cells, they do promote cells fortuitously initiated by ambient environmental factors. Progression results from alterations in the genome which are characterized by translocation, addition, deletion and/or amplification of genes and/or controlling elements in the DNA of the genome of the cell (4). Cells in the stage of progression are capable of incorporating exogenous DNA into their genome such that progeny of such transfections exhibit the phenotypic characteristics of the new genetic information. Thus a thorough understanding of the characteristics and mechanisms of the stages of carcinogenesis can serve to clarify the role of apparently divergent observations such as "epigenetic" carcinogenesis, transfection of oncogenes and the extreme chemical diversity of promoting agents in the development of cancer.

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0056 THE SOS RESPONSE: IMPLICATIONS FOR CANCER, Evelyn M. Witkin, Waksman Institute of Microbiology, Rutgers University, P. O. Box 759, Piscataway, NJ 08854

In Escherichia coli damage to DNA by ultraviolet light, ionizing radiations and many carcinogenic chemicals induces the derepression of at least 16 genes or operons that are otherwise partially or completely repressed. The varied phenotypes coordinately expressed after such treatments are collectively known as the SOS response (reviews and references, 1,2). Some of the induced gene products enhance survival by increasing the cell's capacity to repair DNA, to limit DNA degradation and to maintain coordination between cell division and DNA replication; others promote a transient but significant increase in genetic variability (and possibly in population fitness under stress) by increasing the infidelity of DNA replication, the error-proneness of DNA repair and the efficiency of both homologous and site-specific genetic recombination. In strains carrying integrated prophages, SOS induction triggers the transition from the lysogenic to the lytic state, thus favoring viral rather than host survival. Not all known DNA damage-inducible (din)genes have yet been assigned phenotypes, nor have all inducible phenotypes been matched to known din genes.

Regulation of the SOS response depends upon the protein products of the recA and lexA genes. Genetic studies, supported by in vitro experiments with purified proteins, have established that the lexA protein is the repressor of all known bacterial din genes, binding their operator sites ("SOS boxes") to limit transcription in the undamaged cell. DNA damage generates cofactors (ssDNA, dATP) believed necessary to activate recA protein, which can then promote proteolytic cleavage of lexA protein (or prophage repressors) to derepress the SOS regulon, including the recA and lexA genes themselves. Nucleotide sequence analysis of several cloned din genes has suggested explanations for marked differences in the kinetics of their individual expression. Repression of din genes is rapidly restored once DNA damage is repaired and DNA replication resumed.

Implications for cancer of the bacterial SOS response will be discussed, including: 1) use of various SOS phenotypes in bacterial test systems for detection of carcinogens; 2) evaluation of experimental evidence for and against a comparable regulatory response to DNA damage in eucaryotes, and especially in mammalian and human cells, and 3) theoretical considerations as guides to future research strategies.

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(See also Biochimie 64: 541-850, for proceedings of a conference on Inducible Responses to DNA Damage held in Toulouse in May, 1982)

0057 CELLULAR PROTOONCOGENES: ACTIVATION BY EXCLUSION?, William A. Haseltine, Laboratory of Biochemical Pharmacology, Department of Pathology, Harvard Medical School,*Department of Cancer Biology, Harvard School of Public Health, Dana-Farber Cancer Institute, Boston, MA 02115, Joseph G. Sodroski*

Cellular protooncogenes (c-onc genes) are highly conserved throughout evolution; they are present in animals as diverse as yeast and humans. Such a degree of conservation and their patterns of expression suggest that c-onc genes may encode factors involved in normal cell growth, homeostasis, and differentiation. The association of genes involved in cellular growth and development with transforming genes leads to a question: if c-onc genes are routinely expressed in normal cells, why doesn't transformation of those cells occur?

On answer might be that some cell types are resistant to transformation by certain onc genes and only when these genes are expressed in the "wrong" cell type does neoplastic transformation result. While this may be true in some instances, certain c-onc genes are constitutively expressed in all cell types. Clearly, mechanisms that prevent the routine development of neoplasia as a result of the expression of these genes must exist.

A second answer might be that qualitative and/or quantitative differences between the normal (inactive) c-onc gene and transforming (activated) onc gene account for differences in transforming activity. Recent experimental evidence from many laboratories suggests that qualitative differences play a more prominent role in the activation of most protooncogenes than do differences in levels of expression. A recurring theme is that the exclusion, via deletion or mutation, of particular protooncogene components may be a requisite event in the activation to a transforming state. Thus, the activation of cellular protooncogenes may involve damage to regulatory elements that control the activity of the protein product, or that control levels of expression of the protein by inhibition of transcription or translation. Evidence for and against this hypothesis will be discussed.

0058 GENETIC DEFECTS IN DNA REPAIR AND REPLICATION IN HUMAN CANCERS, James E. Cleaver, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143

Elevated sensitivity to carcinogens involving diverse biochemical mechanisms is a common feature of a large variety of human genetic disease. In a small subset of these diseases, the primary biochemical abnormalities involve DNA repair (xeroderma pigmentosum, XP), or DNA replication (XP variant; ataxia telangiectasia, AT; Cockayne syndrome, CS). Comparison of the clinical symptoms with biochemical abnormalities in each disease allow predictions to be made about possible mechanisms of carcinogenesis. XP and XP variant patients both show similar elevated ultraviolet (UV) light induced skin carcinogenesis; these cells share common properties of enhanced delays in DNA synthesis and increased mutagenesis after irradiation with UV light. Since transient delays in DNA synthesis are thought to induce gene amplification, the cancers in both forms of XP may involve amplification and mutation of critical DNA sequences. Cockayne syndrome, on the other hand, shows increased sensitivity to UV light and persistent radiation-induced delays in DNA synthesis without exhibiting enhanced mutagenesis; cancer in this disease is uncommon. AT cells are sensitive to killing by X rays, but are resistant to radiation-induced delays in DNA synthesis and show no increased mutagenesis, although specific chromosomal translocations are characteristic; patients are immunodeficient and lymphoreticular cancers are common. Blooms syndrome shows no abnormalities in DNA repair, but does show elevated levels of spontaneous mutagenesis and chromosome exchange (sister chromatid exchanges and translocations) and lymphoreticular cancers. The clinical symptoms and cellular and biochemical abnormalities of these varied diseases, suggest that gene amplification and mutation may be important in some carcinogen-induced cancers, whereas translocations more important in others (especially lymphoreticular types). Work supported by U.S. Department of Energy.

0059 GENETIC MECHANISMS OF TUMOR PROMOTION, Nancy H. Colburn, Michael I. Lerman, and Glenn A. Hegamyer, National Cancer Institute, Frederick, MD 21701

That one or more stages of tumor promotion are irreversible suggests that tumor promoters can act on preneoplastic cells to produce stable switches in gene expression that lead to malignancy. We are testing the hypothesis that sensitivity to promotion of neoplastic transformation in JB6 preneoplastic mouse epidermal cells is determined by a gene that is inducible by phorbol esters and other tumor promoters. DNA from promotion sensitive (P+) JB6 clonal cell lines can be transfected into promotion insensitive (P-) JB6 clonal cell lines with resultant transfer of sensitivity to promotion of anchorage independence by the phorbol ester TPA. (1) The DNA containing P+ activity is sensitive to digestion with Bam HI, Eco RI, Hind III and XhoI but not to Bgl I or Bgl II. (2) The P+ gene has been cloned by sib selection and characterization is underway. To date the only biochemical distinction found between P+ and P- cells has been that P+ cell lines consistently undergo a greater than 90% decrease in precursor incorporation into cell surface ganglioside GT1b, while P- cells do not. (3) This suggests the possibility that the decreased net synthesis of GT is due to increased enzymatic degradation and that the P+ gene product is a neuraminidase. Alternatively the P+ gene product and GT could act on separate promotion relevant pathways or the GT decrease could occur prior to the action of the P+ gene on the same pathway. One of the questions being asked regarding the P+ gene function is, does the P+ gene specify sensitivity to nonphorbol tumor promoters that do not bind to the phorbol ester receptor such as free radical generating agents and growth factors, agents that may be important in human tumor promotion. Differences in the mechanism of tumor promotion for benzoyl peroxide and TPA have been suggested by the observations of lack of cross sensitivity to the two compounds by different mouse strains and lack of common sensitivity to retinoic acid as an inhibitor of tumor promotion. JB6 P+ cells that are sensitive to induction of transformation by TPA are also sensitive to benzoyl peroxide, EGF, and certain TGF's while P- cells show cross resistance, suggesting that the P+ gene product may not be compound-specific for its induction, but rather promotion specific. Models are suggested to explain the phenotypes of P+ and P- cells in comparison with tumorigenic transformants.

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The Genetics of the Cancer Cell

0060 CHROMOSOMAL ALTERATION IN CANCER, Peter C. Nowell, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Most tumors have somatic genetic changes demonstrable at the chromosome level. These are usually the same, or related, throughout a tumor, indicating unicellular origin and also the selective growth advantage afforded the cells in the neoplastic clone by the chromosome changes. Many of the alterations are non-random, involving gain, loss, or translocation of genetic material. These are providing clues to the location and altered function of genes important in human neoplasia, including several examples of known "oncogenes." Chromosome changes are often more extensive in advanced tumors, perhaps the result of genetic instability in the neoplastic cells, and apparently reflecting genetic alterations contributing to late stages of tumor development as well as early stages. Data from recent studies with Burkitt's lymphoma, acute and chronic leukemia, and malignant melanoma will be used to illustrate these generalizations.

0061 HUMAN CELL HYBRIDS AND THE ANALYSIS OF MALIGNANCY, Eric J. Stanbridge, Department of Microbiology, University of California, Irvine, College of Medicine, Irvine, CA 92717

An overview of the use of human cell hybrids in the analysis of the genetic control of transformed and tumorigenic phenotypes will be presented. Previous studies have shown that when a tumorigenic cell is fused with a normal cell the resulting hybrid population exhibits the phenotype of transformed cells in culture but has lost the ability to form tumors in nude athymic mice (1). We have established that the nontumorigenic hybrid cells are induced to differentiate in the host animal. Tumorigenic segregant hybrid cells have been isolated which have fully regained their tumorigenic potential. Reacquisition of tumorigenic potential is associated with the loss of specific chromosomes from the hybrid cell.

Recent developments in the study of these hybrid cells include techniques for the identification of tumor-associated antigens and differentiation-specific antigens. Techniques for the transfer of single specific human chromosomes will be presented.

Control of expression of human cancer genes and their possible role in determining the neoplastic phenotype of human hybrid cells will be discussed.

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0062 MOLECULAR DISSECTION OF UNIQUE TUMOR ANTIGENS AND PROGRESSION OF THE NEOPLASTIC PHENOTYPE, Hans Schreiber, Hans J. Stauss, Constance Philipps, Robert S. Goodenow and Minnie McMillan, Department of Genetics, University of California, Berkeley, CA 94720

Tumors induced by chemical or physical agents often express tumor-specific antigens that are unique, i.e., individually specific for a particular tumor even when tumors are of the same histologic type and induced in the same strain by the same carcinogen. The diversity of these unique antigens among different tumors is seemingly endless and has been compared to that of immune receptors. We have dissected the nature and complexity of the unique antigenicity for a single tumor (1-3). Using tumor-specific T cell clones and hybridoma antibodies, we have found that a single malignant cell expressed multiple unique tumor-specific antigens (4,5). Tumor variants selected in vitro showed that these antigens were independent of each other since each T cell clone selected only for loss of the appropriate antigen and the simultaneous loss of two antigens was too rare to be detected. The expression of the tumor-specific antigens was affected by the selection pressures of the normal host's immune system. However, it is curious that these multiple antigens which are all expressed on the tumor at the same time can only be recognized sequentially by the host (4,6). This hierarchy in the host's recognition of these antigens appears to make tumor progression more likely since loss of only a single antigen allows the tumor to temporarily escape the immune defenses. We are presently determining whether the multiple unique tumor antigens are products of a multigene family by analyzing their molecular composition and genetic origins.

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0063 THE ORIGIN AND PROGRESSION OF CANCER METASTASES, Isaiah J. Fidler, Department of Cell Biology, University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

Multiple metastases proliferating in the same host can be heterogeneous with regard to many biological characteristics. This diversity can well be the consequence of both the nature of tumor cell spread and continuous tumor evolution. Since metastases, in general, result from the selective growth of specialized malignant cells that preexist within the parental tumor (1), we determined whether they are clonal in their origin, and can originate from different progenitor cells. We examined the metastases that arose from subcutaneously growing K-1735 melanoma cells exposed to 700 R X-radiation to induce chromosomal breaks and rearrangements. We reasoned that if a metastasis were derived from a single cell, all the spreads examined would exhibit a uniform karyotype. In contrast, if a metastasis were derived from more than one progenitor cell, its consitutent cells would exhibit different chromosomal arrangements. Spontaneous lung metastases were isolated, grown in culture as individual lines, and karyotyped. In 10 of 21 lines, all the chromosome were telocentric, and therefore, these metastases were noninformative. In the other 11 lines, unique patterns of single or multiple marker chromosomes were observed, suggesting that these metastases originated from the progeny of single but different cells (2). To determine whether melanoma metastases arose as a consequence of individual cells surviving in the blood stream, animals were injected with clumps of K-1735 cells consisting of 2 lines. Cells of one line exhibit a stable submetacentric chromosomal marker, whereas cells of the other line have a stable, normal karyotype. Twenty lung metastases were harvested, grown in culture as individual cell lines, and karyotyped. Spreads from each metastasis exhibited a uniform karyotypic pattern, suggesting that they arose from the proliferation of a single cell.

Since tumor progression toward malignancy could well be accompanied by increasing genetic instability of the evolving cells (3), we have examined the rates of mutation to ouabain resistance and/or 6-thioguanine resistance of paired metastatic and nonmetastatic cloned lines isolated from different mouse neoplasms by the fluctuation assay of Luria and Delbrück (4). Indeed we found that the mutation rate to ouabain resistance was 4-7-fold higher, and that to 6-thioguanine was 3-5-fold higher in metastatic clones than in low metastatic clones (5).

Our findings that different metastasis can originate from different progenitor cells accounts for the biological diversity that exists among various metastases. However, even within a solitary metastasis of proven clonal origin, heterogeneity for various characteristics can develop rapidly, possibly resulting from instability of the phenotype of clonal populations (6) and/or from a high rate of spontaneous mutation in cells populating metastases.

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Viral Models for Oncogenes

0064 EXPLORING CARCINOGENESIS WITH RETROVIRUSES, J.M. Bishop, G. Evan, K.-H. Klempnauer, R. Parker, M. Privalsky, G. Ramsay, M. Schwab, G. Symonds and H.E. Varmus, Dept. of Microbiology and Immunology, University of California, San Francisco, Ca. 94143

The oncogenes of retroviruses provide valuable models for the biochemical mechanisms of neoplastic transformation. New procedures have enabled us to study the products of three leukemogenic genes: v-erb-B, v-myb and v-myc. Our findings exemplify the diverse nature of retroviral transforming proteins. The product of v-erb-B (Mr 65-72,000) is a glycoprotein, found principally on dense intracellular membranes and in small quantities on the plasma membrane. The protein has extensive structural homology with other oncogene products that are protein kinases. By contrast, v-myb and v-myc encode nuclear proteins (Mr 45,000 and 58,000, respectively) that appear to be distantly related to each other and to the major product of the E1a transforming region of human adenoviruses. The myc protein resides in the insoluble matrix of the nucleus. Association of the myb protein with the nucleus is more subtle. The protein is readily apparent in the nuclei of myeloblasts transformed by v-myb and in fibroblasts expressing the gene but not transformed by it. When the myeloblasts are caused to differentiate, however, the v-myb protein is still produced in standard quantities, but its location within the cell may change.

Retroviral oncogenes arise by transduction of cellular "proto-oncogenes". Two separate cellular genes (c-erb-A and c-erb-B) have been repeatedly transduced into the genome of avian erythroblastosis viruses, even though c-erb-A and c-erb-B reside on different chromosomes in the chicken. Transduction can inflict very little or very severe damage on cellular genes, two extremes that are embodied in the proteins encoded by v-myc and v-myb.

The capability of unaltered proto-oncogenes to transform cells has been examined with c-src. Expression of c-src to levels in excess of those required for neoplastic transformation by v-src have failed to alter cellular phenotype. We suspect that demonstrated differences between the c-src and v-src proteins at their carboxy termini may account for the tumorigenicity of v-src. In other instances, unaltered proto-oncogenes may at least contribute to tumorigenesis. For example, we have found amplification and enhanced expression of ostensibly normal proto-oncogenes (c-myc, c-Ki-ras and c-myb) in diverse forms of human and murine tumors. In addition, amplification and enhanced expression of a distant kin of c-myc (denoted N-myc) appears to be a common feature of human neuroblastomas. N-myc may be a previously unrecognized proto-oncogene whose amplification could represent both a molecular marker and part of the etiological mechanism for neuroblastomas.

0065 SUBSTRATES FOR TYROSINE PROTEIN KINASES IN VIRALLY TRANSFORMED CELLS.
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To date six classes of acutely transforming retrovirus are known to contain genes coding for tyrosine protein kinases. Cells transformed by these viruses all show 5-10 fold increases in the level of phosphotyrosine in cellular protein. Although there is no rigorous proof, it is believed that transformation by such viruses is mediated at least in part through alterations in the functions of cellular proteins resulting from tyrosine phosphorylation. Based on this assumption we have been trying to identify cellular proteins phosphorylated by this group of viral tyrosine protein kinases with the aim of characterising their functions and the effects of tyrosine phosphorylation on those functions. We have detected a number of proteins which contain increased levels of phosphotyrosine in cells transformed by the relevant viruses. These include the cytoskeletal protein, vinculin, p81, p50, p42, p36 and the glycolytic enzymes enolase, LDH and phosphoglycerate mutase. In general a single tyrosine residue in each protein is modified, but only a small fraction of the total populations of these substrates is phosphorylated on tyrosine at steady state. There is considerable overlap in the spectrum of phosphotyrosine containing proteins in cells transformed by different viruses implying that the viral tyrosine protein kinases have similar specificities. The phosphorylation of vinculin may in some way mediate the shape alterations characteristic of many virally transformed cells. On the other hand none of the three glycolytic enzymes is normally considered rate limiting so it is unlikely that their phosphorylation plays a role in the increased glycolytic flux associated with transformation. Nevertheless because of their potential for understanding how tyrosine phosphorylation might alter protein function, we have put some effort into identifying the phosphorylated tyrosines in enolase and LDH. The tyrosine phosphorylated in LDH is residue 237, which is found close to the active site histidine, but the effect of this modification on LDH function is not yet known. The sequence of the enolase phosphorylation site, R.A.A.V.P.S.G.A.S.T.G.I.Y.E.A.L.E.L.R, is in a highly conserved part of the protein. This sequence differs from other known sites of tyrosine phosphorylation in having acidic residues downstream rather than upstream of the tyrosine. p36 has been found in many systems, but although it is known to be membrane-associated in fibroblasts, its function is still a mystery. A survey of tissues and cell lines shows that p36 is not expressed in all differentiated cell types. In particular it is found at high level in intestinal brush border cells, where it is concentrated in the terminal web region, suggesting a structural function for p36. The effect of phosphorylation, if any, on p36 function is unclear.

Oncogenes In Human Tumors

0066 TRANSFORMING GENES IN HUMAN AND EXPERIMENTALLY INDUCED TUMORS,
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Transforming genes capable of inducing malignant transformation of morphologically normal cell lines have been detected in a significant percentage of human tumors. Most of these transforming genes originated by single mutational events within the coding sequences of a family of genes, generically designated ras. These genes, in their nonmalignant state, are constitutive components of the genome of all eukaryotic organisms and have been highly conserved during evolution. Three members of the ras gene family, H-ras, K-ras and N-ras, have been molecularly characterized in several laboratories. Although their genetic organization is quite different, they code for highly related proteins of 189 amino acid residues designated as p21. Computer analysis of the predicted amino acid sequence of p21 proteins coded for by normal and transforming genes suggests that conformational changes in the tertiary structure of the mutated molecules are responsible for their neoplastic properties. In collaborative studies with Dr. R. Crowl (Hoffman-La Roche) we have achieved efficient expression of both normal and transforming p21 proteins in E. coli. These reagents should prove very valuable in the investigation of the biological function of these proteins.

Identification of ras transforming genes in human tumors has relied on tedious and insensitive biological assays, primarily transfection of NIH/3T3 cells. However, sequence analysis of ras oncogenes has revealed that some of the point mutations responsible for their malignant activation create restriction enzyme polymorphisms which can be utilized to discriminate between normal and transforming alleles. This experimental approach has been succesfully utilized to identify the point mutation responsable for the malignant activation of a K-ras oncogene in a squamous cell lung carcinoma of a 66 year old male. This critical mutational event did not occur in the normal bronchial or parenchymal tissue of the patient nor in his blood lymphocytes. These results demonstrate that malignant activation of a ras oncogene is specifically associated with the development of a human neoplasm.

Efforts aimed at understanding the role of oncogenes in human neoplasia are necessarily hampered by the impossibility of experimental manipulations. Thus, we have investigated whether certain carcinogens may induce tumors in animals by mechanisms involving activation of ras oncogenes. We have established that induction of mammary carcinomas in Buf/N rats by nitroso-methyl-urea involves the activation of the H-ras locus by the same mutational events observed in human tumors. Detailed characterization of these model systems should provide the experimental basis to establish the role of oncogenes in human carcinogenesis.

0067 TRANSFORMING GENES OF SARCOMAS AND OTHER TUMORS, C.J. Marshall, A. Hall and R.J. Brown, Chester Beatty Laboratories, Institute of Cancer Research, London, SW3 6JB, U.K.

By the use of transfection experiments with NIH-3T3 cells an activated transforming gene has been identified in two human sarcoma cell lines HT1080 and RD(1). This transforming gene has been shown to be a member of the ras gene family, N-ras, distantly related to the Harvey and Kirsten viral ras gene (2). By the use of chimeric molecules constructed between part of the normal N-ras gene and transforming alleles in marker rescue experiments the mutation leading to transforming activity in the HT1080 N-ras allele has been determined. The HT1080 allele differs from its normal counterpart by a single amino acid substitution of glutamine by lysine at amino acid 61.

In order to demonstrate that the HT1080 transforming gene is capable of transforming HT1080 cells flat revertants of HT1080 cells have been selected. Introduction of the HT1080 transforming gene into these cells results in morphological transformation indicating that HT1080 cells can respond to their own transforming gene.

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0068 HUMAN TRANSFORMING GENES. Michael Wigler, Ottavio Fasana, Elizabeth Taparowsky, Daniel Birnbaum, Mitchell Goldfarb and Jorgen Fogh*. Cold Spring Harbor, NY and *Sloan Kettering Institute, Rye, NY.

Use of NIH3T3 focus assay has revealed the widespread presence of activated ras oncogenes in human tumor cell DNA. The three ras genes found so far encode proteins with remarkably conserved amino acid sequence. Systematic in vitro mutagenesis indicate that amino acid substitution at mutliple sites lead to the activation of the transforming potential of the normal ras gene product.. We have begun to utilize cotransformation of NIH3T3 cells and tumorigenicity in nude mice as an assay for new human tumor genes. Our initial experinece indicates this assay to be far more sensitive than the focus assay, and reveals a set of new "tumor" genes which are not detectable by previous bioassays.

Cellular Oncogenes: Structure, Function and Pathogenicity

0069 INSERTIONAL MUTATIONS DURING VIRAL ONCOGENESIS: H. Varmus[1], D. Westaway[1], Y.K. Fung[1], C. Moscovici[2], A.A. van Ooyen[3], and R. Nusse[3], [1]Dept. of Microbiology & Immunology, University of California, San Francisco, CA; [2]VA Hospital, Gainesville, FL; [3]Dept. of Virology, Antoni van Leeuwenhoekhuis, Amsterdam, The Netherlands.

Some viruses without oncogenes initiate tumorigenesis by insertion mutations that activate cellular genes. This phenomenon offers a means to identify new oncogenes and to study mechanisms that affect gene expression. (i) During the development of avian leukosis virus (ALV)-induced B cell lymphomas, ALV proviruses cause insertion mutations at the c-myc locus. The mutations are associated with efficient expression of c-myc, regardless of whether the proviruses are upstream from c-myc in either transcriptional orientation or downstream from the cellular gene (Payne, Bishop, and Varmus, Nature 295:209, 1982). Detailed structural analysis of cloned, insertionally-mutated c-myc alleles indicates that secondary mutational events have occurred: both deletion mutations within proviral DNA and nucleotide substitutions in the c-myc coding domain. These mutations may augment expression of c-myc, incapacitate expression of viral genes, or alter the product of c-myc. (ii) Tumorigenesis by the mouse mammary tumor virus (MMTV) is often accompanied by proviral insertions in a host locus called int-1 (Nusse and Varmus, Cell 31:99, 1982). This locus, on mouse chromosome 15, harbors a transcriptional unit that is generally silent, but it is activated to produce small numbers of copies of a 2.6 kb polyadenylated RNA in tumors bearing insertion mutations. Gene activation most commonly occurs by an indirect mechanism, with MMTV proviruses either upstream from the gene in the opposite transcriptional orientation or downstream in the same orientation. The int-1 gene has not been encountered among retroviral oncogenes, but at least part of the transcriptional domain is highly conserved. cDNA clones of int-1 mRNA have been obtained to identify the gene product. (iii) The avian myeloblastosis associated virus (MAV), like ALV, induces B cell lymphomas by insertion mutations within c-myc, but none of several tested oncogenes, including c-myc, is altered in MAV-induced nephroblastomas. At least three tumors contain novel transcripts of cellular DNA promoted by MAV LTRs. However, cloned cellular DNA flanking a single provirus in one nephroblastoma failed to detect rearrangements of the same locus in 14 other tumors. Thus the target(s) for insertion mutations in nephroblastoma has yet to be identified. (iv) In a search for common integration sites in human hepatitis B virus (HBV)-associated hepatomas, we encountered one tumor with a large cellular domain that has been amplified 20-50-fold and harbors an integrated subgenomic component of HBV DNA. None of several tested oncogenes is amplified in this tumor; the significance of the observed amplification is under study.

0070 CELLULAR ONCOGENES AND TUMORIGENESIS BY HTLV, Robert C. Gallo, M.D. and Flossie Wong-Staal, Ph.D, Laboratory of Tumor Cell Biology, National Cancer Institutes, Bethesda, MD 20205

HTLV is a family of related, T-cell tropic exogenous human retroviruses associated with adult T-cell leukemia/lymphoma (ATLL) and possibly with AIDS. The virus geonomes of two distantly related subgroups (HTLV I and HTLV II) have been cloned. Although HTLV lacks a cell derived onc gene, complete nucleotide sequence of one HTLV I showed that. In addition to gag, pol and env genes, there is a potential coding region (pX) of undefined functions. HTLV positive leukemic cells are clonal, consistent with HTLV being a chronic leukemia virus. However, HTLV can uniquely immortalize and transform T-lymphocytes in vitro. We consider this step as a first stage (initiation) of transformation. The frank leukemic cells derived from patients may be at a later stage (maintenance) of transformation. All in vitro infected cells express high levels of viral mRNA, including a 9.0 Kb genomic size, a 4 Kb env mRNA and a 2 Kb pX mRNA. However, fresh leukemic cells express low or undetectable levels of viral mRNA. These results suggest that expression of viral proteins, including pX, may be necessary for initiation but not maintenance of transformation. We also examined possible activation of c-onc genes and growth factor genes by HTLV. All HTLV infected cells express high levels of TCGF receptor but extremely low or undetectable levels of TCGF, thus ruling out simple autostimulation. There was also no evidence for consistent activation of any onc gene. However, many of the in vitro infected cells and cell lines established from HTLV positive tumors express c-sis, a gene not normally expressed in hematopoietic cells. From one of these lines, a sis cDNA was cloned in an expression vector. This clone, which transforms NIH 3T3 cells in vitro, contains 2.7 Kb 3' sequences of the 4.2 Kb sis transcript, with no nucleotide change from the normal gene. Thus, its transforming activity may be due to its truncated structure which is analogous to v-sis. Since c-sis codes for a growth factor (PDGF), the sis product produced in these cells may aberrently stimulate their own proliferation. In summary, the molecular mechanism of HTLV transformation is not understood, but we currently focus on three areas: (1) The TCGF receptor; (2) The c-sis product; and (3) certain viral proteins, the latter two, however, only in initiation of transformation.

0071 THE COMPARATIVE ANATOMY OF VIRAL AND CELLULAR ONCOGENES, Hidesaburo Hanafusa[1], Hideo Iba[1], Tatsuo Takeya[2], Frederick R. Cross[1], Teruko Hanafusa[1], Teena Lerner[1], and Lu-Hai Wang[1], [1]The Rockefeller University, New York, N.Y. 10021 and [2]Institute for Chemical Research, Kyoto University, Kyoto, Japan.

The generation of recovered avian sarcoma virus in chickens following infection with mutants of Rous sarcoma virus (RSV) containing partial deletions in the src gene suggested that the majority of the viral src sequence can be replaced by the cellular src sequence to encode an active transforming protein. The comparison of DNA sequences of the coding regions of the chicken c-src gene and the viral src gene of SR-RSV showed that these two are very similar; however, in addition to 8 single amino acid changes, the 19 carboxy terminal amino acids of c-src were found to be replaced by 12 different amino acids in v-src. The 39 bp sequence coding the carboxy terminal sequence of $p60^{v\text{-}src}$ was found about 1 kb downstream from the termination codon of the c-src gene. The analysis of the genome of the Bryan strain of RSV indicated that its 3' end sequence, from the last 16 bp of src through the LTR, was virtually identical to a region from downstream of env through the LTR in RAV-2 and also in Y73, suggesting that this strain of RSV, which could be the original RSV strain, was derived by recombination between RAV and c-src DNA.

By transfection of chicken cells with RSV DNA in which various portions of v-src were replaced with the corresponding c-src sequence, we found the replacement of v-src either upstream or downstream of the BglI site with c-src sequences did not affect the transforming activity of the resultant virus. However, when the v-src sequence is totally replaced by the c-src sequence, we found the product $p60^{src}$ is expressed without appreciable transformation of cells. Therefore we conclude that the c-src product cannot transform chicken cells in the same way the v-src product does. However, c-src DNA-transfected cultures produced weakly transforming viruses. We have not yet determined whether these weakly transforming viruses carry the unaltered c-src sequence or a mutated one. After passage, however, strongly transforming mutant viruses emerged. These results show that after a proto-oncogene is incorporated into a retrovirus genome, transforming mutants are rapidly generated, and can be selected on the basis of their transforming capacity.

0072 CELLULAR ONCOGENES: STRUCTURE, EXPRESSION AND ROLE IN DEVELOPMENT, I. M. Verma, T. Curran, A. D. Miller, R. Müller and C. Van Beveren, The Salk Institute, San Diego, California 92138

Acutely oncogenic retroviruses cause a host of malignancies in animals and induce cellular transformation of a variety of cell types. They all contain in their genomes sequences which have counterparts in normal cellular DNA. Acquisition of normal cellular sequences [proto-oncogene or cellular oncogene (c-onc)] by retroviruses confer on them the ability to induce rapid neoplasias. I would like to address two issues: 1) the mechanism by which acquired cellular sequences become highly oncogenic, since the c-onc genes themselves are generally unable to induce transformation, and 2) the role the c-onc genes play in the normal development of an organism. Toward these goals, we have developed the fos oncogenes of FBJ murine osteosarcoma virus (FBJ-MuSV) as a model system. The FBJ-MuSV proviral DNA contains 4,026 nucleotides which include two long terminal repeats, 1,639 acquired cellular sequences (v-fos) and a portion of env gene. Using v-fos specific sequences, the cellular homologs (c-fos) from both mouse and human cells were identified and molecularly cloned. Nucleotide sequence analysis reveals that the v-fos sequences have undergone a deletion of 104 bp near their 3'-end as compared to the c-fos gene. Consequently the putative 381 aa v-fos and 380 aa c-fos proteins are nearly identical for the first 332 amino acids but have different C-termini. Both the v-fos and c-fos proteins are localized in the nucleus.

The FBJ-MuSV containing v-fos sequences can transform rat 208 F cells while c-fos gene is unable to induce cellular transformation. Since the proteins encoded by the v-fos and c-fos genes are qualitatively different, it was assumed that cellular transformation by v-fos sequences is a consequence of its C-termini. To delineate the sequences required for transformation, we constructed a number of recombinant plasmids containing portions of v-fos and c-fos sequences. The results indicate i) the c-fos proteins can induce cellular transformation, ii) the c-fos gene needs two manipulations to manifest its transforming potential. These include addition of transcriptional enhancer elements and disruption of 3'-interacting sequences. Analysis of recombinant plasmids unable to induce transformation indicates that c-fos specific transcripts are synthesized but little or no c-fos protein is detectable.

To analyze the role of c-onc genes during development we undertook a systematic study of the expression of c-onc genes during pre- and postnatal development of the mouse. Results obtained using probes homologous to a number of c-onc genes revealed that i) c-onc genes are expressed during prenatal development in a stage- and tissue-specific manner and ii) some c-onc genes are expressed in postnatal tissues. The differential expression of c-onc genes during embryogenesis lends support to a widely held notion that c-onc genes play a role in normal metabolic processes.

0073 THE *DROSOPHILA src, abl* AND *ras* CELLULAR ONCOGENES. B.Z. Shilo[1], H. Hoffman-Falk[1], F.S. Neumann[1], E. Schejter[1], F.M. Hoffmann[2] and Z. Lev[3]. [1]Department of Virology, The Weizmann Institute of Science, Rehovot, Israel, [2]Harvard Biological Laboratories, Cambridge, MA, and [3]Department of Biology, Haifa Technion, Haifa, Israel.

DNA sequences homologous to the vertebrate *src, abl* and *ras* oncogenes were isolated from a *DROSOPHILA MELANOGASTER* DNA library under non-stringent hybridization conditions. The nucleotide sequence of the *DROSOPHILA src* and *abl* genes has been determined. Alignment of the predicted amino acid sequences in the homologous regions shows sequence identities of 54% and 74% for the *src* and *abl* genes, respectively. Comparison of the *DROSOPHILA* and vertebrate sequences identifies multiple amino acids which are essential for the distinct roles of c-*src* and c-*abl*, and places the gene duplication event which has generated the two genes prior to the Chordate-Arthropod divergence.

The different functions of the two genes are also reflected in their different patterns of expression duing *DROSOPHILA* development. The c-*abl* gene produces a single transcript of 6.2 KB which can be detected only in RNA extracted from eggs and 0-4 hour embryos. It is likely to be maternal RNA which is stored in the egg and is required for the early stages of embryogenesis. In contrast, the c-*src* gene is expressed at all developmental stages. It produces three transcripts of 3.1, 4.8 and 5.2 KB. The ratios between these transcripts however, change during development.

The nucleotide sequence of two of the *DROSOPHILA ras* genes has been determined. The gene which shows the greatest homology to v-*ras* (termed c-D*ras*-1) contains multiple introns. A cDNA clone containing the entire coding region was isolated and sequenced. It is over 70% homologous to the v-*ras* amino acid sequence. The second *DROSOPHILA ras* gene (c-D*ras*-2) contains a single intron, and shows 52% homology to v-*ras*. At the carboxy terminus, the major domain of variability among the vertebrate *ras* genes, c-D*ras*-2, is most similar to exon 4B of Kirsten *ras*. The *DROSOPHILA* c-D*ras*-1 and c-D*ras*-2 genes code for a 2.1 and a 1.8 KB transcript respectively, and are expressed at all stages of development.

The identification of cellular oncogenes in *DROSOPHILA* should provide new approaches to study their function by the utilization of the genetics and developmental biology of *DROSOPHILA*. The high degree of sequence conservation suggests that the *DROSOPHILA* oncogenes and their vertebrate counterparts have analogous functions.

Chromosomal Anomalies and Cellular Oncogenes

0074 THE SPECIFICITY OF TRANSLOCATIONS IN HUMAN LEUKEMIA AND LYMPHOMA, Janet D. Rowley, Department of Medicine, University of Chicago, Chicago, IL 60637

Most human leukemias and lymphomas have a nonrandom clonal abnormality that are different in myeloid and lymphoid neoplasms. For many of the myeloproliferative diseases including acute nonlymphocytic leukemia (ANLL), chronic myeloid leukemia (CML) blast crisis, and pre-leukemic states, an extra No. 8 is the most common change; a loss of No. 7 also occurs. The chromosome gains and losses are more variable in malignant lymphoid diseases, however, gains of Nos. 3, 6, 7, or 21 are seen in some of them. In contrast to these aberrations seen in a spectrum of neoplastic hematologic disorders, is the remarkable specificity of certain structural rearrangements usually translocations. The clearest illustration of this specificity is seen in ANLL, which can be divided into various subtypes depending on the morphology of the predominant leukemic cell. Specific structural rearrangements are each uniquely associated with particular subtypes of ANLL. A translocation of Nos. 8 and 21 [t(8;21)(q22;q22)] is observed in acute myeloblastic leukemia (AML, M2), whereas a translocation of Nos. 15 and 17 [t(15;17)(q22;q21)] is seen only in acute promyelocytic leukemia (APL, M3). In monoblastic leukemia (AMoL, M5), the translocation or deletion involves No. 11, usually band q23 but also 11q13; the translocation partner is variable but often involves the short arm of No. 9. An abnormality of No. 16 is seen in patients who have an increase in myeloblasts and monocytes [acute myelomonocytic leukemia, AMMoL (M4)] associated with morphologically abnormal eosinophils that may be increased in number; it involves either an inversion with breaks in band 16q13 and q22 and or a deletion of 16q22. A translocation involving Nos. 6 and 9 [t(6;9)(p23;q34)] was recently identified in patients with either M2 or M4 leukemia with an excess of basophils. In lymphoid neoplasms, the translocations most commonly observed involve Nos. 8 and 14 in Burkitt's lymphoma or B-cell acute lymphoblastic leukemia or Nos. 14 and 18 [t(14;18)(q32;q21)] in follicular small cleaved cell lymphoma.

These specific rearrangements are important because they define the exact chromosome location of two critical genes. The identity of these genes is largely unknown except for Burkitt's lymphoma. It is quite likely, however, that the observations in Burkitt's lymphoma will have general applicability to all of the specific rearrangements described above. The t(8;14) results in the apposition of a proto-oncogene, c myc, near to an immunoglobulin gene which is functionally active in B cells, essentially the only cell type in which a t(8;14) has been identified. Recent studies have shown that c abl on band 9q34, is affected in the t(9;22) in CML. One can, thus, propose that the specificity of the translocation is related to the active function of a critical gene in a specific cell type interacting with a particular growth regulating gene which may be a recognized oncogene, e.g. c myc or c abl, or a growth factor, e.g. platelet derived growth factor which is homologous to another proto oncogene, c sis.

0075 CHROMOSOMAL ANALYSIS OF SOLID TUMORS. Uta Francke, Department of Human Genetics, School of Medicine, Yale University, New Haven CT 06510

Numerous reported chromosome studies of solid tumor tissue and tumor cell lines have documented enormous variability in chromosome numbers (from hypodiploidy to heteroploidy) and in the presence of structurally rearranged chromosomes of identifiable or of undeterminable derivation ("marker" chromosomes). Postulated mechanisms that contribute to the aneuploid tumor cell karyotypes are endoreduplication, nondisjunction, DNA breakage and repair, nonhomologous mitotic recombination, isochromosome formation, and asynchronous and localized repeated unscheduled DNA replication. Resulting phenomena may include unstable rearrangements such as ring chromosomes, dicentrics, acentrics and newly generated chromosomal material in the form of homogeneously staining regions and double minutes that presumably contain amplified DNA sequences. While serial studies of tumor cell karyotypes may establish clonality of an individual cancer and allow one to trace the steps in the evolution of a modal tumor karyotype, there is no clear cut relationship between biological behavior of the cancer and its apparent level of aneuploidy. Whereas marker chromosomes resulting from translocations or deletions appear to involve some human chromosomes more often than others, there are at present no markers that are unique and specific to tumors of any given site or histologic type (1). The best evidence for involvement of specific chromosomal sites exists for two human embryonic tumors that each occur in a sporadic and in a heritable form: Retinoblastoma (RB) and Wilms tumor (WT) (reviewed in 2). A gene of unknown function, located in chromosome band 13q14, predisposes to bilateral (multifocal) RB when mutated, as in the highly penetrant autosomal dominant form of the disease, or when deleted or inactivated due to a cytologically detectable deletion or translocation (2). A second mutational event is required for RB to become manifest. Cytological, gene marker and molecular studies of the same patients' tumor and non-tumorous tissue are now producing evidence that this second event involves the homologous allele on the other chromosome 13. The situation in WT is only partially analogous since deletion of the distal part of band 11p13 does not uniformly lead to development of WT, but only confers a 30% risk. The autosomal dominant form of WT has reduced penetrance and the gene has not been independently mapped (2). The HRAS1 cellular oncogene is located distal to the region deleted in WT patients (3) which makes its direct involvement in tumor formation unlikely. However, the high frequency restriction fragment length polymorphism near the HRAS1 gene makes it a useful marker locus for the detection of deletion and/or mitotic recombination events that lead to hemi- or homozygosity of the as yet unidentified WT predisposing gene in 11p13 in tumor tissues.

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0076 TRANSLOCATION OF AN IMMUNOGLOBULIN κ LOCUS TO A REGION 3' OF AN UNREARRANGED C-MYC ONCOGENE ENHANCES C-MYC TRANSCRIPTION, Carlo M. Croce, The Wistar Institute of Anatomy and Biology, Philadelphia PA 19104.

We have studied somatic cell hybrids between mouse myeloma cells and JI Burkitt lymphoma cells carrying a t(2;8) chromosome translocation for the expression of human chains and for the presence and rearrangements of the human c-myc oncogene and κ chain genes. Our results indicate that the c-myc oncogene is unrearranged and remains on the 8q+ chromosome of JI cells. Two rearranged Cκ genes are detected: the expressed allele on chromosome 2 and the excluded allele that translocates from chromosome 2 to the deleted chromosome 8 (8q+) Because hybrids containing the 2p- chromosome but not chromosomes 2 and 8q+ and hybrids containing the 8q+ but not the 2 and 2p- carry Vκ genes we conclude that the breakpoint occurred in the chromosomal regions carrying Vκ genes and that the Vκ genes are proximal and the Cκ gene is distal to the breakpoint. The high levels of transcripts of the c-myc gene when it resides on the 8q+ chromosome but not on the normal chromosome 8, indicate that translocation of a kappa locus to a region distal to the c-myc oncogene enhances c-myc transcription.

0077 INVOLVEMENT OF c-abl IN THE PHILADELPHIA (Ph') TRANSLOCATION, J. R. Stephenson[1], N. Heisterkamp[1], G. Grosveld[2], and J. Groffen[1], [1]Laboratory of Viral Carcinogenesis, Natl. Cancer Inst., Frederick, Md. and [2]Dept. Cell Biol. and Genet., Erasmus Univ., Rotterdam, The Netherlands.

By somatic cell hybrid analysis, we have localized c-abl to human chromosome 9 and demonstrated its translocation to chromosome 22q- (the Ph' chromosome) in chronic myelogenous leukemia (CML) (1). In two CML patients, the Ph' chromosomal breakpoint has been molecularly cloned and localized within 14 kb and 30 kb, respectively, of the most 5' v-abl homologous region. The possibility that the breakpoint on chromosome 9 may even map within the 5' coding region of the human c-abl locus cannot be excluded, since the position of the most 5' exon in human c-abl has not been determined (2). Although, as yet, we have not localized the chromosome 9 breakpoints of other CML patients, our results establish that the site of the Ph' translocation breakpoint is variable and suggest that breakpoints of other CML DNAs are located 5' of the approximately 80 kb of cloned DNA encompassing the human v-abl homologous sequences. The orientation of human c-abl on chromosome 9, with its 5' region toward the centromere is established by the fact that the breakpoint is 5' with respect to the human c-abl locus. The possible involvement of c-abl in CML is further indicated by its translocation to the Ph' chromosome in the t(9;11;22) and t(1;9;22) complex transactions associated with CML and the specific amplification of c-abl in the Ph' positive K562 cell line (3,4). Using, as a probe, chromosome 22 specific sequences of a Ph' chimeric DNA fragment, we have isolated an extended region on chromosome 22 from non-CML human DNA. The genomic organization of this region of chromosome 22 was investigated in DNAs of a number of CML patients; the majority exhibited abnormal restriction enzyme patterns indicating that in Ph'-positive CML a breakpoint generally occurs within a single well defined region of chromosome 22. These findings strongly implicate c-abl in CML and suggest that a specific breakpoint cluster region of chromosome 22 may play a role in c-abl activation.

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Pathobiology of the Tumor Cell

0078 CELLULAR ORIGINS, DIFFERENTIATION ARREST AND GENE EXPRESSION IN HUMAN LEUKAEMIA. Melvyn F. Greaves, Imperial Cancer Research Fund, London WC2A 3PX.

The composite cellular phenotype of human leukaemic cells can be determined by immunological analysis with monoclonal antibodies and by enzymatic assays. Molecular probes for gene re-arrangements (Ig loci) or abundant mRNA species also provide cell type tags. Cellular phenotypes so established are being integrated into diagnostic and therapeutic programmes.

The consistent phenotypes expressed in leukaemia are heterogeneous and may shift during progression or show some asynchrony. Nevertheless they predominantly reflect the cell lineage and cell type origin of the dominant clone(s) plus the stringency of maturation arrest and proliferative activity. Few, if any, qualitative abnormalities can be identified other than chromosomal translocations. The overall conservation of phenotype in leukaemia is consistent with the view that leukaemogenesis progressively uncouples proliferation from differentiation with resultant stabilisation of immature phenotypes that are normally transient.

Several of the cell surface structures expressed by leukaemic cells have recently been isolated, biochemically characterised and chromosomally mapped by somatic cell genetics; one has been molecularly cloned (the transferrin receptor). Details of these studies will be presented.

Poster Session No. 1

0079 SINGLE-STRANDED REGIONS IN THE 5' FLANKING DNA OF THE DROSOPHILA HSP 70 GENE. Ronald L. Seale, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The hsp 70 gene of Drosophila nuclei was cleaved at two positions by single-strand endonucleases. The cleavage sites were in the 5' flanking region and were not identical to either the DNase I or micrococcal nuclease hypersensitive sites. Plasmids containing the entire coding region plus 1.5 kb of 5' flanking DNA were not cleaved at the positions recognized in chromatin; this was true of supercoiled plasmids, linear plasmids, and DNA incubated under conditions that induce Z-DNA formation. When nuclei were digested in progressive increments of ionic strength, the cleavage sites disappeared between 200 and 300 mM NaCl. Thus, presence of single-strand regions in the control region of hsp 70 is attributed to chromatin structure, possibly due to non-histone proteins, or to higher order configurations.

0080 SPECIFIC DNA REPAIR MECHANISMS MAY PROTECT SOME HUMAN TUMOR CELLS FROM DNA INTERSTRAND CROSSLINKING BY CHLOROETHYLNITROSOUREAS (CNU's) BUT NOT FROM CROSSLINKING BY OTHER ALKYLATING AGENTS. Leonard C. Erickson*, Chana Zlotogorski**, and Neil W. Gibson**, Lab. of Cell Biology and Pharmacology*, Loyola Stritch School of Medicine, Maywood, IL, and Lab. of Molecular Pharmacology**, National Cancer Institute, N.I.H., Bethesda, MD.

Human tumor cells differ in their ability to repair DNA lesions at the O^6-position of guanine. Mer^+ (Methylation repair) cells have been shown to be capable of reactivating Adenovirus which has been damaged by exposure to MNNG (Day et al., CARCINOGENESIS 1:21, 1980), and are capable of removing O^6-methylguanine from their DNA (Day et al. NATURE 288:724, 1980). Mer^- cells are deficient at both of these processes, and have no detectable O^6-methylguanine DNA methyltransferase (Yarosh et al. CARCINOGENESIS 4:199, 1983). The CNU's produce DNA interstrand crosslinks (ISC) in Mer^- cells, but not in Mer^+ cells (Erickson et al., NATURE 288:727, 1980). Recently we have shown that pretreatment of Mer^+ cells with MNNG or MNU apparently inhibits the DNA repair mechanism by which Mer^+ cells prevent CNU ISC formation. Following MNNG pretreatment, CNU produces ISC and cytotoxicity in Mer^+ cells at levels comparable to Mer^- cells (Zlotogorski and Erickson, CARCINOGENESIS 4:759, 1983). We now wish to report that the DNA lesions produced by the other ISC agents cis-Pt, HN_2, L-PAM, and a Cytoxan derivative, are apparently not repaired by this DNA repair system. Following MNNG pretreatment the ISC produced by these agents was not increased, and the cytotoxicity was also unchanged. Collectively these data suggest that adducts at the O^6-position of guanine are repaired by a specific DNA repair system, which does not recognize alkylations at other positions in DNA.

0081 BUTYRATE INDEPENDENTLY INDUCES BOTH TRANSCRIPTION OF THE GONADOTROPIN α GENE AND A CELL CYCLE SPECIFIC ARREST IN ECTOPIC TUMOR CELLS, Robert B. Darnell and Irving Boime, Washington University School of Medicine, St. Louis, Mo. 63110.
Several types of cells that do not normally express the gonadotropin α subunit do so when they are transformed (ectopic production). Somatic cell fusion studies (Stanbridge et al. Science 215:252, 1982) have correlated the ectopic expression of α protein in HeLa cells with the expression of a tumorigenic phenotype. In addition, trophoblast cells of the placenta, which do normally express α protein (eutopic production), have an extremely invasive growth pattern and anaplastic morphology. To address the relationship between expression of the single copy α gene and tumor-like phenotypes, we analyzed the effects of sodium butyrate on α expression in HeLa cells. Butyrate has been shown to stimulate α subunit production in ectopic tumor cells, but to have no effect on α expression in eutopic cells. We found that 5mM butyrate increased transcription of the α gene in HeLa cells about 10 fold. We determined whether this effect might be due to the known general action of butyrate to block cell cycling, or to a specific action on the α gene in ectopic tumor cells. In HeLa cells synchronized by a double thymidine/aphidicolin block, we found that butyrate stimulated α transcription in S phase or interphase cells, and in cells blocked in mid-S phase of the cell cycle. However, butyrate blocked cell cycle progression through G1 by an indirect action, requiring the presence of butyrate specifically late in the previous S phase. Since butyrate appears to directly affect α expression in HeLa cells, these results may indicate that the control of α gene expression in eutopic trophoblast cells and ectopic tumors differs.

0082 GENE AMPLIFICATION AND CARCINOGENESIS, Martin L. Pall, Programs in Genetics and Cell Biology, and Biochemistry/Biophysics, Washington State University, Pullman, WA 99164-4350

Gene amplification of protooncogenes has been proposed by the author to cause cancer (Proc. Natl. Acad. Sci. USA 78:2465, 1981). According to this carcinogenesis model, initiation involves a tandem duplication of a protooncogene. Promotion involves higher level amplification by a series of unequal sister chromatid exchanges in different cell cycles. Nine types of observations on carcinogens, carcinogenesis, gene amplification and cancer cells provide evidence for this model. Three of these were not available when the model was published and can, therefore, be considered experimental verification. These include evidence that tumor promoters stimulate gene amplification in cell culture, evidence for protooncogene gene amplification in cancer cells and evidence that carcinogens induce tandem duplications. Experimental evidence on this last prediction will be presented. Other support for the model includes cytogenetic evidence for gene amplification in many tumor cells (homogeneously staining regions and double minutes) and evidence for stimulation of sister chromatid exchange by carcinogens and tumor promoters. This gene amplification model is proposed as a general explanation for the initiation-promotion pattern found in chemical carcinogenesis.

0083 "IN VITRO" ACTIVATION OF DMH: MODULATION OF MUTAGENICITY AND CHROMATIN DAMAGE. Lidia C. Boffa, Claudia Bolognesi and Gianfranco Fassina, Istituto Nazionale per la Ricerca sul Cancro, I.S.T. ,Viale Benedetto XV n.10 , 16132 Genova, Italy.

The methylating carcinogen DMH (1,2-dimethylhydrazine) is,under certain experimental conditions, organospecific and produces adenocarcinoma of the colon in rodents. The compound, although is a very potent carcinogen, is not a mutagen "per se" since so far has not proven to be active in any of the available "in vitro" mutagenesis assay.
We have tried to access the possibility that DMH could be converted "in vitro" to its mutagenic metabolite(s) by the appropriate use of microsomal enzymes. In particular we have tested liver and colon rat microsomes from animals treated with β-naphtoflavone or phenobarbital, in condition that would maximize the exposure of the test system to volatile metabolites. Mutagenic potency was judged by three different criteria 1)autoradiographic identification of unscheduled DNA synthesis 2)induction of 6-thioguanine resistant mutants in V79 chinese hamster cells 3)formation of adducts of DNA bases (in particular O-6 methylguanine) and nuclear proteins aminoacids ,using ^{3}H-methyl-DMH and the appropriate analytical and detection system. Some induction of mutagenicity was detected (organospecific to some extent) in all three assays but mainly as judged by the characteristic of the DNA adducts formed.

0084 COORDINATE REGULATION OF CYTOCHROME P-450 GENES BY CHEMICAL CARCINOGENS, *Robert H. Tukey, *Steven Okino, +Sirpa Karenlampi, ¶Peter Lalley, and +Daniel Nebert. *Cancer Center, UCSD, La Jolla, CA. 92093; +NIH, NICHD, Bethesda, MD. 20205; ¶Oak Ridge National Laboratory, Oak Ridge, TN. 37830
The adverse biological effects of most chemcial carcinogens, including polycyclic aromatic hydrocarbons (PAHs), are dependent on the formation of reactive metabolites. The oxidative metabolism of such compounds are the result of induction of a specific group of cytochrome P-450s. In mice, the Ah-locus controls the induction of P-450s by PAHs such as 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Two major structural gene products of the Ah-locus are P_1-450 and P_2-450, monooxygenases that are highly inducible with PAHs and best metabolize benzo(a)pyrene and acetanilide, respectfully. Recently, cDNA clones to P_1-450 and P_3-450 mRNA have been characterized and used as probes to study the induction process. Restriction maps of $pP_1$450-57 (1,770 bp.) and $pP_3$450-21 (1,710 bp) are markedly different and clearly are derived from separate genes. With $pP_1$450-57 as a probe to mRNA from PAH treated mice, hybridization is strongest to the 2700 bp P_1-450 mRNA, with slight hybridization to a 2100 bp mRNA. $pP_2$450-21 hybridizes intensely to the induced 2100 bp P_3-450 mRNA with mild hybridization to the 2700 bp mRNA. Both mRNAs are induced coordinately in a dose response and time dependent fashion. When the cloned fragments were hybridized to the P_1-450 structural gene and substantial 3' and 5' flanking regions, a region of homology was present in the 5' portion of the P_1-450 gene. No hybridization of $pP_2$450-21 occured on either side of the P_1-450 gene, suggesting that the two genes do not lie in tandem. When DNA from hamster-mouse somatic cell hybrids that have selectively lost mouse chromosomes were used in Southern blots to map the location of the two mouse genes, the P_1-450 and P_3-450 genes were assigned to chromosome 9. Hence, P_1-450 and P_3-450 are coordinately regulated by the Ah-locus, do not appear to lie in tandem and reside on the same chromosome.

0085 TRANSIENT COMPLEMENTATION OF XERODERMA PIGMENTOSUM CELLS BY MICROINJECTION OF POLY(A)$^+$RNA, R. J. Legerski, D. Brown, C. A. Peterson and D.L. Robberson, Department of Genetics, The University of Texas System Cancer Center, M.D. Anderson Hospital and Tumor Institute, Houston, Texas 77030

Xeroderma pigmentosum (XP) is an autosomal, recessive disease of humans characterized by dermatologic and ocular symptoms and a high predisposition towards skin cancer after exposure to sunlight. Some forms of XP also have associated neurological disorders and some studies have suggested immunological abnormalities. Other studies have shown that fibroblastic tissues taken from XP patients are defective in the repair synthesis of their DNA after exposure to ultraviolet (UV) light. We have developed an assay in which the UV DNA repair deficiency of XP cells is transiently complemented, as measured by unscheduled DNA synthesis (UDS), by introduction through microinjection of cytoplasmic poly(A)$^+$RNA derived from HeLa cells. Cell lines from four different complementation groups of XP have been assayed. Groups A and G show complementation whereas groups D and F do not. Survival of cells in each of the groups subsequent to microinjection is approximately 50%. Approximately 10-20% of surviving cells from groups A and G are complemented as judged by near normal UDS. Fractionation of cytoplasmic poly(A)$^+$RNA on a 15-30% nondenaturing sucrose gradient and subsequent microinjection of the individual fractions revealed that the repair mRNA that complements XP group G sediments at 12S. We believe this assay will be of great utility in the cloning and biochmical analysis of DNA repair genes.

0086 CONVERSION OF DNA REPAIR DEFICIENT MER$^-$ HUMAN TUMOR CELLS TO REPAIR PROFICIENCY BY TRANSFECTION WITH MER$^+$ DNA. Daniel B. Yarosh, Chuck H. J. Ziolkowski and Rufus S. Day, III, National Cancer Institute, NIH, Bethesda, Maryland 20205

A group of human tumor strains which lack the ability to repair O^6-methylguanine (O^6-MeG) in DNA were previously identified by their defect in reactivation of MNNG-treated adenovirus, and have been designated Mer$^-$. These strains are hypersensitive to MNNG-produced cytotoxicity and all 12-strains which have been tested contain little or no O^6-MeG-DNA methyltransferase (O6MT), an enzyme which is present in Mer$^+$ cells and removes the methyl group from O^6-MeG in DNA. Cells from a Mer$^-$ strain were transfected with a mixture of DNA from a Mer$^+$ strain containing O6MT activity and plasmid pSV2neo DNA carrying the Tn5 neo gene, a drug resistance marker. Cells resistant to the neomycin analog G418 were pooled and seven clones were selected based on their increased resistance to MNNG-induced cell killing.

All seven clones retained the drug resistance marker. Their DNAs were all alkylated to the same extent as the parental strains by treatment in vivo with ^{3}H-MNNG, and all seven had greater post-MNNG colony forming ability than Mer$^-$ cells. One of these clones removed O^6-MeG from DNA, contained O6MT protein and activity, and reactivated MNNG-treated adenovirus as well as Mer$^+$ cells. The other six clones did not remove O^6-MeG from DNA, lacked O6MT activity and were as deficient in reactivation of MNNG-treated adenovirus as Mer$^-$ cells. Cells can become more resistant to the lethal effects of MNNG by acquisition of O6MT activity, but other functions allow cells to tolerate damage induced by alkylation.

0087 DNA DAMAGE AND INDUCIBLE REPAIR IN BACILLUS SUBTILIS. **Bradford M. Friedman, Paul E. Love and Ronald E. Yasbin. University of Rochester, Rochester, New York 14642**

DNA damage and its relationship to carcinogenesis has been actively studied in mammalian systems. These studies have, to some extent, relied on information obtained from the E. coli model. We have been involved in characterizing the inducible DNA repair mechanisms of the gram positive bacterium B. subtilis. Through a combination of molecular biological, genetic and recombinant DNA techniques, a control pattern of DNA repair has begun to emerge which varies significantly from that described for E. coli. For example, the overproduction of cloned bacteriophage repressor protein inhibits the induction of homoimmune prophage and also decreases the induction of heteroimmune prophage. A mutant strain has been constructed which causes the induction: (i) of bacterial filamentation, (ii) of prophage, and (iii) of W-reactivation at elevated temperatures. These three phenomena are part of the 'SOS'-like system of B. subtilis. The genetic analysis of this mutant strain revealed that W-reactivation and prophage induction are controlled by genes which are essential for recombination and post-replication repair while filamentation was not controlled by these genes. In addition, the protein inhibitors PMSF and antipain prevented prophage induction but did not affect filamentation and W-reactivation. These results necessitate a re-evaluation of the potential mechanisms which control SOS systems in other organisms and the relationships of these systems to carcinogenesis.

0088 IDENTIFICATION, CHARACTERIZATION AND MAPPING HUMAN TRANSFERRIN COMPLEMENTARY DNA, Yang, F., Lum, J.B., McGill, J.R., Moore, C.M., van Bragt, P.H., Baldwin, W.D. and Bowman, B.H., The University of Texas Health Science Center, San Antonio, Texas 78284.

Transferrin, the major iron transport protein in vertebrates, belongs to a family of homologous proteins that evolved by intragenic amplification of an ancestor gene in prochordates followed by repeated duplications of this gene in vertebrates. Other members of the transferrin family include lactoferrin, a protein in mammalian secretions; ovotransferrin, a protein synthesized in chicken oviducts; p97, a membrane protein antigen in all human melanoma cells and HuBlym-1, a transforming protein present in Burkitt's lymphoma cells. The study described here initiated the characterization of the genes controlling the transferrin family by identifying and characterizing human transferrin complementary DNA and mapping its chromosomal location.

Recombinant plasmids containing human complementary DNA encoding transferrin (Tf) have been isolated by screening an adult human liver library with a mixed oligonucleotide probe. Clones containing inserts have been obtained which span 2.3 kilobase pairs of Tf cDNA. Sequencing the Tf cDNA revealed a probable leader sequence followed by two domains, the amino (N) and carboxyl (C) sequences with 40% homology. Chromosomal mapping by *in situ* hybridization indicated that the *Tf* gene is located on human chromosome 3, strengthening the evidence of linkage of *Tf*, melanoma *p97* and the *Tf receptor*. The transferrin-like oncogene discovered by Diamond et al. in Burkitt's lymphoma and repeated observations of chromosome 3 translocations associated with human cancer and leukemia offer stimulation for exploring this area of the human genome for an association with malignant transformation.

0089 AP (APURINIC-APYRIMIDINIC) SITES IN DNA: INSTRUCTIVE OR NON-INSTRUCTIVE LESSIONS? Daphna Sagher and Bernard Strauss, University of Chicago, Chicago, IL 60637.

Purines, particularly adenine, are preferentially inserted opposite AP (apyrimidinic) sites at the positions of T's by either *E. coli* pol I (large fragment), polymerase α or AMV reverse transcriptase. To examine the possibility of residual information at the damaged site, leading to the incorporation of the correct nucleotide (e.g. A opposite T-derived lesions), we deaminated cytosines in DNA and removed the resulting uracils with uracil-N-glycosylase. With *E. coli* pol I (l.f.), A is incorporated more readily than G opposite both C- and T-derived AP sites. However, Novikoff hepatoma pol β adds G preferentially opposite C-derived sites and A opposite T-derived lesions. The inclusion of dAMP or dGMP in the reaction mixture does not change the specificity of insertion. However the termination pattern with *E. coli* pol I is affected by what we interpret as inhibition of the 3'-5' exonuclease activity. As expected the termination pattern with pol β remains unchanged. Our results stress the role of polymerase in base selection and hint at the complexity of the factors involved in this selection. Work is now in progress to determine the specificity of insertion at apurinic sites.
[Work supported by the NIH and the DOE]

0090 INTERACTION OF DNA POLYMERASE AND ADDUCT CONFORMATION IN SPECIFICITY OF INCORPORATION OPPOSITE CARCINOGENIC LESIONS IN DNA. Bernard Strauss and Samuel Rabkin, University of Chicago, Chicago, IL 60637.

If DNA polymerases add any nucleotide opposite non-instructional sites, that nucleotide is likely to be a purine, particularly adenine. DNA treated with N-acetoxy acetylaminofluorene does not replicate because of the formation at the C-8 position of AAF-dG (acetyl aminofluorene-dG). *E. coli* pol I (large fragment) and polymerase α can insert dC and occasionally dA opposite AAF-dG at certain sites in the DNA. When Mn^{2+} is substituted for Mg^{2+} this specificity is broadened so that T or dG can also be added. Addition of dAMP or dGMP with *E. coli* pol I (l.f.) enhances the stable incorporation of any of the dNTP's. Polymerase α seems to be more relaxed in specificity than polymerase β. At the majority of sites, DNA with a base added opposite the AAF-dG lesion is not a good substrate for further elongation. The data imply that polymerases ordinarily read AAF-dG in the *anti* configuration but that when elongation occurs with AAF-dG in the *syn* position, the rules for non-instructive lesions apply. Frame shift mutations induced by AAF-dG could be the result of slippage at lesions. [From a PhD thesis by S. Rabkin. Work supported by the NIH and DOE.]

0091 REPLICATION OF CARCINOGEN DAMAGED DOUBLE AND SINGLE-STRANDED DNA TEMPLATES IN VITRO. Karen L. Larson, Karel Angelis and Bernard Strauss, University of Chicago, Chicago, IL 60637.

UV and benzo(a)pyrene lesions in single stranded DNA are blocks to DNA polymerases in vitro. We constructed templates from bacteriophage M13 DNA with unique nicks (double stranded) or gaps (single stranded) to evaluate the effect of lesions in double stranded DNA. M13 replicative form I DNA was treated with restriction endonucleases to produce one or two cuts and the resulting linear forms were denatured and reannealed to circular viral strand molecules. DNA polymerases were challenged with lesions induced by UV, psoralen, angelicin or benzo(a)pyrene diol epoxide. In all cases, synthesis catalyzed by E. coli pol I, pol I (large fragment) or AMV reverse transcriptase stops one nucleotide before the presumed lesions. Termination occurs at these sites with polymerases producing either strand displacement or nick translation. Substitution of Mn^{2+} for Mg^{2+} does not change the pattern of termination on double stranded templates. However, when the products of this first stage reaction are extended by polymerase in a second round of synthesis, Mn^{2+} substitution can affect the pattern of elongation on double as well as single stranded templates. As yet we see no evidence that the double stranded template is more permissive.
[Supported by grants from the NIH and the DOE.]

0092 FACTORS INFLUENCING THE INITIATION OF HEPATOCARCINOGENESIS. W.K. Kaufmann and D.G. Kaufman, Univ. of North Carolina, Chapel Hill, NC 27514., J.M. Rice, Frederick Cancer Res. Fac., Frederick, MD 21701, and M.L. Wenk, Micro. Assoc., Inc., Bethesda, MD 20016
During the first cell division cycle following the proliferative stimulus of a 2/3's partial hepatectomy (PH), rat hepatocytes undergo large changes in sensitivity to initiators of carcinogenesis. To further evaluate the factors which underlie the changes in sensitivity, we have treated rats with a single dose of N-methyl-N-acetoxymethylnitrosamine (DMN-OAc) delivered into the portal vein at times when proliferating hepatocytes in regenerating livers were in defined phases of the cell cycle. Hepatic esterase activity which converts DMN-OAc to the proximate carcinogenic species, DMN-OH, was found not to vary significantly during liver regeneration. Preliminary studies also have revealed little variation in binding to DNA after treatment at selected times after PH. When livers were treated with DMN-OAc early in the prereplicative phase of growth there occurred a 9-14 h delay in the onset of S phase but DNA synthesis reached 60% of the control rate. Treating hepatocytes in S initially inhibited DNA synthesis by 80% followed by a modest recovery in rate. After 42 weeks promotion with dietary phenobarbital hepatic tumor incidences and yields were greatest in those rats treated with DMN-OAc when hepatocytes were early in the S phase. Livers treated when hepatocytes were early in G_1 were significantly less susceptible to initiation. Very few hepatic tumors were observed in rats not subjected to PH indicating a strict requirement for cell proliferation to initiate hepatocarcinogenesis. The results reveal an enhanced sensitivity of S phase hepatocytes to initiation and suggest that DNA repair activities operate before DNA replication to reduce cellular sensitivity. Supported by NIH Grant CA32238.

0093 NUCLEOTIDE SEQUENCE HETEROGENEITY IN MITOCHONDRIAL DNAs OF NORMAL AND NEOPLASTIC HUMAN LYMPHOCYTES. Raymond J. Monnat, Jr., and Lawrence A. Loeb, The Joseph Gottstein Memorial Cancer Research Laboratory, Department of Pathology SM-30, University of Washington Seattle, WA 98195.

Until recently, it has not been possible to determine the frequency and spectrum of nucleotide sequence changes occuring in individual eucaryotic genes. We have used recombinant DNA techniques to answer this question. Mitochondrial DNAs were isolated from normal human lymphocytes of 4 individual donors, and from 4 patients with lymphocytic leukemia (2 acute; 2 chronic). The mitochondrial DNAs were double-digested with the restriction endonucleases SacI and XbaI, then ligated into the filamentous phage cloning vector M13mp11. Three of the four predicted mitochondrial DNA fragments were recovered in recombinant plasmids. These fragments were identified and partially sequenced by the dideoxy chain terminator method of Sanger. Data on the frequency and spectrum of nucleotide sequence changes occuring in 50 independently isolated clones from each individual will be presented. Approximately 10,000 nucleotides have been sequenced from each individual. The majority of changes in normal individuals and leukemic patients are single-base substitutions. These changes are clonal; that is, they are present in all members of the population. The implications of the changes identified for neoplastic progression and the monoclonal origin of human neoplasms will be considered.

0094 EFFECT OF DNA REPAIR ON TRANSFECTION ACTIVITY OF φX174 RF DNA MODIFIED WITH CARCINOGENIC AND NONCARCINOGENIC BENZO[a]PYRENE DIOL EPOXIDE ISOMERS.
Moon-shong Tang, Richard P. Doisy and Michael C. MacLeod. The University of Texas System Cancer Center, Science Park, Smithville, TX 78957.
Three metabolically activated benzo[a]pyrene (B[a]P) derivatives -
(±)-7r, 8t-dihydroxy-9t, 10t-oxy-7,8,9,10-tetrahydo B[a]P (BPDE-I),
(±)-7r, 8t-dihydroxy-9c, 10c-oxy-7,8,9,10-tetrahydo B[a]P (BPDE-II), and
(±)-9r, 10t-dihydroxy-7c, 8c-oxy-7,8, 9,10-tetrahydo B[a]P (BPDE-III)
vary in their mutagenicity and carcinogenicity. Of the three isomers, BPDE-I is the most mutagenic in mammalian cells and is the only isomer with carcinogenic activity. The major BPDE-I-DNA adduct has a relatively exposed conformation and causes appreciable disruption of the double helix; in contrast, BPDE-III-DNA adducts are intercalated and therefore, relatively non-exposed. Although these three isomers differ in their reactivity and lability, which may contribute to the differences in their biological activities, the structural variation in DNA adducts per se may also affect their biological potencies. To assess the biological role of DNA adducts induced by these three isomers, we have transfected φX174 RF DNA modified with BPDE-I, -I or -III into three E. coli DNA-repair mutants and wild type cells. We have found that the repair of BPDE-I and -II DNA damage requires uvrA, uvrB and uvrC genes. However, BPDE-III damage, which is much less efficient at reducing φX174 RF DNA transfection activity, requires none of these three genes for its repair. Research supported by NIEHS grant ES 03124 and NCI grant CA 35581.

0095 INFLUENCE OF CHEMICAL CARCINOGENS AND VIRAL ONCOGENES ON THE NEOPLASTIC PROGRESSION OF SYRIAN HAMSTER EMBRYO CELLS. Tona Gilmer, David G. Thomassen, Lois Annab, and J. Carl Barrett, Nat'l Institute of Environmental Health Sciences, Res. Tri. Park, N.C. 27709
We have previously demonstrated that chemical carcinogen induced neoplastic transformation of Syrian hamster embryo (SHE) cells is a progressive, multistep process. To understand the nature of carcinogen-induced events and the role of oncogenes in this process, we have compared the susceptibilities of normal, diploid SHE cells and carcinogen-induced preneoplastic SHE cells to oncogene-induced neoplastic transformation by transfection with genomic clones of either Harvey murine sarcoma virus (HaSV) or Rous sarcoma virus (RSV). Normal SHE cells treated with HaSV DNA were morphologically normal, did not escape cellular senescence, but did grow in agar at a low frequency ($<10^{-5}$). DES-4 cells, a line of preneoplastic SHE cells treated with the human carcinogen diethylstilbestrol, formed progressively growing tumors in nude mice <3 weeks after HaSV DNA treatment while pBR DNA treated cells were nontumorigenic. Tumors formed with DES-4 cells treated with 50ng of DNA, whereas SHE cells treated with 5000ng of DNA were nontumorigenic. Cotransfection with HaSV and pSV2-neo DNAs and selection for antibiotic resistance indicated that SHE cells were recipients for exogenous DNA, but this treatment did not result in transformation of SHE cells. However, 50% of the cotransfected DES-4 cells were neoplastically transformed and expressed elevated levels of HaSV RNA. In addition, DES-4 cells transfected with RSV DNA formed tumors in nude mice while RSV DNA treated SHE cells did not. These results support the hypothesis that Ha-ras and src oncogenes can complete the neoplastic transformation of cells following carcinogen treatment but that these oncogenes alone are insufficient to cause neoplastic transformation of normal cells.

0096 EVIDENCE FOR THREE STEPS IN THE NEOPLASTIC TRANSFORMATION OF SYRIAN HAMSTER EMBRYO CELLS BY CHEMICALS AND VIRAL ONCOGENES, Mitsuo Oshimura, David G Thomassen, Lois Annab and J. Carl Barrett, Natl. Inst. of Environ. Hlth. Sci., Res. Tri. Park, N.C. 27709
Harvey sarcoma virus (HaSV) and Rous sarcoma virus (RSV) DNAs can induce neoplastic transformation of carcinogen-induced preneoplastic cells (DES-4), but not of their normal parental cells (Syrian hamster embryo fibroblasts) (Gilmer et al., UCLA symposium). We present evidence suggesting that oncogene-induced transformation of preneoplastic DES-4 cells involves 2 steps. DES-4 cells formed progressively growing tumors in nude mice after treatment with DNA from a genomic clone of HaSV. The clonal evolution of neoplastic transformation induced by HaSV DNA was examined by cotransfecting cells with pSV2-neo and HaSV DNAs followed by selection for antibiotic (G418) resistance (neo^R) encoded by pSV2-neo. Fifty percent (7/14) of neo^R clones were tumorigenic and 4/4 of those clones tested expressed Ha-ras RNA. Transfection with pSV2-neo DNA alone gave 17/18 nontumorigenic, neo^R clones. There was no correlation between growth in agar and tumorigenicity by HaSV transformed cells. Some grew in agar, expressed high levels of HaSV RNA, but were nontumorigenic. Cytogenetic analyses demonstrated that DES-4 cells are near diploid. No major karyotypic alterations were observed, irregardless of their HaSV expression, in all the 20 neo^R clones analyzed. In contrast, oncogene-induced tumor dervied cell lines at first passage had extensive structural abnormalities and a considerable number of double minute chromosomes. We propose that the Ha-ras oncogene converts DES-4 cells to a near neoplastic state and that additional karyotypic changes are required for cells to form tumors in nude mice. Thus, neoplastic progression of the cells required carcinogen-induced immortalization, expression of Ha-ras oncogene and karyotypic progression.

0097 INDUCTION OF MUTATIONS AND GENE AMPLIFICATION IN HOST CELL DNA BY HERPES SIMPLEX VIRUS INFECTION, J.R.Schlehofer, B. Matz, L. Gissmann, R. Heilbronn and H. zur Hausen, Institut für Virologie, Universität Freiburg and Deutsches Krebsforschungszentrum, Heidelberg, Fed. Rep. of Germany

Initiator-like functions of herpes simplex virus (HSV) infections are evidenced by three different approaches: (i) Partially inactivated HSV induces mutations within the HGPRT gene of the human rhabdomyosarcoma cell line, RD. This is shown by an up to 10fold increase of the mutation rate for the HGPRT gene in these cells after infection with UV- or neutral-red-inactivated HSV. (ii) HSV infection induces amplification (more than 100fold) of SV40 sequences in SV40 transformed hamster cells. It can be shown by inhibitor experiments and by using ts-mutants of virus that the HSV specified DNA polymerase is an essential function for the induction of gene amplification. Other specific cellular genes (yet unidentified) in human cells as well as viral genes (adenovirus type 12) in transformed cells also become amplified after HSV infection. (iii) The HSV mediated gene amplification as well as the induction of mutations can be prevented by coinfection with defective parvoviruses.

Since chemical and physical carcinogens also induce mutations and gene amplification which also can be prevented by infection with parvovirus, HSV shares properties with these initiating agents. This could suggest an unconventional role of HSV in cervical cancer reconciling the seroepidemiological evidence for HSV involvement in genital cancer with the lack of consistent detection of HSV-DNA within tumor biopsies.

0098 NORMAL LIVER CHROMATIN CONTAINS A FIRMLY BOUND AND LARGER PROTEIN RELATED TO THE PRINCIPAL CYTOSOLIC TARGET POLYPEPTIDE OF A HEPATIC CARCINOGEN, Sam Sorof, Stanley A. Vinores, John J. Churey, Joanne M. Haller, Bernice B. Althouse, Susan J. Schnabel and R. Philip Custer, Institute for Cancer Research, Philadelphia, PA 19111

A 14,000-dalton polypeptide was previously reported to be the principal protein target of the carcinogen, N-2-fluorenylacetamide (2-acetylaminofluorene), in liver cytosol at the start of hepatocarcinogenesis in rats. The 14,000-dalton polypeptide was purified to homogeneity according to gel electrophoreses in both $NaDodSO_4$-containing medium and acetic acid-urea, and also by immunogenicity. An immunologically related form of the cytosolic target polypeptide has now been found to be present in the nuclei of normal rat liver as a 17,500-dalton polypeptide that is firmly bound to chromatin. Extraction of the 17,500 dalton polypeptide from isolated liver nuclei or chromatin requires high ionic strength. Alternatively, short term digestion of purified liver nuclei with micrococcal nuclease solubilizes the 17,500-dalton polypeptide. In normal rat liver only nuclei and cytoplasm of hepatocytes contain reactive antigen according to peroxidase-antiperoxidase immunohistochemistry, staining most intensely perilobularly, less in the lobular midzone, and least centrilobularly. The nuclei of the perilobular hepatocytes constitute the strongest staining compartment within all of normal liver. The present findings indicate a direct connection between a chromosomal protein and an immediate principal cytosolic protein target of a carcinogen.

0099 CORRELATION BETWEEN SPECIFIC DNA ADDUCTS AND MUTATION INDUCTION IN CHINESE HAMSTER OVARY CELLS EXPOSED TO N-HYDROXY ARYLAMINES, David T. Beranek, Robert H. Heflich, Suzanne M. Morris and Frederick A. Beland, National Center for Toxicological Research, Jefferson, Arkansas 72079

N-Hydroxy arylamines are proposed to be ultimate bladder carcinogens. The biological significance of specific DNA lesions formed from a series of N-hydroxy arylamines was assessed by using a mammalian mutagenesis assay. Suspension cultures of Chinese hamster ovary cells were exposed for 1 hour to N-hydroxy-2-aminofluorene, N-hydroxy-N'-acetylbenzidine, or 1-nitrosopyrene (the metabolic precursor of N-hydroxy-1-aminopyrene). Aliquots were taken to determine the relative cloning efficiency and the mutations induced at the HGPRT locus, while the remaining cells were used to measure the formation of DNA adducts. Each compound produced a single amine-substituted DNA adduct through C8 of deoxyguanosine. A comparison of relative cloning efficiency and dose indicated that 1-nitrosopyrene was the most effective compound in reducing cell survival. The mutation frequency increased linearly with dose for all three derivatives, with 1-nitrosopyrene being approximately 50-fold more mutagenic than the other two compounds. However, when mutation frequencies were expressed as a function of C8-deoxyguanosine adducts, the relative order of mutagenic efficiency was: N-hydroxy-N'-acetylbenzidine > N-hydroxy-2-aminofluorene $\simeq$ 1-nitrosopyrene. These data demonstrate that a series of structurally-related deoxyguanosine arylamine DNA adducts induce mutations in mammalian cells with different efficiencies.

0100 DNA DAMAGE: COVALENT CROSSLINKING OF NUCLEAR MATRIX PROTEINS TO DNA, Anne E. Cress, and Kim Rainey, Arizona Health Sciences Center, Tucson, Arizona 85724

Malignant transformation of cells in animals and humans can be induced by ionizing radiation. The transformation of cells is most efficient if one uses an efficient inducer of DNA double strand breaks and DNA protein crosslinking. We have been investigating the production of covalent DNA protein crosslinks (DPC) with ionizing radiation. A particular class of non-histone chromosomal proteins becomes covalently bound to the DNA after 5,000 rads of x-rays. We have partially purified these complexes by CsCl density gradient sedimentation. The incorporation of ^{3}H-tryptophan and the use of SDS polyacrylimide gel electrophoresis reveals that the proteins involved in the DPC correspond in molecular weight, solubility, and a predominance to a subset of the nuclear matrix proteins known as the lamins. The proteins involving the DPC are constitutively synthesized since their presence is independent of concurrent RNA transcription and translation. The DNA involved in the DPC is distinct from the majority of the cellular DNA as estimated by agarose gel electrophoresis. The DPC are removed after the repair of double and single stranded DNA scissions is complete as judged by neutral and alkaline elutions. The removal process of the DPC is also refractory to RNA and protein synthesis inhibition. These data are interesting since these proteins have similar characteristics to the proposed chromatin sites for the nuclear attachment of DNA loops involved in the organization of the genome. Alterations in the DNA topology may be an important component for the transformation of cells to a malignant phenotype.

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0101 TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF NONHISTONE CHROMATIN PROTEINS (NHCP) I. PROGENITOR AND RECOMBINANT INBRED (RI) MICE HARBORING LIVER CANCER GENES. Lewis V. Rodriguez, Louis S. Ramagli, Frederick F. Becker, and James E. Womack*, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030 and*Texas A&M University, College Station, TX 77843.

Analysis of human and animal cancers indicate certain genetic states predispose to spontaneous carcinogenesis. Resulting malignancies exhibit alterations in cell function control demonstrating alteration in specific gene modulation. Further, cumulative evidence indicates NHCP catalyze specific gene transcription. Mice in this study consist of 8 RI strains derived from the progenitors C3H/HeN (male) and C57BL/6N (female) and their F1 hybrid B6C3. Progenitor strains vary drastically in spontaneous incidence (0-100%) of primary hepatocellular carcinoma (PHC), genetic constitution, NHCP, and gene expression. While genetic factors and aging influence tumor incidence in progenitor males, genetic analysis was limited since tumor incidence cannot be evaluated in individual mice of segregating F2 and backcross generations. RI strains allow a genetic approach to the number of genes responsible and for changes in gene modulating proteins that may be critical for malignancy. NHCP from aging progenitors, F1 hybrids, RI strains, and PHC's occurring in male C3H, B6C3 and RI mice were compared by high resolution 2D-PAGE. All RI strains exhibited greater than 75% NHCP homology with progenitors and F1 hybrids. Less than 3% of the total NHCP map (out of a minimum of 320 proteins per map) were common to two or more of the RI strains. Analyses of NHCP occurrance, qualitative differences in NHCP and allelic genetic compositions of progenitors and RI strains continues in both aging normal liver and spontaneous PHC's.

0102 ENHANCED TRANSFORMATION OF HUMAN CELLS BY UV IRRADIATED CHIMERIC PLASMIDS, S.A.Leadon, P.C.Hanawalt, G.Spivak, and A.K.Ganesan, Dept. of Biological Sciences, Stanford University, Stanford, California 94305

We have observed an enhancement in the yield of Gpt+ transformants when the chimeric plasmid, pSV2-gpt, was irradiated with UV (254 nm) prior to being introduced into human cells by calcium phosphate coprecipitation. UV doses up to 200 J/m^2 caused a dose dependent increase in the frequency of Gpt+ transformants. Usually 2-4 times, and occasionally 10-20 times, as many transformants were obtained from plasmid DNA irradiated with 200 J/m^2 as from unirradiated plasmid. The amount of increase was similar for an excision proficient recipient cell line (HT-1080) and an excision deficient recipient line belonging to xeroderma pigmentosum complementation group A (XP12RO-SV40). The increase was restricted to irradiated DNA and did not extend to unirradiated DNA introduced into recipient cells concommitantly with irradiated DNA. Both superhelical and linear DNA showed the increase. UV doses above 1 kJ/m^2 caused a dose dependent decrease in the transformation efficiency of pSV2-gpt which appeared to be more pronounced in the excision deficient cell line (XP12RO-SV40) than in the excision proficient line (HT-1080). Our current hypothesis is that UV irradiated DNA can be integrated into the recipient genome more efficiently than unirradiated DNA; however, heavily damaged DNA may not code for an active product as efficiently as undamaged DNA. To test this idea we are determining the number of copies of gpt integrated after various UV doses. Because the pyrimidine dimer content of the DNA should reach a steady state at about 1 kJ/m^2, dimers alone may not reduce the transforming activity of the plasmid. To identify the inactivating lesion(s) we are assaying irradiated DNA for other photoproducts, such as thymine glycols, dipyrimidine (6-4) photoproduct and DNA crosslinks.

0103 INDUCIBLE RESISTANCE TO ALKYLATING CARCINOGENS, Regine Goth-Goldstein, Lawrence Berkeley Laboratory, Berkeley, CA 94720

Chinese hamster ovary cells were treated with a highly toxic dose of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and the surviving population was exposed to a second dose. Survival (as determined by colony forming ability) was increased in the pretreated cells relative to untreated cells and resistance increased as a function of pretreatment dose. Not all alkylating agents caused increased resistance in the progeny population. Only methylnitrosourea (MNU) gave a similar response as MNNG whereas cells pretreated with ethylnitrosourea (ENU) or methyl methanesulfonate (MMS) had the same sensitivity to a second dose of the agent.

A clone isolated from a pretreated population has been characterized further. It has been stable in culture for 10 months without a change in its response to MNNG and it has the same growth rate as the parent line. It is much more resistant to MNNG ($D_0 \sim$ 8 times higher), also more resistant to MNU, slightly more resistant to MMS and has the same sensitivity to ENU as the parent line. The increased resistance to MNNG is not due to decreased drug uptake, because the same amount of ^{14}C-MNNG is bound to acid precipitable material in Cl 3 and parent line.

Our data suggest that resistance is not due to the selection of a preexisting resistant subpopulation, but rather induced by the alkylating carcinogen.

0104 CHROMOSOMAL ANOMALIES IN OVARIAN TUMORS, Marie C. De Blois, Jean M. Bénard, and Guy F. Riou, Institut Gustave Roussy, 94805 Villejuif cedex.

The study of human chromosomes by high banding techniques has shown that chromosomal defects are present in most neoplasia. We have analysed the karyotype of 5 human ovarian tumors (4 papillary adenocarcinomas and 1 dysembryoma). Fifty mitotic cells were studied for each cellular material. One ovarian adenocarcinoma exhibited two markers constantly detected in all the cells whatever the passages of culture (1rst, 5th, 20th) and in nude mice : a paracentric inversion beared by the short arm of chromosome 3 with break points located in 3q13 and 3q26 and a translocation between chromosomes 2 and 5, t(2,5)(q37, q32). The other tumors showed the following features : a rearrangement of chromosome 7, an elongation of one achrocentric chromosome (presumably chromosome 13), and a third unidentified marker. All the examined cells showed these three markers. An hepatic metastasis corresponding to one of these adenocarcinomas presented the same markers. Other ovarian tumors are under current analysis. The translocation t(6,14) was not observed (Wake, N. et al., Cancer Res. 40, 4512-4518, 1980). In some tumors, chromosomal abnormalities could be correlated to the presence of cellular oncogenes. Investigations are in progress to characterize oncogene sequences in these ovarian tumors.

0105 MECHANISMS OF DNA METHYLATION IN NORMAL AND ONCOGENICALLY TRANSFORMED CELLS. Steven S. Smith, Mary Ellen Tolberg, and Michael Henke, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

*Hpa*II/*Msp*I restriction analysis coupled with high-performance liquid chromatography have been used in conjunction with Southern blotting with probes of several types to compare DNA methylation patterns in normal and oncogenically transformed cells. Some, but not all, oncogenically transformed cells that we tested were found to be hypomethylated relative to their normal counterparts. Apparent hypomethylation was observed in the DNA of the mature nondividing lymphocytes of patients with chronic lymphocytic leukemia. Thus, it is not correlated with the rapid cell division often associated with neoplasia. Studies of the hypomethylation observed in Friend-cell DNA suggest that it may be the result of an accumulation of discrete alterations in methylation state concertedly affecting widely dispersed but closely related sequences as groups. In the Friend cell line, the observed patterns are invariant during hexamethylene-bis-acetamide induction of differentiation, and appear to be stable during normal cell division in culture. Taken together, the results suggest that the altered methylation pattern of these cells may set new limits on their transcriptional potential and may be an important somatic determinant of the transformed phenotype that they exhibit.

0106 SYNTHESIS OF SIMIAN VIRUS 40 CHROMOSOMES IN NUCLEAR EXTRACT FROM DIHYDROXYANTHRAQUINONE-TREATED CELLS, Robert T. Su and Xiaojun Ma, University of Kansas, Lawrence, KS 66045

The effect of dihydroxyanthraquinone(DHAQ), a new antitumor drug, on mammalian chromosome replication was investigated using simian virus 40(SV40) as a model system. The maximum effect of inhibition on viral DNA synthesis was observed within 30-40 minutes after the addition of the drug. The extent of inhibition of viral DNA synthesis appeared to directly related to the number of viral replicons which interact with DHAQ molecules in vivo.

No apparent strand breakage of SV40 DNA was observed in infected cells treated with DHAQ ranging from 0.3 μM to 10 μM. However, strand breakage was induced upon cell lysis presumably by released nuclease. Repair of the damaged SV40 chromosomes in vitro resulted in the synthesis of completed supercoiled SV40 DNA. This repair synthesis was mostly confined to the region containing the replication origin of SV40 DNA as judged by the digestion of DNA with restriction endonucleases Hind II and Hind III. Since SV40 DNA sequences close to the origin of replication are not complexed with histones to form a nucleosome structure, the results suggested that DHAQ may disturb chromosome structure by interacting preferentially to the nucleosome-free regions and causing the aberrant gene duplication and expression.

0107 FIBROBLASTS FROM FANCONI'S ANEMIA PATIENTS ARE ABNORMALLY SENSITIVE TO THE 7,8-DIOL-9,10-EPOXIDE OF BENZO(a)PYRENE, BUT NOT TO ULTRAVIOLET RADIATION. Veronica M. Maher, Masami Watanabe, and J. Justin McCormick, Michigan State University, E. Lansing, MI 48824.

We compared fibroblasts derived from skin or lung tissue from a series of such patients to cells from a series of normal persons for sensitivity to the mutagenic and/or cytotoxic effect of a reactive metabolite of benzo(a)pyrene, 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE). Cells from all six FA strains tested were more sensitive than cells from normal newborns to the killing effect of this agent, but all showed a normal sensitivity to the killing effect of UV 254nm radiation. Three of the FA strains were from fetal material (one from lung, two from skin). Therefore, we also compared the response of skin and lung fibroblasts from a normal fetus. These cells proved just as sensitive as the 6 FA strains to killing by BPDE, but showed normal sensitivity to UV. We then compared 3 FA and 3 normal strains for sensitivity to the mutagenic action of BPDE. There was no significant difference between any of the 6 strains. The mechanism responsible for the increased sensitivity of the FA cells and the fetal cells is under investigation. Supported by NCI grant CA 21253 and DOE Contract 0459.

0108 CELLS FROM PATIENTS WITH HEREDITARY CUTANEOUS MALIGNANT MELANOMA ARE ABNORMALLY SENSITIVE TO THE MUTAGENIC EFFECT OF SIMULATED SUNLIGHT AND 4-NITROQUINOLINE-1-OXIDE. J. Justin McCormick, Jeffery N. Howell, Rebecca Corner, and Veronica M. Maher, Michigan State University, E. Lansing, MI 48824 and Mark H. Greene, Environmental Epidemiology Branch, National Cancer Institute, Bethesda, MD 20205

Fibroblasts derived from patients with an hereditary form of malignant melanoma of the skin and the dysplastic nevus syndrome (HCMM/DNS) were compared with cells from normal persons for sensitivity to the mutagenic and/or cytotoxic effects of simulated sunlight and 4-nitroquinoline-1-oxide (4NQO). All ten HCMM/DNS cells tested were significantly more sensitive than normal to the killing effect of 4NQO (slopes of survival curves were 2- to 3-fold steeper); but only one strain out of 5 strains tested was hypersensitive to killing by Sun Lamp radiation. Two HCMM/DNS strains, one from the most sensitive group (3072T) and one with intermediate sensitivity (3012T) were compared with several strains of normal cells for the response to the mutagenic effect of these agents. Both proved significantly more sensitive than normal to the mutagenic effect of 4NQO (slopes of mutation curves, 7-fold steeper than normal). 3072T was more sensitive than 3012T. Only strain 3072T showed an abnormally high frequency of mutants induced by simulated sunlight. Evidence that the increased sensitivity to 4NQO in HCMM/DNS results from a greater ability to metabolize this compound to the reactive intermediate 4-HAQO comes from our finding that the HAQO survival curves of strains 3072T and 3012T are superimposable on those of normal fibroblasts. These results suggest that hypersensitivity to mutagens contributes to risk of melanoma and support the somatic cell mutation hypothesis for the origin of cancer. Supported by NCI-CP-FS-11029-63.

0109 DNA STRAND BREAK AND CROSS-LINK REPAIR IN TWO CANCER PRONE DISORDERS. Harris S. Targovnik, Hatsumi Nagasawa, John B. Little and Richard J. Reynolds. Harvard School of Public Health, Boston, Ma. 02115.

Gardners Syndrome (GS) and Basal Cell Nevus Syndrome (BCNS) are autosomal dominant inherited disorders which predispose to cancer. There has been considerable speculation that genetic susceptibility to cancer might be related to a DNA repair defect. Only in the case of xeroderma pigmentosum, has a defect been identifed in a specific molecular repair process.

Cultured diploid fibroblasts from GS and BCNS patients have been extensively studied in our laboratory with respect to their response to physical and chemical agents. GS fibroblasts display sensitivity to 254 nm UV light, x-rays and Mitomycin C (MMC) while BCNS fibroblasts are only mildly sensitive to these three agents (J.B. Little et al. Mutation Res. 70 (1980) 241-250; T. Kinsella et al. JNCI 68 (1982) 657-701; H. Nagasawa and J.B. Little Carcinogenesis 4 (1983) 795-798; G.L. Chan and J.B. Little Amer. J. Path. 111 (1983) 50-55).

Using the technique of alkaline elution we have examined the induction of DNA strand breaks following ionizing radiation and cross-links (DNA-DNA and DNA-protein) following treatment with MMC. No difference has been observed for the induction or repair of single strand breaks at doses up to 600 Rads when compared to normal human fibroblast controls. Double-strand break repair is currently being investigated. The production of both DNA-DNA and DNA-protein cross-links is identical in BCNS and normal human fibroblasts at the concentrations tested (5×10^{-7} M to 10^{-5} M MMC). BCNS fibroblasts appear to have no defect in the removal of DNA-DNA cross-links following 5×10^{-6} M MMC. Data for the repair of DNA-protein cross-links will be presented.

This work supported by Grant Nos. CA-09078 and CA-11751 from the N.C.I.

0110 ACTIVATION OF SILENT PROVIRAL ONCOGENE EXPRESSION BY MUTAGENS, L.P. Turek and L. Cooling, Dept. Pathology, VAMC and Univ. of Iowa College of Medicine, Iowa City, IA

DNA mutagens are frequently carcinogenic. While the molecular mechanism underlying chemical carcinogenesis in most specific systems is not understood, activation of the ras gene family is frequently associated with point mutations resulting in an altered peptide product. Here we describe activation of structurally non-defective, yet transcriptionally silent, v-onc genes in response to physical and chemical mutagens. We have derived and characterized a set of non-transformed ("flat") normal rat kidney (NRK) cell clones which contain either a silent v-src of the avian sarcoma virus or silent v-fes of the feline sarcoma virus. In contrast to v-onc transformed cells, the v-fes or v-src mRNA levels are below detection in the silently infected cell clones. The unexpressed v-onc genes are biologically active in transforming virus rescued from these cells. These flat cells, however, segregate typical transformants which express the respective v-onc gene; the conversion occurs at rates predicted for somatic mutation. We have therefore examined the effect of known physical mutagens (UV light, X-rays), and several alkylating agents on the transformation rate. The silently infected clones were highly susceptible to mutagen transformation. The response was dependent, and different mutagens exhibited different efficiency in individual clones. Transformation was accompanied by activation of either v-fes or v-src mRNA transcription to levels found in control virus-transformed cells. We are currently studying DNA structure and postreplicational modification of the unexpressed and activated v-onc genes, and their adjacent viral and cellular sequences.

0111 CHROMOSOMAL ALTERATIONS ASSOCIATED WITH AMPLIFICATION OF THE GENE FOR ASPARAGINE SYNTHETASE, Irene L. Andrulis and Louis Siminovitch, Hospital for Sick Children, Toronto, Ontario, Canada. M5G 1X8.

Certain tumors express little or no asparagine synthetase activity and are sensitive to the chemotherapeutic drug, asparaginase. Those cells which are resistant to treatment with asparaginase often have elevated levels of asparagine synthetase. A model system has been developed in cultured Chinese hamster ovary cells to study the regulation of asparagine synthetase activity. Using the amino acid analog, albizziin, resistant lines have been isolated with up to 300-fold elevations in the levels of asparagine synthetase activity. Karyotypic analyses of the albizziin-resistant lines show chromosomal alterations including breaks and rearrangements affecting the long arm of chromosome 1. Highly resistant lines contain chromosomes with homogeneously staining regions at the break points of translocations. The homogeneously staining regions are most likely the sites of amplification of the gene.

Poly A^+ RNA from the highly resistant lines was used to prepare cDNAs. Recombinant plasmids containing cDNAs for asparagine synthetase were identified by differential hybridization and immunoprecipitation of asparagine synthetase translated from hybrid selected mRNA. Using the asparagine synthetase recombinant DNA as probe the albizziin resistant lines were examined and shown to contain amplified copies of the gene. We are now in the process of examining the copy number and arrangement of the human gene for asparagine synthetase in cells from unaffected normals and patients with acute lymphocytic leukemia.

0112 PREFERENTIAL BINDING OF AROMATIC AMINES TO RAT REPETITIVE DNA SEQUENCES IN VIVO.
Ramesh C. Gupta and Nutan R. Dighe, Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030.

We have examined the distribution of adducts in the repetitive DNA sequences in rats following a single i.p. dose of the carcinogen N-hydroxy-2-acetylaminofluorene (N-OH-AAF) or N-hydroxy-2-acetylaminophenanthrene (N-OH-AAP). Repetitive fragments (82, 125, 179, 225, and 370 bp) were isolated by restriction of hepatic DNA with Hind III endonuclease and gel electrophoresis. Adducts were analyzed by a previously described ^{32}P-assay. The levels of N-OH-AAF-induced adducts, after 24 h treatment, in 179, 225, and 370 bp sequences were found to be 13.6, 2.0, and 3.0 times higher, respectively, than in total DNA, while the other two sequences were modified to similar extents. The relative distribution of specific adducts was significantly different in the various sequences. After 9 d treatment, all the DNA repeats showed 1.3 - 1.7 times higher binding than in total DNA. For N-OH-AAP, adduct levels in 179, 225, and 370 bp sequences, after 24 h, were 2.8, 1.7, and 1.8 times higher and after 9 d, 1.3, 1.6, and 1.3 times higher, respectively, than in total DNA. In contrast to the in vivo results the specific and total adducts were found to be distributed randomly when DNA was reacted in vitro with N-acetoxy-AAF. Taken together, these results suggest that the enrichment and differential excision of adducts in the DNA repeats may be a function of hetrochromatin in which they are organized. (Supported by USPHS Grant CA30606).

0113 CLEAVAGE AT IONIZING RADIATION-ALTERED PYRIMIDINES BY GAMMA ENDONUCLEASE OF MICROCOCCUS LUTEUS.
Patricia Hentosh[1], William D. Henner[2], and Richard J. Reynolds[1], [1]Harvard University, School of Public Health and [2]Dana Farber Cancer Institute, Boston, MA 02115

Micrococcus luteus extract contains endonuclease activity which is capable of cleaving gamma-irradiated DNA (Paterson and Setlow, P.N.A.S., U.S.A. 69: 2927, 1972). We have used DNA fragments of defined sequence and DNA sequencing techniques to study the sites (sequence specificity) of cleavage by this activity. ^{32}P end-labelled DNA restriction fragments of pBR322 were gamma-irradiated under N_2 and in the presence of KI, conditions which minimize the ratio of strand breaks to enzyme sensitive sites. Gamma-irradiated DNA or control unirradiated DNA fragments were treated with M. luteus extract and analyzed by electrophoresis on denaturing polyacrylamide gels. The M. luteus extract cleaved irradiated DNA with strong specificity for sites of cytosine and thymine. Little or no cleavage occurred at purine sites.

Strand breaks produced in gamma-irradiated DNA by M. luteus extract contain a 5' phosphate terminus. The presence of a 5' phosphate terminus was confirmed by the alteration in electrophoretic mobility observed after phosphatase treatment of 3' end-labelled DNA fragments.

These results suggest that both radiation-altered cytosines and thymines may be important sites for excision repair after gamma-irradiation of M. luteus. (Supported by NIH grant No. CA 09078).

0014 CLOSELY OPPOSED PYRIMIDINE DIMERS ARE NONRANDOMLY INDUCED
Lun H. Lam, John B. Little and Richard J. Reynolds
Harvard School of Public Health, Boston, MA 02115

Irradiation of DNA with far-UV light induces lesions that are converted to double-strand breaks by incubation with enzymatic activities from Micrococcus luteus. These enzymatically-induced double-strand breaks are quantified by velocity sedimentation through neutral sucrose density gradients. No double-strand breaks are detected if the UV-irradiated DNA is first photoreactivated in vitro with E. coli photoreactivating enzyme. In addition, no double-strand breaks are detected if only one of the complementary strands of reannealed DNA duplexes has been UV-irradiated. These results suggest that the lesions which give rise to the enzymatically-induced double-strand breaks are closely opposed pyrimidine dimers.

Many more closely opposed dimers are induced at low fluences of UV light than are predicted by models which are based on the assumption that individual dimers are randomly induced. This suggests that closely opposed dimers might be induced in a nonrandom manner. To test this hypothesis, several UV-irradiated ^{32}P end-labeled DNA restriction fragments of defined sequence were each incubated with the M. luteus enzyme preparation and electrophoresed through nondenaturing polyacrylamide gels. A random distribution of closely opposed dimers would have resulted in long smears in the corresponding lanes on autoradiographs. Instead, discrete bands of characteristic and reproducible patterns were observed, demonstrating that the closely opposed dimers were distributed in a nonrandom manner in each of the DNA sequences.
(Supported by NIH grant No. CA 09078)

0115 AFLATOXIN B_1-DNA ADDUCT FORMATION IN NORMAL RAT AND HEPATOMA TISSUE CULTURE CELL AND IN HUMAN LIVERS FROM FETAL, REYE'S SYNDROME AND HEPATOCELLULAR CARCINOMA

Stringer S. Yang[1], Rama Modali[1] and George C. Yang[2] [1]Laboratory of Cellular Oncology, NCI, Bethesda MD 20205 and [2]Division of Chemistry And Physics, FDA, Wash., DC 20204

Alatoxin B_1 (AFB_1), an Aspergillus flavus toxin, is known to be highly carcinogenic, mutagenic and teratogenic in numerous animal species including trout, mouse, rat, hamster, dog, primate and human. Of striking interest is its extreme potency in hepatocarcinogenesis (1). AFB_1 binds to the N^7 of guanine in both DNA and RNA to form adducts (Fig. 1)(2). AFB_1 were detected in human primary hepatocellular carcinoma samples, and in liver, blood and urine of patients with Reye's syndrome. The characteristic of the DNA fragments preferentially targetted by AFB_1 and the mechanism by which neoplastic transformation is induced are the focus of this communication. AFB_1 binding to DNAs prepared from (A) a human fetal liver obtained by autopsy, (B) a liver sample of a patient with Reye's syndrome, and (C) MAH hepatocellular carcinoma was investigated. AFB_1-DNA adducts were analyzed by polyacrylamide gel electrophoresis. Small DNA fragments bound to [^{3}H]-AFB_1 were readily detected. Their estimated molecular sizes were 430 bp (c), 310 bp (d), and 185 bp (e). Some AFB_1 binding with larger DNA fragments, 1080 bp (a) and 680 bp (b), were also observed. When analyzed by DNAase and RNAase hydrolyses the majority of the adducts was DNAase sensitive (75%) and only a minor fraction (25%) was RNAase sensitive. We are currently isolating these AFB_1-DNA adducts for resolving their nucleotide sequences and for studying the possible biological activity in the cell since AFB_1 was considered an initiating agent for neoplastic transformation. (1) Miller, E.C. & Miller, J. A. (1981) Cancer 47, 2327-2345. (2) Bennett et.al. (1981) Cancer Research 41,650-654.

0116 DECREASED ENDOGENOUS DNA REPAIR SYNTHESIS AND RESTRICTION OF TEMPLATE CAPACITY IN CHROMATIN AND NUCLEI ISOLATED FROM 2-ACETYLAMINOFLUORENE INDUCED PRENEOPLASTIC NODULES.

John Y. H. Chan and Frederick F. Becker, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

In attempting to identify the genomic alterations associated with chemical induced neoplasia, in vitro DNA and RNA synthesis was compared in chromatin isolated from preneoplastic liver nodules induced by 2-acetylaminofluorene (AAF) and liver from control rats. In the nodular chromatin, the endogenous DNA synthesis was 3 to 4-fold lower than that of normal liver, while endogenous RNA synthesis was only 20% lower. When the template capacity of these chromatins was assayed with rat liver DNA polymerase β , E coli DNA polymerase 1 (Pol.1), and E coli RNA polymerase, the nodular chromatin was extremely repressed in its template capacity with polymerase β , but less so with Pol.1, and not repressed at all with RNA polymerase. Similar differences in DNA repair synthesis in vitro were also observed in nuclei isolated from these tissues when assayed in the presence of aphidicolin to inhibit DNA polymerase α activity. This defect was neither due to a difference in the polymerase activity nor did it correspond to the amount of nucleases present. It is apparently due to the restriction of template capacity of the nodular DNA by associated chromosomal components since the isolated DNA did not exhibit such differences. The results also indicate that the nodular chromatin contains inaccessible regions for repair enzymes, which may then account for the increase in DNA damage previously observed.
Supported by NIH grant CA 20657.

0117 THE TRANSFORMING ACTIVITY OF SV40 AND THE EJ BLADDER CARCINOMA GENE IN HUMAN FIBROBLASTS,

Robert J. Zimmerman and Peter A. Cerutti, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland

We have investigated the frequency of in vitro oncogenic transformation as measured by foci formation above a monolayer, or anchorage independent (AI) growth induction, in human fibroblasts from normal, Fanconi's anemia (FA), and Bloom's syndrome (BS), following transfection of an origin-defective SV40 containing plasmid. Both normal and FA cells were transformed by both criteria at frequencies of approximately 20 colonies/μg plasmid/10^6 transfected cells. BS cells, on the other hand, were found to transform at 10 to 15-fold lower efficiencies, which was not due to resistance to the uptake of $CaPO_4$-precipitated DNA. Further, the transformed phenotype was unstable in BS cells. We hypothesize that the inherent chromosomal instability of BS affects the integration and expression of the transfected DNA.

In parallel experiments, we have transfected constructions containing either the normal or the mutated cellular proto-oncogene from the EJ bladder carcinoma, with or without the dexamethasone inducible LTR from MMTV linked upstream of this c-Ha-ras 1 gene. We have selected colonies after co-transfection with the pSV-neo plasmid and assayed these cells for either foci formation or AI growth in the presence or absence of dexamethasone. We have not been able to demonstrate either foci formation or AI using any of the EJ constructions in these human fibroblasts. We conclude that the c-Ha-ras 1 gene alone, in contrast to SV40 T-antigen, is insufficient to induce transformation, even in fibroblasts from patients genetically predisposed to cancer development.

0118 DEGREE OF PLOIDY ABNORMALITY AND GRADE OF MALIGNANCY, A. Zetterberg[1,2] and G. Auer[1], [1]Department of Tumor Pathology, Karolinska Institute, 10401 Stockholm, Sweden and [2]Department of MCD Biology, University of Colorado, Boulder, CO 80309

In a systematic retrospective study on human tumors we have investigated the relationship between "gross ploidy level", as determined from DNA-cytophotometry, and clinical course in terms of metastasis formation and death from the tumor disease. We have optimized methods for large scale quantitative cytophotometric DNA-analysis of individual tumor cells in old cytological slides, which were originally used in the routine diagnostics, and which have been stored in our files for up to 20 years. We have analysed nearly one thousand tumors. A very close correlation between the cytophotometric DNA-data and clinical course was found. Low-grade malignant tumors, with no or very slow progress over a 10-20 year period were almost invariably characterized by having predominantly diploid or combined diploid-tetraploid DNA-contents. Metastases from the low-grade tumors were similar to the primary tumors in this respect, and the diploid-tetraploid "DNA pattern" appeared to be stable, since it was most often unchanged in metastases appearing decades after the diagnosis of the primary tumor. High-grade malignant tumors, that rapidly developed metastases, and killed the patients within 1-3 years, were almost invariably characterized by having a very small (or no detectable) proportion of tumor cells with diploid DNA-contents. Instead these tumors contained increased and highly variable amounts of DNA, suggesting a considerably higher degree of aneuploidy in these more aggressive tumors, than in the low-grade variants. Taken together these data seem to reflect the importance of genomic instability, at the chromosomal and/or gene level, for the rate of progression of the tumor disease.

0119 HYPERSENSITIVITY AND HYPOMUTABILITY OF LYMPHOBLASTS FROM FAMILIAL MELANOMA PATIENTS FOLLOWING UV IRRADIATION, Robert G. Ramsay, Wendy Relf and Chev Kidson
Queensland Institute of Medical Research, Brisbane, Australia, 4006.

Cell lines from familial melanoma (FM) patients have been shown to be UV sensitive (1,2). Those cell lines from sporadic melanoma patients (SM) do not differ from normals in their response to UV.

Attempts to show defects in excision and post replication repair have been unsuccessful. While induced sister chromatid exchange frequencies following UV treatment were normal, chromosome aberrations were higher in FM cell lines. This was also found following 4NQO and mitomycin C treatment, but not after γ-irradiation where the frequency of induced aberrations was normal.

When cell lines were assayed for UV induced mutagenesis, xeroderma pigmentosum cell lines (gps A, D & V) were most mutable and SM cells were induced to a similar degree as normals. Seven FM cell lines however were refractory to UV induced mutagenesis. When treated with EMS, FM cell lines were found to be as mutable as controls and SM cells by this agent. The analogies that exist between the response of FM cell lines and mutants of the SOS repair pathway *E. coli* (eg. *lex A ind*$^-$ mutants) are compelling. Therefore, in these skin cancer prone individuals UV sensitivity is not associated with higher UV induced mutagenesis. This challenges the view that solar radiation operates only as a mutagen in UV sensitive individuals.

(1) Ramsay *et al* (1982) Cancer Res. *42* pp 2909-2912.
(2) Smith *et al* (1982) Int. J. Cancer, *30* pp 3945.

0120 ADP-RIBOSE IN THE REPAIR OF DNA DAMAGE AND IN CARCINOGENESIS.

Sydney Shall, University of Sussex, Brighton, Sussex, England.

ADP-ribosylation of chromatin proteins is a component of efficient DNA excision repair, probably because it regulates DNA ligase activity. Nuclear ADPRT is entirely dependent on DNA for its activity because it has a specific requirement for ends of DNA strands. DNA damage activates nuclear ADPRT, stimulates the synthesis of $(ADP\text{-}ribose)_n$ and lowers cellular NAD levels, these responses seem to be in proportion to the number of sites in the DNA being repaired by strand breakage. The inhibitors of nuclear ADPRT especially benzamides retard DNA excision repair, and synergistically potentiate the cytotoxicity of many DNA-damaging agents, including both chemicals and radiation. In addition, these enzyme inhibitors substantially increase the frequency of sister chromatid exchanges. Transformation of NIH 3T3 cells by SV40 or gpt plasmids is esentially blocked by 3-aminobenzamide. The conclusions derived from inhibitor studies have been extended by the isolation, after mutagenesis and selection, of cell variants of mouse leukaemic L1210 cells. These cell variants confirm the regulation of DNA ligase by ADPRT activity. Experiments indicate that human tumour cells may be deficient in this nuclear enzyme system. We suggest that inadequacies of the ADP-ribose system may enhance the frequency of translocations and chromosomal aberrations. Thus, defects in the nuclear ADP-ribose system may be part of the genesis or progression of human tumours.

0121 THE NATURE OF PERSISTENT CARCINOGEN-CHROMATIN DAMAGE
Arend Kootstra, Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges s/Lausanne, Switzerland.

Excision repair of a variety of carcinogenic lesions invariably leads to persistent damage in the intact eukaryotic cell. When human lung epithelioid cells were treated with Benzo(a)-pyrene diol epoxide (anti), analysis of the distribution of the carcinogen was analysed by DNase I digestions of nuclei at various points during the excision repair process. The data obtained strongly suggests that the majority greater than 90% of the persistent DNA adducts are associated with the inactive region of the chromatin. These results imply that for bulky carcinogen-DNA adducts a preferential binding occurs in the active fraction of the genome and that the excision repair mechanism may be largely concerned with the removal of potentially cytotoxic and mutagenic lesions in the functionally active region of the genome.

Poster Session No. 2

0122 HEMATOPOIETIC CELL TRANSFORMATION BY A MURINE RECOMBINANT RETROVIRUS CONTAINING THE SRC GENE OF ROUS SARCOMA VIRUS, Jacalyn H. Pierce, Stuart A. Aaronson, National Cancer Institute, Bethesda, Maryland 20205 and Steven M. Anderson, Merck, Sharp & Dohme Research Laboratories West Point, Pennsylvania 19486

A recombinant murine retrovirus (MRSV) containing the src gene of avian Rous sarcoma virus (RSV) was shown to induce hematopoietic colonies in infected mouse bone marrow. MRSV-induced colony formation followed single-hit kinetics and required mercaptoethanol in the agar medium. Cells from the colonies induced by MRSV could be established as continuous cell lines which demonstrated unrestricted self-renewal in vitro and tumorigenicity in vivo. The transformants, all of which expressed high levels of the Rous sarcoma virus transforming protein, pp60^{src}, appeared to be at an early stage in lymphoid cell differentiation. They lacked Fc receptors and detectable immunoglobulin mu chain synthesis, markers normally associated with committed B cells. The majority of the MRSV-transformed cell lines contained high levels of terminal deoxynucleotidyl transferase, an enzyme present in lymphoid progenitor cells committed to the T cell lineage. One cell line expressed Thy-1 antigen, but none expressed Lyt-1 and Lyt-2, markers of more differentiated T cells. These findings represent the first demonstration that the src gene is capable of transforming cells of hematopoietic origin.

0123 SV40 SURFACE T ANTIGEN: REACTIVE SITES MAPPED WITH MONOCLONAL ANTIBODIES
Roger Hand and Lori Whittaker, McGill Cancer Centre, Montreal, H3G 1Y6

T antigen transforms animal cells from several species. Recent studies show it is on the cell surface. As in other tumor virus systems, this may be important for transformation. We have used a radioimmunoassay to map epitopes on living and formaldehyde-fixed transformed cells with six different monoclonal antibodies to T antigen. Irrelevant monoclonal antibodies of the same subclass served as controls. With the transformed mouse line SVT2, antibodies PAb 101 and PAb 423, which bind to the C-terminal region of T antigen, reacted with fixed and living cells. Of the antibodies against the N-terminus, PAb 430 did not react, while PAb 416, reacted with living cells only. PAb 1700, a new antibody, reacted similarly to PAb 416 and therefore is directed at or near the same epitope. PAb 100, directed against the central region of T antigen, did not react. Untransformed 3T3 cells, from which SVT2 cells are derived, did not react specifically with any of the monoclonal antibodies. The SV40-transformed human cell line SV80, and the hamster cell line CHLwt reacted similarly to SVT2. We conclude that the C-terminus and the N-terminal region containing the reactive site of PAb 416 are on the outer surface of the cell membrane. The central region and the N-terminal region containing the site for PAb 430 are buried in the membrane or blocked. Fixation blocks the site detected with PAb 416. Since lines from three species gave similar results, T antigen is most likely inserted in the membrane of all transformed cells in a specific manner.

0124 THE PHOSPHORYLATION OF RIBOSOMAL PROTEIN S6 IS INCREASED FOLLOWING MICROINJECTION OF $pp60^{v\text{-}src}$ INTO XENOPUS LAEVIS OOCYTES, Jordan G. Spivack, Raymond L. Erikson* and James L. Maller, University of Colorado School of Medicine, Denver, CO 80262 and *Harvard University, Cambridge, MA 02138.

Rous sarcoma virus (RSV) is able to transform cells through the expression of a single gene product, termed $pp60^{v\text{-}src}$, which is a protein kinase that phosphorylates tyrosine residues. One clue to the interaction between $pp60^{v\text{-}src}$ and the host cell is that ribosomal protein S6 is phosphorylated in RSV-transformed cells even in the absence of serum (Decker, S., PNAS 78: 4112, 1981). Normally, S6 is not phosphorylated in serum-starved cells in culture, but is rapidly phosphorylated in response to many stimuli that promote protein synthesis and cell division. We have microinjected purified $pp60^{v\text{-}src}$ into Xenopus oocytes and observed an increase in S6 phosphorylation on serine residues. Two-dimensional gel electrophoresis of ribosomal proteins confirmed an increase in the absolute phosphate content of S6. Since the $pp60^{v\text{-}src}$ preparation lacked S6 kinase activity in vitro, we propose that $pp60^{v\text{-}src}$ directly or indirectly activates a kinase and/or inactivates a phosphatase for S6. Microinjection of $pp60^{v\text{-}src}$ into oocytes also led to a 2-3 fold increase in the level of phosphotyrosine and a slight elevation in total protein phosphorylation. Additionally, $pp60^{v\text{-}src}$ dramatically shortened the time course of progesterone-induced maturation of oocytes, but appeared unable to cause maturation by itself. These experiments suggest that microinjection of oocytes may prove useful in identifying the pathway(s) by which growth is regulated by $pp60^{v\text{-}src}$ and the role of tyrosine phosphorylation in early development.

0125 QUAIL EMBRYO FIBROBLASTS TRANSFORMED BY FOUR V-MYC-CONTAINING VIRUS ISOLATES SHOW ENHANCED PROLIFERATION BUT ARE NONTUMORIGENIC, Steven Palmieri[1], Patricia Kahn, and Thomas Graf, European Molecular Biology Laboratory, 6900 Heidelberg, West Germany and [1]NIH, NIAID, Rocky Mountain Laboratories, Hamilton, MT 59840

Quail embryo fibroblasts infected with any of the four natural avian myc gene-containing virus strains (MC29, CMII, OK1Ø and MH2) or with the myb, ets-containing E26 acute leukemia virus, were examined for their expression of several transformation-associated parameters. All myc-containing viruses, but not E26 or Rous sarcoma virus (used as a control) induced a dramatic stimulation of cell proliferation. In addition, the myc-virus transformed cells exhibited prominent nucleoli, possibly as a consequence of their increased proliferation. Cells transformed by MC29, OK1Ø, MH2, and E26 were capable of growing in semisolid medium and showed a loss of actin cables and, except for the E26-transformed cells, of an ordered fibronectin network. The CMII-transformed cells resembled normal cells in these properties but exhibited an abnormal fibronectin distribution. All of the myc-virus transformed fibroblasts, as well as the E26-transformed cells, were unable to form tumors in nude mice, indicating that the myc gene (and the myb/ets genes) are not sufficient for the induction of a fully malignant phenotype in fibroblasts.

0126 ADENOVIRUS TYPE 12 REGION E1 SUPPRESSES THE EXPRESSION OF MHC CLASS 1 GENES IN TRANSFORMED RAT CELLS, René Bernards, Peter Schrier, Alex van der Eb. University of Leiden, Holland.

We have found that oncogenic and non-oncogenic human adenoviruses differ in their potential to modulate the expression of cellular genes in transformed cells. At least two genes are specifically suppressed by the highly oncogenic Ad12 but not by the non-oncogenic Ad5. These proteins are also expressed in untransformed rat cells. One of these proteins has been identified as the heavy chain of the class 1 transplantation antigens encoded by the major histocompatibility complex. Decreased expression of this protein is brought about by the product of the 13S E1a mRNA at the level of mRNA synthesis. The virtual absence of class 1 antigens in Ad12 transformed cells results in a drastic decrease in susceptibility to cytotoxic T-lymphocytes in vitro as compared to Ad5 transformed cells. Experiments designed to further understand this switching proces will be presented.

0127 PROTEIN PRODUCTS ASSOCIATED WITH HUMAN & MOUSE MAMMARY CARCINOMA TRANSFORMING GENES, Dorothea Becker

The major aspect of my research concerns the identification and characterization of antigens associated with NIH 3T3 cells transformed by DNAs from various cellular neoplasms. Up to date four proteins have been identified as being either encoded by or associated with expression of neoplasm transforming genes. Transforming genes representing members of the ras gene family encode proteins of 21 kds. Blym, the transforming gene of chicken B cell lymphomas and human Burkitt's lymphomas encodes a protein of 8 kd. A 185 kd phosphoprotein has been shown to be associated with the expression of the transforming gene of rat neuroblastomas. Previous investigations in our laboratory led to the identification of two glycoproteins (gp 86 and gp 72) and two nonglycosylated proteins (p 70 and p 19) being associated with the transforming genes of human and mouse mammary carcinomas. Most recent studies indicated that all four proteins represent plasma membrane antigens, with gp 72 being expressed on the cell surface. Furthermore, we were able to show that gp 86, gp 72 and gp 70 are neither structurally related nor do they interact in the form of a high molecular weight complex. On the other hand, studies with respect to the characterization of p 19 suggest, that p 19 is most likely complexed to gp 72. Current investigations such as hybrid-selection are carried out using a molecular clone which contains the sequence of the transforming gene of human mammary carcinomas. These studies should allow determination which if any of these four proteins represents the direct protein product encoded by the transforming genes of human and mouse mammary carcinomas.

0128 **MONOCLONAL ANTIBODIES TO THE SRC PROTEIN OF ROUS SARCOMA VIRUS DETECT ENZYMATICALLY ACTIVE C-SRC FROM RODENT AND HUMAN CELLS, Sarah J. Parsons, Deborah J. McCarley, Constance M. Ely, David C. Benjamin, and J. Thomas Parsons, University of Virginia, Charlottesville, VA 22908.**

Transformation of cells by Rous sarcoma virus (RSV) is mediated by the product of the RSV src gene, pp60^{src}. Twenty-four hybridoma cell lines producing monoclonal antibodies to pp60^{src} have been isolated from mice immunized with immunoaffinity-purified pp60^{src} from RSV-transformed field vole cells (SR-1T) and with p60^{src} purified by preparative gel electrophoresis from extracts of E. coli expressing the src gene product. The monoclonal antibodies have been characterized as to their cross-reactivity with tyrosine protein kinases specified by several acute transforming viruses of avian, murine, and feline origin. In addition, their ability to recognize the normal cellular homologue of v-src (i.e., c-src) present in avian and mammalian species, including human, has been studied. Five monoclonal antibodies immunoprecipitate in vivo radiolabeled c-src from rodent and human cells. However, only two monoclonal antibodies immunoprecipitate enzymatically active forms of c-src. The antigenic determinants recognized by all of the monoclonal antibodies have been localized to the amino terminal one-third of the src molecule by testing the ability of the antibodies to immunoprecipitate mutant src proteins containing deletions in different regions of the molecule. These monoclonal antibodies should prove useful in studying the structure and function of viral and cellular src proteins.

0129 Inhibition of Transformation in a Cell Line Infected with a Temperature Sensitive Mutant of Murine Sarcoma Virus (MuSV) by Cytoplasmic Microinjection of Purified IgG from an Antisera Generated Against a Synthetic v-mos Peptide. R.B. Arlinghaus[1], G.E. Gallick[2], D.B. Brown[2], E.C. Murphy[2], and J. Sparrow[3]; [1]Scripps, Dept. of Mol-Biol., La Jolla, CA 92037; [2]M.D. Anderson Hospital, Houston, TX, 77030; [3]Baylor College of Med., Houston, TX,

The 6m2 cell line infected with a temperature sensitive Moloney MuSV mutant (tsl10) produces easily detectable levels of an 85,000d gag-mos fusion protein. Indirect immunofluorescence studies with antisera to a synthetic peptide, comprising amino acids 37-55 (cyclized through cysteine residues) of the predicted MuSV 124 v-mos sequence, indicated that p85$^{gag-mos}$ appeared to be a soluble cytoplasmic protein. To analyze whether or not the purified antibody could interact with gag-mos proteins, it was microinjected into the cytoplasm of 6m2 cells at the permissive and non-permissive temperatures. Fixed cells were stained with fluorescein-conjugated goat anti-rabbit IgG and counterstained with Evan's blue stain. Microinjected cells fluoresced bright green, whereas uninjected cells fluoresced red. Of the approximately 90% of cells which survived, injection with anti-ovalbumin IgG (as a control) had little or no effect. In contrast, injection of anti-mos IgG into 6m2 cells at the permissive temperature resulted in dramatic changes in many cells, e.g. the cells flattened and displayed extended psuedopodia. Similarly, microinjection of cells at the non-permissive temperature with anti-mos antibody prevented onset of the transformed phenotype in a large percentage of cells. These results strongly suggest that cytoplasmic injection of purified IgG from rabbits immunized with a v-mos peptide can inhibit the function of the transforming protein.

0130 PROMOTER ACTIVATION MEDIATED BY SV40 T ANTIGEN. J.M. Keller and J.C. Alwine, Department of Microbiology/G2, University of Pennsylvania; Philadelphia, PA., 19104.

We have analyzed the activation of the SV40 late promoter using chloramphenicol acetyltransferase (CAT) transient expression vectors. These studies were done under conditions where viral DNA synthesis could not occur, thus eliminating effects caused by genome amplification or DNA replication. This was accomplished by either mutating the origin of replication to render it nonfunctional or by transfecting plasmids into cell lines which produce mutant T antigens which are unable to replicate viral DNA. The results indicate that T antigen directly stimulates the late promoter in the absence of, and independent of, genome amplification or DNA replication. The ability of mutant T antigens to stimulate the late promoter correlates well with the maintenance of DNA binding activity. The up regulation of the late promoter, at a time when T antigen is down regulating the early promoter, indicates that the two SV40 promoters are quite different in their mechanism of activation. In addition, the early promoter is enhancer driven, while no enhancer-like elements have been identified with the late promoter. Thus the late promoter may define a class of RNA polymerase promoters which are activated by the specific action of certain proteins. T antigen would be such a protein as well as the adenovirus E1A protein. Using our transient expression vectors we have determined that the E1A protein will effectively substitute for T antigen in activating the late promoter. This apparently similar promoter stimulatory function of T antigen and E1A protein may be involved in the turn on of cellular genes which results in transformation.

0131 CHARACTERIZATION OF HOST SEQUENCES PRESENT IN SHOPE FIBROMA VIRUS, A TUMORIGENIC POXVIRUS OF RABBITS, C. Upton, W. Block, A.M. Delange, C. Macauley and G. McFadden, University of Alberta, Edmonton, Canada T6G 2H7

The Shope Fibroma Virus (SFV) genome consists of a 160 kb linear ds DNA molecule with covalently closed termini and inverted repeat sequences at the terminal 12 kb. In Southern blotting experiments using high complexity rabbit DNA probe the presence of viral sequences homologous to the host DNA probe was detected in each of the two terminal repeat sequences contained in BamHl fragments C and E. In reciprocal blotting experiments restriction enzyme digests of rabbit, monkey, human and mouse DNAs were probed with SFV BamHl E fragment: the resulting profiles indicated that the mammalian DNA sequences homologous to the SFV E clone are highly conserved. A part of these host sequences homologous to the SFV probe were found to reside within a novel 4.8 kb plasmid species, and the remainder are integrated cellular sequences. The human counterpart of these latter sequences has been isolated from a human lambda library at a frequency consistent with it being present as a single copy per haploid genome. The involvement of these SFV host-related sequences in progression of viral infection and tumorigenesis is suggested by characterization of the newly discovered tumorigenic poxvirus, Malignant Rabbit Virus (MRV). The genome of MRV has been mapped and appears to be identical to that of a related leporipoxvirus, Myxoma, except for the presence of these host-related DNA sequences homologous to SFV E probe in the MRV terminal repeats. We conclude that SFV has acquired in each terminal repeat approximately 5 kb of its sequences from the host and that acquisition of these same sequences by MRV is responsible for the phenotypic differences between MRV and Myxoma.

0132 CHARACTERIZATION OF THE HUMAN c-myc GENE PRODUCT. G. Ramsay, G. Evans, J.M. Bishop and R. Lerner*, Department of Microbiology and Immunology, University of California, San Francisco, CA 94143, *Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The cellular oncogene c-myc is believed to be involved in the genesis of many types of human tumor. Elucidation of the mechanism by which human c-myc elicits tumorigenesis requires identification of the c-myc protein. To this end we have synthesized a series of peptides corresponding to 7 regions from the c-myc gene and used these to raise antisera that reacts with myc protein. The sera immunoprecipitate the viral protein $p110^{gag-myc}$ from MC29 transformed quail cells and the $p58^{c-myc}$ protein from chicken lymphoma cells. In a variety of human cell types a candidate c-myc protein of 60,000 daltons was detected which could be localized to the nucleus by immunostaining. We are mapping the protein in order to confirm its relationship with chicken $p58^{c-myc}$. Currently under investigation is the role c-myc protein plays in cell lines containing amplified or translocated c-myc genes.

0133 A cDNA CLONE CONTAINING THE C-TERMINAL CODING REGION OF THE HUMAN MYB GENE. L. Souza, T. Boone, M. Cline* and D. Slamon*. Amgen, Thousand Oaks, CA 91320; *UCLA School of Medicine, Division of Hematology/Oncology, Los Angeles, CA 90024

Poly A^+ mRNA was isolated from circulating myeloblasts taken from a patient with acute myelogenous leukemia and from normal thymus tissue taken from another patient. Northern blot analysis of both mRNA preparations showed a band of approximately 3.4kb when probed with viral myb sequences. Similar analysis of mRNA from chicken thymus tissue placed the size of the chicken c-myb gene at approximately 3.6kb. cDNA was prepared using the Okayama and Berg technique from the leukemic myeloblast mRNA because this was the most abundant source of human c-myb message. Viral myb sequences were nick translated with ^{32}P-dCTP and used as a probe to screen 40,000 cDNA clones. One clone, Hu-mybl, was isolated and sequenced by the dideoxy method using M13/Hu-mybl clones as templates. The sequence we obtained contained the following features: 1) an open reading frame extending 831 bases from the 5' end of the clone, 2) a 3' untranslated region of about 200 bases containing a poly adenylation signal, and 3) about 100-200 bases of poly A. Translating the 831 base open reading frame yielded the carboxyl terminal 277 amino acids (a.a.) of the human c-myb protein. Comparison of the hu-mybl protein sequence with that of the viral protein sequence (Rushlow et al. 1982. Science 216:1421) showed the following: 1) there is 84% homology in the 245 a.a. of overlap between the two sequences, 2) there are regions (up to 72 a.a. in length) where the homology between the two proteins is as low as 57%, and 3) the human myb sequence terminates prior to the termination of the v-myb sequence. The results of the final point are consistant with the differences in length noted for the human and chicken c-myb mRNAs.

0134 A PROTEIN KINASE IN MICROTUBULES PHOSPHORYLATES TYROSINE ON MICROTUBULE ASSOCIATED PROTEINS, Arthur H. Lockwood, Maryanne Pendergast and Michael Dennis, Temple University School of Medicine, Philadelphia, PA 19140

Tyrosine specific protein kinases appear to be essential for oncogenic transformation by many tumor viruses. They may also mediate cellular responses to polypeptide growth factors such as EGF. Transforming viruses and growth factors elicit reorganization of the actomyosin and microtubule cytoskeleton concomitant with alterations in cell morphology and adhesion. Cytoskeletal proteins are thus likely substrates for phosphorylation by cellular or viral tyrosine kinases. We have found that microtubules isolated from mammalian brain contain substantial levels of a tyrosine-specific kinase. This enzyme phosphorylates specific microtubule associated proteins (MAPs) in a cAMP independent manner. Several of the MAPs which are substrates for the tyrosine kinase are also phosphorylated by an endogenous cAMP-dependent protein kinase. Phosphorylation of the same set of MAPs by either cAMP dependent or tyrosine specific protein kinases may therefore regulate the assembly and function of cellular microtubules.

0135 The Subgenomic mRNA Encoded by V-Rel and the Quantitation of Rel Expression in Various Cell Lines, Tissues and Organs

Norbert D. Herzog, William J. Bargman, Barbara E. Moore, Nancy R. Rice and Henry R. Bose, Jr.

REV-T is a replication-defective avian acute leukemia virus that transforms immature lymphoid cells of the B cell lineage in vivo and in vitro. The genome of REV-T contains a unique helper-independent sequence, designated v-rel, which is presumed to be responsible for transformation. Nucleotide sequence data has localized the 1.5 kb v-rel sequence as an insertion into the env gene. Furthermore, the sequence has revealed a long open reading frame that would be transcribed as a subgenomic mRNA of approximately 3.0 kb. Using a molecularly cloned rel specific DNA probe, we have identified a single subgenomic 3.0 kb, mRNA in REV-T transformed-nonproducer (NP) cell lines. The cytoplasmic poly A^+ RNA from REV-T transformed NP cell lines all appear to harbor high molecular weight RNA species that hybridize with rel cDNA suggesting the accumulation of incompletely processed c-rel mRNA. Marek's disease transformed cells poly A^+ RNA was analyzed to identify a 4.0 kb c-rel mRNA but no high molecular weight precursor TNA that hybridizes with rel cDNA. Dot hybridization is used to quantitate the levels of rel mRNA expression.

REV-T transformed cell lines express rel TNA at various levels, generally 4 - 10 fold greater than c-rel expression in Marek's disease transformed cells. The expression of c-rel normal uninfected chickens can be detected in all organs of the hematopoietic system.

0136 COMPLEMENTATION OF ADENOVIRUS REGION E1a DELETIONS BY ONCOGENES, J.L. Bos* and A.J. van der Eb**, *Institute of Public Healt, Bilthoven and **Sylvius Laboratories, Leiden The Netherlands.

We have studies complementation of defects in the E1a region of adenovirus 12 in transformation of BRK cells by cellular and viral oncogenes. The mutant adenovirus DNA used in this study is pSVR11, a plasmid containing the E1 region of Ad12 with a deletion in the E1a region (897-1006), as well as the SV40 enhancer element. This plasmid has a low transforming capacity in primary BRK cells. The transformed cells have lost their tumorigenic potential although they normally express region E1b. We have cotransfected BRK cells with pSVR11 and various oncogenes. It was found that the ras^H gene (E.J 6.6) increased the transformation frequency at least tenfold, and that the transformed colonies appear much faster and have an altered morphology as compared to cells transformed by pSVR11 or intact region E1 of Ad12 (the ras^H gene alone does not transform the primary rat cells).These results suggest that the ras^H oncogene can complement the defect of the Ad12 E1a region in transformation of primary BRK cells. The results will be discussed in relation to the tumorigenic properties of the transformed cells.

0137 OLIGONUCLEOTIDES AS SPECIFIC TRANSLATION INHIBITORS, Richard H. Tullis, Robert N. Bryan and Vladimir Mackedonski, Molecular Biosystems, Inc., San Diego, CA 92121.

Hybrid arrest of translation discovered by Hastie and Held and Kuff, Patterson and Roberts has been used in number of laboratories to identify specific translation products. Using synthetic oligonucleotides capable of spontaneously hybridizing to specific mRNA sequences under physiological conditions of salt, temperature and pH, we have been investigating the idea that oligonucleotides might be capable of selective protein synthesis inhibition in vitro. We have synthesized several oligonucleotides designed to inhibit the translation of SV-40 T-antigen mRNA. Under certain conditions, these oligonucleotides selectively inhibit the synthesis of immunoprecipitable T-antigen protein in the rabbit reticulocyte cell free protein synthesis system. At a 1000 fold calculated sequence excess of oligomer over T-mRNA in total poly A mRNA from SV-40 infected monkey cells, protein synthesis can be strongly suppressed. The oligomers tested show little apparent effect on normal cellular mRNA translation. The oligonucleotides tested, although stable in the reticulocyte lysate, are rapidly degraded in serum and after uptake into cells. In order to stabilize these molecules to nuclease digestion and to increase their lipophilicity, we have developed methods for the synthesis and purification of specific sequence phosphotriester and methyl phosphonate DNA oligonucleotides. Our eventual goal is to selectively inhibit the synthesis of specific proteins in vivo.

0138 Evidence For In Vivo Phosphorylation of DNA Polymerase α in Rous Sarcoma Virus Transformed Cells. Robert W. Donaldson and Eugene W. Gerner. Division of Radiation Oncology, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Previous work has demonstrated that DNA polymerase α activity is modulated by phosphorylation. Rous Sarcoma virus (RSV) mediated cell transformation has been shown to affect a variety of cellular functions. Studies in this laboratory have demonstrated that RSV transformation alters cell cycle progression as well as rates of DNA synthesis. Rat embryonic fibroblasts infected with a mutant of RSV(tsLA24) were used for these studies. Rat-1 (tsLA24/RSV) are permissive for transformation at 35°C and nonpermissive at 39°C. Using alkaline phosphatase, a phosphotyrosine prefered phosphatase, we show a concentration dependent differential inhibition of DNA polymerase α activity in RSV transformed cells. The results suggest that in RSV transformed cells, DNA polymerase α, or an accessory protein is phosphorylated by $pp60^{src}$ or another transformation specific phosphotransferase. Highly purified preparations of monoclonal antibodies to DNA polymerase α were used to investigate the in vivo phosphorylation state of Rat-1 (tsLA24/RSV) DNA polymerase α. Purification utilizing either rapid immunoprecipitaion or affinity chromatography followed by SDS-PAGE reveals that DNA polymerase α copurifies with several other proteins. Phosphoprotein coresponding in apparent molecular weight to DNA polymerase α was observed. These results suggest that, in RSV transformed cells, DNA polymerase α is phosphorylated in vivo. Since alkaline phosphatase mediated dephosphorylation inhibits enzyme activity, it is likely that this observed phosphorylation serves to modulate the activity of DNA polymerase α in vivo. (Supported by USPHS Grant CA18273).

0139 GENERATION OF ANTIBODIES TO THE *ras*^{Ha} GENE PRODUCT USING SYNTHETIC PEPTIDES, Dennis J. Slamon*, Robert Stein†, Takeo Tanaka*, Edward M. Scolnick† and Martin J. Cline*, *UCLA School of Medicine, Los Angeles, CA 90024; and †Merck, Sharp & Dohme Research Labs, West Point, PA 19486

Antibodies directed against synthetic peptides derived from the known nucleic acid sequence of a variety of genes have been shown to be useful in identifying proteins encoded by the genes. Using this approach we synthesized 6 peptides based on the predicted amino acid sequence of the v-*ras*^{Ha} gene. Two of these peptides, *ras*^{Ha} #2 (amino acid positions 160-179) and *ras*^{Ha} #6 (amino acid positions 29-44) generated rabbit polyclonal antisera that were capable of immunoprecipitating a p-21 protein from cells infected with the Harvey murine sarcoma virus, as well as cells transfected with the cloned *ras*^{Ha} gene. A p-21 protein was also easily identified by Western blotting techniques using the same antisera. The interaction between the antisera and the p-21 molecule could be competitively blocked by the synthetic peptides. These antisera were also capable of immunoprecipitating a purified mammalian *ras*^{Ha} p-21 as well as a *ras*^{Ha} p-21 produced in a bacterial promotion system. Examination of the effects of these antisera on the biologic activity of the gene product revealed that neither appeared to interfere with GTP binding, implying that these sites, i.e., amino acid positions 29-44 and 160-179, may not be involved in the GTP binding site.

0140 TRANSFORMING PROTEINS OF HUMAN ADENOVIRUSES, Philip E. Branton, David T. Rowe, Siu-Pok Yee, Michel L. Tremblay and Frank L. Graham, McMaster University, Hamilton, Canada

Transformation by human adenovirus type 5 (Ad5) is induced by products of two early transcription units, E1A and E1B, located at the extreme left end of the genome. E1A proteins appear to regulate transcription of certain viral and cellular genes and probably act to immortalize cells while products of E1B are needed for establishment of fully transformed cells. We have used both anti-tumor and anti-synthetic peptide sera to characterize E1 polypeptides. The E1A region produces two mRNA's of 1.1 and 0.9 kilobases, each of which produces two major and two minor phosphoproteins. Fluorescent antibody staining and cell fractionation experiments indicate that the E1A products are located both in the nucleus and the cytoplasm. The nuclear species represent only a subset of the E1A proteins and are stable for up to two hours. All the E1A species are present in the cytoplasm, with one subset in a membrane fraction and the other detectable in cytoskeletal preparations, and all of these species have a half life of less than 30 min unless protein synthesis is inhibited. We believe that the nuclear species regulate transcription whereas the cytoplasmic polypeptides carry out another function perhaps involving the stabilization of mRNA. E1B encodes two major products, a 58K phosphoprotein found both in the nucleus and the perinuclear region of the cyotplasm, and a 19K membrane-associated protein. Analysis of a large series of Ad5-transformed cell lines indicated that only the E1A proteins and E1B-19K are necessary for the maintenance of oncogenic transformation. We believe that transformation by Ad5 is a two-hit process in which the E1A proteins endow cells with immortality and one or more E1A proteins in combination with the E1B-19K polypeptide provide functions required for oncogenesis.

0141 MAPPING OF GENES INVOLVED IN EBV-INDUCED LYMPHOCYTE TRANSFORMATION, David J. Volsky, Thomas Gross, Ronald Bartzatt, Charles Kuszynski, Barbara Volsky and Faruk Sinangil, U.N.M.C., Dept. of Pathology and Eppley Inst. for Cancer Res., Omaha, NE 68105.

Identification of Epstein-Barr virus (EBV) genes responsible for inducing lymphocyte immortalization and contributing to the process of lymphomagenesis has been hampered by the lack of viral mutants. In an alternative approach, we study the expression of purified EBV DNA and cloned DNA fragments in normal human lymphocytes, and evaluate their effect on lymphocyte immortalization in relation to the endogenous lymphocyte transforming (c-onc) genes. Since isolated EBV DNA and subgenomic fragment cannot be efficiently introduced into lymphocytes by the known DNA-transfer techniques, we use our recently developed gene transfer method based on reconstituted Sendai virus envelopes (RSVE). We report here that the RSVE-transferred 7.9 kbp SalIF-1 and a smaller 4.8 BamHI-K fragments of EBV DNA induced the EBV-determined nuclear antigen (EBNA) in 2-4% of lymphocytes. The antigens associated with the lytic cycle of the virus, EA and VCA, were not observed. No stimulation of cellular DNA synthesis or promotion of cell immortalization was detected with these fragments, suggesting that the EBNA-coding gene(s) is not an autonomous transforming gene of EBV. In contrast, 7.8 kbp BamHI-D-1, or BamHIXand H (1.55 and 6.15 kbp, respectively) fragments of EBV DNA stimulated lymphocyte DNA synthesis, but no viral antigens could be detected, and immortalization was not achieved. Our results suggest that cell tranformation by EBV requires collaboration between several viral and perhaps cellular genes. Identification of these genes is in progress.

0142 EFFECTS OF src GENE MODULATION ON CELLULAR PHENOTYPE, Edward B. Jakobovits, John E. Majors and Harold E. Varmus, Dept. of Microbiology & Immunology, University of California, San Francisco CA 94143

Our interest has been in determining whether quantitative modulation of a transforming gene within a transfected cell can lead to the generation of intermediate transformed phenotypes. For this purpose, we have constructed a hybrid expression vector in which a cloned v-src gene sequence has been linked to the glucocorticoid inducible promoter of the mouse mammary tumor virus. Transfection of this vector into rat fibroblasts led to the isolation of a number of cell lines in which modulatable v-src expression could be demonstrated in response to hormone induction. Within these lines, distinctive levels of phenotypic transformation could reproducibly be demonstrated. Our results strongly indicate that the transformed state can be titrated by regulation of oncogene expression and is not a simple on-off phenomenon.

0143 An Adenoviral Superinfection System Used to Study the Induction of a Viral Early Region Gene and Cellular Genes by EIa. Roland W. Stein and Edward B. Ziff. Kaplan Cancer Center and Dept. of Biochemistry, New York University Medical Center, New York, NY 10016. We assayed in HeLa cells the EIa dependent activation of transcription of adenovirus early region genes in the mutant dl 312 (an EIa deletion mutant) by EIa products provided by a superinfecting helper virus, dl 327 (an E3 deletion mutant). We also analyzed the effect on transcription from several cellular genes i.e. β-tubulin, β-actin, β-globin. Transcriptional activity in E3 of dl312 was assayed after superinfection with a probe pXba D (78.5-84.3 map units), and EIa activity from the dl 327 helper with a probe pA6 (0-4.5 map units). EIa and E3 transcription following superinfection are similar to that found for a wild-type infection. This implies that the transcriptional regulation pattern of the dl 327 helper can be superimposed on dl 312 promoters. Cellular gene transcription was determined by hybridization of pulse labeled nuclear RNA to homologous human cDNA probes. β-Tubulin transcription was transiently induced after dl 327 superinfection with kinetics that approximated viral early gene expression. In contrast, β-globin, which is not transcribed in HeLa, was not induced. And β-actin, which is actively transcribed in uninfected HeLa, continued at the same high level. The E3 transcription plateau shifts to earlier times with increasing multiplicities of helper virus, but the maximal rate of transcription was unchanged. In contrast, increasing helper multiplicity increased the maximal rate of β-tubulin transcription. These studies suggest that a viral function, potentially EIa, can stimulate transcription of certain cellular genes by a mechanism similar to that by which viral early gene transcription is activated.

0144 THE NUCLEAR AMV AND E26 ONCOGENE PRODUCTS ARE ALTERED FORMS OF A CYTOPLASMIC PROTO-ONCOGENE PRODUCT, W.J. Boyle, M.A. Lampert, J.S. Lipsick, and M.A. Baluda, UCLA Jonsson Comprehensive Cancer Center, Department of Pathology, UCLA School of Medicine, Los Angeles, California 90024

The two defective leukemia viruses avian myeloblastosis virus (AMV) and E26 exclusively cause acute leukemias. Both of these viruses share a common 0.8 kb sequence present within a cellular insert derived from a highly conserved cellular gene, proto-amv. Interestingly, this normal cellular progenitor is expressed only within immature hematopoietic tissues. The oncogenes of AMV and E26 differ from their cellular proto-oncogene in gene structure, transcript structure, and gene product. The product of the AMV oncogene, $p48^{amv}$, is encoded by short viral terminal sequences flanking a transduced, 1.2 kb segment (amv) of proto-amv. The E26 oncogene product, $p135^{gag\text{-}amv\ ets}$, is encoded by a significant portion of the viral structural gene (gag), 0.8 kb of sequences representing the core of the AMV cellular insert (amv), and additional E26-specific transduced cellular sequences (ets). Both the $p48^{amv}$ and $p135^{gag\text{-}amv\text{-}ets}$ oncogene products have been localized within the nucleus of cells transformed by these viruses. In contrast, their normal cellular homolog, $p110^{proto\text{-}amv}$, has been localized in the cytoplasm of both normal and transformed hematopoietic cells.

Thus, leukemogenesis induced by these two related viruses appears to be mediated by functional differences in the proteins coded for by structurally altered, transduced proto-amv sequences. Also, leukemogenesis is independent of the expression of the normal proto-amv gene.

0145 **V-MOS EXPRESSION IN CELLS INFECTED WITH A MUTANT OF MUSV IS ACTIVATED BY A TEMPERATURE-SENSITIVE RNA SPLICING EVENT**, Micheal A. Nash, Bill Brizzard and Edwin C. Murphy, Jr. Dept. Tumor Biol., UTSCC M.D. Anderson Hosp., Houston, TX 77030

NRK cells infected with a ts mutant of wild-type MuSV-349, designated MuSVts110, appear transformed at 33° but normal at 39°C. At 39°C, infected cells contain a single truncated viral gag gene polypeptide, $P58^{gag}$, and a 4.0 kb viral RNA. At 33°C, however, a gag-gene/mos-gene fusion protein, $P85^{gag-mos}$, appears in concert with the appearance of a 3.5 kb viral RNA. S-1 nuclease mapping and primer extension sequencing of the MuSVts110 RNA mos gene revealed the presence of two viral RNAs each with distinct gag and mos gene deletions. MuSVts110-infected cells grown at 39°C contain an RNA in which 1488 bases, bounded by 6-base direct repeats, of the wild type sequence between the p30 coding of the gag gene and the N-terminal end of the mos gene have been deleted. In this RNA, the gag and mos genes are fused out of frame at positions corresponding to nucleotides 2404 and 3892 in the wild type sequence. MuSVts110-infected cells grown at 33°C, however contain another RNA in which gag gene nucleotide 2017 and mos nucleotide 3935 are now fused, an excision of 430 bases. The gag-mos junction in this RNA corresponds to in-frame splice donor and acceptor sequences which could produce a gag-mos fusion protein. Our data support a model in which a MuSVts110 4.0 kb RNA, containing an out of frame fusion of the gag and mos genes, is the primary transcript of the MuSVts110 viral DNA at both 39°C and 33°C. However, at 33°C a 430 base 'intron' can be spliced out of the 4.0 kb RNA, producing an in frame fusion of the gag and mos genes, and allowing the translation of the transforming protein, $P85^{gag-mos}$.

0146 SRC INDUCED ENHANCED SELF RENEWAL OF STEM CELLS IN THE ABSENCE OF CELL TRANSFORMATION, David Boettiger and T. Michael Dexter, University of Pennsylvania, Philadelphia, PA 19104, and Paterson Laboratories, Christie Hospital, Manchester, U.K.

A recombinant murine leukemia carrying the src gene from Rous sarcoma virus was used to infect hemopoietic stem cells using the long term murine bone marrow culture system which maintains the active proliferation of these cells for several months. The stem cells produced from the infected cultures were assayed in the standard spleen colony assay. The infected spleen colony forming cells exhibited reduced seeding efficiency in the spleen, suggesting an altered cell surface, a common property of Rous sarcoma virus infected cells. The infected cultures produced 20 to 50 fold the level of CFU-S cells produced by control cultures. These infected CFU-S could be serially passaged in vitro or in vivo longer than normal CFU-S, implying a higher self renewal capacity.

The spleen colonies produced by the infected CFU-S contained only normal cell types and the cells were capable of reconstituting potentially lethally irradiated mice. Leukemia studies revealed no evidence of leukemic cells over 4 to 6 months by examination and by attempts at in vivo passage and in vitro culture.

0147 HIGH LEVEL PRODUCTION IN E. COLI OF BIOLOGICALLY FUNCTIONAL ADENOVIRUS EIA PROTEIN, B. Ferguson[1], B. Krippl[2], H. Westphal[2], N. Jones[3], J. Richter[4] and M. Rosenberg[1], [1]Dept. of Molecular Genetics, Smith Kline and French Labs, Philadelphia, PA; [2]NICHHD, NIH, Bethesda, MD; [3]Purdue Univ., W. Lafayette, IN; [4]Univ. of Tenn., Knoxville, TN

The human adenovirus EIA gene encodes a product which positively regulates the transcription of viral and certain cellular genes and is involved in the transformation of mammalian cells. We have designed and constructed a plasmid vector for the controlled, high level expression in E. coli of the product of the human type C adenovirus EIA 13S mRNA. The E. coli-produced EIA protein has been purified to near homogeneity in 10-100 mg quantities. The purified EIA protein was shown to be as effective as the EIA gene in its ability to induce expression from the EIA responsive Ad5 E3 promoter in microinjected Xenopus oocytes. The EIA protein, microinjected into somatic cells, was found to activate the adenovirus E2A gene and to complement the EIA deletion mutant d1312. Following microinjection into the cytoplasm, the EIA protein was shown to migrate rapidly and quantitatively to the nucleus. Experiments are in progress to determine whether the purified E1A protein interacts with DNA or affects transcription in an in vitro system. Purified E1A has been used to obtain a high titre antiserum from rabbits and attempts to affinity purify cellular factors which interact with EIA are now in progress. We have in addition isolated several E. coli-produced mutant EIA proteins. Experiments are in progress to determine the effect of these structural alterations on the ability of the protein to be transported to the nucleus and to function as a positive transcriptional regulator.

0148 EXPRESSION, PURIFICATION AND CHARACTERIZATION OF HUMAN Ras PROTEINS PRODUCED IN E. COLI, M. Gross, R. Sweet, G. Sathe, S. Yokoyama and M. Rosenberg, Smith Kline and French Labs., Philadelphia, PA 19101, O. Fasano, J. Feramisco, M. Goldfarb and M. Wigler, Cold Spring Harbor Labs, Cold Spring Harbor, NY 11724

cDNA clones of the T24 bladder carcinoma transforming gene and its normal homolog have been described (Fasano et al. J. Mol. Appl. Genet. 2, 173-180 ('83)). Utilizing these cDNA clones, sequences precisely encoding the normal and transforming p21 proteins have now been inserted into a derivative of the pAS-1 (Rosenberg et al., Meth. Enz. 101, 123-138 ('83)) E. coli expression vector. The T24 construct retains 3T3 transforming activity whereas no foci are observed with the plasmid bearing the normal gene. We obtained high-level expression of both polypeptides and have purified the T24 product to greater than 90% homogeneity in a soluble form. This protein has a MW of 24,000 by SDS/PAGE, is immunoprecipitable by monoclonal antibodies raised against the Harvey p21 and by tryptic mapping contains the carboxy-terminal "variable" peptide. Purification of the normal gene product is in progress. We have also constructed plasmids to express both amino- and carboxy- truncated T24 polypeptides. We will report on the in vitro and in vivo, via microinjection, activities of these proteins.

0149 EXPRESSION AND CHARACTERIZATION OF THE c-MYC PROTEIN, Rosemary Watt, Allan Shatzman and Martin Rosenberg, Department of Molecular Genetics, Smith Kline and French Laboratories, Philadelphia, PA 19101

The human c-myc oncogene has been overexpressed in E. coli in an effort to understand the role which c-myc might play in neoplastic development. The entire c-myc coding region was removed from a cDNA clone and inserted into the pOTS5 expression vector under the transcriptional and translational control of the efficient phage λ regulatory sequences. The expression of c-myc in this thermo-inducible system is efficient, since the majority of the ^{35}S methionine incorporated into proteins in a pulse labeling experiment appears in the c-myc gene product. Analysis of the total protein synthesis by Coomassie Blue staining of SDS polyacrylamide gels shows that the c-myc gene product accumulates to at least 10% of the total cellular protein. This high level synthesis has permitted us to purify large quantities of the E. coli-synthesized c-myc protein. The biochemical properties and interactions of the purified protein will be examined in cell free extracts of human cells and in vivo following microinjection. These studies will be facilitated by antibody being raised against this protein. Additionally, antisera will be tested for the ability to immunoprecipitate the $p110^{gag-myc}$ fusion product of MC29 virus and the c-myc protein in human cell lines. Positively responding sera will be used to monitor c-myc expression during normal differentiation and in neoplasia.

0150 MODULATION OF THE EXPRESSION OF v-src AND v-myc AFTER TRANSFECTION INTO MAMMALIAN CELLS. Manuel Grez, MichaelKarin and Peter K. Vogt. USC Medical School, Department of Microbiology, Los Angeles CA 90033

Expression of various oncogenes under the control of an inducible promotor should allow the study of the cellular events which result in neoplastic transformation. For this purpose we have constructed recombinant plasmids carrying the v-src and v-myc coding sequences under the transcriptional control of the human metallothionein-II (hMT-II) promotor, a promotor known to be induced by dexamethasone and heavy metals after transfection into mammalian cells. Transfection of hMT-II/v-src plasmid into established mammalian cell lines results in only a two-fold increase in the number of foci present in the induced plates. The low level of induction was due to a very high constitutive level of expression of v-src and to the toxic effects of v-src when expressed at high levels in the induced plates. Therefore,recombinants were constructed which contained only a small portion of the hMT-II promotor which is still inducible by heavy metals. Co-transfection of this plasmid together with pSV2-neo into Rat I cells resulted in several hundred G-418 resistant colonies. Analysis of some of them revealed the presence of 1 to 20 copies of the v-src recombinant plasmid. Some of these clones showed a transformed morphology after induction with 10-6 M Cd^{++}. Further characterization of these clones is under way. Co-transfection of mammalian cells with the hMT-II/v-myc recombinat DNA and pSV2-neo resulted in several hundre G-418 resistant colonies some of which showed an altered morphology with or without induction. Analysis of these clones showed a fusiform cell shape and a limited ability to grow in soft agar.

0151 CHROMOSOMAL LOCALIZATION OF CELLULAR ONCOGENES IN THE MOUSE, Christine A. Kozak and M. David Hoggan, LVD, NIAID, National Institutes of Health, Bethesda, MD 20205

The retroviral oncogenes have cellular homologs which are highly conserved in a variety of species, and these sequences have been implicated in various naturally occurring neoplastic diseases. Because of the association of some cellular oncogenes with chromosomal regions involved in tumor-specific chromosomal abnormalities, we have continued our efforts to map these sequences within the mouse genome. Hamster-mouse somatic cell hybrids were generated which contain different complements of mouse chromosomes. High molecular weight DNA extracted from each hybrid line was analyzed by Southern blot hybridization for the presence of the mouse sequences homologous to various oncogenes. Using this approach, we determined the chromosomal locations for the oncogenes c-fes and c-Ha-ras (J. Virol. 47:217), c-sis (Science 221:867), c-raf (J. Virol., In press), and c-akt (In preparation). More recently, these hybrids were analyzed using cloned probes representing a 0.45 kb PstI fragment of the v-fms gene of SM-FeSV and a 1.0 kb HaeII-XbaI fragment of the v-myb gene of AMV. Unique c-fms and c-myb fragments were identified in mouse and some hybrid cell DNAs restricted with HindIII or BamHI, and the chromosomal locations of both oncogenes were determined.

0152 ~~THE RAF ONCOGENE: NUCLEIC ACID SEQUENCE, EVOLUTION, AND REQUIRED STRUCTURES FOR~~ TRANSFORMATION, G.E. Mark, M.D. Goldsborough, T.I. Bonner and U.R. Rapp, National Cancer Institute, Frederick, MD 21701

A new replication-defective, acute transforming retrovirus (3611-MSV) was recently isolated from mouse and molecularly cloned. The nucleotide sequence of 1.5 kilobases encompassing the transforming gene (v-raf) revealed a single open reading frame, continuous with the gag gene, which is terminated by an amber codon 180 nucleotides from the 3' end of the inserted cellular sequences. Comparison of the deduced v-raf amino acid sequence with those of other oncogenes revealed significant homologies only with members of the "src family" of transforming proteins. Although these homologies align exclusively with the kinase half of the "src family" progenitor we have been unable to demonstrate a tyrosine-specific kinase activity with v-raf. This is consistent with raf having diverged from the ancestral gene prior to the latter's acquisition of tyrosine-specific kinase activity. The unique nature of the raf gene is also seen in its Drosophila homolog (D-raf-1) which is separate from those loci described for abl, src, and fps. The restriction map of D-raf-1 and its nucleotide sequence will be presented, as will restriction maps for the raf homologs in yeast and Dictyostelium. Finally, those sequences required for the transformation of NIH 3T3 have been determined by transfection of these cells with subgenomic, or Bal-31 resected, cloned DNAs. Subgenomic fragments required either a 5' LTR or co-transfection with helper virus DNA to induce transformed foci. Removal from the gag-raf carboxy terminus of a predicted -helical peptide structure (common to all "src family" members) abolished transforming activity.

0153 THE *mil/raf* FAMILY OF AVIAN AND MURINE ONCOGENES. Klaus Bister*, Hans W. Jansen* and Ulf R. Rapp+, * Max-Planck-Institut für Molekulare Genetik, Otto-Warburg-Laboratorium, D-1000 Berlin 33, Fed. Rep. Germany, + Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, Maryland 21701.

The avian retrovirus Mill Hill 2 (MH2) induces in chickens a high incidence of liver and kidney carcinomas in addition to sarcomas and leukemias. The genome of MH2 contains two unrelated and independently expressed oncogenes, v-*mil* and v-*myc*(1,2). The oncogene v-*myc* is shared with three other avian retroviruses which induce acute leukemias, sarcomas and various visceral tumors. Oncogenic specificities of MH2 may be due to the expression of two different but possibly cooperative oncogenes, v-*mil* and v-*myc*. The murine retrovirus 3611-MSV induces fibrosarcomas in newborn mice and transforms fibroblasts and epithelial cells in culture. The genome of 3611-MSV contains a cell-derived transformation-specific sequence, v-*raf* (3). The human homologue of v-*raf* is located on chromosome 3. We show now that the two independently transduced oncogenes v-*mil* and v-*raf* are closely related. This was demonstrared by hybridization between cloned DNAs, heteroduplex analysis, complete nucleotide sequence comparison, and hybridization of both probes with the same fragments of either chicken c-*mil* or human c-*raf* clones. We conclude that v-*mil* and v-*raf* were derived from homologous genetic loci of avian and mammalian species.

1) Jansen, H.W., Patschinsky, T. & Bister, K.(1983) J. Virol. 48: 61-73.
2) Jansen, H.W., Rückert, B., Lurz, R. & Bister, K.(1983) EMBO J. 2: 1969-1975.
3) Rapp, U.R. et al. (1983) Proc. Nat. Acad. Sci.USA 80: 4218-4222.

0154 DELETION MUTANTS OF SV40 REVEAL A LATE FUNCTION FOR LARGE T ANTIGEN AND THE EXISTENCE OF AT LEAST TWO SEPARABLE FUNCTIONAL DOMAINS. C.N. Cole[1,2], J. Tornow[1], M. Polvino-Bodnar[1] and G.M. Santangelo[1] [1]Department of Human Genetics, Yale University, New Haven, Ct.06510 and [2]Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03756.
Several SV40 mutants with small deletions (3-20 bp) at Dde I sites have been prepared and characterized. Both frameshift and in-phase deletions were obtained at many sites within the early region of SV40. One, dlA2459, lacking 14bp at 0.193 map units, did not plaque in CV-1p cells, but was positive for viral DNA replication and late mRNA production. The level of capsid proteins was reduced at least 50-fold when compared to wildtype SV40. The mutant was defective for adenovirus helper function. The properties of this mutant are similar to those of human adenoviruses in monkey cells. These results suggest that adenovirus helper function is required by SV40 for productive infection. The defect of dlA2459 could be complemented intracistronically by co-infection with any SV40 group A mutant having either an in-phase deletion or a point mutation. Frameshift deletions at any location were unable to complement dlA2459. Inversion of the Bam HI to Bcl I fragment of SV40 yielded a viable mutant, inv2408, which produced large T antigen and VP1 polypeptides which have exchanged their carboxy-termini. Complementation analysis of additional mutants prepared from inv2408 indicated that the VP1 polypeptide carrying the C-terminus of large T antigen could complement dlA2459. Therefore, the C-terminus of large T constitutes a functional domain separable from the remainder of the polypeptide. All mutants have been tested for viral ATPase, transformation of mouse cells, complementation properties and adenovirus helper function.

0155 TYROSYL PROTEIN KINASES IN HUMAN SERUM, Gail M. Clinton, Ming-Fing Lin, Tina Q. Tan, and Pat Lobelle-Rich, Louisiana State Medical Center, New Orleans, LA 70112.
Human serum was tested for the presence of tyrosyl-protein kinases in order to evaluate their expression during normal human development and to evaluate possible changes in their expression in malignant diseases. Tyrosyl kinase activity was detected in human serum using the peptide angiotensin II, pp60src specific IgG, or endogeneous proteins as substrates. Analyses of tyrosyl kinase activities in over 150 serum samples indicate that levels of the enzyme relative to serum volume or relative to overall protein kinase activity vary in different age groups. High activity was observed in serum obtained from the cord blood of newborns. The levels dropped during early development and increased again in the third age decade. These results point to a role for these enzymes in human development. Fractionation of the serum enzymes by column chromatography revealed the presence of at least three tyrosyl kinases. We are currently determining which of these enzymes are differentially expressed in the serum from individuals of different ages. These studies were supported by Grant CA-34517 (GC).

0156 THE STRUCTURAL RELATIONSHIP BETWEEN HUMAN PLATELET-DERIVED GROWTH FACTOR AND THE TRANSFORMING PROTEIN OF SIMIAN SARCOMA VIRUS. Ann Johnsson, Christer Betsholtz, Åke Wasteson, Bengt Westermark, Carl-Henrik Heldin, Jung San Huang, Thomas F. Deuel, Geoffrey T. Scrace, Nigel Whittle, Paul Stroobant & Michael D. Waterfield. University of Uppsala, Sweden, Washington University, St. Louis, U.S.A. and Imperial Cancer Research Fund, London, U.K.

Platelet-derived growth factor (PDGF) is the major mitogen for connective tissue cells. Recent studies have revealed an extensive amino acid sequence homology between human PDGF and the transforming protein $p28^{sis}$ of simian sarcoma virus (SSV)(Waterfield et al. Nature, 304, 35-39, 1983; Doolittle et al. Science, 221, 275-279, 1983). PDGF is a 30 kDa protein which is composed of two disulphide-linked polypeptide chains, designated A and B. In the present study we have extended the amino acid sequence analysis on isolated PDGF A and B chains, prepared by reverse-phase HPLC of reduced and alkylated PDGF. An amino terminal stretch of 110 residues of the B chain show virtual identity with residue 67 through 176 with only three amino acid substitutions. Further, the 54 amino terminal residues determined in the A chain show about 60% homology with $p28^{sis}$ (corresponding to residues 73 through 126). A potential glycosylation site has been identified in the A chain. SSV has arisen by recombination of the replication competent simian sarcoma associated virus (SSAV) with woolly monkey cellular gene sequences. Our sequence data suggest that SSV has obtained transforming capacity by acquiring gene sequences which encode the PDGF B chain or part thereof. The data further demonstrates a structural homology between the PDGF A and B chains implying that these are coded for by sequences of a common ancestral origin.

0157 CELL CYCLE-LINKED EXPRESSION OF LARGE-T IN TYPE N FR 3T3-SV 40 TRANSFORMANTS, STABILIZATION OF LARGE-T IN TYPE A TRANSFORMANTS, Jean-Jacques LAWRENCE*, Jean IMBERT°, François COULIER°, Françoise BIRG°, *Laboratoire de Biologie Moléculaire et Cellulaire, DRF/CEN-G Grenoble, France, ° U.119 INSERM, MARSEILLE, France.

The instability of large-T at 32°C (half-life : 30 min) the low level (20 o 30%) of T-Ag positive cells after immunofluorescent staining strongly suggested that T-Ag in FR 3T3-SV40 type N transformants may exhibit a cell cycle-dependent expression of this protein. This was investigated using a fluorescence activated cell sorter and a combination of stainings for cellular DNA (DAPI) and T-Ag (fluorescein-labeled IgG). Such an analysis revealed that in 3 lines out of 6 (2 N transformants and one WT transformant isolated in the conditions leading to an N phenotype), T-Ag could be detected only in cells in the G2 phase of the cycle. In the 3 other lines (2 A and one WT), the accumulation of T-Ag appeared to be independent of the position of the cell in the cycle. This cycle-dependent expression is controlled at the transcriptional level (accumulation of viral RNA in cells in G2 in N transformants, SV40 RNA detected in similar amounts in G1, S and G2 cells in the case of A transformants). In both types of cells, the stability of the associated cellular p53 protein (determined by labeling with [^{35}S] methionine followed by immunoprecipitation with monoclonal antibodies) appears to follow that of large-T.

0158 THE INTERACTION OF POLYOMA MIDDLE T ANTIGEN WITH THE CELLULAR HOMOLOGE OF pp60^{src} RESULTS IN AN ENHANCEMENT OF TYROSINE SPECIFIC PHOSPHOTRANSFERASE ACTIVITY. W. Yonemoto, J. Brugge, M. Israel and J. Bolen*, Dept. of Microbiology, SUNY, Stony Brook, NY 11794 *The Pediatric Branch, NCI, NIH, Bethesda, MD 20205

Recently evidence has been presented that the middle T antigen of polyoma virus is associated with the cellular homologe of the Rous sarcoma virus transforming protein, pp60^{src}. This result suggests that pp60^{c-src} could be partly, if not wholly, responsible for the polyoma middle T associated protein kinase activity. Our laboratory has developed a battery of monoclonal antibodies (MAbs) directed against pp60^{src} and we have utilized these MAbs to determine if the interaction between pp60^{c-src} and middle T alters the protein kinase activity of pp60^{c-src}. These MAbs are useful reagents for this study since 1) they are monospecific antibodies, 2) they recognize the mammalian cellular src protein, 3) they allow pp60^{c-src} to autophosphorylate, 4) they allow immune-complex bound pp60^{src} to phosphorylate exogenous substrates. We have examined the autophosphorylation of pp60^{c-src} and pp60^{c-src}-mediated tyrosine-specific phosphorylation of exogenous substrates in a variety of polyoma infected and transformed cells. Although there was no change in the level of pp60^{c-src} after polyoma transformation, there was a 5-10 fold enhancement in autophosphorylation of pp60^{c-src} as well as a 5-10 fold increase in the phosphorylation of the exogenous substrates, casein and angiotensin. These results intimate that the association of pp60^{c-src} and polyoma middle T ean result in an increase in the specific activity of pp60^{c-src}. It is not known whether this enhanced protein kinase activity is required for transformation by polyoma virus.

0159 SV40 LARGE T ANTIGEN DNA BINDING STUDIES, Daniel G. Tenen, Dana Farber Cancer Institute, Boston, MA 02115.

SV40 large T (tumor) antigen is a DNA binding protein which is required for regulation of viral messenger RNA synthesis, initiation of viral DNA synthesis , stimulation of cellular DNA synthesis, and neoplastic transformation of cells in culture. These diverse functions may be mediated by the DNA binding properties of large T. In order to better understand these processes, large T antigen was purified from SV40 infected cells and used in DNA "footprinting" assays to analyze the specific nucleotide binding sites in DNA sequences located at or near the SV40 origin of replication. Using wild type SV40 and a series of deletion mutants, it can be shown that T binds sequentially to a strong affinity site and a second adjacent site (site 2) which includes the origin of replication, although the binding to sites 1 and 2 does not appear to be cooperative. Binding to the origin site (site 2) was sequence specific, and occurred in 2 stages, with binding to the "early" half (site 2A) preceding binding to the "late" side of site 2. Further experiments were performed to look at the effect of a stem-loop structure within the SV40 replication origin upon T antigen binding. Such structures have been postulated to play a role in origins of replication. Formation of heteroduplexes between SV40 origin-containing fragments of wild-type and a mutant containing an 18 base pair deletion in the origin results in a stem loop structure, and T binding studies to these structures shall be discussed.

0160 STRUCTURE AND EXPRESSION OF PLATELET-DERIVED GROWTH FACTOR/c-sis GENE, Richard K. Barth, Paul Tempst, Astar Winoto, Lance Force and Lee Hood, California Institute of Technology, Pasadena, CA 91125

Platelet-derived growth factor (PDGF) stimulates the growth of a variety of cells of mesenchymal origin. PDGF has been demonstrated to be produced by the human osteosarcoma line U-2 OS. Recently PDGF has been shown to be homologous to p28 sis, the transforming protein of Simian sarcoma virus. Thus the autocrine production of PDGF by U-2 OS may be important for the establishment or maintenance of the transformed state of this tumor. We have been studying the expression of the PDGF/sis gene in U-2 OS and a variety of normal tissues. We have detected two equimolar PDGF/sis transcripts, 3.9 and 3.4 kilobases, in U-2 OS cells. The length of these transcripts is at least four times that required to encode PDGF; therefore, PDGF may be part of a large precursor polypeptide similar to epidermal growth factor. PDGF/sis transcripts have also been detected in bovine pituitary and murine submaxillary gland but not in liver kidney spleen, brain or thymus. We have isolated cDNA and cosmid genomic clones corresponding to PDGF/sis and are engaged in a structural analysis of the gene encoding this growth factor.

Poster Session No. 3

0161 DETECTION OF MAMMARY CELL TRANSFORMING GENES BY TRANSFECTION AND INDUCTION OF TUMORS IN NUDE MICE, Bernd Groner, Sara Kozma and Nancy E. Hynes, Ludwig Institute for Cancer Research - Bern Branch, Inselspital, CH-3010 Bern, Switzerland

A combination of DNA-mediated gene transfer into cultured cells and cellular transplantation into nude mice has been used to probe the existence and identity of genes responsible for the tumorigenic phenotype of mammary tumor cells. To test the validity of this approach nontumorigenic rat-2 cells and NIH/3T3 cells were transfected with genomic DNA from cells transformed by RSV, SV40 or the human EJ-ras oncogene. Transfected cells whose growth was anchorage-independent were selected. They are able to induce tumors in nude mice within 2 weeks after injection of 1×10^6 cells. Genomic DNA from primary human and mouse mammary tumors as well as from lung metastases of mouse mammary tumors was introduced into rat-2 cells. The highest incidence of tumors in nude mice was induced by cells which had received the DNA isolated from the mouse mammary metastases. The identity of the oncogene responsible is being investigated. Recent results relating acceptor cell phenotype and oncogene activity suggest that multiple steps of activation of distinct genes are required to elicit the tumor cell phenotype. We have used an epithelial cell line derived from the milk of an apparently normal female (HBL-100) as an acceptor cell for the human EJ-ras oncogene and for genomic DNA from mammary tumor lines (HS 578 T and BT-20). HBL-100 is rapid-growing and nontumorigenic. Transfected HBL-100 cells are being investigated for tumorigenic growth and an acquired oncogene.

0162 TRANSFORMING GENES OF HUMAN HEMATOPOIETIC TUMORS, Alessandra Eva and Stuart A. Aaronson, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20205

We have analyzed a wide variety of human hematopoietic tumors and tumor cell lines for DNA sequences able to transform NIH/3T3 cells by DNA mediated gene transfer techniques. We have demonstrated that the oncogene of one intermediate T-cell leukemia was the activated human allele of c-kis. In contrast, n-ras transforming genes were identified in two additional cell lines of intermediate T-cell leukemia, a CML derived cell line and an AML primary tumor. In efforts to detect additional human transforming DNA sequences in human tumors, we have detected an oncogene in a primary human poorly differentiated diffused lymphoma. This oncogene induces a distinct morphologic alteration of NIH/3T3 cells and has been transmitted through several cycles of DNA transfection. Moreover, it shows no detectable homology with members of the ras family or other available oncogenes. We are now in the process of cloning this transforming gene, and data will be presented about its biological and molecular characterization.

0163 MONOCLONAL ANTIBODIES REACTIVE WITH A 185K PHOSPHOPROTEIN ASSOCIATED WITH THE TRANSFORMING GENE(S) OF SEVERAL RAT NEUROBLASTOMAS. Jeffrey A. Drebin, David Stern*, Robert A. Weinberg*, and Mark I. Greene. Department of Pathology, Harvard Medical School, and *The Center for Cancer Research, Massachusetts Institute of Technology.

High molecular weight DNA from several rat neuroblastoma cell lines is capable of conferring the neoplastic phenotype upon susceptible non-transformed cells following DNA transfection. Cells transformed by these DNAs contain a common 185K phosphoprotein (pp185) when immunoprecipitated with sera from tumor immune mice, whereas cells transformed by a number of other oncogenes do not (Cell 28:865, 1982). Tunicamycin treatment of pp185 positive cell lines results in a loss of immunoprecipitable pp185 and the appearance of a new band at about 165K, suggesting that pp185 is heavily glycosylated. Pulse-chase experiments also demonstrate an initial protein at about 170K and subsequent increasing amounts of pp185. Following adsorption of pp185 immune ascites fluid with normal NIH3T3 cells we have found significant cell surface fluorescence on rat neuroblastoma DNA transformed NIH3T3 cells but no reactivity with either NIH3T3 cells or H-ras transformed NIH3T3 cells, as determined on the Fluorescence Activated Cell Sorter (FACS). FACS analysis of a number of different cell lines has shown absolute concordance between immunofluorescent staining with pp185 immune ascites fluid and the presence of immunoprecipitable pp185. We have recently developed hybridomas which secrete monoclonal antibodies reactive with pp185, as determined by FACS analysis and immunoprecipitation. Our results suggest that these antibodies react with a novel oncogene-related glycoprotein at least partially localized on the extracellular face of the plasma membrane.

0164 HUMAN Ha-ras POLYMORPHISM ANALYSIS IDENTIFIES LOCUS INSTABILITY AND UNUSUAL ALLELES IN SOLID TUMORS, T.G. Krontiris and D.R. Parkinson, Tufts Univ. School of Medicine

We have characterized the system of polymorphic alleles at the human Ha-ras locus by Southern blot analysis of DNA preparations from normal white cells. The identity and frequency of appearance of normal alleles was thus established. In addition, we determined that the basis for this polymorphism was a region of variable tandem repetition (VTR) and that the VTR was homologous to the repetitive DNA which generates allelic diversity at both the human insulin and ζ-globin loci. We then analyzed polymorphic alleles of Ha-ras in tumor tissue. Our results demonstrated that (a) the locus was unstable in solid tumors, with alteration of the normal 1:1 allelic ratio occurring frequently; (b) alleles not present in the normal population appeared relatively frequently in tumor DNA; and (c) in some instances these unusual alleles were present in the patient's normal DNA. We are continuing studies with paired DNA samples to determine if tumor rearrangement of Ha-ras occurs, as suggested by our preliminary data from several carcinomas. Oncogene polymorphisms may be useful in assessing an individual's risk of tumor development and in analyzing genomic instability during tumor progression.

0165 AMPLIFICATION OF c-myc AND c-myb ONCOGENES IN HUMAN COLON CARCINOMA CELLS Kari Alitalo[1], Manfred Schwab[2], C.C. Lin[2], Donna George[3], and J. Michael Bishop[2] Department of Virology[1], University of Helsinki; Department of Microbiology, University of California in San Francisco[2], Department of Human Genetics, University of Pennsylvania[3]

We have recently found a 30-40 fold amplification of the c-myc oncogene in homogenously staining regions (HSRs) of a HSR marker chromosome of human colon carcinoma cells COLO 320 (Alitalo et al. PNAS 80, 1707, 1983). Our further studies confirm that the HSR chromosome originates from an X-chromosome, since it retains an X-linked repeated DNA marker. Double minute chromosomes (DM:s) preceded the HSR marker in COLO 320 cells from the colon carcinoma patient. We have now obtained over 30-fold enrichment of c-myc in purified DM:n and we therefore propose that the translocation of c-myc from its resident site in chromosome 8, band q24, occurred through a DM intermediate.

The c-myb oncogene was found about 10 fold amplified in COLO 201 and 205 colon carcinoma cells that do not show cytogenetic signs of DNA amplification. In contrast to the amplified c-myc in COLO 320 cells, restriction endonuclease mapping shows no apparent rearrangements in the amplified c-myb locus. Amplification of c-myb is refelected as elevated expression of c-myb RNA in these cells. We have studied the chromosomal localization of the amplified c-myb copies by in situ hybridization. Other carcinomas or sarcomas were not found to express detecteable levels of c-myb RNA.

Amplification and enhanced or inappropriate expression of cellular oncogenes may contribute to the genesis and/or progression of at least some naturally occurring cancers.

0166 IN VITRO MALIGNANT TRANSFORMATION OF PRIMARY CELLS BY ACTIVE ONCOGENES, Neil M. Wilkie and Demetrios A. Spandidos, Beatson Institute for Cancer Research, Glasgow G61 1BD, Scotland.

Using eukaryote expression vectors with differing transcription control sequences and dominantly selectable genetic markers, active oncogenes have been introduced into primary cells from different tissues of a number of animal species. The mutant ras-1 gene derived from the T24 human bladder carcinoma cell line is capable of inducing malignant transformation in adult mouse muscle, kidney and lung cells, baby rat muscle, skin, kidney and lung cells, adult chinese hamster lung cells and feline embryo cells. The co-introduction of other active oncogenes is not required. The ability to transform primary cells depends on high expression levels of the T24 ras-1 gene, achieved by eukaryote enhancer sequences. Transformed cells show reduced serum requirement and anchorage independent phenotypes and induce metastatic tumours in nude mice. In some cases evidence has been obtained for the rapid induction of karyotype instability and the presence of marker chromosomes. This approach is being extended to other potentially active oncogenes from a variety of sources.

0167 EXPRESSION OF A PROTO-ONCOGENE (PROTO-MYB) IN HEMOPOIETIC TISSUES OF THE MOUSE. Diana Sheiness, and Minnetta Gardinier, LSU Medical School, New Orleans, LA 70112

This study addressed the possibility that proto-myb, the cellular homologue of an avian retroviral transforming gene, plays a role in maturation of T lymphocytes.

Cells from the thymus, an organ where T cell differentiation takes place, were separated into maturational subclasses in two ways. First, mice were injected with hydrocortisone, which kills cortical (immunologically immature) thymic lymphocytes; surviving cells consisted primarily of medullary (essentially immunocompetent) thymocytes. The second procedure exploited the selective agglutination of cortical thymocytes by the lectin peanut agglutinin (PNA). Both cortical and medullary thymocytes were recovered and analyzed after PNA fractionation. Total cellular RNA was serially-diluted, aliquots applied to nitrocellulose sheets, then hybridized to ^{32}P-labelled myb-specific DNA. Relative amounts of proto-myb RNA were determined by comparison of signals obtained with RNA to signals obtained with plasmid DNA standards. Results indicated that two to three-fold more proto-myb RNA was present in cortical than in medullary thymocytes. Proto-myb expression was about ten-fold lower in lymph node cells, which contain of 65% mature T cells, than in unfractionated thymus cells.

In contrast to the ubiquitous expression of other proto-oncogenes, proto-myb expression is confined primarily to hemopoietic lineages that descend from a pluripotential stem cell. Proto-myb transcription may be turned on in the stem cell, then switched off selectively as cells in the several hemopoietic lineages branch off and/or progress towards maturity. In support of this proposal, we found that proto-myb expression decreased significantly in T cells taken from compartments corresponding to successive degrees of maturation.

0168 TRANSFORMING ACTIVITY OF DNA FROM HUMAN MELANOMA, NEUROBLASTOMA, BREAST CARCINOMA CELLS AND FROM NORMAL HUMAN LYMPHOCYTES, Reinhold Schäfer, Sabine Griegel, Peter Knyazev, and Klaus Willecke, Institute of Cell Biology (Tumor Research), University of Essen, D-4300 Essen 1, Federal Republic of Germany

DNA (>20 kilobases) prepared from 21 different human tumor biopsies and cell lines was assayed for transforming activity by transfection of preneoplastic mouse NIH/3T3 cells. Transformants were selected in semi-solid agar medium. DNA from human melanoma, neuroblastoma, and breast carcinoma cells induced neoplastic transformation in NIH/3T3 cells with high efficiency ($\geq$ 1 transformant/ug of DNA/10^6cells). The transformants were analyzed for the presence of human repetitive sequences (plasmid BLUR-8 used as a probe) and of exogenous sequences related to known transforming genes (plasmids harboring v-Ha-ras, v-Ki-ras, and N-ras transforming genes used as probes). All transformants from human melanoma, neuroblastoma, and breast carcinoma cells harbored exogenous transforming genes of the ras-Harvey type.

Sonicated DNA (0.5-2.0 kilobases) prepared from normal human lymphocytes was able to transform NIH/3T3 cells with low efficiency. In a second cycle of transfection, the efficiency was increased approximately 100fold with high molecular weight DNA from primary transformants. These results confirm previous data obtained with normal mouse and chicken DNA (Cooper et al., Nature 284, 418, 1980). Human repetitive DNA was detected in three independent secondary transformants using BLUR-8 DNA as a probe. Primary transformants did not form tumors in newborn BALB/c mice, whereas secondary transformants were highly tumorigenic.

0169 A GENE RELATED TO THE myc ONCOGENE (N-myc) IS AMPLIFIED IN CHROMOSOMAL ABNORMALITIES, TRANSLOCATED AND OVEREXPRESSED IN HUMAN NEUROBLASTOMA, Manfred Schwab[1], Karl-Heinz Klempnauer[1], Jay Ellison[1], Kari Alitalo[1], Harold Varmus[1], J. Michael Bishop[1], Jeffrey Trent[2], and Allan Sakaguchi[3], [1]University of California, San Francisco, CA. 94143, [2]University of Arizona, Tucson, AZ. 85724, [3]Roswell Park Mem. Inst., Buffalo, NY 14263.

Cells of human neuroblastomas contain at high consistency conspicuous karyotypic abnormalities, "double minutes" (DMs) and "homogeneously staining chromosomal regions" (HSRs). In our initial survey we detected that DNA related to the myc oncogene was amplified. Further studies involving restriction endonuclease mapping and DNA sequencing of molecular clones revealed that the amplified DNA was not the previously isolated prototypic version of c-myc but rather a distant kin which we have provisionally denoted N-myc. We have found amplification varying from 10 to 150 fold in 9/10 neuroblastoma cell lines and 5/5 fresh tumors. Cytoplasmic mRNA contains an N-myc transcript of approximately 3.7 kb. N-myc, when amplified, is abundantly expressed, it is expressed at low levels in the neuroblastoma line not showing amplification, and its expression has not been detected in RNA of cells of other cell lineages. In situ hybridization localizes amplified N-myc to HSRs, which are located on different chromosomes in the different cell lines. Our studies with mouse x human somatic cell hybrids showed that N-myc is normally localized on the short arm of chromosome 2 (2p23→ pter). Thus amplification, translocation and overexpression of N-myc are associated at high consistency with human neuroblastoma.

0170 ROLE OF N-ras ONCOGENES IN HUMAN LYMPHOID NEOPLASMS, Michele Souyri and Erwin Fleissner, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

DNA of several human T-cell leukemia cell lines exhibited transforming activity in transfection assays on mouse NIH 3T3 cells.

Southern blot analysis of DNAs from secondary and tertiary transformed foci, after digestion by EcoRI or BamHI, revealed conserved fragments containing Alu sequences. Similar blot hybridizations with a probe specific for the human N-ras oncogene detected a 9 kb EcoRI fragment in all cases.

The transforming gene of one of the cell lines was molecularly cloned in bacteriophage lambda; the cloned gene showed a strong focus-inducing activity in transfection assays. Restriction map analysis indicated that the central part of the gene is similar to the N-ras clone obtained by Shimizu et al.[1] Thus the N-ras oncogene appears to be active in these human leukemias of T-cell origin.

Further investigations on the expression of the N-ras genes in human leukemias will be presented.

[1]Proc. Natl. Acad. Sci. 80, 383-387 (1983).

0171 REARRANGEMENTS CAN OCCUR AT EITHER SIDE OF THE c-myc ONCOGENE IN BURKITT'S LYMPHOMA CELLS, Susan Malcolm, Mary Davis and Terry Rabbitts, MRC Human Genetic Diseases Research Group, Queen Elizabeth College London W8 7AH and MRC Laboratory for Molecular Biology, Cambridge CB2 2QH

Specific chromosome translocations and abnormalities exist in tumour cell lines. In Burkitt's lymphoma cells, translocations are found between the long arm of chromosome 8 (8q24) and either the long arm of chromosome 14, the short arm of chromosome 2 or the long arm of chromosome 22. The breakpoint on chromosome 8 is close to the site of the cellular oncogene c-myc and the other breakpoints are all associated with immunoglobulin gene loci. Heavy chain genes are located at 14q32, κ light chains at 2p12 and λ light chains at 22q11. We have shown, using in situ hybridisation to tumour cell lines, that c-myc is translocated from chromosome 8 to chromosome 14 in Daudi (an 8:14 type Burkitt lymphoma). However, in two lines we have examined which contain an 8:2 translocation, J1 and LY91, the c-myc gene is found on both the normal and translocated chromosome 8. As clones have been isolated from 8:14 type Burkitt lymphoma lines (Taub et al., 1982; Proc. Nat. Acad. Sci. USA 79, 7837-41) which contain a c-myc gene rearranged at the 5' end and joined to the Cμ region, the rearrangement in these cells with the 8:2 translocation must occur on the 3' side of the gene. We have shown, also by in situ hybridisation, that at least two members of the V_κ group of genes remain on the translocated chromosome 2.

0172 INDUCTION OF CELLULAR ONCOGENES IN POLYOMAVIRUS OR SV-40 TRANSFORMED CELLS OR TUMORS. Michele M. Fluck and Larry Winberry. Michigan State University, East Lansing, Mi. 48824-1101

The transcripts levels of a number of cellular oncogenes have been determined in tissue culture transformed Fischer rat fibroblasts and compared to the levels found in normal cells. The oncogenes tested include: abl, erb-A, erb-B, Ha-ras, Ki-ras, mos, myc, src, fps, and sis. So far, no significant differences have been observed between the normal and transformed cells, for all cases tested, with the exception of one case of elevated sis in one SV-40 transformant. We are in the process of screening more SV-40 transformants for sis expression. Furthermore, we are beginning to analyze a series of SV-40 induced tumors, including many different types, for expression of the oncogenes listed above. The results of these experiments will be presented.

0173 ALTERATIONS IN DNA METHYLATION OF GENES OF HUMAN COLON CANCERS AND POLYPS, Andrew P. Feinberg, Bert Vogelstein, and Susan Goelz, Johns Hopkins Oncology Center, Baltimore, MD 21205

In a continuing investigation of alterations in DNA methylation in human cancer, we have compared the methylation pattern of a panel of genes in a large number of human colonic adenocarcinomas with that of adjacent normal mucosa. DNA was purified from fresh surgical specimens, digested with methylcytosine-sensitive restriction endonucleases, and transferred to nitrocellulose. The filters were hybridized with gene fragment probes "oligolabeled" to high specific activity (3×10^9 dpm/ug). The probes included genes unexpressed in normal colon, genes implicated in ectopic hormone production, and several cellular oncogenes. Substantial hypomethylation of a wide variety of genes was observed in the cancers. In addition, benign colonic adenomas also demonstrated substantial hypomethylation of the examined genes. Tumor heterogeneity was reflected by variations in gene methylation within primary tumors and among metastases. These observations suggest a possible role for alterations in DNA methylation in tumorigenesis and tumor progression.

0174 THE HUMAN CELLULAR HOMOLOGUES OF THE raf/mil ONCOGENE, Ulf Rapp, Pramod Sutrave, George Mark and Tom Bonner*, NCI/NIH, Frederick, MD 21701 and *NIMH, Bethesda MD 20205

We have obtained human DNA clones covering 27-31 kb from each of two loci in the human genome which are related to both the v-raf and v-mil oncogenes. These two loci are the only v-raf related genes detected in human DNA at moderately low stringency. The sequence of one locus, c-raf-2, demonstrates that it is a processed pseudogene. The second locus, c-raf-1, has 9 exons spanning more than 16 kb which are homologous to v-raf and v-mil. The exon sizes appear to be indentical to those of the chicken c-mil, while the intron sizes are quite different. Overall, c-raf-1 show 85% DNA sequence homology to the murine v-raf and 97% amino acid sequence homology. At the points in the c-raf-1 sequence, corresponding to the helper virus-oncogene junctions of v-raf, there are short sequence homologies to the Moloney leukemia virus sequence, suggesting that the v-raf sequence was transduced by homologous recombination. The 5'end of v-raf is within an exon of c-raf-1 while the 3' end is in the 3' untranslated portion of the gene. There is at least one more exon at the 5' end of the gene which hybridizes to the pseudogene.

The biological activity of c-raf-1 has been demonstrated by transfection using DNA constructs in which portions of v-raf have been replaced by human c-raf-1 sequence. One construct makes use of a conserved SphI site to replace the 3' two thirds of v-raf with exons 5-9 of c-raf-1. This construct transforms NIH3T3 with low efficiency, but when foci are examined, they contain the construct in unaltered form and express the expected polyprotein. A complimentary construct which replaces all but 20 nucleotides of the v-raf sequence 5' of the Sph site is being studied.

0175 Isolation of Transforming Sequences of Two Human Lung Adenocarcinomas:

Hirofumi Nakano, Fumiichiro Yamamoto, Craig Neville and Manuel Perucho
State University of New York at Stony Brook, Stony Brook, NY 11776

We have molecularly cloned over 40 kb of human DNA sequences from NIH3T3 transformants derived from DNA of two human lung tumors, PR371 and PR310. This allowed us to perform a comparative analysis of the mechanisms by which the c-k-ras oncogene has been independently activated in two naturally occurring human lung carcinomas. We have found that the oncogene of PR371 has been activated by a single base substitution in the first exon similar to that of T24-EJ bladder carcinoma oncogene while the PR310 oncogene contains a base substitution at position 61 of the second coding exon. These results strongly suggest that the mechanism of activation of the same proto-oncogene (c-K-ras) can occur by different mutational events in human lung carcinomas.

We are now studying the transcription of the c-K-ras oncogene and the mechanisms which regulate its expression in human normal and tumor cells, and in mouse and rat fibroblasts transformed by the oncogene.

0176 ISOLATION AND CHARACTERIZATION OF A TRANSFORMING GENE DERIVED FROM A CHEMICALLY TRANSFORMED HUMAN CELL LINE, M. Park, M. A. Tainsky, D. G. Blair, NCI-Frederick Cancer Research Facility, Frederick, MD 21701, and C. S. Cooper, Dana-Farber Cancer Institute, Boston, MA 02115

We have previously reported that DNA from the chemically-transformed human cell line, MMNG-HOS, will transform NIH3T3 cells in DNA transfection assays. Analysis of the alu hybridizing restriction fragments in secondary transfectants revealed a common pattern. No homology to either v-Ki ras, v-Ha ras, N-ras, v-mos or v-myc was detected in the human sequences present within these cells. Restriction analysis of several overlapping lambda clones spanning 40 kb of the transferred human DNA confirm that the transforming sequences are distinct from the known members of the ras oncogene family. Southern analysis, utilizing subcloned fragments of the MMNG-HOS derived clones as probes, identify unique restriction fragments within human placental DNA. These probes also detect a unique species of polyadenylated RNA isolated from MMNG-HOS transfectants. Comparison of DNA from the parental cell line (HOS), human placental tissue and from several human tumors revealed that there has been no apparent rearrangement of this DNA within the MMNG-HOS cell line, or within secondary transfectants derived from it. Activation of this transforming gene following MMNG treatment, may therefore be by mutation within the coding sequence of this gene or through aberrant or enhanced expression.

0177 C-MYB TRANSCRIPTS IN HUMAN SMALL CELL LUNG CARCINOMA CELL LINES. Constance A. Griffin, Barry D. Nelkin, and Stephen B. Baylin, Johns Hopkins Oncology Center, Baltimore, MD. 21205

Oncogenes have provided the first major group of genes which can be expected to play a role, as yet unclear, in the initiation or maintenance of human cancers. Small cell cancer of the lung comprises about 20% of human lung cancers. Biologically an interesting and aggressive tumor, it is initially quite sensitive to chemotherapy and radiation, only to relapse and become refractory to all treatment within a year in most patients. We have been studying the structure, amplification, and expression of known oncogenes in small cell lung carcinoma cell lines. Our results suggest a possible role for the myb gene in this disease. Although mRNA complementary to v-myb has not been detected in most normal and tumor cell types examined previously (Westin et al, PNAS 79;2194-2198,1982; Griffin and Baylin, unpublished)we have observed myb-specific poly(A) RNA of 3.5 kb in two small cell lines. The transcript is absent in four non-small cell lines and one variant small cell line, and we are currently testing the hypothesis that activation of c-myb correlates with either the early or late phases of the disease. This involves testing of additional established small cell lines as well as fresh tumor lung cancer specimans. Cells containing active myb genes did not show evidence of c-myb amplification or rearrangement. We are currently attempting to determine if the 3.5 kb RNA in these cells is translated into a myb-related protein.

0178 TRANSFECTION OF MALIGNANT PHENOTYPE BY DNA FROM RAT KIDNEY TUMORS INDUCED BY DIMETHYLNITROSAMINE (DMN), S.K. Shore, L.T. Bacheler and P.N. Magee, Fels Research Institute, Temple U. Schl. Med., Philadelphia, PA 19140 and S.W. Needleman and S.A. Aaronson, National Cancer Institute, NIH, Bethesda, MD 20205

A single injection of the rapidly metabolized alkylating and mutagenic chemical carcinogen dimethylnitrosamine (DMN) will produce mesenchymal and/or epithelial kidney tumors in virtually 100% of Wistar rats preconditioned on a low protein, high carbohydrate diet. DNA isolated from these primary tumors was assayed for transforming activity by transfection into NIH/3T3 cells. Transformation was induced with an efficiency of $\sim$ 0.001 transformants per μg tumor DNA. Transfection of kidney, liver, and brain DNA from untreated rats yielded no transformants. DNA from primary transformants was shown to contain rat sequences by hybridization to a cloned repetitive rat DNA sequence. Transformation efficiency was increased to $\sim$ 0.005 transformants per μg DNA in secondary transfection experiments. Hybridization to viral N-ras, K-ras, and H-ras probes suggested an increased level of H-ras sequences in NIH cells transformed by the rat tumor DNA. The detailed pathogenesis of the DMN induced renal tumors and the pattern and persistence of initial methylation of kidney DNA have been extensively studied. These tumors should, therefore, be useful models for future investigation of oncogene activation by this important class of chemical carcinogens.

0179 FREQUENT ACTIVATION OF ONCOGENES IN PATIENTS WITH ACUTE LEUKEMIAS, Christoph Moroni, Christof Gambke and Hanspeter Senn*, Friedrich Miescher-Institut and Pharmaceuticals Division, CIBA-GEIGY*, Basel, Switzerland

We are studying the relationship between oncogene-activation and the clinical course of acute human leukemias. Transfection experiments indicate that oncogene activation is a frequent event as DNA from 6/8 cases induced foci on NIH3T3 cells. The positive cases included 3/4 acute myeloblastic leukemias, 1/2 acute lymphoblastic leukemias, 1 non-Hodgkin lymphoma and 1 monoblastic leukemia.

In one AML case, we identified the activated gene as N-ras as indicated by Southern blot analysis of primary and secondary transfectants. Transfectants contained elevated levels of protein p21. Cloning experiments are in progress to compare the activated N-ras gene with its alleles from the patients fibroblasts. Also we are currently trying to identify the oncogenes activated in the remaining positive leukemias.

0180 REGULATION OF ras-RELATED PROTEINS IN THE LIFE CYCLE OF DICTYOSTELIUM DISCOIDEUM, AND IN MAMMALIAN CELLS. Tony Pawson, Nellie Auersperg and Gerald Weeks.
Dept. Microbiology, Univ. British Columbia, Vancouver, B.C. V6T 1W5, Canada.
During the life cycle of the slime mould Dictyostelium Discoideum cell growth and differentiation can be mutually exclusive. D. discoideum amoebae grow rapidly and are indifferent to one another; upon starvation cell division stops and cells synchronously aggregate and differentiate into a slug and then a fruiting body. Cell proliferation is halted throughout differentiation with the exception of a wave of DNA synthesis in the pre-spore cells of the early slug. D. discoideum amoebae contain a 23,000 M_r protein (p23) that is antigenically and structurally related to rat $p21^{ras}$. P23 is apparently the processed form of a p24 protein which we speculate corresponds to the primary translation product of a D. discoideum ras gene. Absolute levels of p23 drop up to 10-fold during differentiation as cell division is arrested, but a transient rise is seen around 15 hours concommittant with reported DNA synthesis in the slug. The marked decrease in p23 levels during differentiation argue that the protein's function is in the regulation of cell growth or division and is only negatively involved in differentiatin. Synthesis of p23 continues throughout differentiation, and it is not yet clear whether its level is regulated by transcriptional or translational controls or by specific protein degradation. Similar to the residual synthesis of p23 in arrested differentiating D. discoideum, we have identified synthesis of rat $p21^{ras}$ in primary cells such as those from the ovarian granulosa with little or no proliferative capacity in culture. Rat and human ovarian granulosa cells synthesize a 27,000 M_r protein which is immunoprecipiatated by anti-p21 antibodies, as do many primary cells and cell lines. The function of this ras-related protein is under investigation.

0181 CHICKEN CELLULAR DNA SEQUENCES HOMOLOGOUS TO THE fps GENE OF FuSV: ISOLATION OF c-fps AND TWO RELATED SEQUENCES WITH LIMITED HOMOLOGY TO THE TYROSINE KINASE PORTION OF THE fps GENE, David A. Foster, George Q. Daley, Masabumi Shibuya, and Hidesaburo Hanafusa, The Rockefeller University, New York, New York 10021

A library of chicken cellular DNA was screened for sequences homologous to the transforming gene (fps) of Fujinami sarcoma virus. Three distinct sequences were isolated and partially characterized. One of the cellular sequences is highly homologous to the entire viral fps gene and presumably is its cellular progenitor (c-fps). Cellular sequences spanning 25kb contained all v-fps sequences (2.3kb) within a 6.8kb region. Heteroduplex and restriction mapping suggested an interrupted gene structure with a minimum of 6 introns. The transforming ability of this DNA is being analyzed. Two other cellular sequences were found that were weakly homologous to fps. The region of shared homology between these two fps-related sequences and v-fps is limited to the 3' portion of v-fps which is responsible for the tyrosine kinase activity. This is the same region that shares homology with other transforming genes such as src and yes. Consistent with this we have detected weak homology between the fps-related sequences and src, yes, and ros gene sequences. Whereas we have detected expression of c-fps in bone marrow cells, we have been unable to detect significant levels of expression of the fps-related sequences in these cells or in several others tested.

0182 EXPRESSION OF THE C-SRC PROTO-ONCOGENE DURING EMBRYOGENESIS OF THE HAWAIIAN SEA URCHIN. Alan F. Lau, Tom Rayson, and Tom Humphreys, University of Hawaii, Honolulu, HI 96813

Cellular proto-oncogenes, homologous to retroviral transforming genes have been detected in various vertebrates, and some invertebrates such as sponge and Drosophila. Expression of several proto-oncogenes has been reported to occur in a developmentally-regulated fashion in vertebrate tissues. This report describes the identification of the c-src proto-oncogene and its protein product in invertebrate sea urchin embryos. A c-src clone has been isolated from a λ Charon 4A library of sea urchin DNA. Results of restriction enzyme mapping of this clone and "Northern" blot analysis of sea urchin RNA to determine the transcriptional activity of the c-src proto-oncogene will be presented. The putative sea urchin c-src protein was immunoprecipitated by tumor bearing rabbit (TBR) serum and was initially identified by its tyrosine-specific protein kinase activity in vitro. Expression of this activity appeared to be developmentally-regulated as it was highest in egg, decreased approximately 5-fold through the gastrula stage, then subsequently increased 3-fold to the final pluteus stage embryo. Phosphoamino acid analysis demonstrated that the expected amino acid, tyrosine, was the phosphate-acceptor in the immunoglobulin substrate. Direct identification of the sea urchin c-src protein was also accomplished by immunoprecipitation of [^{35}S]-methionine-labeled cell lysates using TBR serum. Its apparent molecular weight was approximately 53-55,000 daltons. The primary structure of the sea urchin c-src protein, pp60$^{v\text{-}src}$ from avian sarcoma virus-transformed cells and pp60$^{c\text{-}src}$ from normal vertebrate cells is being compared by partial proteolytic peptide mapping and these results will also be presented.

0183 TRANSFORMING GENES OF COMMON ACUTE LYMPHOCYTIC LEUKEMIAS (ALL), Geoffrey R. Kitchingman, St. Jude Children's Research Hospital, Memphis, TN 38101

The genes involved in the transformation of early B cell precursors to malignancy are being determined by the use of the NIH-3T3 cell transformation assay. DNAs isolated from the leukemic bone marrow cells of patients with undifferentiated or common ALL have been used to obtain primary transformants of NIH-3T3 cells. To date, 7 of 7 common ALL and 1 of 1 undifferentiated ALLs have yielded transformants. Secondary transformations are in progress utilizing representative primary transformants. The primary transformants are being analyzed for the presence of known oncogenes. Thus far, we have identified human n-ras genes in some, but not all, of the primary transformants of 6 out of 6 of the ALL DNAs examined thus far. The identification of the transforming genes in the other primary transformants is in progress.

The association of n-ras with common ALLs is intriguing in light of the fact that these leukemic cells share a number of cell surface antigens with neuroblastoma, the original source where n-ras was identified.

0184 IS *C-ERB A1* REARRANGED IN THE 15q+;17q- CHROMOSOME TRANSLOCATION ASSOCIATED WITH ACUTE PROMYELOCYTIC LEUKEMIA? Denise Sheer, Lynne R. Hiorns, Nigel Spurr, Ellen Solomon.

Analysis of interspecific somatic cell hybrids containing the 15q+/17q- translocation chromosomes has enabled us to localize the translocation breakpoints to 15q22 and 17q12-21 (Sheer et al,Proc. Natl. Acad. Sci. USA,1983,80,5007). Of particular interest is our finding that the *c-fes* oncogene which maps to chromosome 15 is not present on the 15q+ translocation chromosome and therefore probably moves to the 17q- chromosome. Furthermore, we have mapped the *c-erb A1* oncogene to 17p11-17q21, and shown that it is not present on the 15q+ translocation chromosome and therefore probably remains on the 17q- chromosome. (Spurr et al, EMBO J.,1984, in press). We are currently analyzing DNA from APL patients showing this translocation for rearrangements of the *c-erb A1* gene. These results will be presented.

0185 PROTOPLAST FUSION TRANSFECTION OF ONCOGENES INTO HUMAN BRONCHIAL CELLS, G.H. Yoakum, J.F. Lechner, E.Gabrielson, B.E. Korba, L.Malan-Shibley, J.C. Willey, T. Seeley and C.C. Harris, National Cancer Institute, Bethesda, MD 20205

To investigate the role of oncogenes in human cell carcinogenesis, we have developed a protoplast fusion method for the efficient transfection and stable integration of genes into human cells. For example, the pBR322 plasmid (clone H1) carrying the cDNA of viral Harvey ras (vHa-ras) has been transfected by protoplast fusion into normal human bronchial epithelial cells. Although the normal bronchial epithelial cells are induced by 2% blood-derived serum (BDS) in LHC-4 medium to terminally differentiate, the transfected cells were resistant and are still proliferating after more than 100 cell generations. Between passages 10 and 15, cell clones were isolated that grew as densely packed, multilayered foci. DNA hybridization studies using Ha-ras gene specific and Ha-LTR specific DNA probes indicate that these cells have integrated Ha-ras genes into their nuclear DNA and contain differing amounts of mRNA. Ha-ras gene product (p21) labeled with either ^{35}S-met or ^{32}P was detected in cell extracts following immunoprecipitation with vHa-ras specific anti-p21 monoclonal antibody and SDS gel electrophoresis. These cell lines are also being evaluated for tumorigenicity in athymic nude mice, growth in semisolid medium, and cell surface antigenic markers. (We thank Dr. Esther Chang for providing the plasmid H1 and Dr. Mark Furth for the monoclonal antibody Y13-238).

0186 ISOLATION AND CHARACTERIZATION OF C-MYC GENES FROM HL-60, Russel E. Kaufman and Joni Nichols, Duke University, Durham, NC 27710

The human cellular analog of the avian mc-29 viral transforming gene is known to be amplified in several malignant cell lines in which the expression of c-myc is enhanced. In one of these cell lines, HL60, the c-myc genes reside on restriction endonuclease fragments which are different from those present in DNA derived from non-malignant cells. Despite the large number of c-myc genes in HL60 cells, accumulation of RNA transcripts from these genes can be diminished by induction of differentiation to neutrophils with retinoic acid or dibutylcyclic AMP, or to macrophages with PMA. We have attempted to study those c-myc genes which remain under some regulatory control in HL60. To do so we have isolated a large number of bacteriophage recombinants with sequence homology to MC29 from a bacteriophage library prepared from HL60. The majority of these recombinants (90%) have very limited sequence homology to MC29 while the remainder have moderate to very extensive homology. This indicates that amplification of the gene may result in a large number of incomplete c-myc genes or fusion genes and may not be active transcriptionally. As such they would not contribute to the increased c-myc transcripts in HL60. We are analyzing the apparently normal cloned c-myc sequences to determine if they function normally in short-term expression systems and are under regulatory control by inducers of differentiation.

0187 DIFFERENT ONCOGENE REARRANGEMENTS IN HUMAN HEMATOPOIETIC NEOPLASIAS, Riccardo Dalla-Favera[+], Luisa Lanfrancone[+], Michel Brathwaite[+], Stefano Martinotti[+] and Edward P. Gelmann[x], [+]Department of Pathology, Kaplan Cancer Center, New York University School of Medicine, New York, NY 10016, [x]Medicine Branch, National Cancer Institute, Bethesda, MD 20205.

Recent studies have suggested that cellular oncogenes are involved in chromosomal abnormalities which are specifically associated with certain types of neoplasias. In particular the c-myc locus has been shown to be directly involved in translocations typical of undifferentiated B cell lymphomas and amplified in several tumor cell lines. In an attempt to expand these observations we have surveyed a number of different hematopoetic neoplasias for rearrangements affecting different oncogene loci. Our studies indicate that rearrangements, amplifications or both, affecting the c-myc and c-myb loci are detectable in lymphoid and myeloid neoplasias. These findings are detectable in both cell lines and primary pathologic material. In some cases genomic rearrangements correlate with new cytogenetic alterations whereas in other cases no alteration is detectable suggesting that genomic rearrangements may be more frequently associated with malignant cells than currently detectable with ordinary cytogenetic analysis. Oncogene rearrangements are associated with different alterations in mRNA structure and levels of expression. Work is in progress to generate antibodies against oncogene protein products in an attempt to correlate the effects of different oncogene rearrangements on the structure or expression of onc-proteins.

0188 ACTIVATION OF DIFFERENT TRANSFORMING GENES IN BALB/3T3 CELL LINES TRANSFORMED BY THE SAME CHEMICAL CARCINOGEN, BENZO(a)PYRENE, Margherita Bignami, Eric Westin, Michael I. Lerman, Takeo Kakunaga, Edward M. Kaighn and Umberto Saffiotti, National Cancer Institute, Frederick, Maryland 21701.

In order to investigate whether more than one transforming gene could be activated by carcinogens in the same cell system, DNAs obtained from 16 different BALB/3T3 cell lines, transformed in vitro by different chemical and physical carcinogens, were transfected into NIH/3T3 cells. Transforming activity was obtained from seven different cell lines, indicating that these cells contained alleles that act dominantly in the NIH/3T3 system. The transforming genes activated in 3 benzo(a)pyrene-transformed lines were investigated for their sensitivity to digestion with 4 different restriction endonucleases. The transforming DNA from each of the 3 lines showed a different pattern of sensitivity, indicating that different transforming genes were activated in these 3 lines transformed in vitro by the same chemical carcinogen. Hybridization experiments indicated that these transforming genes are different from the Harvey and Kirsten ras oncogenes. Karyotypes of the parent and the transformed BALB/3T3 cell lines were analysed and several chromosomal markers were identified, but the presence of these markers was not correlated with transforming ability as detected by transfection in the NIH/3T3 cells.

0189 EXPRESSION OF RAS GENE PRODUCTS IN HUMAN LUNG AND COLON TUMORS, Gary E. Gallick, Razelle Kurzrock, Jan C. Liang, William S. Kloetzer, Edwin C. Murphy, Jr., Ralph B. Arlinghaus, and Jordan U. Gutterman, M.D. Anderson Hospital, Houston, Texas 77030

Although expression of cellular oncogenes is usually measured by mRNA levels in "Northern" analyses, the level and activity of the protein products of these genes may best correlate with the events in malignant transformation. Therefore, we have begun to systematically examine human tumors for steady-state levels of oncogene proteins. Initially, we are comparing the ras gene products (p21) of colon and lung tumor to adjacent normal tissue. Extracts of lyophilized tissue were immunoblotted with a rat monoclonal antibody to the p21 of the v-ras^{ha} gene. Further, to increase the amount of p21 which could be analyzed, extracts which had been immunoprecipitated with the monoclonal antibody prior to immunoblotting were also examined. By this procedure, p21 was detected in all tissue extracts analyzed. However, 5 of 15 colon tumors and 2 of 4 lung tumors expressed at least five-fold elevated p21 when compared to adjacent normal tissue. To determine if chromosomal aberrations correlate with ras gene expression, we have cultured cells from several tumors. Preliminary cytogenetic analysis of cells from one of the lung tumors with elevated p21 indicated trisomy 12 in approximately 20% of metaphase spreads examined. Similar cultured cells from histologically normal lung tissue adjacent to the tumor, in which the level of p21 was not elevated, did not exhibit trisomy of chromosome 12. These studies indicate that elevated ras proteins can be found in some human colon and lung tumors. Ongoing analyses may reveal the role of these elevations in tumor development.

0190 NORMAL RAT CELLS ARE ONCOGENICALLY TRANSFORMED BY A SINGLE TRANSFECTION OF RAT TUMOR DNA, Stephen A. Schwartz, Peter Freebeck, and Charles F. Shuler, The University of Chicago, Chicago, Illinois, 60637

A recent controversy has arisen regarding the number of activated oncogenes required to transform target cells in-vitro. Although single, activated oncogenes obtained from spontaneous tumors efficiently transform 3T3 cells, relevance to the true neoplastic condition has been challenged, inasmuch as 3T3 cells are a permanent and established cell line. More recently, it was reported that two or more interacting, activated oncogenes were required to transform normal cells. Therefore, we attempted to transfer malignant potential from a spontaneously-appearing rat sarcoma to normal rat cells in-vitro. High molecular weight tumor DNA was isolated and transfected to low-passage, normal rat embryo fibroblasts. Within a few months, normal DNA-transfected control cultures underwent senescence and died; tumor DNA-treated cells continued to grow in monolayer as well as in 0.3% agar. All of the normal target cells were diploid (42 chromosomes); the "oncogene"-treated cells contained from 40-135 supernumerary and abnormal chromosomes. Furthermore, malignant tumors identical to the original rat sarcoma were generated within 3 months in normal, unaltered newborn rats injected subcutaneously with transformed cells. Preliminarily, the tumor cells synthesized [^{32}P]-containing proteins corresponding to 21kd and 28kd in amounts greater than control fibroblasts. Molecular evidence for mRNA corresponding to v-Ras-Ki could not be detected. Therefore, our early data indicate that a single rat oncogene is indeed capable of stably transforming a malignant phenotype to normal rat cells in-vitro and in-vivo after a single transfection.

0191 MOLECULAR CLONING OF A NEW TRANSFORMING GENE INDUCED BY TUMOR PROMOTING 12-O-TETRADECANOYLPHORBOL-13-ACETATE (TPA) IN MOUSE EPIDERMAL PRENEOPLASTIC CELLS, Michael I. Lerman[1], Glenn A. Hegamyer[2] and Nancy H. Colburn[2], Laboratory of Experimental Pathology[1] and Cell Biology Section, Laboratory of Viral Carcinogenesis[2], National Cancer Institute, Frederick, MD 21701

Recently Colburn et al. (Mol. Cell. Biol., 3: 1182-1186, 1983) demonstrated by DNA-mediated gene transfer the existence of DNA sequences conferring anchorage independent growth on clonal lines of mouse JB6 cells in response to TPA.

Now we have molecularly cloned the responsible gene in a plasmid vector by using Lederberg's sib-selection technique. The gene termed *pro* is found in two clones containing inserts of about 3 kb and 12 kb respectively. The *pro* gene exists in a transcriptionally active configuration in non-treated preneoplastic cells and is fully expressed in TPA transformed cells. The transformed cells also contain another active transforming gene distinguishable from *pro* by restriction enzyme sensitivity. The *pro* gene is not related to any of 11 known viral oncogenes, as shown by RNA hybridization on expression blots. The structure and function of the *pro* gene is under investigation.

0192 CLONED DNA TRANSFORMING RAS RELATED GENE FROM TUMORIGENIC GUNIEA PIG CELLS INITIATED IN VITRO, Jay Doniger and Joseph A. DiPaolo, Lab. of Biology, NCI, Bethesda, MD 20205

An oncogene derived from a tumorigenic MNNG induced guinea pig cells has been cloned. This 11 Kb sequence, isolated from an 8 to 12 Kb Eco R1 cut sub-genomic library from a second round transformant of 3T3 cells, was detected by hybridization to v-bas under relaxed conditions. Similar oncogenes have also been identified by DNA transfection and Southern blot analysis with v-bas in 4 other tumorigenic guinea pig cell lines (Sukumar et al. Laboratories of Biology and Cellular and Molecular Biology). Of the 5 tumorigenic guinea pig cell lines studied, 4 were initiated in vitro and the fifth in utero; 4 different chemical carcinogens were used. The guinea pig carcinogenesis model is being studied because it is characterized by distinct stages in the transition from non-transformed to neoplastic growth induced by carcinogen treatment. Because oncogenes were detected only after in tumorigenic cells but not in cells at preneoplastic stages, the aquisition of tumorigenic properties and activation of the ras oncogene could have occurred by a common mechanism. To ascertain the genetic changes responsible for transformation, the cloned oncogene sequence will be compared to that of its proto-oncogene derived from non-treated normal guinea pig cells. The oncogene will also be used to investigate the susceptibility of guinea pig cells at different pre-neoplastic stages to transformation by this gene.

0193 ENHANCEMENT OF TRANSFECTED GENE ACTIVITY IN NORMAL HUMAN CELLS, R. Reeves*, C.M. Gorman#, and W.R. Clevenger*, Washington State Univ.*, Pullman, WA 99164 and Imperial College of Sci. and Tech.#, London, UK SW72AZ

Compared to rodent cells, primary cultures of normal diploid human cells are very inefficient in the uptake and expression of foreign genes in transfection experiments. We have recently developed techniques for markedly stimulating the expression, both transient and stable, of various bacterial genes (CAT, NEO, GPT) transfected into "established" lines of mammalian tissue culture cells (Gorman et al., 1983; Sci. Sci. 221:551; Gorman et al., in the press). These optimized transfection procedures, particularly the exposure of newly transfected cells for a short period of time to sodium butyrate at appropriate concentrations, are also found to enhance the expression of foreign genes introduced into normal human cells. For example, butyrate treatment stimulates the expression of the chloramphenicol acetyltransferase (CAT) gene by a factor of greater than 20-fold in primary cultures of newborn human foreskin fibroblasts transfected with the vector pSV2.cat and over 8-fold in cells transfected with pRSV.cat. Similarly, normal human cells transfected with selectable marker genes such as GPT and NEO form 10-15 times more stably transformed colonies under selective conditions after exposure to butyrate than do untreated cells transfected with the same genes. And, finally, primary foreskin fibroblasts transfected with the human bladder carcinoma oncogene T24c-Ha-ras are also stimulated 8-10 fold in their levels of oncogene mRNA production following butyrate exposure. Butyrate enhancement of transfected gene activity appears to require the presence of DNA "enhancer" sequences and may be partially the result of the foreign genes being assembled into an active form of chromatin structure after transfection.

0194 ABOUT CELLULAR IMMORTALIZATION, Jean Feunteun, Maryvonne Gardes and Hélène Jacquemin-Sablon, Institut de recherches scientifiques sur le cancer, Villejuif, France

Cellular immortalization is defined by the acquisition of an unlimited growth potential in vitro and may represent an early step in vivo tumorigenicity. Immortalized cell lines belong to three main classes : i) post crisis cells ; ii) tumor cells ; iii) virus transformed cells. We have developped a transfection assay capable of detecting dominant immortalization genes. By transfecting genomic DNAs from SV40 transformed cell lines we can transfert immortality to precrisis rat embryo cells. We are currently using the assay to investigate the nature of immortality in different classes of established cell lines.

0195 STRUCTURAL ANALYSIS OF THE *int-1* MAMMARY ONCOGENE. Albert van Ooyen, Laura van 't Veer, Harm van Heerikhuizen* and Roel Nusse. The Netherlands Cancer Institute, Amsterdam; *Free University, Amsterdam.

A region of the mouse genome, called *int-1*, is occupied by MMTV proviruses in many different mammary tumors. A sequence within this region is transcribed into a 2.6 Kb poly $(A)^+$ RNA in mammary tumors with a provirus integrated at *int-1*, but not in normal tissue. Restriction enzyme mapping on the configuration of the inserted proviruses has revealed that virtually all proviruses are either on the 5' side of the *int-1* gene in the opposite transcriptional orientation or on the 3' side of the gene in the same orientation indicating that activation occurs by an indirect mechanism such as the provision of a transcriptional enhancer. Only one case of a promoter insertion has been found.
We have subjected the *int-1* gene and the transcript found in mammary tumors to a detailed structural analysis: the transcriptional unit consists of at least 5 exons, most of which are confined to the region in between the inserted proviruses. In some tumors insertion has occurred in the last exon, giving rise to *int-1* transcripts that are continuing in the MMTV LTR.
We have cloned the human homologue of *int-1*, and have used heteroduplex mapping with the mouse gene to confirm the intron-exon assignment.

Poster Session No. 4

0196 K562 HUMAN ERYTHROLEUKEMIA CELLS DEMONSTRATE COMMITMENT, Peter T. Rowley, Betsy M. Ohlsson-Wilhelm, and Barbara A. Farley, University of Rochester, Rochester, NY 14642
Commitment, i.e. the decision to express a differentiated phenotype and to terminate proliferation irreversibly in the absence of inducer, was investigated in K562 human erythroleukemia cells. Cells were cultured for 0, 1, 2, 3, or 4 days with inducer and then plated in methylcellulose without inducer. Daily following plating, hemoglobin content was scored by benzidine staining and growth was assessed by estimating cell number per colony. With all inducers used, three types of colonies were found, those containing only benzidine positive cells, those containing only benzidine negative cells, and those containing both benzidine positive and benzidine negative cells (mixed colonies). Thymidine produced a progressive increase in the percentage of positive and mixed colonies and a progressive fall in the percentage of negative colonies. Whereas negative colonies grew at an exponential rate with a generation time of about 20 hours, positive colonies reached an average maximum size of 16 cells, representing a total of four divisions. Butyrate had a similar effect except that the rise was greater for mixed colonies than for positive colonies and the plateau in positive colony size was less evident. In contrast, CO_2 depletion or hemin treatment induced benzidine positivity which rapidly reverted upon removal of the inducing condition. Thus, of the four conditions, thymidine and butyrate caused commitment whereas hemin and CO_2 depletion did not. Thus K562 cells, like Friend cells, demonstrate commitment, but, unlike Friend cells, demonstrate a significant rate of commitment in the absence of inducer and hence form a significant percentage of mixed colonies with or without inducer.

0197 A NOVEL ROLE OF VIRAL GENE EXPRESSION IN DETERMINING THE TUMOR-INDUCING CAPACITY OF TRANSFORMED CELLS. Arthur S. Levine, James L. Cook, Cephas T. Patch, Janet Hauser, and Andrew M. Lewis, Jr., NICHD and NIAID, Bethesda, Md. 20205
The lack of a consistent association between cell transformation in vitro and tumor induction in vivo is well documented, but virus-transformed cells that are non-oncogenic are immortal and share other properties with oncogenic cells. Non-oncogenic transformed cells are usually considered to be more immunogenic than oncogenic cells. However, we and others have found, using adenovirus (Ad)-transformed rodent cells, that there is no correlation between immunogenicity (as measured by the detection of TSTA in bioassays with sensitized hosts) and oncogenic potential. In contrast, we found an excellent correlation between the oncogenicity of DNA virus-transformed hamster cells and their in vitro resistance to lysis by activated macrophages and natural killer cells. Using a variety of DNA virus-transformed hamster cell lines, we have identified a spectrum of tumorigenic phenotypes defined by the immunologic maturity and immunologic relatedness of the challenged host. To develop this model, which dissociates transformation and oncogenesis, we formed somatic cell hybrids of Ad2 (weakly oncogenic) and SV40 (highly oncogenic) transformed hamster cells. Hybrid cells which express both Ad2 and SV40 T antigens possess the morphology, oncogenicity, and sensitivity to lysis (by naive effector cells) of the Ad2 parental line; thus, the phenotype of these hybrid cells is governed by Ad2 gene expression. Since these large differences in biological behavior appear to reflect the differential expression of a small number of viral genes, our model should facilitate studies to distinguish between immortalization, morphologic transformation, and oncogenesis at the molecular level.

0198 UNRESTRICTED (H-2) GROWTH OF A MALIGNANT TUMOR OF MOUSE ORIGIN. Guillermo Alfaro, Enrique Escandón, Veronica Yakoleff and Gabriel Nava. National University Mexico. México, D. F. 04510, México.

We have identified a malignant murine tumor of lymphoblastic origin capable of overcoming H-2 restriction when injected in the peritoneal cavity of mice. This ability allowed the malignant cells to progress with high efficiency in mice of various haplotypes H-$2^{b,d,k}$, without any detectable difference either in the capacity to produce metastasis (usually very poor) or in time required to kill the mice. An interesting property of this tumor is that the cells were not only resistant to cytotoxic T lymphocytes raised against different H-2 products; but also resistant to NK cells. On the other hand, antisera against these cells which had been raised in two different species (rabbit and mice), indicated that this tumor shared some antigenic determinants with other tumors, such as LW.1 (originally thought to be L5178Y). The molecules recognized by these two antisera were not H-2, since specific anti-H-$2^{b,d}$ or ^{k} failed to react with these cancer cells.

0199 ONCOGENE EXPRESSION DURING HL-60 DIFFERENTIATION, François G. Dautry and Robert A. Weinberg, I.R.S.C., Villejuif, France and M.I.T. Cancer Center, Cambridge, MA 02139

At least two genes are altered in the human promyelocytic cell line HL-60 : c-myc is amplified to about 30 copies and there is a transforming allele of N-ras. It is possible to induce differentiation of HL-60 in granulocytes or monocytes and in both cases cell proliferation stops. We have studied transcription of myc and N-ras as well as several other c-onc during differentiation in both lineages. The dominant feature is the transcriptional shut off of c-myc and the precise correlation between c-myc expression and cell proliferation. N-ras transcription is unaffected by granulocytic differentiation while it shows a complex regulation during monocytic differentiation. Nevertheless N-ras is still expressed in mature monocytes. Taken together these results suggest a central role for c-myc in cell proliferation.

0200 PARTIAL CHARACTERIZATION OF HTLV ASSOCIATED SURFACE PROTEINS FROM AN HTLV INFECTED TUMOR CELL LINE. K.T. Schultz*, T.H. Lee*, T. Homma*, M. Essex* and M.I. Greene+
*Harvard School of Public Health +Harvard Medical School

The cell line Hut 102 was derived from a leukemia patient who was infected with the human T cell leukemia virus (HTLV). Using membrane immunofluorescence, sera from patients with adult T cell leukemia react specifically with cell membrane antigens (HTLV-MA) expressed on Hut 102 and numerous other lines of HTLV-infected lymphocytes. Two glycoproteins of approximately 61 kd and 45 kd are regularly immunoprecipitated from Hut 102 cells with HTLV-MA positive human sera, but the same proteins are not detected with anitserum to HTLV gag-gene related core proteins nor with human sera negative for antibodies to HTLV-MA. The gp61 and gp45 proteins have been partially purified using a combination of lentil-lectin affinity chromotography and immunoaffinity chromatography. Detailed characteristics of these proteins will be described.

0201 HUMAN T CELL HYBRIDS EXPRESSING NATURALLY OCCURRING HLA-DR RESTRICTED RECEPTORS THAT RECOGNIZE EBV-INFECTED B CELLS. Mary A. Valentine, Constantine D. Tsoukas, John H. Vaughan, and Dennis A. Carson. Scripps Clinic and Research Foundation, La Jolla, California 92037.

Receptor interactions of T cells that react with Epstein-Barr Virus (EBV) infected B cells are not well defined. Several types of recognition have been described in cells during the course of EBV infection, but clonal examination of naturally occurring receptors showing the actual requirements for HLA restriction has not been reported. Therefore, we utilized somatic cell hybridization to create cells whose surface molecules would pertain directly to those expressed in vivo during various phases of EBV infection. The parent lymphoma was a double mutant of the human cell line, JM, a cell which can release IL-2 on mitogenic stimulation. Patient cells used for direct fusion with JM were acquired from Viral Capsin Antigen (VCA) negative donors, or from a patient during the acute and convalescent phases of mononucleosis. Fusion products were then characterized as hybrids by phenotypic and karyotypic analysis, as well as by growth in selective medium and the acquisition of functional receptors. Receptor activity was monitored by production of IL-2 following co-culture with irradiated autologous or allogeneic B cells which were Epstein-Barr Nuclear Antigen (EBNA) positive or negative. The derived hybrid clones recognized specifically autologous EBV-infected B lymphoblasts, or allogeneic EBNA positive cells sharing HLA-DR antigens. Stimulator cells with no shared HLA-DR or evidence of EBV infection could never induce IL-2 release. These recognition requirements clearly define a population of T cells that arise in vivo during acute phase EBV infection that interact with EBV transformed cells in a HLA-DR restricted manner.

0202 CHEMICALLY-INDUCED CHANGES IN THE ANTIGEN EXPRESSION OF PRELEUKEMIC AND LEUKEMIC THYMOCYTES FROM THE RF STRAIN OF MICE, Kelly Switzer-Timmons, Marie Metlay, and Frank Lilly, Albert Einstein College of Medicine, Bronx, New York 10461

Skin painting of RF/J mice with the chemical 3-methylcholanthrene induces T cell lymphomas within 8-12 weeks of treatment. We have been studying the effect of this carcinogen on the expression of various surface antigens on both preleukemic and leukemic thymocytes. In addition, we have compared and contrasted our results with those observed in spontaneous lymphomas which occur in the AKR strain of mice. The RF strain of mice was initially selected as a low-leukemic strain from the same outbred stock as AKR. In previous studies we have shown that there is a specific loss of expression of Lyt-1 and Lyt-2 antigens from the surface of thymocytes as they become transformed. Size-gated analysis of thymocyte subpopulations in the preleukemic thymus reveals that there is no obvious target cell for this transformation. In contrast to the AKR lymphomas in which there are multiple Lyt phenotypes expressed on the tumors, in the RF system there is a consistent trend toward loss of expression of both of these antigens. There is also a shift in the specific endogenous viral cell surface antigens which are expressed on these thymocytes. However, this phenomenon, the disappearance of one viral antigen and the appearance of another is an age-related effect. There is no difference either qualitatively(specific size protein) or quantitatively (enhancement of expression) between age-matched control mice and MCA-treated mice. Therefore, in contrast to the spontaneous lymphomas observed in AKR mice, there is no evidence that the mechanism responsible for chemically-induced lymphomas in mice of the RF strain involve gross changes in viral antigen expression.

0203 RSV ASSOCIATED TUMORIGENESIS MAY REQUIRE ADDITIONAL FACTORS FOR TUMOR FORMATION David Dolberg and Mina Bissell, Lawerence Berkeley Laboratory, Berkekey, CA 94720

We have inoculated over 1500 embryos in the limb bud on day 4 of development with up to 10^5 ffu of SRD-RSV. The limb as well as peripheral tissues were evaluated 10 days later. In contrast to its effect in newborn chicks (palpable tumors within a week), the virus was remarkably non-pathogenic in young embryos. Based on morphologic and histologic examinations we have found: 1)no evidence of teratogenesis; 2)no evidence of tumorigenesis; 3)occasional presence of hemorrhagic lesions (endothelial blood blisters). These results confirm observations made over fourty years ago. In addition we have found:1)the virus was integrated and expressed; 2)the embryos were viremic and all tissues contained virus; 3)the recovered virus formed foci in culture; 4)all tissues contained an elevated level of viral pp60src associated phosphokinase activity; 5)this activity was detectable as early as 2 days after inoculation; 6)the amount of kinase activity in the limb bud was comparable to that found in 10 day old tumor tissue. This suggests that, in early embryos, kinase activity may be necessary but not sufficient for tumorigenesis. Furthermore, cells dissociated from infected embryonic limbs and placed in culture initially behaved as normal cells with respect to morphology and 2-deoxyglucose uptake; but began to assume a transformed phenotype within 24 hours, even though kinase activity was elevated in the intact tissue. This suggests that embryonic cells aquire the ability to express the transformed phenotype subsequent to culturing. Taken together these results indicate that multiple factors are involved in RSV associated malignancies in embryonic tissues. Lastly, evidence will be presented which suggests that Multiple factors may also be involved in tumor formation in hatched chicks Supported by US DOE and NIH.

0204 GLUCOCORTICOID REGULATION OF SV40 LARGE T ANTIGEN EXPRESSION, †Delano V. Young, †Hasi R. Das, and *Mitchell Finer, †Boston University, Boston, Mass. and *Harvard University, Cambridge, Mass.

Although glucocorticosteroids stimulate growth in 3T3 mouse fibroblasts, they suppress proliferation of SV40 transformed 3T3 cells. This inhibition is specific for glucocorticoids, receptor-mediated, reversible and not cytotoxic, and requires 24-42 hours before the cessation of cell division is complete. The cells appear to be arrested in G_2 of the cell cycle, since DNA amounts are elevated and cell division occurs rapidly after removal of the steroid. Accompanying the growth inhibition are striking changes in morphology, especially at the cell surface.

The SV40 oncogene is the large T antigen. As part of their anti-tumor action, glucocorticoids reduce the amount of T antigen found in these cells. This was shown by indirect immunofluorescence, using a mouse monoclonal antibody against T antigen and a fluorescein-conjugated, goat, anti-mouse antibody. This phenomenon is currently being explored by measurements of the amount of T antigen by immunoprecipitation from labelled cell proteins and of the relative amounts of T antigen mRNA in control and steroid-treated cells.

0205 ALTERATIONS IN GAG PROTEINS IN MuLV-INDUCED CELL LINES FROM H-2-CONGENIC MICE. J.H. Wolfe, D.M.Murasko, K.J.Blank, Univ. Pennsylvania Sch. Medicine, Phila., Pa. 19104

Certain H-2 haplotypes, notably $H\text{-}2^k$, are associated with susceptibility to endogenous and some exogenous (e.g. Friend and Gross) retrovirus-induced leukemias, whereas others, primarily $H\text{-}2^b$, are associated with resistance. In vivo, both immune responses to viral antigens and levels of virus replication have been shown to be influenced by genes linked to H-2. In order to examine the possible effects of H-2 on viral replication in the absence of any indirect host/virus interaction, e.g. immunoselection, cultured cell lines have been established from tumors induced by either Gross or Friend virus in mice congenic at H-2. Of the cell lines studied thus far, cells expressing the $H\text{-}2^k$ haplotype continue to produce infectious, oncogenic virus for years in tissue culture, while those of the $H\text{-}2^b$ or $H\text{-}2^d$ genotypes stop producing infectious virus after a few months in vitro. Both Gross and Friend virus-induced cell lines that have become non-producers may nonetheless continue to produce MuLV-encoded proteins. These cell lines harbor various defects in gag protein expression. The best chracterized of these is the BbGV1 line which produces a morphologically mature virion that is neither infectious nor oncogenic. This cell line contains a truncated gag precursor polyprotein of 47Kd which does not appear to contain p15. The P47 is also found in small amounts in the virion along with other uncleaved proteins. This cell line produces functional reverse transcriptase but not Pr180gag-pol. In several new Friend virus-induced lines derived from $H\text{-}2^b$ and $H\text{-}2^d$ mice, changes in gag proteins are seen after several months in culture. Additionally, cell lines from F1 (k x b), have been established and are being analysed. Cells are also being examined for differences in viral mRNA which might account for these protein changes.

0206 THE DETECTION AND IDENTIFICATION OF A HUMAN B-CELL LEUKEMIA VIRUS (HBLV), Frederick A. Garver, Daniel P. Kestler, Donald Beezhold, and Charles Kiefer. Medical College of Georgia, Augusta, GA 30912

Immunological, biochemical, and morphological evidence will be presented that demonstrates the association of a retrovirus with a human B-cell leukemia. This virus has been identified directly from primary B-cell leukemia tissue culture fluids of patients' cells which have not been induced by IUDR or any other induction method. Xenoantisera against both the intact and disrupted virus have been obtained. Retroviral antisera provided by NIH have been shown to cross-react with this virus by a variety of immunological assays. In addition, electron microscopic examination of this virus reveals that the morphology is consistent with that of known retroviruses provided by NIH and used as controls in this study.

0207 EPIGENETIC SUPPRESSION OF TUMORIGENESIS MEDIATED BY 5-AZACYTIDINE
Cheryl Walker, Jerry W. Shay, University of Texas Health Science Center, Dallas, TX 75235.

Recent models for the origin of cancer have suggested alterations in patterns of DNA methylation, as well as somatic mutation, in the initiation and promotion of neoplasia. However, the exact relationship between the extent of DNA methylation and oncogenic transformation remains unclear, with various investigators reporting increases, decreases or no change between normal and transformed cells in total levels of methylation. We have examined the effect of the hypomethylating agent 5-azacytidine (5-azaC) on a highly tumorigenic murine cell line, T984-15. After treatment with 5 μg/ml 5-azaC, 6 individual clones were isolated and examined for tumorigenic potential. While 16 out of 16 untreated T984-15 clones produced tumors when injected at 10^6 cells into nude mice, 5 out of 6 of the 5-azaC treated clones displayed suppressed tumorigenicity under identical conditions. In addition to suppressing tumorigenicity, 5-azaC also induced the differentiation defective T984-15 cells to differentiate myogenically. Tumorigenic suppression, however, was independent of the induction of myogenesis as 1 tumorigenic clone and 2 of the clones whose tumorigenic potential was suppressed remained non-myogenic. The fact that 5-azaC has been classified as a possible carcinogen has raised concern over the use of the cytosine analog in the treatment of patients with sickle cell anemia and β-thalassemia. The evidence that 5-azaC can also act to suppress tumorigenicity may be important in evaluating the possible theraputic value of the use of 5-azaC in future treatments.

0208 SUCCESSFUL MAINTENANCE OF NORMAL AND CML PERIPHERAL BLOOD HEMOPOIETIC PROGENITORS ON SUBCULTURED HUMAN MARROW ADHERENT CELL LAYERS. Allen C. Eaves, Dagmar K. Kalousek and Connie J. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C. V5Z 1L3

The most primitive hemopoietic progenitor cell types detectable by colony assays can now be routinely maintained in normal human long-term marrow cultures for periods in excess of 2 months. The majority of these progenitors typically remain in the adherent layer and it has been suggested that close proximity to other "non-hemopoietic" cell types may be important for their long-term survival in vitro, although very little is known about the phenotype, origin or role of the cells that may function in this supportive capacity. As a first approach to their characterization, we developed a procedure for reconstructing long-term marrow cultures from subcultured stem cell depleted adherent layers on to which peripheral blood cells of the opposite sex are then seeded. Such systems have been found to sustain primitive hemopoietic progenitors of the peripheral blood genotype for periods in excess of 4 weeks. Of particular interest is the observation that circulating Philadelphia chromosome positive progenitors from patients with chronic myeloid leukemia (CML) are also maintained in such reconstructed cultures in contrast to the usual rapid disappearance of the Philadelphia chromosome positive population in conventional long-term cultures initiated with Philadelphia chromosome positive CML marrow cells.

0209 CELLULAR GENES IN THE MOUSE REGULATE IN TRANS THE EXPRESSION OF ENDOGENOUS RETROVIRUSES. V. L. Traina-Dorge, J. K. Carr, B. Taylor, J. Bailey-Wilson, R. Elston, and J. C. Cohen, Louisiana State University Medical Center, New Orleans, LA 70112, and The Jackson Laboratory, Bar Harbor, ME

The transcription of genetically-transmitted mouse mammary tumor virus (MMTV) sequences was examined in the lactating mammary gland of recombinant inbred (RI) mouse strains. These data indicated that expression of viral genes was not dependent upon either the presence of any one provirus nor upon the number of proviruses present. The parity of the individual animal was the only environmental factor found to influence virus-specific RNA levels. In the BXD and BXH RI strains used in these studies, 74 and 55 viral and nonviral loci are polymorphic, respectively. Linkage analyses between the level of viral RNA and each of these alleles were performed. These data revealed the specific association of viral gene expression with mouse loci unlinked to MMTV proviruses. In one case the linkage analysis revealed the association of MMTV expression with a host gene known to have a pleiotropic effect on gene expression. Data suggest that this locus identified by linkage analysis is associated with mammary carcinogenesis in the mouse. Thus, the expression of endogenous MMTV is regulated by a multigenic mechanism involving trans-acting mouse regulatory loci.

0210 AMPLIFICATION, CLONING AND EXPRESSION OF THE MOUSE ORNITHINE DECARBOXYLASE GENE, Philip Coffino, Lisa McConlogue, University of California, San Francisco, San Francisco, CA. 94123

Ornithine decarboxylase (ODC) is the first and rate limiting enzyme in the pathway of polyamine synthesis. Its activity is responsive to multiple effectors that modulate cellular proliferation, including mitogens, tumor promotors, oncogenes and hormones. Biochemical and molecular biologic analysis of ODC is hampered by its low abundance. We have used an inhibitor of ODC, difluormethylornithine, to select mutants of S49 mouse lymphoma cells that overproduce the enzyme several hundred fold. The mutant cells make more than 15% of their protein as ODC. In vitro translation experiments demonstrated that an increased mRNA pool size accounts for the increased synthesis. The mutants have been used as a source of ODC mRNA to form cDNA, and ODC-specific cDNA clones thus isolated. These have been used to study regulation of ODC. In mouse kidney, ODC induction by androgen was shown to result, at least in part, from an increase in ODC mRNA levels. Southern blot analysis of DNA from wild type and resistant S49 cells showed that both have multiple equivalent Eco R1 and Bam H1 fragments homologous to ODC cDNA. Only one fragment produced by each enzyme is amplified in the mutant cells.

0211 TRANSCRIPTIONAL AND TRANSLATIONAL CONTROL OF c-myc EXPRESSION IN HL60 CELLS, Nancy J. Butnick[1], Chikara Miyamoto[2], Richard Chizzonite[2], Grace Ju[2] and Anna Marie Skalka[2], [1]Roche Institute of Molecular Biology, Nutley NJ 07110, [2]Department of Molecular Genetics, Hoffmann-LaRoche Inc., Nutley NJ 07110.

Our laboratory is interested in the regulation of expression of the c-myc oncogene in HL60 cells in which c-myc is amplified to 16-20 copies. The c-myc gene contains three exons but it is unusual in that the first AUG initiation codon appears in exon 2. Therefore, the c-myc mRNA contains a leader sequence of over 500 bases. The function of this leader is unknown but it has been suggested (Saito et al., PNAS, in press) that it serves to regulate c-myc expression at the translational level. We have isolated two c-myc cDNA clones from HL60 cells. One clone contains the three exons whereas a second clone initiates in the intron between exons 1 and 2. The clones are being used to study the relationship between mRNA structure and c-myc expression. The two cDNAs have been subcloned into expression vectors and c-myc protein has been detected immunologically in COS cells. Quantitation of protein and mRNA levels can be used to determine the correlation between the expression of c-myc protein and the presence of exon 1 in the c-myc mRNA.

0212 STABLE PHENOTYPIC REVERSION OF MOUSE CELLS TRANSFORMED BY HUMAN ras ONCOGENE, FOLLOWING INTERFERON TREATMENT. Dvorit Samid, Esther H. Chang, and Robert M. Friedman, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814

Treatment of NIH 3T3 cells with interferon (IFN) at different times after transfection with c-Ha-ras1 or v-Ha-ras oncogenes caused an inhibition of Ha-ras induced oncogenic transformation. When the cells were co-transfected with c-Ha-ras1 and with pSV Neo (which renders the cells both oncogenic and resistant to neomycin analogue G418), the inhibitory effect of IFN on the oncogenic transformation was significantly greater than the effect on the biochemical transformation.

The effect of IFN on oncogene expression of an established tumor line was also studied. A tumor line of NIH 3T3 ($RS485N^{+}$) was transformed by human c-Ha-ras1 activated by the viral long terminal repeat, and by pSV Neo. Prolonged treatment of the tumor cells with IFN was associated with a progressive appearance of reverted flat colonies which exhibited a normal phenotype with respect to morphology and growth. The revertants were well spread, contact inhibited cells. They did not grow in soft agar and were not tumorigenic in nude mice, yet they grew in selective medium containing G418; therefore the cells reverted with respect to oncogenicity, but retained expression of the neo gene. This indicated a selective effect of IFN on onc gene expression. Revertants grown in the absence of IFN for over 8 passages retained their normal phenotype, although they contained an active human c-Ha-ras1 and produced elevated levels of the encoded protein p21 as compared to NIH 3T3 cells.

0213 PROCESSING OF MOUSE MAMMARY TUMOR VIRUS MEMBRANE GLYCOPROTEINS DEPENDS ON CELL SHAPE, David Kabat, Department of Biochemistry, Oregon Health Sciences University, Portland, OR 97201

MMTV infected hepatoma (epithelial) cells were grown in culture in the presence of dexamethasone and were examined for viral encoded cell surface glycoproteins. Surprisingly, the MMTV glycoproteins were heterogeneously distributed on surfaces of different cells and this heterogeneity was determined by cellular shape rather than by genetic factors. In round cells, the glycoprotein precursor is partially cleaved to form the membrane anchor gp36 plus gp52 and the complex is transferred to the cell surface. In flat relatively adherent cells, gp36 is degraded intracellularly and the gp52 is subsequently released as a secretory glycoprotein. When cells change shape, a lag precedes remodeling of the plasma membranes. This relationship between cellular shape and attachments and MMTV glycoprotein processing could be involved in the cellular dysplasia which initiates progressive MMTV induced tumorigenesis.

0214 VIRAL ONCOGENES THAT CAUSE PROGRESSIVE LEUKEMOGENESIS: MEMBRANE GLYCOPROTEINS OF THE SPLEEN FOCUS FORMING VIRUSES, R. Bestwick, C. Machida and D. Kabat, Oregon Health Sciences University, Portland, OR 97201.

Analyses of Friend and Rauscher SFFV mutants with abnormalities in their gp54-55 membrane glycoprotein genes have established that this gene is essential for initiation of the erythroblastic hyperplasia which progresses to form erythroleukemia These are the first oncogenes known to be capable of causing progressive tumorgenesis. Moreover, the mutants contain major lesions affecting different portions of the glycoprotein. We have now molecularly cloned the wild-type and mutant R-SFFVs and have determined the nucleotide sequences of the env gene and LTR regions. Moreover, we have found that pathogenic activity of the mutant viruses is surprisingly dependent on host age. Specifically, mutations which alter or delete major regions of the glycoprotein still produce erythroleukemia (and never other types of tumors) in newborn mice. These results support the hypothesis that the env genes of leukemogenic dual tropic class retroviruses are oncogenes but that tissue tropism may be determined by the LTRs.

0215 ON THE MECHANISM OF AMPLIFICATION OF THE DIHYDROFOLATE REDUCTASE GENE IN A METHOTREXATE - RESISTANT HUMAN CELL LINE, Robert J. DeLap*, Srinivasan Srimatkandada, Mark D. Carman, and Joseph R. Bertino, Yale University School of Medicine, New Haven, CT 06510. (*Present address, Clinical Oncology Research Program, Warner-Lambert/Parke-Davis, Ann Arbor, MI 48105).

Methotrexate resistance has previously been induced in a human myelogenous leukemia cell line (K562) by exposure to gradually increasing levels of this drug. The resistant line (K562R-4) is maintained in 10^{-4}M methotrexate, has amplified the gene for dihydrofolate reductase (DHFR) approximately 200-fold, and has three chromosomal HSRs. Using repeated CsCl-ethidium bromide equilibrium density gradient centrifugation, we have now found that a small proportion of the K562R-4 amplified DHFR genes appear to exist in circular, supercoiled DNA. These extrachromosonal genes have the same structure, including introns, as chromosomal DHFR genes.

Other chromosomal DNA segments have also been identified in extrachromosomal, supercoiled DNA, in a variety of eukaryotic systems (data from this and other laboratories). It is reasonable to suppose that most or all chromosomal DNA segments can be represented in extrachromosomal DNA. We believe that an equilibrium exists over evolutionary time between the chromosomal and extrachromosomal gene pools. Since extrachromosomal genes may segregate unequally at mitosis, maintenance of an extrachromosomal gene pool may allow for relatively rapid up or down modulation of gene copy number in a cell population subjected to a changing environment. Oncogene activation (via "self-selection", with more rapid proliferation of cells with increased oncogenes), aging, and gene conversion could be other manifestations of this process.

0216 EFFECTS OF TRANSFORMATION OF CELLS BY HERPESVIRUSES, Joan C.M. Macnab, Alex Orr and Morag Park, Medical Research Council Virology Unit, Institute of Virology, University of Glasgow, Church Street, Glasgow, G11 5JR

Cultured rodent cells transformed by UV inactivated, ts mutant, sheared DNA or molecularly cloned fragments of the herpes simplex genome do not maintain the transformed state by retention of viral DNA either of the transforming fragment or of other viral DNA sequences at the level of one copy per cell. The transformed cells cause metastasising tumours. Investigation of the cellular DNA extracted from transformed and tumour cells reveals that certain cell sequences are both amplified and rearranged. In addition, there are quantitative and qualitative differences in the cellular proteins expressed in transformed cells compared with control cells. These changes will be compared with those seen in similar cells transformed by other viruses.

0217 INTERFERON INDUCED ONCOGENE REGULATION: THE GENE BANK HYPOTHESIS. L. J. Krueger J. Bresser, P. J. Andryuk, and D. H. Gillespie. Cancer Inst.,Hahnemann U.,Philadelphia

The interferons, a family of related proteins, are currently in clinical trials to determine conditions for maximizing the effectiveness of interferon therapy in cancer treatment. The role of interferon in the antitumor response appears to be complex. Recent data confirmed earlier ideas that interferon(IFN) exposure induces a cascade of biological events which culminate in a radical phenotypic change. IFN induced alterations include regulation of the immune response, modulation of growth and differentiation,augmentation of NK cell activity and an increase in resistance to the spread of infection.

We investigated gene expression alterations associated with the IFN induced antiproliferative response. A 4 hr pulse of low-dose IFN caused the Daudi Lymphoblastoid cell line to become "super-sensitive" to subsequent IFN exposures. This potentiated cell, designated IFN^{ss} was resistant to viral infection but demonstrated normal growth parameters in the absence of additional IFN. A second miniscule dose(1.0 IRU/ml) caused very rapid growth cessation and prolonged cytostasis. A reduction in the quantity of mRNA complementary to the Moloney sarcoma virus oncogene(v-mos) occurred after the second IFN pulse, but not after the first. Several other oncogenes, expressed at various levels in the Daudi cell, remained unchanged after IFN-induced growth arrest. It has long been known that a single low-dose IFN pulse causes protein changes associated with an antiviral state, independent of growth modulation. Here, we show specific gene regulation associated with growth arrest, but not the antiviral state.Consequently we propose a 2-stage process in the IFN-induced growth arrest. This occurrs through the regulation of at least 2 gene banks during each of the key transitions in the antiproliferative state.

0218 STUDIES ON THE HTLV INFECTION IN VITRO IN THE PRESENCE OF INTERFERON AND THYMOSTIMULIN, C.Grandori,B.Reads and E.Bonmassar, Lab.of Tumor Cell Biology, NCI, Bethesda 20205, MD

It is known that HTLV positive leukemic human cells or in vitro infected lymphocytes do not show natural or antigen-elicited cell-mediated immunity. Therefore, it was of interest to investigate if the functional activity of normal lymphocytes infected in vitro could be affected by immunomodulators agents. Ficoll-separated mononuclear cells from normal human umbilical cord blood were treated with different doses of Interferon (β-fibroblast, IF) or Thymostimulin (TP-1) for 16-18 hrs. The cells were then extensively washed and cocultivated with an HTLV-producing cell line. Functional cytotoxic activity of the cocultivated lymphocytes was tested in a ^{51}Cr-4hr release assay against both the infecting donor cells and the natural killer susceptible K562 line. The results indicated that IF (1000 U/ml) and TP-1 (25ug/ml) markedly improved the killing activity of the infected lymphocytes, the increase ranging from 50 to 100%. The effect persisted for over 3 week period tested. The improvement of the cytolytic activity correlated with the corresponding decrease of cells expressing the P19 viral protein. Therefore we are currently determining whether pretreatment with these agents interferes with the virus integration or expression. Preliminary data suggest that IF-treated cocultures show little or no detectable HTLV proviral DNA. These results provide further explanation for the IF modulation of immunological parameters during HTLV infection in vitro, in addition to the well known immunoregolatory activity at the cell level.

0219 VARIATION IN Ph^1-POSITIVE PROGENITOR MAINTENANCE IN LONG-TERM CML MARROW CULTURES, Ian D. Dubé, Dagmar K. Kalousek, Connie J. Eaves and Allen C. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C. V5Z 1L3 Canada

Previous studies of long-term marrow cultures established from 11 patients (7 untreated, 4 treated) with Philadelphia chromosome (Ph^1) positive chronic myeloid leukemia (CML) have shown that the Ph^1-positive population is typically not maintained even though conditions within the cultures are adequate to support residual Ph^1-negative hemopoietic progenitors for periods of at least 6 weeks. We now report a different result obtained in long-term cultures initiated with marrow cells from a 39-year-old chronic phase male patient studied 43 months after diagnosis and initiation of chemotherapy. Methylcellulose assays revealed relatively high numbers of primitive hemopoietic progenitors to be present in this patient's cultures even by comparison to normal marrow cultures handled in a similar fashion. Cytogenetic analysis of individual erythroid and granulocyte colonies produced in 3 and 6 week assays revealed exclusively Ph^1-positive metaphases. These findings indicate that poor survival in vitro is not an intrinsic property of all Ph^1-positive stem cells but may reflect as yet undefined differences between CML clones or other marrow parameters that may be secondarily related to clonal amplification in vivo.

0220 ASSOCIATION OF RECOMBINANT MuLVs WITH SPONTANEOUS PRE B-CELL LYMPHOMA IN CURLY WHISKER MICE (CWd/J).

Christopher Y. Thomas and Beverly J. Boykin. Division of Hematology-Oncology, Departments of Medicine and Microbiology, University of Virginia Hospital, Charlottesville, VA 22908

We have studied CWd/J mice that not only carry a recessive mutation for curly whiskers (cw) but also develop spontaneous lymphoma. By Kaplan-Meier analysis, we estimate a 50% incidence at 18 months of age. The lymphoma is predominantly splenic with rare thymic involvement and has lymphoblastic morphology on histologic sections. Southern blot analysis of tumor DNAs showed that 6 of 8 tumors contained rearranged heavy chain genes (J_H), with one having a rearranged light chain gene (J_K). Thus, most tumors appeared to be of early B-cell origin. Rearrangement of the c-myc oncogene was not detected. In addition, we have isolated murine leukemia viruses (MuLV) from both leukemic and non-leukemic mice. T_1-oligonucleotide fingerprinting and mapping of viral RNAs revealed these mice produce ecotropic viruses derived from two endogenous proviruses Emv-1 and Emv-3, located on chromosome 5 and 9, respectively. Recombinant polytropic viruses were recovered from leukemic cells from the spleen. The viral genomes of two isolates studied showed complete substition of env and some U3 region sequences relative to the ecotropic viruses. Interestingly, the pattern of the substituted sequences differs from known recombinant MuLV that cause thymic lymphomas. Leukemogenicity testing of the CWd viral isolates and immunologic phenotyping of the lymphomas is in progress.

0221 CELLULAR FUNCTIONS ASSOCIATED WITH THE EXPRESSION OF C-MYC AND N-RAS IN HL60 CELLS.

Carole A. Heilman, Robert Maguire, Larry Linder and Snorri S. Thorgeirsson. National Cancer Institute, Bethesda, Maryland 20205

The human promyelocytic leukemia cell line, HL60, has been widely used as an in vitro model for studying differentiation along the myeloid/monocyte pathway. Exposure of HL60 cells to phorbol esters such as 12-O-tetradecanoyl phorbol 13-acetate (TPA) results in an irreversible commitment of the cells to differentiate, both functionally and morphologically, into mature monocytes. Recently, using an irreversible inhibitor of polyamine synthesis, difluoromethyl ornithine (DFMO), it has been demonstrated that TPA-induced monocytic differentiation was independent of the decrease in cell proliferation also associated with TPA treatment. Thus, treatment of the HL60 cell line with TPA, DFMO, or the combination allows for separation of the differentation specific and growth specific functions. Using this approach we have examined the association of two oncogenes that are expressed in HL60 cells c-myc and N-ras with cellular function(s) characteristic for either differentiation or cellular proliferation. We report here that the c-myc specific decrease in gene expression associated with TPA treatment of HL60 cells correlates to the differentiation of the promyleocytes into mature monocytes and is not associated with the decrease in cell proliferation. Conversly, N-ras expression appears to be associated with the rapid growth of untreated cells during log phase and is markedly decreased when the cells are maintained in the presence of DFMO or exposed to TPA at concentrations preventing cellular proliferation.

0222 GENES CONTROLLING HYBRID RESISTANCE AND SUSCEPTIBILITY TO BALB/C PLASMACYTOMAS, Julia M. Phillips-Quagliata, Mark Marsili and Mary Clare Walker, New York University Medical Center, New York, N.Y. 10016.

F_1 hybrids between BALB/c mice and various members of the C57 family of strains resist subcutaneous challenge with numbers of viable MPC-11 cells sufficient to kill 100% of BALB/c mice. Different numbers of genes are, however, involved; for example, C57BL/10Sn (B10) mice provide one gene for resistance whereas C57BL/6J (B6) mice provide two. One of these two genes appears to have been lost in B6.G_{1X}+ mice, suggesting that it might be linked or allelic to either Gv-1 or Gv-2, two 129/J genes bred onto the B6 background to make the G_{1X}+ congenic strain. The B10 gene for resistance is dominant and autosomal and not linked to the H-2^b haplotype. Its possible mechanism of action has been analysed by comparing the anti-MPC-11 cellular cytotoxicity of H-2 matched resistant (BALB/c X B10)F_1 and susceptible (BALB/c X BALB.B)F_1 hybrids. Resistance is not explained by either enhanced natural killer or antibody-dependent cellular cytotoxicity on the part of resistant hybrids. Moreover both resistant and suceptible hybrids mount high primary in vitro cytotoxic T lymphocyte (CTL) responses to MPC-11. The CTL response of susceptible hybrids diminishes, however, with time after tumor challenge, the diminution appearing first in the draining lymph nodes and subsequently in the spleen. The splenic diminution correlates with the appearance of suppressor cells with cytoxan-sensitive precursors. The suppressors include both Thy 1^+ and Thy 1^- cells and are predominantly adherent. The cause of the diminution in lymph node CTL activity is unknown. The main site of gene action in this model of hybrid resistance appears to be the regional lymph nodes where resistant hybrids maintain a CTL response long enough to prevent tumor escape and metastasis. (Supported by American Cancer Society grant IM 213E)

0223 MODULATION OF KERATIN GENE EXPRESSION IN MOUSE EPIDERMIS BY TUMOR PROMOTERS, Rune Toftgard, Dennis R. Roop and Stuart H. Yuspa, NIH, Bethesda, M.D. 20205.

To monitor the influence of tumor promoters on epidermal differentiation we studied the expression of keratin genes using cDNA clones. The $50,55_b$and 60 kd keratins are predominantly synthesized in the proliferating basal cells and the 55,59 and 67 kd keratins are synthesized in differentiating cells. TPA treatment caused a decrease in the relative levels of RNA corresponding to the 55,59 and 67 kd keratins.At 12 and 24h after TPA, less than 20% of the level for these mRNAs in untreated epidermis was observed.By 48h mRNA for the 67 kd keratin had returned to control values while the 55 kd keratin remained low. mRNA for the 59 kd keratin remained lower than control for the first 72h after treatment but returned to normal after 7 days.The mRNAs for the $50,55_b$and 60 kd keratins were elevated above control at 12,24,48 and 72h after treatment.Other hyperplastic agents which are complete or incomplete promoters caused the same changes.The decrease in mRNAs corresponding to the differentiation associated keratins may reflect a promoter mediated acceleration of terminal cell maturation. The priority return of the 67 kd mRNA before the 59 and 55 kd mRNA is reversed compared to that observed normally and suggests that an altered differentiation program results from promoter treatment, perhaps by accelerating the transit time to the upper layers where the 67 kd keratin is synthesized.The increase in the $50,55_b$and 60 kd keratins indicates expansion of the basal cell compartment by compensatory hyperplasia or retardation of maturation in a subclass of basal cells.The 55,59 and 67 kd keratin mRNAs are very low in epidermal carcinomas whereas in papillomas they are essentially the same as in untreated epidermis. The mRNAs for the $50,55_b$and 60 kd keratins are apparently not altered in either papillomas or carcinomas.

0224 ONCOGENE EXPRESSION IN CHEMICAL CARCINOGENESIS, Dennis R. Roop, Rune Toftgard and Stuart H. Yuspa, NIH, Bethesda, MD 20205

The induction of epidermal tumors in mouse skin by sequential treatment with initiating and promoting agents proceeds in well-defined stages. An occult population of initiated cells clonally expands to form a benign papilloma which may progress to carcinomas albeit at a low frequency. Recent studies indicate a minimum of two genetic changes are required for the process to proceed to the malignant end point. Squamous cell carcinomas induced by chemicals applied to mouse skin have been shown to contain an activated cellular homologue of the Harvey-ras (ras^H) oncogene by DNA transfer into NIH/3T3 fibroblasts. We wanted to determine if altered expression of this or other oncogenes could be detected in epidermis exposed to tumor promoters or in papillomas and carcinomas. RNA was isolated from control adult back epidermis, or epidermis at 12, 24, 48, 72h and 7d after treatment with the tumor promoter TPA. RNA was also isolated from papillomas and carcinomas. The RNA was analyzed by the dot-blot technique using probes corresponding to the following oncogenes: ras^H, ras^K, myc, myb, fes, fos and abl. Only minimal changes (less than 2-3 fold) in transcript levels for these genes were observed after TPA treatment at any time point. Similar results were obtain for RNA from papillomas and carcinomas as compared to controls. These results suggest that TPA exposure or the hyperplasia which ensues is not associated with marked alterations in expression of these oncogenes. Similarly the acquisition of the neoplastic phenotype does not require a large increase in transcription of these genes. If the oncogenes assayed, other than ras^H, are involved in the induction of papillomas and carcinomas they are probably activated by a mutational event rather than increased expression.

0225 SYNTHESIS, CLONING, AND EXPRESSION OF GENES CODING FOR HUMAN EPIDERMAL GROWTH FACTOR AND RAT TYPE I TRANSFORMING GROWTH FACTOR IN E.COLI, David L. Hare+, Margery Nicolson*, Allen Banks+, +Amgen Development Inc., Boulder CO 80301, *Amgen Inc., Thousand Oaks CA 91320.

Human epidermal growth factor (hEGF) is a 53 amino acid protein with in vitro cell proliferative activities. Rat type I transforming growth factor (rTGF) is a 50 amino acid polypeptide with structural features and biological properties similar to hEGF but little amino acid sequence homology. We have synthesized structural genes for both hEGF and rTGF which incorporate the flexibility of recombining the two genes to construct hybrid genes capable of expressing hybrid hEGF/rTGF proteins. Using direct expression vectors we are able to express hEGF and rTGF at high levels in E.coli. Both proteins compete equivalently with ^{125}I-labeled mouse EGF for EGF receptors on human A431 cells. In addition, both proteins are fully active in both ^{3}H-thymidine uptake assays using human foreskin fibroblasts and in soft agar colony formation assays using NRK cells. hEGF is also active in causing premature eyelid opening in neonatal mice. hEGF/rTGF hybrids have been constructed and expressed in E.coli at high levels. We anticipate that these hybrids will have novel biological properties reflective of their composite structural features.

0226 TRANSLOCATIONS THAT HIGHLIGHT CHROMOSOMAL REGIONS OF DIFFERENTIATED ACTIVITY. Ilan R. Kirsch, Judith Brown, Cynthia Morton, Stanley Korsmeyer and Gregory Hollis, NCI-Navy Medical Oncology Branch/DCT/NCI/NIH, Bethesda, MD 20814 and Medical College of Virginia and Harvard Medical School and Metabolism Branch, NCI.

The frequent translocation of the oncogene c-myc into the immunoglobulin loci in tumors of B-lymphocytes prompted us to ask whether disease associated chromosomal translocations in other cell types would also involve regions of the genome that encoded important differentiation specific products made by these cells. A cytogenetic analysis of two patients with erythroleukemia and a commonly available erythroleukemia cell line, K562 (late passages), show translocations within the regions where the genes that encode alpha and beta globin reside. The translocation in K562, not seen in early passages of these cells, has been shown to correlate with a change in globin inducibility. When cloned B-lymphocytes from a patient with ataxia-telangiectasia are analyzed, a translocation into the regions encoding the immunoglobulin light and heavy chain genes are observed in distinction to the translocation seen in T-lymphocytes from the same patient. Both kappa and lambda producing cells manifest this translocation. These examples provide insight into the mechanism of chromosomal translocation in both cancerous and noncancerous conditions and lead to the speculation that genomic activity is a necessary, if not sufficient, factor for the occurrence of such translocations.

0227 C-MYC IS EXPRESSED IN AVIAN TUMOR CELL LINES EXHIBITING TWO DISTINCT CELLULAR PHENOTYPES. Timothy W. Baba and Eric H. Humphries, Department of Microbiology University of Texas Health Science Center at Dallas, Dallas, Texas 75235.

Infection of susceptible chickens with avian leukosis virus (ALV) results in the production of lymphomas. The primary tumor develops in the Bursa of Fabricius, a specialized organ in birds within which B-lymphocyte differentiation occurs. Tumor cells express immunoglobulin of the IgM isotype. It has been demonstrated that the virus acts as an insertional mutagen, integrating upstream from the cellular oncogene c-myc and enhancing its transcription. Recent studies of chromosomal translocations in murine plasmacytomas and human Burkitt's lymphomas also have implicated c-myc in tumorigenesis.

We have adapted three independently derived ALV-induced tumors to in vitro growth. These cell lines, like the primary tumors, contained an integrated provirus located 5' to c-myc and expressed increased levels of c-myc RNA. FACS analysis revealed the expression of cell-surface IgM. Electrophoretic analysis of lysates prepared from metabolically labelled cells demonstrated the synthesis of both heavy (mu) and light chains. However, IgM was secreted by only one of these cell lines. This cell line displayed a plasmacytoid phenotype and accordingly exhibited increased intracellular complexity, as evidenced by flow cytometry and electron microscopy. Therefore, these cell lines represent two distinct cellular phenotypes. These findings provide the first evidence for the involvement of the c-myc oncogene in two distinct differentiation stages of B-cell derived tumors within a single animal species.

0228 HORMONE EFFECTS ON CLONOGENIC GROWTH OF BREAST TUMOR CELL LINES ARE INDEPENDENT OF SPECIFIC RECEPTORS. Verena Hug, Gary Spitzer, Benjamin Drewinko and George Blumenschein. M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

We determined the effects of two peptide hormones (epidermal growth factor (EGF), insulin) and of two steroid hormones (hydrocortisone, 17- -estradiol) on the clonogenic growth of four established human breast tumor cell lines (MCF-7, MDA-231, MDA-453, MDA-468). Functional receptors for all four hormones are present in MCF-7 cells, but have not been demonstrated in the other cell lines. Our own assay demonstrated 16 fmol/mg cytosol protein of estrogen receptor in MCF-7; no estrogen receptor protein was detected in the other three cell lines. Dose-dependent stimulatory effects for all hormones were observed on all lines, regardless of their receptor status and at similar effective dose ranges: 25-250 ng/ml for EGF; 10-75 g/ml for insulin; 10^{-7}-10^{-5} M for 17- -estradiol and 2.5-12.5 g/ml for hydrocortisone. Clonogenic growth could be increased by 60-70% with each of the hormones alone, and this improvement was only slightly superior in the receptor-containing MCF-7 cell line. Horse serum, a known source of growth factors for human breast carcinoma cell lines, at concentrations of 15-30% also increased clonogenic growth, but not to the degree obtained with the four hormones. We conclude that the absence of demonstratable specific receptors does not interfere with the mitogenic effects of these four hormones.

0229 RETROVIRUSES SPECIFICALLY ALTER THE GROWTH AND DIFFERENTIATION OF MOUSE EPIDERMAL KERATINOCYTES, Bernard E. Weissman and Stuart A. Aaronson, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20205

A model system for transformation of epithelial cells using a mouse epidermal keratinocyte cell line has been developed. This cell line, designated Balb/MK, is totally dependent upon the presence of epidermal growth factor (EGF) for its continued proliferation. It also undergoes terminal differentiation in response to high levels of extracellular calcium and displays well-known epithelial cell markers such as keratin synthesis and desmosome formation. Infection of these cells by a variety of different mammalian sarcoma viruses leads to an abrogation of the requirement for EGF as well as a block in the calcium induced terminal differentiation. We will demonstrate by both morphological and biochemical markers that each retrovirus blocks terminal differentiation of Balb/MK cells in a different point in the pathway. Thus each viral onc gene has a specific effect on the normal differentiation of epidermal keratinocytes. These results bear a remarkable similarity to those observed with retrovirus induced alterations in hemapoetic cell differentiation. Our findings extend the range of retrovirus effects to cells of epithelial origin and may provide useful insights into the relationship between differentiation and transformation.

0230 GLUCOCORTICOIDS INHIBIT C-SIS GENE EXPRESSION AND CELL GROWTH IN THE ANDROGEN-RESPONSIVE DDT_1MF-2 SMOOTH MUSCLE CELL LINE, R.G. Smith, A.J. Syms, L.E. Cornett, J.W. Hardin and J.S. Norris. Dept. of Urology, Baylor College of Medicine, Houston, TX and Dept. of Medicine & Physiology, Univ. of Arkansas School of Medical Science, Little Rock, Arkansas.

Recently our laboratories have undertaken a series of studies designed to ascertain the mechanisms involved in androgen stimulation and glucocorticoid inhibition of growth of the well characterized androgen responsive DDT_1MF-2 smooth muscle cell line. When DDT_1MF-2 cells are treated with 10 nM triamcinolone acetonide (TA) or dexamethasone they are rapidly blocked in the G1 phase of the cell cycle. Androgens (10 nM) administered simultaneously with TA failed to prevent the G1 block. Northern blot analysis of total cellular RNA or poly A^+-RNA revealed that control or testosterone treated (10 nM) cells expressed an RNA species with close homology to a v-sis nick translated probe. The v-sis gene has a close homology to that of platelet derived growth factor (PDGF) a known mesenchymal mitogen; furthermore these cells secrete PDGF-like activity into their culture medium and contain receptors for this growth factor (D. Bowen-Pope-personal communication). Cells treated with TA either in the presence or absence of testosterone fail to express the v-sis homologous RNA. While TA induces synthesis of several new proteins in these cells, a protein of similar molecular weight (31,000) and isoelectric point (9.5) to PDGF is decreased. Evidence from flow cell cytometric analysis indicates that exogenously added PDGF (2.5 units/ml) overcomes the glucocorticoid induced G1 block. These date suggest that glucocorticoids inhibit cell growth by suppressing the production of a c-sis coded growth factor.(Supported by NIH CA36264, AM27450 and NSF ISP8011447.)

0231 IMMUNOGLOBULIN GENE REARRANGEMENTS MARK UNIQUE CLONAL POPULATIONS IN LYMPHOID NEOPLASIA, Andrew Arnold, Ajay Bakhshi, Jeffrey Cossman, Elaine S. Jaffe, Thomas A. Waldmann, and Stanley J. Korsmeyer, NCI, NIH, Bethesda, MD 20205

Germline immunoglobulin (Ig) genes must undergo somatic DNA recombinations during B-cell development, each such rearrangement being specific for a given B-cell and its progeny. Rearrangement of both heavy plus light chain Ig genes is strongly B-lineage associated. Southern hybridization can sensitively detect specific clones in lymphoid tissues lacking definitive surface markers, and was used to (1) distinguish lymphoma from undifferentiated carcinoma in a malignancy of uncertain histogenesis, (2) demonstrate clonal B-lymphocytes in lymphomas with numerically predominant T-cells, (3) find clonal B-cell populations within "null" lymphomas and within an atypical follicular hyperplasia, (4) show that the malignant B-cell precursors in a CML lymphoid blast crisis retained the capability of further Ig gene rearrangement, which permitted distinguishing between the malignant subclones of two separate crises in a single patient. Detection of Ig gene rearrangement provides a sensitive, uniquely specific clonal marker which is applicable on most clinical specimens and is not dependent on expression of the genes. Its capabilities include early detection of recurrent or persistent tumor, and the illumination of the pathogenesis and natural history of neoplasia by marking clonal cells early in this process. Similar molecular marking of non-lymphoid tumors with chromosomal translocations will be possible as appropriate probes become available.

0232 THE ALTERATION OF TUMORIGENICITY OF TRANSPLANTABLE MOUSE TUMORS BY TREATMENT WITH INTERFERON, +P.M. Pitha-Rowe, *V.E. Vengris, +G. Glasgow and *N.A. Wivel, +The Johns Hopkins University Oncology Ctr., Baltimore, MD 21205 - *National Institute of Health, Bethesda, MD 20205

A virus-free methycholanthrene transformed cell line (Meth A and CU-7) and B cell lines established from NFS and AKV NFS congenic mice were grown in vitro and treated with purified α, β and γ interferons prior to testing for oncogenicity in host animals. Use of interferon in vitro caused growth inhibition, but not cytopathic effects, which was fully reversible upon removal of interferon. There was a significant decrease in tumor incidence in mice challenged with interferon-treated cells, which was not observed after transplantation of interferon-treated cells into nude or immunosuppressed mice. The effect of interferon treatment on the alteration of expression of tumor and viral antigens, retrovirus production and expression of oncogenes present in these cells, has been examined and correlated with the observed tumor rejection.

0233 TRANSFORMATION OF TUMOR CELLS BY MoMuSV INDUCES TRANSITION FROM CARCINOMA TO SARCOMA TYPE. Ulrich Scherdin, Sadatoshi Sakuma and Fritz Hölzel, University of Hamburg, FRG.

The aim of the investigation was to study the effects of sarcoma virus information introduced additionally into the genome of cell lines derived from chemically (DMBA) induced rat mammary tumor. Cells of the fibroblast-like sarcoma line HH-16-2/1 and the adenocarcinoma line HH-16-4 were transformed by Moloney murine sarcoma virus (MoMuSV). The transformed cell clones were characterized by morphology, growth properties, virus production and malignancy in vivo. Whereas the parental HH-16-2/1 cells were sensitive to glucocorticoids which reversibly alter the morphology and induce contact inhibition, the transformed cell clones are resistant to glucocorticoids. Upon inoculation of rats, the transformed cells produce tumors of anaplastic morphology in contrast to fibrosarcomas observed after injection of HH-16-2/1 parental cells. HH-16-4 adenocarcinoma cells were transformed by MoMuSV, yielding cell clones with unchanged epitheloid or fibroblast-like morphology. The fibroblast-like cell clones produced sarcomas upon injection into rats. Southern blot analyses demonstrated the MoMuSV genome integrated in multiple copies and at different sites in the genome of the transformed cell clones. Virus production was not correlated with changes of morphology in vitro nor with alterations of the tumor type. The data provide evidence that the introduction of the MoMuSV sequences into the tumor cell genome can induce the complete change from carcinoma to sarcoma type, and from sarcoma to anaplastic type. The system makes it possible to investigate the influence of endogenous and exogenously provided oncogene information at the molecular level. (Supported by Deutsche Forschungsgemeinschaft, SFB34)

0234 CHEMICAL TRANSFORMATION OF EPSTEIN-BARR VIRUS (EBV) IMMORTALIZED HUMAN B LYMPHOCYTES. Dana J. Kessler, Robert Maguire, Carole A. Heilman and Snorri S. Thorgeirsson. National Cancer Institute, Bethesda, Maryland 20205.

Both EBV positive and negative Burkitt lymphoma cell lines have been extensively used to study the relationship between expression of retroviral oncogenes, chromosomal abnormalities, and cellular transformation. The role of chemical carcinogens in this process has not been fully investigated to date. Therefore, we have employed a model human B cell system to investigate the potential correlation of oncogene expression and chromosomal abnormalities to chemical induction of tumorigenesis. EBV immortalized cord blood lymphocytes and B cells derived from patients with infectious mononucleosis were treated with N-acetoxyacetylaminofluorene (1 to 15 µg/ml in 0.5% DMSO), a potent ultimate carcinogen and frame shift-inducing mutagen. Twenty population doublings post treatment, treated and untreated cells (5×10^6) were subcutaneously injected into athymic mice. Rapidly proliferating tumors giving rise to high grade B cell lymphomas were noted after one week in the treated lines. Karyotyping revealed modal chromosome numbers ranging from diploid (untreated) to tetraploid (treated), with one tumorigenic line displaying a 6p-16p translocation. Oncogene expression of c-myc, c-myb, c-fes and H-ras, K-ras and N-ras were measured by Northern blot analysis, revealing no qualitative or quantitative differences in the level of expression. These findings are in keeping with reports from other sources indicating no up regulation of ras family genes in tumorigenic cells. Differences in DNA arrangements are presently being examined by Southern blot analysis.

0235 LDH_K, a Kirsten associated protein expressed in normal retina.

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LDH_K is a 56,000 dalton antigen found in diverse cells transformed by the Kirsten sarcoma virus and in rat cells expressing VL30 RNA. This antigen copurifies with a novel lactate dehydrogenase activity. This antigen is also found in cells transformed by the Harvey sarcoma virus, and a related enzyme activity is found in Rous transformed cells and in a majority of human carcinoma.

We have mapped LDH_K to human chromosome 11, in the region 11 p11 - 11 p13. This region is also known to contain the c-ras-H1 locus. This may relate to the sequence homology shown between the ras p21 and conventional isozymes of lactate dehydrogenase.

Biochemical characterization of LDH_K has clarified its direct regulation by oxygen. K_m values for substrate binding are 10^3 fold higher under aerobic assay conditions than under anaerobic conditions. Oxygen additionally appears to function as an electron acceptor.

In studies of normal tissue expression of LDH_K, we find mammalian retina expresses a high level of this activity, although mammalian brain expresses negligible amounts. Retina tissue from lower vertebrates contains much lesser amounts of LDH_K, equivalent to brain levels. This might relate to the fact that mammalian retina exhibits high aerobic glycolysis similar to that seen in most malignant tumors, while retina of lower species does not.

0236 HUMAN ALPHA-INTERFERON GENE FAMILY: ORGANIZATION AND EXPRESSION, Arthur P. Bollon, Motohiro Fuke and Richard Torczynski, Wadley Institutes of Molecular Medicine, Dallas, TX 75235

Several human alpha-interferon genes have been isolated from a human genomic library using two 17-base synthetic probes. One of the isolated genes is a pseudogene since it contains a termination codon in the signal sequence and another is a new functional alpha-interferon gene not previously described. DNA sequencing by the Maxam and Gilbert technique, as well as the M13 dideoxy technique, has permitted a comparison of the gene sequences with sequences of the limited number of other genomic alpha-interferon sequences and about ten cDNA sequences previously described. The functional alpha-interferon gene has been engineered in bacteria for expression and the human alpha-interferon is being characterized in terms of physical properties and anti-viral and anti-proliferative activities. The pseudogene structural sequence is being engineered for expression by removal of the termination sequence by hybrid-signal construction. Expression and activity of the pseudogene product is being compared to the normally expressed genes. Flanking putative promotor sequences and repetitive inserts homologous to oncogene sequences of the normal and pseudogenes will be compared. This work is supported in part by grants from NIH, GM28090 to APB and the Meadows Foundation.

0237 REGULATION OF THE DIFFERENTIATED PHENOTYPE IN NORMAL AND RSV TRANSFORMED CHICK CHONDROCYTES, Gionti, E., Pontarelli, G., Capasso, O., Quarto, N. and Cancedda, R., Istituto di Biochimica Cellulare e Molecolare, 2nd Medical School, 80131 Naples, Italy.

Chondrocytes obtained from chick embryo tibiae 2 days before hatching synthesize in culture specific markers of differentiation i.e. type II collagen , sulphated proteoglycans, chondronectin. In addition these cells synthesyze and incorporate into their extracellular matrix a new type of collagen with a molecular weight of 64 K. This collagen can be converted in vitro by limited pepsin digestion in a protein of lower molecular weight; pulse-chase experiments failed to show any similar maturation in culture, but demonstrated a reduced half-life of the 64 K collagen with respect to the type II.

Cultured chondrocytes can be infected and transformed by RSV. Transformation represses the expression of chondrocyte specific markers (type II collagen, 64 K collagen and chondronectin). Moreover we observed an enhancement of fibronectin expression and a switch on of type I collagen, at variance with the observed reduced expression of these proteins in RSV transformed fibroblasts. The establishment of a permanent cell line of RSV transformed chick embryo chondrocytes will be also discussed.

0238 HTLV-II DELETION MUTANTS AND ALTERED GROWTH CHARACTERISTICS OF THE MO CELL LINE,
William Wachsman, Irvin S.Y. Chen, Kunitada Shimotohno, and David W. Golde, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024

Human T-cell leukemia virus, type II (HTLV-II), was isolated from the Mo cell line established by primary culture of splenic tissue obtained from a patient with T-cell hairy-cell leukemia. Continued passage of the Mo cell line resulted in altered growth characteristics, such that late passage Mo cells became capable of growing in serum free medium and clone spontaneously at limiting dilution. Utilizing molecular clones of HTLV-II proviral DNA we determined that late passage Mo cells contain the 8.7 kb replication-competent genome of HLTV-II as well as two HTLV-II deletion mutants. The early passage Mo cell line contains the wild type HTLV-II, but neither deletion mutant. We are investigating the relation of these HTLV-II deletion mutants to the altered growth characteristics of the late passage Mo cell line. Restriction enzyme mapping of the 3 proviral DNA clones was performed. In addition to conservation of the LTRs, both the 3.5 kb and the 6.6 kb mutants conserve 1.3 kb of sequence at the 3' end. This conserved region contains the pX locus, which may be important in HTLV-II induced transformation. The extent and location of deletions in mutant provirus DNA make it unlikely that either is replication competent. S_1 mapping and DNA sequencing of mutant and wild type HTLV-II have been performed to determine the precise location of the deletions. The relative amounts of HTLV-II RNA of the 3 HTLV-II genomes are being measured to test the possibility that increased transcription of the pX region is related to the altered growth characteristics of late passage Mo. These studies provide insights into the mechanism of HTLV-II-induced leukemogenesis.