GENOMIC INSTABILITY AND CANCER

Organizers: Curtis Harris, Philip Hanawalt and Janet Rowley

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Plenary Sessions Page

Poster Sessions

Structure and Unique Features of the Mammalian Genome

J 001 REGULATION OF DNA REPLICATION IN MAMMALIAN CHROMOSOMES, Joyce L. Hamlin, James P. Vaughn, Pieter A. Dijkwel, Tzeng-Horng Leu, and Chi Ma, Department of Biochemistry, University of Virginia School of Medicine, Charlottesville, VA, 22908.

The major research interests in our laboratory are the regulation of replication of mammalian chromosomes, and the way in which chromosome architecture influences replication and transcription processes. As a model system, we have developed a methotrexate-resistant Chinese hamster ovary cell line (CHOC 400) that has amplified a 240 kb sequence from the dihydrofolate reductase (DHFR) locus ~1,000 times. The multiple DHFR amplicons are located in three stable, abnormally-banding chromosome regions. Previous in vivo labelling studies on synchronized cells showed that replication initiates preferentially somewhere within a 28 kb zone mapping just downstream from the DHFR gene. This result suggested the presence of a fixed origin in this region, analogous to the origins of microorganisms. However, more recent two-dimensional replicon mapping studies suggest that replication initiates at multiple random sites scattered over the entire 28 kb zone. A model is suggested in which interaction of a trans-acting factor with a cis-acting origin induces melting of a large chromosomal domain prior to the actual polymerization of DNA chains; once the region is melted, DNA chains then initiate anywhere within the single-stranded region.

J 002 DNA TOPOISOMERASES AND GENOME STABILITY. Raymond A. Kim and James C. Wang, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

We have reported recently that in a yeast top1 top2 ts mutant devoid of DNA topoisomerase I and containing a temperature-sensitive DNA topoisomerase II, the ribosomal gene cluster is unstable and extensive excision of rDNA genes as extrachromosomal rings occurs when these cells are propagated at a permissive temperature. This observation has been interpreted in terms of intramolecular recombination stimulated by the supercoiling of the actively transcribing rDNA cluster under conditions of low intracellular levels of DNA topoisomerases. Christman et al (1) has also reported an increase in the frequency of recombination within the rDNA cluster in yeast top1 mutants, or in top2 ts mutants at semipermissive temperatures. Mutations in the yeast TOP3 gene have also been shown to stimulate recombination within rDNA cluster and between repetitive sequences, and the sequence of the TOP3 gene suggests that it encodes a third DNA topoisomerase which is homologous to E. coli DNA topoisomerase I (2). These recent findings point to a paradox: if the product of TOP3 affects recombination through its relaxation of supercoiled DNA in vivo, why are top3 mutants hyper-recombinogenic in the presence of wildtype DNA topoisomerase I, a strong relaxation activity? In order to answer this question, genetic and biochemical experiments were carried out to assess the relative activities of these enzymes. It is shown that the yeast TOP3 product possesses a relaxation activity specific for negatively supercoiled DNA. This activity appears to be much weaker than that of yeast DNA topoisomerase I, however. It is plausible that the TOP3 product might have a specific role in the resolution of plectonemically interwould DNA strands in recombination intermediates.

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(2). Wallis et al. (1989). Cell 58, 409.

J 003 TOPOISOMERASE II: AN EXPLOITABLE TARGET OF ANTICANCER DRUGS, Leonard A. Zwelling, Department of Medical Oncology, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030

DNA topoisomerase (topo) II is required for chromosome segregation and thus for cell division. Topo II is also the intracellular target through which a number of active antineoplastic drugs effect their cytocidal actions. Among the most convincing pieces of evidence supporting this is the finding that some cells that are resistant to the cytocidal actions of topo II-reactive drugs possess a drug-resistant form of the enzyme. The topo II in one line of human leukemia cells (HL-60/AMSA) is such a drug-resistant topo II. HL-60/AMSA topo II as well as the cells themselves are specifically resistant to topo II-reactive DNA intercalating agents (amsacrine), but sensitive to nonintercalating topo II-reactive drugs (etoposide). Using novel active and inactive analogs of amsacrine we have identified some of the structural parameters determining drug activity or inactivity. We have computer-simulated the drug structures and used these models to speculate on the way the drug-enzyme interaction might occur. To examine the validity of these models will necessitate a better picture of the structure of the drug-sensitive and drug-resistant forms of topo II. We have cloned the topo II genes from the drug-sensitive and drug-resistant human leukemia cell lines and have begun to identify mutations that might underlie topo II's sensitivity or resistance to these agents. Additionally, we have begun to identify specific DNA structures such as DNA bends that might influence the activity of topo II activity. Only through the examination of each of the 3 parts of the DNA-drug-topo II interaction will a clearer understanding of the biochemical and molecular determinants of the action of topo II-reactive drugs emerge. We are applying the biochemical and molecular assays of topo II to clinical samples in an effort to develop a therapy-specific predictive assay for the success or failure of the treatment of adult leukemia with topo II-reactive drugs.

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Genomic Maintenance and Modulation of Genomic Alterations

J 004 DNA REPAIR FINE STRUCTURE, Philip C. Hanawalt, Stanford University, Stanford, California 94305.

The mutagenic and carcinogenic consequences of unrepaired DNA damage depend upon its precise location with respect to the relevant genomic sites. It is therefore important to learn the fine structure of DNA damage processing in particular protooncogenes and other DNA sequences that may be implicated in tumorigenesis. Both the introduction and the repair of some types of DNA lesions are heterogeneous with respect to chromatin structure and/or gene activity (1,2). Thus, for example, cyclobutane pyrimidine dimers are more efficiently removed from an expressed than a silent protooncogene in UV irradiated mouse 3T3 cells (3). Persisting damage in non-transcribed domains may account for genomic instability in those regions, particularly during cell proliferation as lesions are encountered by replication forks. Efficient replicative bypass of bulky lesions such as psoralen photomonoadducts (but not interstrand cross-links) occurs in human cells although with predictable high error frequencies (4). The preferential repair of pyrimidine dimers in the transcribed strands of active genes results in a bias toward mutagenesis from dimers in the non-transcribed strands (2). In terminally differentiated rat PC12 cells or L8 myoblasts overall DNA repair levels are attenuated while some tissue specific genes are selectively repaired (5). In human HL60 promyelocytic cells repair in the *c-myc* protooncogene is markedly reduced when transcription is down-regulated during differentiation to granulocytes (A. Islas, to be published). The cancer-prone phenotype of xeroderma pigmentosum (group C) correlates with a deficiency in repair of unexpressed DNA sequences while in Cockaynes syndrome (also characterized by sunlight sensitivity) a defect in the preferential repair of expressed genes is not accompanied by cancer susceptibility (6). The above examples will be reviewed and incorporated into a testable hypothesis.

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6. Hanawalt PC "Role of gene expression in the fine structure of DNA damage processing" in *Biotechnology and Human Genetic Predisposition to Disease* (eds C Cantor, *et al.*) Wiley-Liss, Inc., New York, pp 135-145 (1990)

J 005 MISMATCH REPAIR, SEQUENCE POLYMORPHISM AND GENETIC STABILITY, Miroslav Radman, Institut Jacques Monod, 75251 - Paris -05, France. The general mismatch repair system which recognizes a variety of base-pair mismatches has been studied in great detail in bacteria (1,2). This multi-enzyme system edits both DNA replication and recombination processes thus assuring the Hi-Fi reproduction of DNA and preventing recombination between similar but diverged DNA sequences such as repetitive DNA elements (3,4) and genomes of related species (5). How fast can this genetically stabilizing sequence divergence take place? There is evidence from fungal genetics that a process of homologous pairing (occuring exclusively in the haploid germ-line phase of reproduction) targets extensive DNA cytosine methylation (5-meC) and the subsequent 5-meC -> T mutagenesis to sequences of high homology within the genome, i. e. to duplications (6,7). This process appears to transform repetitive/transposable elements into functionally and recombinationally inert "junk" DNA thereby providing the principal source of sequence polymorphism in higher eucaryotes (8) which may represent the basic structural feature of the chromosomal stability and of the genetic barrier in sympatric speciation processes (9).

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Recombination I

J 006 CONTROL OF RECOMBINATION EVENTS DURING LYMPHOCYTE DIFFERENTIATION.

Frederick W. Alt, Lori Covey, Gary Rathbun, Paul Rothman, Suzanne Li, Eugene Oltz, Ami Okada, Jianzhu Chen, Beverly Gorham and Monica Mendelsohn. The Howard Hughes Medical Institute and Departments of Biochemistry and Microbiology, College of Physicians and Surgeons of Columbia University, New York, New York. 10032

Ability of VDJ recombinase to assemble particular gene segments in appropriate cell types and stages within lymphoid lineages is effected by modulating accessibility of substrate gene segments to a common VDJ recombinase. Accessibility has been correlated with transcription of targeted unrearranged gene segments. To elucidate controlling elements, we created transgenic mice that carry a hybrid antigen receptor gene mini-loci in which germline TCR or Ig heavy chain variable region gene segments (V, D, and J) were combined with various Ig or TCR receptor transcriptional regulatory elements including downstream Ig heavy chain, Ig light chain, or TCR enhancer elements and upstream TCR or IgH promoter elements. Consistent with the postulates of the accessibility model, we find that such transcriptional control elements can dominantly target rearrangement of the associated gene segments in a lineage specific fashion. We are also using new approaches to further address these issues including the development of novel cell lines with higher (more physiological) levels of VDJ recombinase activity as recipients for transfected recombination substrates and the use of ES cell/somatic chimera technology to assay substrates in an in vivo setting.

We have defined transcription units that initiate upstream of class-switch recombination target sequences of four different germline H chain genes including $\gamma 1$, $\gamma 2b$, $\gamma 3$, and ϵ. Treatment of pre-B cell lines or normal splenic B cells with LPS or LPS plus IL-4 differentially induces transcription from these germline C_H promoters followed by induction of switch recombination to the corresponding genes. These results imply a role for transcription of germline CH genes in heavy chain class-switching. Such a role could include targeting of appropriate class switch sequences for recombination and /or the generation of germline transcripts for substrates in putative trans-splicing mechanisms of class-switching. We have identified DNA regions that are involved in regulating transcription of germline C region genes and are analyzing the effects of dominant germline transcription vectors that were introduced into cells and animals to test postulates of the potential functions described above.

J 007 Mutation of the *TOP3* gene from *Saccharomyces cerevisiae*, which encodes a bacterial-like type I topoisomerase, causes hyper-recombination and genomic rearrangements.

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We have isolated a mutation in the yeast *Saccharomyces cerevisiae* that causes increased recombination in a variety of repeat sequence assays, including recombination between components of the yeast retrotransposon, *Ty*, and as well as recombination in the rDNA multiple repeat array. Recombination is also increased between the genes encoding two AdoMet synthetase isozymes, *SAM1* and *SAM2*, which are 83% identical at the DNA level and map to chromosomes XII and IV, respectively. In addition, mutant cells exhibit slow growth, poor diploid formation and a sporulation deficiency. The wild-type gene was cloned by complementation of slow growth and hyper-recombination. DNA sequence analysis of the clone revealed that the gene is homologous to both bacterial type I topoisomerases, TopA and TopB. It is not homologous to the two known yeast topoisomerases, *TOP1* or *TOP2* or any other known eukaryotic topoisomerases. Therefore we named this new yeast gene *TOP3*. Triple mutant strains (*top1 top2 top3*) are not viable. Over-expression of bacterial TopA in *top3* mutants suppresses poor growth and partially suppresses the sporulation defect. However, the hyper-recombination phenotype is not suppressed to wild type levels. The mating defect of *top3* mutants stems from the inappropriate expression of mating-type regulated genes in *MAT*α cells. In addition, regulation of the glucose-repressible gene, invertase, is altered in both mating types. These observations suggest that *TOP3* plays a role in the regulation of gene expression at diverse loci and that as a result of the altered expression, enhanced genomic rearrangements are observed.

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Recombination II

J 008 FUNCTIONS AND DYSFUNCTIONS OF CHROMOSOMAL LOOP ATTACHMENT SITES,

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A family of AT-rich sequences termed MARs (matrix association regions), or SARs (scaffold attached regions) mediate chromosomal loop attachment to the nuclear matrix. Previously we provided evidence for a dysfunction of MARs in illegitimate recombination, since these sequences are sometimes found at points of DNA insertion, deletion, and translocation (1). Several MARs both specifically bind and contain multiple sites of cleavage by topoisomerase II (topo II), a major protein of the mitotic chromosomal scaffold. Since "hotspots" of enzyme cutting coincide with the breakpoints of a previously described chromosomal translocation event, we proposed that topo II may mediate illegitimate recombination at MARs. Interestingly, topo II has more recently been shown to be required for the resolution of meiotic recombination products (2), suggesting that points of chromosome disentanglement may be centered at MARs. Currently, we are studying the function of chromosomal loop attachment in transcription. We are utilizing the HO-endonuclease system in yeast to create double-stranded DNA breaks at selected sites, thereby disrupting loop organization. We find that linearization inhibits the transcription of a CUP1:LacZ fusion gene. This observation may permit engineering an approach to map the boundaries of topological units in the genome. Research supported by grants from NIH and the Robert A. Welch Foundation.

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J 009 GENOMIC STABILITY AND INSTABILITY: SEVERAL FACES OF GENOMIC IMPRINTING, Charles D. Laird, Departments of Zoology and Genetics, University of Washington, NJ15, and Fred Hutchinson Cancer Research Center, Seattle, WA 98195. A stable, epigenetic inactivation of a gene or group of genes can change a chromosomally diploid cell into one that is functionally haploid (or deficient) for part of its genome. Chromosomal or genomic imprinting, as some examples of such inactivation are called, is a form of partial genome silencing. Principles of genomic imprinting are well described for *Drosophila* and the mouse, although the molecular details of imprinting processes are as yet unkown. Genomic imprinting has also been proposed as an explanation for several unusual patterns of inheritance in other organisms, including maize and yeast.

Some events of genomic imprinting appear to reflect normal cellular processes that occur during gametogenesis or embryogenesis. In such situations, a mutation that is expected to be recessive in a diploid cell is sometimes observed to be dominant. Such dominance is thought to result from inactivation of a normal allele of, for example, a tumor-suppressing gene, thereby forcing a cell to depend solely on the function of a mutant allele.

Other events of genomic imprinting reflect abnormal cellular processes. Proposed examples of abnormal imprinting in humans include the fragile-X syndrome, Huntington's disease, Prader-Willi syndrome and Angelman syndrome. The genetic and epigenetic peculiarities expected from abnormal genomic imprinting are illustrated by these human syndromes, as are the novel properties of signals that control imprinting. The stability of the imprinted state may be inferred for one of these human disorders, the fragile-X syndrome. Information concerning the stability of genomic imprints in other human diseases may be useful in understanding the molecular basis of, and in contemplating treatments for, diseases of genomic imprinting.

Methylation and Gene Amplification

J 010 DE NOVO GERMINAL MUTATIONS IN MAN: WHEN AND HOW DO THEY ARISE? Ann C Chandley, MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, Scotland, UK.

Studies into parental origins of chromosome rearrangements and the germinal mutations in diseases such as retinoblastoma, Prader-Willi syndrome and Wilms tumour show a preferential bias towards a paternal origin. One general belief is that more errors arise in men because of the numerous mitotic divisions which occur during a lifetimes proliferation of germ cells from stem-cell spermatogonia, oocytes in females being finite in number and arrested at the dictyate stage throughout adult life. But while such an explanation may hold true for point mutation, it is unlikely to explain the preponderance of structural aberrations which also arise paternally. Mutagenesis studies carried out in Drosophila and the mouse in the 1950's and '60's, provide strong evidence for germinal selection operating to eliminate most chromosome rearrangements which arise in spermatogonia as the cells carrying them pass through subsequent mitotic and meiotic divisions. Deletions and translocations are much more frequently recovered from treated late spermatocytes and spermatids, cell types which do not have to survive such barriers on their passage to the sperm stage. Chromosome spatial arrangements and repair capabilities appear to favour structural rearrangement in these meiotic and post-meiotic stages. These studies also show a lower overall mutagen sensitivity of females, which may be applicable in humans. Current investigations into the DNA sequences surrounding the breakpoints of deletions and translocations in humans are providing clues to the mechanisms by which such rearrangements might arise. The same factors which act to promote rearrangement between or within chromosomes in germ cells may equally predispose specific chromosomes or chromosome regions to exchange in somatic cells, this being an important first step in neoplastic transformation.

J 011 IDENTIFYING TARGETS OF ALTERED DNA METHYLATION IN NEOPLASTIC TRANSFORMATION, Andrew P. Feinberg, Shirley Rainier, Peter J. Lee, Robin Pena, Stephen E. Kuehn, Muneesh Tewari, Laura Bonetta*, Annie Huang*, Bryan R.G. Williams*, David J. Law, Howard Hughes Medical Institute and Departments of Internal Medicine and Human Genetics, University of Michigan Medical Center, 4520 MSRB I, Ann Arbor, MI 48109-0650, *Hospital for Sick Children, Toronto, Ontario M5G 1X8

The earliest change we have found in multistep colorectal carcinogenesis is widespread hypomethylation of DNA, involving one third of tested single copy genes. Altered DNA methylation appears ubiquitously in human malignancies, and it occurs early in multistep carcinogenesis, involving, for example, premalignant colorectal adenomas. In order to better understand the role of altered DNA methylation in multistep carcinogenesis, we have developed a novel strategy to isolate cells treated with 5-aza-2'-deoxycytidine, which causes hypomethylation of DNA, before the cells have become morphologically transformed, anchorage independent, and tumorigenic in nude mice.[1] This model of biological determination for transformation should enable identification of potential targets of altered DNA methylation in neoplastic transformation.

We have also identified a CpG island "archipelago", including several islands associated with transcribed sequences, in the Wilms tumor gene region of 11p13. Three of these islands are at least partially methylated, and thus may be involved in either normal developmental regulation or imprinting of the genes associated with them.[2]

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J 012 OVEREXPRESSION OF RAS, MOS, FOS AND MYC INDUCES CHROMOSOMAL ALTERATIONS KNOWN TO BE CAUSED BY DNA DAMAGING AGENTS, Susanne van den Berg, Sabine Mai, Bernd Kaina, Hans J. Rahmsdorf and Peter Herrlich, Kernforschungszentrum Karlsruhe, Institute of Genetics and Toxicology, P.O.Box 3640, D-7500 Karlsruhe 1, FRG.

DNA damaging agents are known to induce in mammalian (and other) cells point mutations, chromosomal aberrations, recombinational events and gene amplifications (1,2). No doubt, many cellular enzymes must participate in order to catalyze these various structural alterations of DNA. Two extreme views could be taken: The enzymes are constitutively present and the events are simply triggered by the "substrate", the DNA damage; or the enzymes are induced by the DNA damage which elicits a signal transfer (3,4) to specific transcription factors. In the absence of this induction, structural changes such as amplifications, breakage and religation, and replication "errors" do not occur.

We have made attempts to dissociate DNA damage from the processes that lead to structural DNA changes. These attempts are based on the assumption that the chain of events leading from DNA damage to particular endpoints must include physiological components that can be stimulated by physiological factors or by artificial overexpression and thus generate an elevated flow of communication. Earlier we have reported that UV irradiation causes synthesis and secretion of growth factors and other extracellular mediators which in turn can induce mutations (5,6). We have now manipulated the levels of putative elements of signal transduction and have been able to induce the same genetic alterations which are introduced by damaging DNA directly.

A number of individual clones of 3T3 and CHO cells stably transfected with either one of several dexamethasone, cadmium or heatshock inducible oncogene constructs were analyzed for their rate of mutagenesis. We compared control cells with a very low level of oncogene expression, transfectants with low spontaneous and high induced expression and cells treated with specific antisense oligonucleotides. For instance, elevated levels of MYC protein cause gene amplification. Activated RAS, viral MOS and FOS (a mutant with prolonged half life) induce chromosomal aberrations. RAS and MOS act through FOS. The most dramatic increase occurred with FOS and ouabain resistance as the endpoint. FOS increased the yield of resistant colonies 8 to 60 fold. These changes occurred in the absence of any detectable increase in proliferation. The experiments document some of the features of induced mutagenesis and gene amplification and suggest significant contribution of an induced genetic program.

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J 013 GENE AMPLIFICATION AND TUMOR PROGRESSION, Thea D. Tlsty, Lineberger Cancer Research Center, Department of Pathology and Curriculum in Genetics, University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599-7295

It has been hypothesized that genomic instability is an important component of tumorigenesis. Our recent studies have examined the relationship between tumorigenicity and one marker for genetic instability, gene amplification. Gene amplification is frequently observed in tumors and transformed cell lines. This phenomenon is known to contribute to the generation of drug-resistant tumor cells and quantitation of the event is believed to have prognostic value in several types of neoplasia. Our previous work has reported a correlation between the ability of a cell to amplify endogenous genes and their ability to form a tumor when injected in the appropriate animal. We exposed cells to N-(phosphonoacetyl)-L-aspartate (PALA), a drug which specifically inhibits the aspartate transcarbamylase activity of the multifunctional CAD enzyme and selects for amplification of the CAD gene, and observed a striking parallel between the ability of these cell lines to become resistant to this drug and the ability of these same cells to form tumors after injection into day-old syngeneic rats. Molecular analyses of independent PALA-resistant subclones confirmed that, in each case, this resistance was due to amplification of the CAD gene. We found that highly tumorigenic cell lines demonstrate a frequency of gene amplification that can be greater than 100 times that seen in nontumorigenic cell lines. These frequencies range from 10^{-3} in highly tumorigenic cell lines to 10^{-6} in nontumorigenic but immortalized cell lines. Further studies, using the Luria-Delbruck fluctuation analysis showed that the amplification is a spontaneous event and that the rate measurement parallels the frequency measurement previously reported. The rate of gene amplification is high compared to the rate of point mutations usually reported in mammalian cells, and its potential contribution to the tumorigenic process could be tremendous. In stark contrast, we find that gene amplification is undetectable ($<10^{-9}$) in primary, diploid cell populations. These results demonstrate that a dramatic difference exists between primary diploid cell populations and immortalized populations in their ability to amplify genomic sequences and suggests a significant difference in genetic stability between these two cell types.

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Viral Carcinogenesis

J 014 TRANSCRIPTIONAL ACTIVATION OF HOMOLOGOUS AND HETEROLOGOUS GENES BY HEPATITIS B VIRUS, William S. Robinson, Jane Wu and Jesse Twu, Department of Medicine, Division of Infectious Diseases, Stanford University School of Medicine, Stanford, Ca. 94305. Epidemiologic evidence shows a strong association of persistent hepadnavirus infection with development of hepatocellular carcinoma (HCC) in man, woodchucks, ground squirrels. Except for a small number of HCC in which viral integrations have been found to be within DNA domains of cellular genes which could play a role in the pathogenesis of HCC, in the over whelming majority of hepadnavirus associated HCC experimental findings do not support the possibility of a viral insertional mutagenesis mechanism and a specific role for the virus in development of HCC in unclear. Of the four genes of these small viruses, one (hbx) can transactivate transcription regulated by several unrelated cis-acting sequences of different viruses and cellular genes. Thus hbx has the potential to regulate the expression of certain cellular genes and thus alter cell growth or function. Cis-acting sequences that have been shown to be transactivated by hbx include regulatory sequences of hepatitis B virus (HBV) and unrelated heterologous regulatory sequences in the long terminal repeat (LTR) of human immunodeficiency virus type 1 (HIV-1) and Rous sarcoma virus, and regulatory sequences of Simian virus 40 and the human ß interferon gene. The diversity of regulatory sequences that appears to be activated by hbx suggests that the hbx protein acts by a general mechanism that is not sequence specific. The hbx protein expressed in E.coli and highly purified was therefore tested for mechanisms known to activate cellular transcription factors and it was found to have protein kinase activity and to activate transcription directed by the HIV-1-LTR in a cell free system including a nuclear extract of HepG2 cells. There was no detectable DNA binding of the hbx protein. The hbx protein was found to catalyze phosphorylation of serine/threonine residues in protein and it was present in hepatitis B virions, and thus appears to represent the serine/threonine kinase activity previously found in virions. These results are consistent with the hbx protein regulating viral and cellular gene expression by direct covalent modification (phosphorylation) of cellular (and/or viral) transcription factors. It will be important to investigate whether HBV by this mechanism can effect the course of infections by HIV or other hbx responsive viruses and the associated diseases during coinfection in vivo, and whether hbx plays a role in development of any hepatocellular carcinomas by activating cellular genes.

J 015 ANALYSIS OF THE ACTIVITIES OF THE GENITAL HPV E6 AND E7 PROTEINS.
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National Cancer Institute, Bethesda MD, USA.

HPV 16 and HPV 18 are often detected in cervical carcinomas, while HPV 6, although frequently found in benign genital lesions, is only rarely detected in cervical cancers. The apparent oncogenic risk potential of these viruses is reflected in the in vitro activities of their E6 and E7 proteins. The E6 and E7 open reading frames of HPV 16 or HPV18, which are selectively retained and expressed in cervical carcinomas, can cooperate to induce immortalization of normal human foreskin keratinocytes but E6 and E7 of HPV 6 cannot. Changes in cellular gene expression are probably also required for immortalization since all of the keratinocyte lines examined were aneuploid.

The E7s of HPV6, HPV16 and HPV18 can each independently induce anchorage independent growth in murine NIH3T3 cells. However, the activity of the E7 from the latter two high risk viruses is much greater and correlates with the ability of the E7 proteins to bind to the retinoblastoma tumor suppressor protein in vitro and serve as a substrate for casein kinase II phosphorylation.

One of the striking features of the high risk types is their potential for expressing both a full-length and truncated E6* proteins, translated from spliced mRNAs. To examine the question of whether an E6* protein contributes to the apparent oncogenic potential of the high risk types, we have constructed clones to express only the full-length HPV 16 E6, only E6* or both, and tested their biological activity in two assays. The clones expressing only the full-length E6 or both E6 and E6* were equally able to complement E7 in immortalizing keratinocytes while the clone expressing only E6* was inactive. We have also determined that the clones expressing only E6 were able to trans-activate the adenovirus E2 promoter and transform NIH3T3 cells while the clone that could express only E6* was defective. These results suggest that the expression of an E6* protein may not be an important determinant of pathogenicity, but rather that the splicing of the E6 mRNA may function to facilitate the translation of the E7 ORF, which is translated from the same polycistronic message.

Werness et al.(Science 248,76-79, 1990) have reported that the HPV16 and HPV18 E6 bind p53 in vitro while the E6 of HPV 6 does not. We have therefore examined the activity of p53 in our keratinocyte immortalization assay. Mutant p53, but not wild type p53, was able to fuctionally substitute for E6 but not for E7. These result support the hypothesis that E6 and E7 act through different pathways and that E6 fuctions, at least in part, by inactivating wild type p53.

Chemical and Physical Carcinogenesis

J 016 THE SEQUENTIAL ACTION OF THE RAS ONCOGENE AND p53 TUMOUR SUPPRESSOR GENE IN MULTISTAGE SKIN CARCINOGENESIS, Allan Balmain, Phil Burns and Chris Kemp, Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD.

Cell growth is controlled by both positive and negative signals, the balance of which ensures that tissue size and turnover are maintained in the correct homeostatic equilibrium. Disruption of these controls occurs during tumour development, either by activation of oncogenes or inactivation of tumour suppressor genes. Mouse skin carcinogenesis constitutes an excellent system to study such changes, since the stages of tumour development are very well defined. Initiation involves, at least in certain cases, the induction of H-ras gene mutations. Further alterations of the H-ras locus occur during the development of benign tumours and at the transition to malignancy. These lead to net over-representation of the mutant ras allele and/or loss of the corresponding normal allele (1). The latter is seen predominantly in highly undifferentiated invasive spindle cell carcinomas. Loss of heterozygosity at the P53 tumour suppressor gene locus is seen in at least 30% of carcinomas, but not to date in any premalignant tumours, suggesting that alterations at this locus occur during tumour progression. A number of p53 mutations have been characterised both in carcinoma cell lines and in primary tumours. This system therefore offers the possibility to examine the specific roles of both ras and p53 genes in tumour development.

(1) Bremner, R. and Balmain, A. (1990) CELL 61, 407-417.

J 017 CELLULAR SENESCENCE AND CANCER, J. Carl Barrett, P. Andrew Futreal, Lois A. Annab, Holly Bivins, Hartmut Richter, and Mitsuo Oshimura, Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC 27709, Tottori University, Yonago-City, Tottori-Ken 683, Japan.
Normal cells exhibit a finite life span in culture and then undergo a process termed cellular senescence, which results in a stable, postreplicative state. Most tumor cells, in contrast, exhibit an indefinite life span in culture; immortal cells that have escaped cellular senescence have a predisposition for neoplastic transformation and hence represent a preneoplastic stage in cell transformation models. Senescence is a genetically programmed process. This conclusion is based on the observation that the senescent phenotype is dominant in normal cross immortal cell hybrids and that the defect(s) in the normal senescence program can be complemented by fusion of different immortal cell lines. Introduction of specific chromosomes from normal cells has also been shown to induce senescence in immortal cells. Our previous work showed that chromosome 1 induced senescence of certain immortal hamster and human cells. The putative senescence gene for immortal hamster cells has now been mapped on human chromosome 1 to the region 1q23-1q31. We are also studying the control mechanisms for the growth arrest state in senescent cells. We examined the expression and phosphorylation status of the RB protein in senescent Syrian hamster embryo cells. Both phosphorylated and unphosphorylated forms of the RB protein were observed in cells in early passages; however, only unphosphorylated RB protein was found in senescent cells. Also, senescent cells did not show any phosphorylation of the RB protein in response to serum. These results show that unphosphorylated RB is a new marker for cellular senescence and suggest that the RB protein plays a role in cellular senescence with phosphorylation status determining this role. Factors controlling this phosphorylation, which are potential key factors in controlling cellular life span in culture, are currently under study.

J 018 ROLE OF ONCOGENES, TUMOR SUPPRESSOR GENES, AND ANTIMETASTASIS GENES IN HUMAN LUNG CARCINOGENESIS, Teresa A. Lehman, Kathleen Forrester, Robert A. Metcalf, William P. Bennett, Ainsley Weston, Andrea M.A. Pfeifer, Brenda I. Gerwin, and Curtis C. Harris, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892.
Five families of activated protooncogenes, ras, raf, jun, neu, and myc have so far been associated with human bronchogenic carcinoma. Normal human bronchial epithelial (NHBE) cells and SV40 T "immortalized" NHBE cells (BEAS-2B) have been utilized for in vitro investigations of the functional roles of specific oncogenes, tumor suppressor genes (e.g., p53), and antimetastasis genes (e.g., nm23) in carcinogenesis and tumor progression. Transfection of the oncogenes Ha-ras, N-ras, or Ki-ras into BEAS-2B cells causes neoplastic transformation and resistance to inducers of terminal differentiation (e.g., transforming growth factor-β_1, TGF-β_1). In addition, the combined transfection of c-myc and c-raf-1 into BEAS-2B cells produces neoplastic transformation and the expression of neuroendocrine markers found in small cell lung carcinomas. Therefore, these oncogenes dysregulate pathways of growth and differentiation of human bronchial epithelial cells during lung carcinogenesis.

The functions of putative tumor suppressor genes and antimetastasis genes are also being studied. Somatic cell hybrids between NHBE cells and HUT-292DM, a lung carcinoma cell line, have a finite lifespan in vitro and undergo senescence. Loss of allelic heterozygosity in non-small cell bronchogenic carcinoma is more frequent among squamous cell carcinomas than among adenocarcinomas. Allelic deletion of chromosome 17p was found in 89% of squamous cell carcinomas and only 18% of adenocarcinomas. Direct DNA sequencing and co-immunoprecipitation with heat shock protein 70 indicate the presence of p53 mutations and the expression of the mutant protein in human lung carcinoma cell lines. Studies of transfected mutant versus wild type p53 in BEAS-2B cells indicate that expression of the mutant p53 gene enhances growth and decreases cell responsiveness to the growth inhibitory effects of serum or TGF-β_1. Lung carcinoma cell lines transfected with wild type or mutant p53 selectively retain expression of the mutant gene and eliminate the wild type. Thus, the p53 gene may affect the neoplastic potential of human bronchial epithelial cells by means of regulation (wild type) or dysregulation (mutant) of cell growth and differentiation.

J 019 PERMANENT CONVERSION OF MOUSE AND HUMAN CELLS TRANSFORMED BY ACTIVATED *RAS* TO NORMAL PHENOTYPE BY TREATMENT WITH THE ANTIBIOTIC, AZATYROSINE. Nobuko Shindo-Okada, Wlodzimierz J. Krzyzosiak, Mitsuo Izawa, Hisako Teshima, Osamu Makabe and Susumu Nishimura. Biology Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, and *Central Research Laboratories, Meiji Seika Kaisha, Ltd, Yokohama, Japan.

Azatyrosine [L-ß-(5-hydroxyl-2-pyridyl)alanine], an antibiotic isolated from *Streptomyces chibanensis*, inhibited the growth of NIH3T3 cells transformed by the activated human c-Ha-*ras* but did not significantly inhibit the growth of normal NIH3T3 cells. In addition, upon treatment with azatyrosine for 6 days, most of the transformed cells apparently became normal. These apparently normal cells, named revertant cells, grew in the presence or absence of azatyrosine and stopped growing when they reached confluence, and their normal phenotype persisted during prolonged culture in the absence of azatyrosine. The revertant cells did not grow in soft agar and scarcely proliferated in nude mice. The human c-Ha-*ras* gene present in transformed NIH3T3 cells was still present in the revertant cells and was expressed to the same extent as in the original transformed cells, producing the same amount of activated p21.

Treatment with azatyrosine caused similar conversion of NIH3T3 cells transformed by activated c-Ki-*ras*, N-*ras*, c-*raf* or *neu* to apparently normal cells, but NIH3T3 cells transformed by *hst* or ret were not exclusively converted by azatyrosine.

The genes which are specifically expressed in the revertant cells were identified by differential screening of 15,000 recombinants from λgt10 cDNA library, prepared from azatyrosine treated NIH3T3 cells. Nucleotide sequence analysis of the cloned cDNA indicated that they most likely belong to some new type of genes or gene families, showing variable homology to several mammalian DNA sequences.

Human pancreatic adenocarcinoma cells (PSN-1), which are known to contain an amplified activated c-Ki-*ras* and amplified c-*myc*, were also converted to flat and giant revertant cells by treatment with azatyrosine. The similar result was also obtained with human colon cancer cells (HCT116).

Shindo-Okada, N., Makabe, O., Nagahara, H., and Nishimura, S., (1989) Mol. Carcinogenesis **2**: 159-167.

Tumor Suppressor Genes

J 020 DELETION OF THE INTERFERON GENES ON THE SHORT ARM OF CHROMOSOME 9 IN LEUKEMIA AND SOLID TUMORS. Manuel O. Díaz, Department of Medicine, University of Chicago, Pritzker School of Medicine, Chicago, IL 60637.

Interstitial deletions and unbalanced translocations are frequent mechanisms of chromatin loss in malignant cell clones from leukemia and solid tumors. Even if these abnormalities show no clustering of breakpoints, their recurring nature is evident in the overlap of the lost chromatin segments over a small chromosomal region. In the case of retinoblastoma, this minimum region of overlap contains a tumor suppressor gene which inactivation or deletion seems to contribute to malignant transformation.

A complex of deletions and unbalanced translocations involving the short arm of chromosome 9, is associated with acute lymphoblastic leukemia, gliomas, melanomas and other solid tumors. These abnormalities result in hemizygous or homozygous loss of chromosomal material with a minimum region of overlap at band 9p22, and frequently are submicroscopic. Somatic cell hybridization experiments have provided evidence for the presence of a tumor suppressor gene in human chromosome 9, and syntenic regions of mouse chromosome 4. It is possible that loss of this tumor suppressor gene provides the selective force for the clonal expansion of cells with deletions and unbalanced translocations of 9p. Further experiments with somatic cell hybrids are been used to test this hypothesis. These deletions frequently, but not always, include part or all of the interferon gene cluster located on 9p. This raises the possibility that the interferon genes are tumor suppressor genes. Nevertheless, mapping of the minimum region of overlap of the deletions between the interferon gene cluster, and the methylthioadenosine phosphorylase gene, located closer to the centromere, does not support this hypothesis.

J 021 CHROMOSOME 3p DELETIONS IN CANCER, Susan L. Naylor, M. Elizabeth Wolf, and Ann M. Killary, Department of Cellular & Structural Biology, The University of Texas Health Science Center at San Antonio, 78284-7762 and Division of Laboratory Medicine, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

Virtually every case of small cell lung cancer examined shows a loss of heterozygosity of human chromosome 3 short arm (3p) markers. Chromosome 3p deletions have been observed in many types of cancer including renal cell carcinoma, ovarian, breast, and other lung cancers. Deletion of material from 3p suggests a tumor suppressor gene may lie in this region. To assay suppression of tumor formation, we marked human chromosome 3 with neo and transferred it first into mouse A9 cells and subsequently into a small cell lung cancer line (H592) by microcell transfer. The addition of a normal chromosome 3 to H592 resulted in a partial suppression of tumor formation in nude mice with the tumors averaging about one tenth the volume of H592 tumors. In addition, chromosome 3 in the mouse A9 cells showed an altered morphology. Mouse A9 cells produce a rapidly growing fibrosarcoma in nude mice. When three independent monochromosomal hybrids containing chromosome 3 injected into nude mice, the only very small tumors were formed. A9 hybrids which had segregated chromosome 3 and were negative for 52 chromosome 3 markers were fully tumorigenic. Other human chromosomes did not suppress tumor growth in nude mice. One hybrid segregant which contained a small fragment of chromosome 3p translocated to a mouse chromosome suppressed A9 tumor formation in nude mice. This fragment of 3p is from band 3p21, the same region thought to be involved in small cell lung cancer. Approximately 2 Mb of human DNA is contained in the fragment. This cell clone should allow us to isolate the gene responsible for the suppression and test its activity on small cell lung cancer. We then will be able to test whether a single locus or multiple loci are involved in the genesis of these human cancers that show allele loss on chromosome 3p.

Tumor Progression I

J 022 THE NEUROFIBROMATOSIS TYPE 1 GENE: cDNA SEQUENCE, GENOMIC STRUCTURE AND MUTATIONS, Ray White*, Richard Cawthon*, David Viskochil+, Gangfeng Xu*, Arthur Buchberg#, Robert Weiss*, John Carey+, Roger Wolff*, Ray Gesteland*, Fuyu Tamanoi, Kazuma Tanaka, Frank McCormick, George Martin and Peter O'Connell*, *Department of Human Genetics and Howard Hughes Medical Institute, +Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT and #BRI-Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, MD. The NF1 gene has been identified and cloned. Mutations in this gene have been detected in NF1 patients and characterized. Pulsed field gel and standard Southern blot analysis of patient DNAs have revealed deletions of 190 kb, 40 kb and 11 kb of DNA. DNA sequence derived from a contig of overlapping cDNA clones shows that the NF1 gene is interrupted by the deletions and by both the t(1;17) and t(17;22) translocations found in NF1 patients. The t(17;22) breakpoint has been examined at the DNA sequence level and reveals that the translocation occurred between regions of repeating AT dimer present in both chromosomes. Furthermore, PCR amplification of a subset of the exons, followed by electrophoresis of denatured product on native gels, identified variant conformers specific to NF1 patients, indicating base pair changes in the gene. Sequencing revealed that one mutant allele contains a T->C transition changing a leucine to a proline; another NF1 allele harbors a C->T transition changing an arginine to a stop codon. Comparison of the deduced amino acid sequence of the NF1 gene to the sequence databases revealed strong similarities to the mammalian GAP proteins and the yeast IRA proteins. Biochemical and genetic tests have confirmed that the NF1 gene interacts with both mammalian and yeast ras proteins to accelerate their rate of GTP hydrolysis.

Tumor Progression II

J 023 GENE AMPLIFICATION IN TUMORS: BIOLOGICAL CONSEQUENCES, June L. Biedler, Esther Bossart, Tien-ding Chang, and Barbara A. Spengler, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

Increase in gene copy number generated by amplification is a now well established mechanism for increasing the amount of an encoded protein product. In several instances it is clear that amplification and overexpression of proto-oncogenes have adverse consequences for tumor progression and patient prognosis, e.g., the MYCN gene in neuroblastoma and the HER-2/neu gene in breast and ovarian cancer. It is reasonable to suppose that, in such instances, decrease in number of amplified genes and/or in specific mRNA would result in decrease in malignant potential. To investigate this possibility, we are attempting to eliminate amplified MYCN genes carried extrachromosomally on DMs (double minutes) or to reduce expression of genes chromosomally integrated as HSRs (homogeneously staining regions). Many human neuroblastoma cell lines contain amplified MYCN genes. Amplification levels tend to be high, about 25 to 150 gene copies, and expression level tends to be commensurate with copy number. We have demonstrated that human neuroblastoma cells in culture often have the capacity to undergo spontaneous phenotypic interconversion (transdifferentiation) between two morphologically and biochemically distinct cell types (1,2). One is neuroblastic (N) in appearance, with functional features of noradrenergic neurons. The other is substrate-adherent (S) and has features of Schwannian/glial/melanocytic cells. S-type cells are less or non-tumorigenic as compared to N counterparts (3). A current question is whether this difference in malignant behavior is associated with differences in amplification and/or expression of N-myc. N and S clones isolated from the DM-containing lines CHP-234 (150-fold MYCN-amplified) and SMS-KCN (100-fold) have been examined by Southern analysis. In both cases, amplification level in S cells was less than 10% of that in N cells, indicating that transdifferentiation from N to S resulted in a loss of MYCN genes. Further, neuronal differentiation of N-type SMS-KCN cells induced by retinoic acid resulted in an approximately 25% lower copy number. N-type CHP-234 cells did not visibly respond to retinoic acid and showed no change in MYCN amplification level. Experimental modulation of MYCN gene amplification and expression level appears feasible and may provide further understanding of the role of this gene in human neuroblastoma cell transformation and differentiation.

1. Ross et al., J. Natl. Cancer Inst. 71:741-747, 1983.
2. Ciccarone et al., Cancer Res. 49:219-225, 1989.
3. Biedler et al., Advances in Neuroblastoma Research 2. A.E. Evans et al., eds., Alan R. Liss, Inc., 1988, pp. 265-276.

J 024 TRANSLOCATIONS INVOLVING 11Q23 IN TREATMENT RELATED ACUTE LEUKEMIA: RESPONSE TO TOPOISOMERASE II INHIBITORS? Janet D. Rowley, Manuel O. Diaz, Rafael Espinosa, Sheryl Zieman, Yogesh Patel, Elizabeth Davis, Michelle M. Le Beau, Department of Medicine, University of Chicago, Chicago, IL 60615

Acute myeloid leukemia is an unfortunate consequence in about 10% of patients following treatment with chemotherapeutic drugs and/or radiation for another malignant disease. The most frequent chromosome abnormalities in the malignant cells are loss of chromosomes 5 and/or 7. More recently, translocations involving chromosome band 11q23 have been identified, particularly in patients treated with topoisomerase (topo) II inhibitors, such as the epipodophyllotoxins, adriamycin, and actinomycin D. In acute leukemia de novo, a number of different rearrangements involving 11q23 occur in both lymphoblastic and monoblastic as well as in other myeloblastic types. The 11q23 aberrations account for more than 2/3 of all abnormal karyotypes in infant leukemia. Critical questions include (1) is the breakpoint the same in de novo and treatment related leukemia, (2) is the breakpoint the same in different translocations, (3) what gene(s) at 11q23 is involved, and (4) what is the role of topo II inhibitors in the translocation process? We have shown that a yeast artificial chromosome containing a 330 kb human insert including the CD3G gene is split by the 4;11, 6;11, 9;11, and 11;19 translocations in human acute leukemia. This YAC is split in 9;11 translocations from both de novo and treatment related leukemia. Cloning of the breakpoint(s) will help to answer the questions outlined above.

Late Abstracts

SITE SPECIFIC BREAKAGE AND RECOMBINATION AT CHROMOSOMAL FRAGILE SITES, Thomas W. Glover, Bryan K. Hall, Eric Legius, Paula E. Gregory and Catherine R. Begy, Departments of Pediatrics and Human Genetics, University of Michigan, Ann Arbor, Michigan 48109

Chromosomal fragile sites are points on chromosomes that appear cytologically as gaps or breaks when DNA synthesis is perturbed. At least some fragile sites predispose to a high frequency of sister chromatid exchanges and deletions or translocations in somatic cell hybrid test systems. The conditions under which fragile sites are expressed are similar to those used to induce gene amplification. Both rare and common or constitutive fragile sites have been described. The only fragile site that has clearly been shown to be associated with a human genetic disorder is the fragile X. However, based on their location and potential for chromosome instability, it has been suggested that other fragile sites may play a role in chromosome rearrangements or deletions involved in birth defects or cancer. This hypothesis has been difficult to test in part because fragile sites are, at present, a cytogenetic phenomenon. Virtually nothing is known about the molecular basis for chromosome instability at these sites.

Our laboratory has been conducting experiments to define fragile sites at the molecular level focused on the fragile site at 3p14.2 (FRA3B), the site most "fragile" in the human genome. Two general strategies are being pursued. First, based on the high frequency of induced chromosomal recombination at this site, we are testing the hypothesis that a selectable exogenous DNA sequence (the neo^r gene) can be inserted into the fragile site by directed recombination during fragile site expression. In order to enrich for cells with integration of pSV_2neo at FRA3B, we have used somatic cell hybrids containing human chromosome 3 for these experiments and produced subsequent radiation-reduced hybrids from transfected cell populations. Radiation hybrids containing both 3p14 loci and the neo^r gene are candidates for integration at the fragile site. A second strategy is based on reverse genetics, or positional cloning, approaches to identify the fragile site. Clonal cell lines have been isolated with deletions or translocations at or very near FRA3B. These will serve as markers for cloning sequences at the fragile site. A long range physical map of the region is being constructed to identify the breakpoints. As a valuable source of new probes for this map, we are characterizing a microdissection library estimated to contain 5000 to 20,000 clones from the 3p14 region.

ROLE OF ONCOGENES, TUMOR SUPPRESSOR GENES, AND ANTIMETASTASIS GENES IN HUMAN LUNG CARCINOGENESIS, Teresa A. Lehman, Kathleen Forrester, Robert A. Metcalf, William P. Bennett, Ainsley Weston, Andrea M.A. Pfeifer, Brenda I. Gerwin, and Curtis C. Harris, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892.

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IDENTIFICATION OF A CANDIDATE TUMOR METASTASIS SUPPRESSOR GENE, NM23. Patricia S. Steeg, Laboratory of Pathology, National Cancer Institute, Bethesda, MD 20892.
The *nm23* gene was identified by differential colony hybridization in a series of murine K-1735 melanoma cell lines; *Nm23* RNA levels were ten-fold greater in two low metastatic K-1735 cell lines than in five, related highly metastatic K-1735 cell lines. In human cells two distinct *nm23* genes have been identified, *nm23-H1* and *nm23-H2*. In human breast carcinomas, allelic deletion of the *nm23-H1* gene was observed in 64% of informative cases. Tumors from patients with involved lymph nodes at surgery contained quantitatively less *nm23* RNA than tumors from lymph node negative patients; Analysis of *nm23-H1* versus *nm23-H2* RNA levels in a limited set of breast carcinomas indicated that the two genes were independently regulated, in that *nm23-H1* expression was decreased in highly metastatic tumor cells to a greater extent than *nm23-H2*. Using immunoperoxidase staining, which detects both *nm23* gene products, 57 breast tumors were separated in a blinded study into low and high *nm23* expression sets; Patients with low *nm23* content tumors had a significantly reduced survival times than high *nm23* content tumors. Allelic deletion of *nm23-H1* has also been observed in human renal, colorectal and non small cell lung carcinomas. The relationship of allelic deletion to protein expression remains to be established in these cell types. A homozygous deletion of *nm23-H1* was observed in a lymph node metastasis of a colorectal carcinoma, indicating that *nm23-H1* can be recessively inactivated. *Nm23-H1* mapped to chromosome 17, near the centromere. No significant concordance was observed between *nm23-H1* and *p53* allelic deletion. The consistent reduction in expression of *nm23* in murine melanoma and *nm23-H1* in human breast carcinoma support the hypothesis that *nm23* may suppress certain aspects of the tumor metastatic process in these cell types. Transfection experiments, in progress, will test this hypothesis.

SOURCES OF SPONTANEOUS MUTATIONS, Lawrence A. Loeb, Keith C. Cheng, Thomas M. Reid, and Timothy J. McBride, Department of Pathology, University of Washington, Seattle, WA, 98195. Evidence indicates that the mutation frequency in somatic cells is 1.4×10^{-10} mutations/nucleotide/cell generations. We have analyzed three potential sources of spontaneous mutations. These are: 1) chemical hydrolysis of the glycosylic bond in DNA, 2) damage to DNA by oxygen free radicals; and 3) misincorporations by DNA polymerases. Each process, if not repaired, could generate >3000 mutations/cell/day. 1) Spontaneous chemical hydrolysis of the glycosylic bond in DNA generates abasic sites that direct the perferential incorporation of deoxyadenosine. 2) Replication of DNA damaged by oxygen free radicals results a spectrum of nonrandom single-base mutations; most frequently c->T and G->T substitutions. Studies with bacteria containing mutations in uracil glycosylase indicate that the C->T substitutions are not the result of cytidine deamination to uridine and base-pairing with adenine. Studies using site specific mutagenesis indicate that the G->C substitutions, presumably mediated by G:G mispairs, do not result from the production of 8-hydroxyguanosine. Thus, two prominent DNA lesions produced by oxygen free radicals may not be highly mutagenic. 3) Misincorporation by different DNA polymerases yields mutations that are characteristics of each of the enzymes and comprize a variety of singlebase substitutions.
A comparison of this data with the published spectrum of spontaneous mutations in different genes indicates that abasic sites are not a major premutagenic event but rather that replication errors or oxygen free radicals are major sources of spontaneous mutagenesis.

p53 GERMLINE MUTATIONS IN LI-FRAUMENI PATIENTS AND THEIR ROLE IN GENOMIC INSTABILITY AND IMMORTALIZATION, Michael A. Tainsky, Department of Tumor Biology, University of Texas, M.D. Anderson Cancer Center, Houston, Texas 77030. Li and Fraumeni have described a form of familial cancer syndrome that is characterized by multiple primary tumors, early age of onset, and interestingly marked variation in tumor type. Williams and Stron (1) demonstrated that at least 7% of childhood soft tissue sarcoma patients had family histories that were not only suggestive of the syndrome, but most readily explained by a highly penetrant autosomal dominant gene. To characterize the mechanism for genetic predisposition to many tumor types in these familes, we have studied genetic alterations in fibroblasts, a target tissue from patients with the Li-Fraumeni Syndrome (LFS). We have observed spontaneous changes in initially normal dermal fibroblasts from LFS patients as they are cultured in vitro. The cells acquire an altered morphology, chromosomal anomalies, the ability to exhibit anchorage independent growth; they enter a growth crisis from which they escape and grow as cell lines (2). The aberrant behavior of fibroblasts from LFS patients has never been observed in fibroblasts from normal donors. Immortal LFS fibroblasts can be transformed by activated ras oncogenes (3). A tumor suppressor gene called p53 has been previously associated with immortalization of cells in culture and cooperation with ras in transfection assays. Therefore, we tested patients' fibroblasts and normal DNA for point mutations in p53. We found that patients with LFS have inherited certain point mutations in one of their p53 genes (4). The mutations all cluster in conserved domain IV of the p53 protein near amino acid 250. Five out of five families tested had p53 germline mutations and four different mutations were observed. The presence of the germline p53 mutations cosegregates with the inheritance of cancer and in two tumors from families members, the remaining normal p53 allele was lost. The presence of the germline p53 mutation predisposes to cancer and aneuploidy of the fibroblasts in culture. The altered fibroblasts described above from LFS patients were found to have lost the remaining wild type p53. While the loss of the second allele correlates with immortalization it is not sufficient for cells to become susceptible to transformation by an activated H-ras oncogens.

1) Williams, et al. J. Natl. Cancer Inst. 79:1213, 1987.
2) Bischoff, et al. Cancer Research 50:7979-7984, 1990.
3) Bischoff, et al. In Press ONCOGENE, Feb 1991.
4) Malkin, et al. Science, 250:1233-12238, 1990.

DNA Damage and Repair

J 100 EFFECT OF DNA REPAIR GENES ON THE PROCESSING OF TRANSPOSON-INDUCED DNA DAMAGE IN DROSOPHILA, Satnam S. Banga, Antonia Velazquez and James B. Boyd, Department of Genetics, University of California, Davis, CA 95616

We have devised a genetic system to identify host genes responsible for repairing DNA lesions generated during mobilization of P elements. Application of this system to repair deficient mutants has revealed that the *mei-41* and *mus302* genes are necessary for recovery of P-bearing chromosomes undergoing transposition. Because mutants at these loci are deficient in postreplication repair, P element mobilization may generate intermediates similar to those formed when DNA is replicated on a damaged template. Mutants deficient in excision repair, on the other hand, have no detected effect on the repair of transposition-induced lesions. These observations support recent studies implicating double strand DNA breaks as intermediates in P transposition, because the *mei-41* gene has been genetically and cytologically associated with the repair of interrupted chromosomes. Analysis of this system has also revealed a striking stimulation of site-specific gene conversion by P transposition. This result supports a recently developed model for a conversion-like mechanism of P transposition. Involvement of the the *mei-41* and *mus302* genes in the repair of both P element-induced lesions and postreplication repair points to a commonality in the mechanisms of these processes.

J 101 USE OF A MODIFIED ALKALINE-AGAROSE GEL ELECTROPHORESIS TO EVALUATE NUCLEOSOMAL DNA DAMAGE BY A DIRECTLY ALKYLATING CARCINOGEN, Lidia C.Boffa, Maria Rita Mariani, Elisabetta Carpaneto, Department of Chemical Carcinogenesis, National Cancer Institute, IST, 16132 Genova, Italy.

As we have previously described, nucleosomes containing transcriptionally inactive sequences (class 1)can be separated from those of active ones(class 2 and 3)on organo-mercurial agarose column. We have examined the damage on HeLa cells(in suspension culture)exposed to the directly methylating carcinogen MNU(N-methyl-N-nitrosourea)at concentrations up to 1mM, for the half life of the compound (30'). Nucleosomes were analyzed with a modified alkaline-agarose electrophoretic tecnique: 2% agarose, running buffer 10%(v/v)TEA(tetraethylammonium hydroxyde)pH 12.3 EDTA 40 mM. In these extreme, although buffered, alkaline conditions DNA double strands separate, allowing a spontaneous depurination of the chemically alkylated bases that, in turn, induces DNA breakage at the damage sites.
Fragmented DNA is electrophoretic separated, in the above described conditions, from intact nucleosomes and quantitated by densitometric analysis with a laser scanning equipped with integrator. We here intend to show that a)fragmentation occurs in a higher amount within "active" nucleosomes (class 2 and 3) and b)preferentially breaks transcribed gene sequences.

Supported by a AIRC grant to L.C.B.

J 102 IDENTIFICATION OF A NUCLEASE ALTERATION IN MUTANTS HYPERSENSITIVE TO DNA CROSS-LINKING AGENTS FROM THREE HIGHER EUKARYOTES, James B. Boyd[a], Kengo Sakaguchi[b] Manuel Buchwald[c], Malgorzata Z. Zdzienicka[d] and Paul V. Harris[a], [a]Department of Genetics, University of California, Davis, CA 95616 USA: [b]Dept. of Applied Biological Science, Science Univ. of Tokyo, Japan: [c]Hospital for Sick Children, Toronto, Canada: [d]State University of Leiden, Dep. of Radiation Genetics, The Netherlands. Mutants that are hypersensitive to DNA cross-linking agents but not to monofunctional alkylating agents have been identified in Drosophila, man and chinese hamster. We have recently shown that all six available mutant alleles of the *mus308* gene exhibit an alteration in a mitochondrial nuclease (Boyd et al, 1990 Genetics 125: 813-819). That observation has been extended to three unrelated cases of Fanconi's anemia. The coincidence of four phenotypes (mutagen sensitivity, elevated chromosomal aberrations, lack of mutagen sensitivity of DNA synthesis and nuclease alteration) in mutants of both organisms suggest that the Drosophila *mus308* gene provides a model for this human disorder. Extension of the enzymological observations to Chinese hamster lines that have been selected for hypersensitivity to DNA crosslinking agents has identified a related function in a third metazoan organism. Further analysis of these mutations is expected to provide an improved understanding of the mechanism employed by higher eukaryotes to resist DNA cross-links.

J 103 PRODUCTS OF *MU* EXCISION FROM THE *BRONZE1* GENE OF *ZEA MAYS*.
Anne Bagg Britt* and Virginia Walbot† *Current address: The Plant Growth Lab, U. C. Davis, Davis, CA 95616 †Dept. of Biological Sciences, Stanford University, Stanford, CA 93405

Somatic excision products of the maize transposable element *Mutator* from the mutable *bz::mu1* allele were selectively amplified from cob tissue by PCR. The sequence of these "footprints" often included deletions of up to 34 bp of target site DNA, suggesting that substantial exonucleolytic degradation occurs upon excision of the element. Single base insertions, as well as *Mu1* sequences, were also found. The isolation of an excision product including a four bp inverted duplication of the target site provides evidence that the double stranded chromosomal break generated by *Mu* excision is, at least in some instances, terminated by a covalently closed hairpin structure. Most excision products, however, do not include such inverted duplications of target site sequences, suggesting that such structures are the result of occasional repair activities, rather than an essential step in the mechanism of *Mu* excision.

J 104 STUDIES OF AN 800-KILOBASE DNA STRETCH OF THE DROSOPHILA X CHROMOSOME: COMAPPING OF A SUBCLASS OF SCAFFOLD-ATTACHED REGIONS WITH SEQUENCES ABLE TO REPLICATE AUTONOMOUSLY IN SACCHAROMYCES CEREVISIAE, Christine Brun, Raymond Miassod, Laboratoire de Génétique et Biologie Cellulaires, CNRS, Case 907, 13288 Marseille cedex 9, France.
We have previously mapped scaffold attached regions (SARs) on an 800 kb DNA walk from the Drosophila X chromosome. We have also previously shown that the strength of binding, i.e. the ability of SARs to bind to all nuclear scaffolds, or only to a fraction of them, varied from one SAR to another one. In the present study, 71 of the 85 subfragments that bind scaffolds, and 38 fragments that do not bind scaffold, were tested for their ability to promote autonomous plasmid replication (ARS activity) in yeast. Sixteen SAR-containing fragments from the chromosome walk were also examined for association to yeast nuclear scaffolds in vitro. All identified ARSs (a total of 27) were present on SAR-containing fragments except 2, which were adjacent to SARs. There is thus a correlation between ARS and SAR activities and this correlation defines a SAR subclass. Moreover, the presence of an ARS on a DNA fragment appeared to be highly correlated with the strength of binding. The binding activity was highly conserved, from Drosophila to yeast. These data suggest that Drosophila DNA sequences responsible for binding to components of the nuclear scaffold from either Drosophila or yeast may be involved in the process of heterologous extrachromosomal replication in yeast.

J 105 EVIDENCE FOR TRANSCRIPTION-DEPENDENT AND INDEPENDENT DNA EXCISION REPAIR PATHWAYS IN HUMAN CELLS, Madeleine Carreau and Darel J. Hunting, Department of Nuclear Medecine and Radiobiology, Université de Sherbrooke , Sherbrooke, Québec, Canada, J1H 5N4
In cultured mammalian cells, cyclobutane dimers induced by ultraviolet radiation are repaired more rapidly in several transcriptionally active genes than in the genome as a whole. Although chromatin accessibility may be involved, this preferential repair appears to be limited to the transcribed strand both in mammalian cells and in E. coli. To determine if transcription is required for repair, we have used alpha-amanitin to inhibit transcription by RNA polymerase II in UV-irradiated normal human fibroblasts, and in xeroderma pigmentosum group C (XP-C) cells. We find that alpha-amanitin has little effect on either repair synthesis or incision in normal cells but almost completely inhibits both processes in XP-C cells. Inhibition of protein synthesis with cycloheximide has no effect on repair in either cell type. Cot analysis demonstrates that repair occurs in all classes of DNA sequence in both normal and XP-C cells. Thus, the functional UV repair pathway in XP-C cells is dependent on transcription, even though repair also occurs in highly repetitive, non-transcribed sequences. Supported by the MRC of Canada.

J 106 EFFECTS OF DNA TOPOLOGY AND CONFORMATION ON EUKARYOTIC TOPOISOMERASE I REACTION, M. Caserta, G. Camilloni, P. Venditti, R. Perini and E. Di Mauro, Centro Acidi Nucleici CNR, Università di Roma "La Sapienza", Italy.
Eukaryotic genome is highly compacted in the nucleus. The entangled organization of the chromosome needs to be cleared up at certain locations and times, for gene expression and replication. As a consequence, the accessible sites become attackable by many different proteins: some of them will recognize the DNA by virtue of specific contacts with certain bases; some others will be attracted by the conformation and topology of certain sequences. We have shown the relevance of DNA torsional strain to regulate the activity of topoisomerase I, by analyzing the in vitro interaction between the enzyme and various single DNA topoisomers. Moreover, we found that intrinsically curved regions are preferred by the enzyme relative to random sequences. The analysis of the specific sites at which topoisomerase I cleaves its substrate reveals the importance of the local conformation of DNA. Curved DNA sequences have been shown to be preferential substrates also for other strand transferases. When a chromosomal domain undergoes replication or transcription, relaxation by DNA topoisomerase I will facilitate those processes and will restrain the action of enzymes involved in recombination.

J 107 INDUCTION AND OVEREXPRESSION OF DAMAGED-DNA BINDING PROTEINS IN CISPLATIN-RESISTANT HELA CELLS, Chuck C.-K. Chao[1], S.-L. Huang[1], L.-F. Shyur[1], L.-Y. Lee[1], P.-W. Cheng[1], M.H.L. Young[1] and Sue Lin-Chao[2], [1]Tumor Biology Laboratory, Departments of Biochemistry and Medicine, Chang Gung Medical College, Taoyuan, Taiwan 33332 and [2]Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Republic of China
To understand the molecular basis for cisplatin resistance (CPR), we examined extracts of cultured cells for factors that interact with cisplatin-modified DNA (CMD). A human HeLa cell line resistant to cisplatin showed a fourfold higher host cell reactivation of bacterial chloramphenicol acetyltransferase gene carried by a transfected plasmid in comparison with nonresistant cells, suggesting a crucial role for DNA repair in CPR. Using a modified Western blotting, we have identified in CPR cells two groups of CMD binding factors [b_{130} (~130 kD) and b_{95} (~95 kD)], both of which showed at least a tenfold amplification following treatment of the cells with cisplatin. A cisplatin-suppressible b_{25} (~25 kD) nuclear factor that interacts with unmodified DNA was absent in CPR cells. At least some of the CMD binding factors appear to be different from the binding factors specific for UV-modified DNA. Taken together, our findings suggest that the CMD binding factors we have identified in CPR cells may play a specific role in the repair of cisplatin-modified DNA and in the development of CPR phenotype.

J 108 MITOCHONDRIAL DNA DELETIONS IN NORMAL ADULT HUMANS DETECTED BY PCR, G.A. Cortopassi and N. Arnheim. Molecular Biology Section, Univ. of Southern California, Los Angeles, CA 90089-1340 USA

The exquisite sensitivity of the polymerase chain reaction (PCR) suggests it could be used for assays of the frequency of in vivo somatic mutation. The inherent problem is to design a PCR assay that amplifies mutant DNA specifically. We have designed such an assay to detect a particular deletion (Δ1) of mitochondrial DNA (mtDNA) even when the non-deleted form is present in great excess. The Δ1 deletion occurs frequently among patients with the rare diseases Kearns-Sayres Syndrome and Progressive External Opthalmoplegia, and is thought to be the result of an mtDNA replication error. The specificity of our assay is based on the preferential amplification of Δ1 over normal, longer mtDNA by successive rounds of rapid temperature cycling. Several experimental tests support the notion that the amplified products are the result of a deletion mutation generated in vivo, rather than in vitro during PCR.

We find that in heart and brain from normal adult individuals, Δ1 is present at a frequency of about one per 1,000 normal mtDNAs. Adult tissues that contain mitotic cells (spleen, skin, and intestine) have much lower levels of Δ1. The frequency of Δ1 in fetal heart and brain is at least 1000-fold lower than in the adult, which suggests the higher adult levels are the result of an accumulation of this particular somatic mutation with age.

J 109 REPAIR OF UV-INDUCED PYRIMIDINE DIMERS IN THE INDIVIDUAL GENES GART, NOTCH AND WHITE FROM *DROSOPHILA MELANOGASTER* CELL LINES.

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The excision repair of UV-induced pyrimidine dimers was investigated in three genes: *Gart*, *Notch* and *white* in a permanent Drosophila cell line *Kc*, derived from wild type *Drosophila melanogaster* embryonic cells. In this cell line *Gart* is an actively transcribed gene, whereas *Notch* and *white* are not expressed. In all three genes UV-induced pyrimidine dimers were removed at the same rate and to the same extent: 15 to 25% after 8 hours, 60% removal in 16 hours upto 80-100% after 24 hours. The same repair kinetics was measured in the genome overall. No preferential repair of the actively transcribed *Gart* gene was found. A similar result was obtained using a different wild type cell line, *SL 2*. In these cells the actively transcribed *Gart* gene and *Notch* gene were not preferentially repaired when compared to the *white* gene and to the genome overall. The repair in the *SL2* cell line appeared somewhat slower: upto 70% after 24 hours.

J 110 THE COORDINATE DEVELOPMENT OF m-AMSA RESISTANCE $(mA)^R$, RADIORESISTANCE AND INCREASED POLY(ADP-RIBOSE) POLYMERASE ACTIVITY IN HAMSTER irs-2 CELLS. Dethlefsen, L., Barley, L., Marston, J., Zhou J-F., and Smith, H. Sect. of Expt. Oncol., Univ. of Utah, S.L.C., UT 84132.

The parental V79 and radiosensitive mutant irs-2 cells were grown for 9+ mo. in increasing concentrations of m-AMSA and each subline developed significant drug resistance with both $(mA)^R$ sublines now grown continuously in $2x10^{-8}$ M m-AMSA. Also the cellular radiation response of the irs-2$(mA)^R$ cells returned from the sensitive irs-2 response to that of the parental V79 cells while the V79$(mA)^R$ was unchanged (i.e. $D_o \simeq$ 1.52, 0.80, 1.53, and 1.51 Gy resp.). Topo II activity (kDNA decatenation) was down-regulated appreciably (a factor $\simeq$ 10 in both V79$(mA)^R$ cells and irs-2$(mA)^R$ cells and Topo I activity (pBR322 relaxation) was only reduced modestly (a factor $\simeq$ 2 in each subline). Moreover, the cellular DNA sensitivity to damage by m-AMSA (double strand breaks as measured by neutral elution with pro K) in the irs-2$(mA)^R$ cells was only $\simeq$ 12% (16 μM for 30 min) of that in the irs-2 cells. In addition, the endogenous poly(ADP-ribose) polymerase activity was about the same in irs-2 and irs-2$(mA)^R$ vs V79 cells but the response to X-irradiation (45 or 60 Gy) was more dynamic in the $(mA)^R$ cells (a factor of $\simeq$ 2.8 vs 2.2 resp.). The $(mA)^R$ cells are cross-resistant to adriamycin, vinblastine, and VM-26, but not camptothecin. Our hypothesis is that these three nuclear enzymes interact in some coordinate manner, currently not understood, to modify the DNA/chromatin higher-order structure, thereby modifying radiosensitivity as well as m-AMSA sensitivity.

Supported in part by CA 22188.

J 111 **MOLECULAR ANALYSIS OF DNA REPAIR IN ASPERGILLUS NIDULANS,**

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One approach to investigate DNA repair systems is the isolation of mutants which are sensitive to DNA damaging agents; these mutants are likely to represent defects in repair pathways. We have isolated a number of mutants of Aspergillus nidulans which are sensitive to alkylating agents, sag, (Swirski et al., Mut.Res. 193: 255-268,1988).

By complementation of a number of these mutants with a wild-type Aspergillus DNA cosmid genebank, we have isolated two sequences that affect the response to damage by the alkylating agent N-methyl-N'nitro-N-nitroso-guanidine (MNNG). These two clones have been shown to be unrelated by restriction analysis and hybridisation studies, and have not undergone rearrangement on cloning. One of the sequences transforms the sagA1 strain back to wild-type phenotype and has been investigated further by restriction analysis and sub-cloning.

J 112 CLONING OF A GENE INVOLVED IN AN ERROR-PRONE DNA REPAIR PATHWAY IN MAMMALIAN CELLS, D.B.Godfrey, S.D.Bouffler and R.T.Johnson, CRC Mammalian Cell DNA Repair Research Group, Department of Zoology, University of Cambridge, Downing Street, Cambridge. CB2 3EJ U.K.

SVM, the SV40 transformed Indian muntjac cell line, is by comparison with a wild type line, hypersensitive to a number of DNA damaging agents including ultraviolet light (UV), methylnitrosourea (MNU) and dimethylsulphate (DMS). The basis for the UV sensitivity is the limited ability to synthesize DNA on a damaged template. The basis for the MNU sensitivity is, in part, the lack of O^6methylguanine alkyltransferase activity.

The sensitivity of SVM to UV and MNU, (but not DMS), has been partially corrected by transfection with sheared genomic mouse DNA. The increased resistance to both UV and MNU is associated with an increased frequency of induced mutation, but not with an increase in alklytransferase activity.

The DNA sequence involved in this error-prone repair pathway has been cloned by a cosmid rescue technique and further molecular characterisation of the gene is in progress.

J 113 RADIORESISTANT MELANOTIC CLOUDMAN S91 MOUSE MELANOMA CELLS ELABORATE A FACTOR THAT MAKES CLONALLY RELATED AMELANOTIC RADIOSENSITIVE CELLS BECOME RADIORESISTANT, Helene Z. Hill, George J. Hill and Zoltan Trizna, Departments of Radiology and Surgery, New Jersey Medical School, Newark, NJ 07103

The radiation resistance to gamma rays of clonally related Cloudman S91 mouse melanoma cells increases with increasing melanin content. Survival of the most radiosensitive cell line, S91/amel, depends on the number of cells plated. When large numbers of heavily irradiated cells (HRCells) were preplated and incubated overnight, the D_o of viable cells plated and irradiated 3 hours later was greater by a factor of 1.5 than that of viable cells similarly irradiated but in medium devoid of HRCells. At a dose of 3 Gray, increasing numbers of Amel-HRCells increased the surviving fraction of the target viable cells up to a maximum of 1.6 fold, while I3-HRCells improved survival up to a maximum of 7 fold. Medium that had been conditioned by I3-HRCells similarly improved the survival of S91/amel cells by a factor of approximately 3 - less than the increase produced by 15 fold fewer I3-HRCells present in the medium throughout their incubation. Medium conditioned by similar numbers of Amel-HRCells had little effect on survival. The presence of HRCells and conditioned medium does not significantly affect the plating efficiency of the S91/amel cells at 0 dose. I3-HRCells have little effect on the survival of viable I3 cells or of EMT6 mouse mammary carcinoma cells. The results indicate that the most radioresistant of these clonally related melanoma cell lines elaborates a diffusible factor that can alter the radiation response of the most radiosensitive of these lines. The relationship appears to be indirectly related to melanization.

J 114 A GENE PRODUCT FROM YEAST ASSOCIATED WITH THE REPAIR OF DAMAGED DNA ENCODES A PROTEIN KINASE, Merl F. Hoekstra, Anthony DeMaggio, and Namrita Dhillon, Molecular Biology and Virology Lab, The Salk Institute, P.O. Box 85800, San Diego CA 92186-5800.

A wide variety of functions have been identified for repairing genotoxic damage. Such functions include the enzymatic and nucleolytic components involved in DNA repair while other functions are regulatory and presumably control the process. In smaller eukaryotes like *S. cerevisiae*, the *RAD* series of mutants are required for repairing UV and X-ray induced lesions and several *CDC* genes have also been implicated in DNA repair. We have used a genetic screen to identify genes in *S. cerevisiae* involved in repairing a site-specific double-strand break. One of these mutants, *hrr25-1*, is described in this report and shows a pleiomorphic phenotype. *Hrr25-1* strains are sensitive to agents that cause DNA double-strand breaks, resistant to UV, proficient for mitotic recombination, and unable to proceed through meiosis. Deletion of the *HRR25* coding region result in additional phenotypes including cell cycle defects and aberrant cellular morphology. Surprisingly, the *HRR25* gene encodes a novel protein with two functional regions. The N-terminus of the protein encodes the kinase homology unit of approximately 250 residues. The C-terminus contains a repeated motif of 50 prolines and glutamines through the last 100 amino acids. The C-terminal domain appears to confer substrate recognition and/or intracellular localization. Potential activity of the Hrr25 kinase and its species distribution is being characterized.

J 115 THE SPECTRUM OF SPONTANEOUS MUTATIONS IN A HPRT cDNA IS DIFFERENT AT DIFFERENT POSITIONS IN THE HUMAN GENOME, Elgin Lichtenauer-Kaligis, Hans den Dulk, Judith Tasseron-de Jong and Micheline Giphart-Gassler, Department of Molecular Genetics and Department of Radiation Genetics, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

To address the question whether the instability of the genome varies among different positions we have introduced a retroviral vector containing a HPRT cDNA and a neo marker gene into the DNA of TK6 lymphoblastoid human cells. Five cell lines each containing one HPRT cDNA at a different position have been studied for the loss of HPRT activity. By selecting for HPRT$^-$ mutants in the presence of G418 we were able to isolate only the mutant class with a mutation within the HPRT gene. The rate at which such mutations arise varies from 0.4 to 5.10^{-6} per cell per generation indicating an up to 10 fold difference in spontaneous mutation within the gene for the various viral integration sites. The change in DNA sequence from 30 mutants of each different cell line has been determined using PCR and direct DNA sequence analysis of the PCR products. The overall data show that small deletions are the most frequent mutations followed by base-pair substitutions and small duplications. Among the base-pair substitutions transitions are more frequent than transversions. For the individual cell lines, however, the distribution of the mutant classes is different. For instance two cell lines show more base-pair substitutions than other mutations whereas mutants of another cell line are predominantly caused by small deletions. These cell lines therefore seem suitable to study the molecular basis of differences in genome instability.

J 116 DNA DOUBLE-STRAND BREAK MEASUREMENT BY FILTER ELUTION: CALIBRATION OF THE EFFECTS OF PORE DENSITY AND PORE DIAMETER ON ELUTION RATE. Christopher S. Lange, Peter J. Mayer, and Matthews O. Bradley*, Department of Radiation Oncology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203 and *Merck Institute for Therapeutic Research, West Point, PA 19486

The biophysical mechanism of neutral filter elution is unknown. Two mechanisms have been suggested: sieving, pore diameter dependent, and tug-of-war, pore density dependent. We examined the effects of these parameters on elution behavior (pH 9.6) of genomes of known size and shape: coliphage T4c (M_r=1.15 x 10^8), E. coli (M_r=2.7 x 10^9), and Chinese hamster lung fibroblasts (V79, M_r=2-4 x 10^{10}). DNA eluted through 15% sucrose atop the filter in a biphasic pattern. Elution rate (ER) of the initial component correlated (r>0.97) exponentially with $1/M_r$ for monodisperse samples of DNA eluted through pore sizes 0.1-3.0 um. Using this relationship between ER and M_r, we estimated M_n of polydisperse, (253 Gy) X-irradiated samples of DNA from E. coli or V79 cells to be 3.15 $\pm$ 1.46 and 1.42 $\pm$ 0.33, respectively, compared to expected values of 2.93 and 3.52 (all x 10^8 Da). Calibrations at 2.0 and 3.0 um pore diameters (same pore density) in units of (10^8 Da)/(log percent DNA eluted/min) were: 1.02 $\pm$ 0.11 for T4c DNA, 2.62 $\pm$ 0.24 for X-irradiated E. coli DNA fragments, and 3.09 $\pm$ 0.47 for X-irradiated V79 cell DNA fragments. These significantly different calibrations do not support the tug-of-war model. For pore sizes 0.1-3.0 um, pore density best predicts ER for DNA from T4c, intact and X-irradiated V79 cells, whereas pore diameter best predicts ER for DNA from X-irradiated E. coli. Funded by Mathers & NCI RO1CA39045

J 117 CONSTRUCTION OF AN EPSTEIN-BARR VIRUS-BASED EXPRESSION VECTOR FOR THE ISOLATION OF SELECTABLE MARKERS IN HUMAN CELLS, Randy J. Legerski and Carolyn Peterson, Department of Molecular Genetics, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.

Progress in cloning DNA repair genes by transformation of human cells has been extremely slow despite intensive efforts concentrated on repair deficient diseases such as xeroderma pigmentosum, ataxia telangiectasia, cockayne's syndrome, fanconi's anemia, and Bloom's syndrome. The principal reasons for the slow progress is the typical low transformation frequency of human cells and difficulties encountered in rescuing integrated sequences after a transformant is obtained. We have constructed an episomal vector designated pEBS7 that transforms human cells at high efficiency, rearranges at low frequency, and is easily rescued from stably transformed cells. Large scale cDNA libraries are conveniently prepared in this vector and reconstruction experiments indicate the efficacy of this approach for the isolation of selectable markers.

J 118 SELECTIVE METAL BINDING TO CYS-78 WITHIN ENDONUCLEASE V CAUSES AN INHIBITION OF CATALYTIC ACTIVITIES WITHOUT ALTERING NONTARGET AND TARGET DNA BINDING, R. Stephen Lloyd, Melissa A. Prince, Benjamin Friedman, Elliott A. Gruskin, and Robert D. Schrock, III, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

T4 endonuclease V is a pyrimidine dimer-specific DNA repair enzyme which has been previously shown not to require metal ions for either of its two catalytic activities or its DNA binding function by virtue of its ability to function in the presence of metal chelating agents. However, we have investigated whether the single cysteine within the enzyme was able to bind metal salts and influence the various activities of this repair enzyme. A series of metals (Hg^{++}, Ag^{+}, Cu^{+}) were shown to inhibit both endonuclease V's pyrimidine dimer-specific DNA glycosylase activity and the subsequent apurinic nicking activity. The binding of metal to endonuclease V did not interfere with nontarget DNA scanning or pyrimidine dimer-specific binding. The Cys-78 codon within the endonuclease V gene was changed by oligonucleotide site-directed mutagenesis to Thr-78 and Ser-78. The mutant enzymes were able to confer enhanced ultraviolet light (UV) resistance to DNA repair-deficient *Escherichia coli* at levels equal to that conferred by the wild type enzyme. The C78T mutant enzyme was shown to be catalytically active on pyrimidine dimer-containing DNA. The catalytic activities of the C78T mutant enzyme were demonstrated to be unaffected by the addition of Hg^{++} or Ag^{+}. These data suggest that the cysteine is not required for enzyme activity but that the binding of certain metals to that amino acid block DNA incision by either preventing a conformational change in the enzyme after it has bound to a pyrimidine dimer or sterically interferring with the active site residue's accessibility to the pyrimidine dimer.

J 119 PARTIAL CORRECTION OF THE ATAXIA DEFECT, H.D. Lohrer, Gray Laboratory, Mount Vernon Hospital, Northwood, Middlesex HA6 2JR, U.K.

In an attempt to clone the "Ataxia gene" the cell line AT5BI-VA was transfected with the plasmid pSV2neo and the isolated subclone ATs4neo1 was fused with gamma-irradiated cells of the X-ray sensitive CHO cell line xrs-2. After selection in G418 containing medium the colonies were subjected to a X-ray selection procedure which only the clone atxbc survived.

The survival of the clone atxbc after treatment with bleomycin or exposure to X-ray, proved to be nearly as high as for normal CHO or human cell lines. The relative DNA synthesis after treatment with bleomycin or X-ray was intermediate when compared with the parental AT5BI-VA, xrs-2, CHO wildtype and normal human cell lines. Southern blot analysis of atxbc DNA revealed only very little CHO specific hybridisation with no specific banding. Experiments to amplify CHO-repetitive elements by Taq DNA polymerase reaction are under way.

The isolated clone atxbc is intermediate in its survival after X-ray and bleomycin, and its relative DNA synthesis is sensitive to both DNA damaging agents. Unlike other phenotypic AT-revertants, atxbc does not show a split of the phenotype of X-ray sensitivity and X-ray resistant DNA synthesis. This clone therefore should be a good source for cloning the complementing CHO sequences.

J 120 TWO NCKING ENZYME SYSTEMS SPECIFIC FOR MISMATCH-CONTAINING DNA IN NUCLEAR EXTRACTS OF HUMAN CELL LINES, A-Lien Lu, Yang-Chuen Yeh and Dau-Yin Chang, Department of Biological Chemistry and Program of Molecular and Cell Biology, University of Maryland at Baltimore, Baltimore, MD 21201

Besides the T/G mismatch-specific nicking enzyme system (Wiebauer, K., and Jiricny, J., Nature, 339, 234-236) we have identified two novel enzyme systems in human HeLa nuclear extracts that can nick at specific sites of DNA molecules with base mismatches. One enzyme (called all-type) can nick all eight base mismatches with different efficiencies. The other (A/G-specific) nicks only DNA containing an A/G mismatch but not other mismatches. The all-type enzyme can be separated from the T/G-specific and A/G-specific nicking enzymes by Bio-Rex 70 chromatography. Further purification by a DEAE-5PW column, the A/G-specific nicking enzyme was eluated at different position from that of the T/G-specific nicking enzyme. Therefore, at least three different enzyme systems are able to cleave mismatched DNA in HeLa nuclear extracts. The two novel enzymes work at different optimal salt concentration and cleave at different sites within the mismatched DNA. The all-type enzyme can only cleave at the first phosphodiester bond 5' to the mispaired bases. This enzyme shows nick disparity to only one DNA strand and may be involved in genetic recombination. The A/G-specific enzyme simultaneously makes incisions at the first phosphodiester bond both 5' and 3' to the mispaired adenine but not the guanine base. This enzyme may be involved in an A/G mismatch-specific repair similar to the E. coli mutY (or micA)-dependent pathway.

J 121 STRUCTURAL AND FUNCTIONAL ANALYSIS OF cDNA FOR THE HUMAN ERCC5 NUCLEOTIDE EXCISION REPAIR GENE, Mark A. MacInnes and John S. Mudgett Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545

Nucleotide excision repair proteins recognize and excise a wide variety of DNA lesions in all eucaryotic cells. We have previously isolated functional clones of the human excision repair gene *ERCC5*, via cross-species complementation of the hamster UVC sensitive mutant, UV-135. Four different DNA probes obtained from the central and putative 5'-end regions of the gene were found to hybridize to a mRNA size class of about 4,600 nt. A pCD2 human cDNA library (from H. Okayama) was screened extensively (3 x 10^6 colonies) for clones hybridizing with one or more probes. About forty positive clones were isolated which possessed inserts of sizes from 2.3 to 4.4 kbp. None of the clones so far tested are functional for complementation of UV-135. They appear incomplete both in size, and lack of hybridization to the putative extreme 5'end ERCC5 probe. However, cDNAs complemented UV sensitivity when cotransfered into UV-135 cells with a single cosmid containing only the 5' exons and promoter region. Functional minigenes were probably reconstructed via intracellular homologous recombination. Sequence organization and structure of the functional cDNAs and minigene product will be presented. (Supported by DOE program F331/B04717, and a DoE/OHER, Alexander Hollaender Distinguished Fellowship, to JSM).

J 122 GEL MOBILITY-SHIFT ASSAY FOR BENZO(A)PYRENE DIOL EPOXIDE-MODIFIED DNA, Michael C. MacLeod, Bruna P. Brylawski, Marila Cordeiro-Stone and David G. Kaufman, Department of Carcinogenesis, University of Texas M.D. Anderson Cancer Center, Smithville, TX 78957 and Pathology Department, University of North Carolina, Chapel Hill, NC 27599-7525.

The binding of carcinogens to DNA in intact cells is modulated not only by the primary sequence of the DNA, but also by its environment and functional state, and high resolution methods are needed to study the distribution of carcinogen-DNA adducts among DNA sequences. Towards this end, a rabbit polyclonal antibody raised against DNA adducted with (±) 7r,8t-dihydroxy-9t,10t-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE-I) was tested for its ability to alter the electrophoretic mobility of adducted DNA through agarose. The migration of BPDE-I-adducted DNA but not unmodified DNA was retarded by the IgG fraction of the antiserum, and adduct densities as low as 0.6 adducts per 1000 nucleotides were effective in causing complete retardation of 3.2 kbp fragments. This method may be useful in analyzing preferential binding and/or repair in specific genes which are, for example, associated with the nuclear matrix, transcriptionally active, or in the process of replicating. Supported by USPHS/NIH grants P01 CA42765 and R01 ES 03602.

J 123 HYDROPHOBIC AND ELECTROSTATIC CONTRIBUTIONS TO THE STABILITY OF UVRA-DNA AND UVRAB-DNA COMPLEXES, Sharlyn J. Mazur, Department of Chemistry, The American University, 4400 Massachusetts Ave. NW, Washington DC 20016

The UvrABC endonuclease of *Escherichia coli* initiates the repair of a wide variety of aberrant structures in DNA including photoproducts formed by ultraviolet light and adducts formed from polycyclic aromatic hydrocarbons, mitomycin C, and aflatoxins. The lack on a common structure among the damage repaired suggests that the enzyme recognizes a distortion of the DNA helix rather than a specific structure. The mechanism of damage recognition is not well understood and is under investigation by several laboratories. Recently proposed models of damage recognition by the Uvr system have been constructed from experiments performed at different temperatures and different salt concentrations, without any evaluation of the effect of these changes on the mechanism. The systematic investigation of the effect of variation of temperature and salt concentration on the stability of DNA-protein complexes presented here provides a new perspective on previously proposed models. Specific binding of UvrA to damaged DNA requires dimerization. This process is promoted by physiological temperatures. The stability of complexes formed between UvrA and DNA damaged by ultraviolet light is strongly affected by the concentration of salt, typical of most DNA-protein complexes. Salt-jump experiments show that the UvrB-uvDNA interaction is not very sensitive to the salt concentration.

J 124 CLONING AND CHARACTERIZATION OF THE HUMAN URACIL-DNA GLYCOSYLASE GENE, Susan Muller, Diane Worrad, and Sal Caradonna, Dept. of Molecular Biology, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine, Stratford, NJ 08084.
Cancer appears to result from the accumulation of mutations in genes which control cellular growth. Therefore, we rely on the efficient and accurate repair of damaged DNA to protect our cells from transformation. Our research has focused on one aspect of human excision repair; the removal of uracil from DNA by the enzyme uracil-DNA glycosylase. We have generated antibodies against purified human uracil-DNA glycosylase and used them to isolate the corresponding cDNA. The 1.25 kb uracil-DNA glycosylase cDNA encodes a functional protein with a predicted molecular weight of 35,351. Analysis of human-hamster somatic cell hybrids revealed that the human uracil-DNA glycosylase gene if located on chromosome 5. Preliminary studies indicate that uracil-DNA glycosylase activity is stimulated under conditions of serum starvation. To investigate the mechanisms by which uracil-DNA glycosylase is regulated, the 1.25 kb cDNA was used to isolate a 12.5 kb genomic clone. The clone was then mapped and uracil-DNA glycosylase coding sequence was found to be contained within 2.8 and 1.2 kb SST-1 fragments. Sequence analysis revealed a putative AP-2 binding site within the promoter region. The effects of phorbol esters and cAMP on uracil-DNA glycosylase expression are currently being investigated.

J 125 SUBSTITUTION OF BASIC AMINO ACIDS WITHIN ENDONUCLEASE V ENHANCES NONTARGET DNA BINDING, Courtney Nickell, Wayne F. Anderson and R. Stephen Lloyd, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232
Several DNA-interactive proteins, including the DNA repair enzyme T4 endonuclease V, have been shown to locate their target recognition sites utilizing an electrostatically mediated facilitated diffusion mechanism. Previous work indicates that a decrease in the affinity of endonuclease V for nontarget DNA results in an increased nontarget dissociation rate. This study was designed to investigate the effect of an increase in the affinity of endonuclease V for nontarget DNA. Using a working structural model of the enzyme as a guide, the electrostatic character of endonuclease V was altered. Substitution of Thr-7 with Lys-7 resulted in an enzyme with wild type *in vitro* characteristics. Mutations which increased the positive charge along a proposed solvent-exposed α-helical face had significant effects. The mutants Ala-30, Val-31 → Lys-30, Leu-31 and Asn-37 → Lys-37 displayed wild-type *in vitro* apurinic-specific and dimer-specific nicking activities. Although the processive dimer-specific nicking rate of the Lys-37 mutant resembled that of wild type, the rate of the Lys-30, Leu-31 mutant was reduced by 60%. In addition, the salt concentration range over which these mutants processively nick dimer-containing DNA has been greatly expanded. Both mutants are shown to have an increased affinity for nontarget DNA.

J 126 ENHANCED TRANSLATION OF DNA DOUBLE STRAND BREAKS INTO CHROMOSOME ABERRATIONS IN ATAXIA-TELANGIECTASIA , Tej K. Pandita and Walter N. Hittelman, The University of Texas M.D.Anderson Cancer Center, Houston, Texas 77030 USA
Cells derived from individuals with ataxia-telangiectasia (AT) exhibit increased sensitivity to ionizing radiation as well as to certain drugs e.g., bleomycin, neocarzinostatin and etopside, as evidenced by decreased survival and increased chromosome aberrations when compared to normal cell lines. To better understand the basis of this sensitivity, three AT and three normal lymphoblastoid cell lines were enriched at different phases of the cell cycle by centrifugal elutriation and examined for their survival, relative initial levels of DNA damage (double strand breaks by neutral filter DNA elution) and chromosome damage (premature chromosome condensation). Ataxia cells were shown to exhibit decreased levels of survival throughtout the cell cycle, however early G1 was shown to be especially sensitive compared to normal cell lines. While AT and normal cells exhibited slight differences in the amount of initial DNA double strand breaks (DSBs) throughout the cell cycle, the AT cells showed a nearly two-fold increase in the initial levels of chromosome damage in G1 and G2 phase cells. One out of three normal cell lines (3498 P) was found to be relatively radiosensitive and it exhibited higher levels of chromosome damage in G1 and G2 phases as compared to other two normal cell lines. These results indicate that AT cells and radiosensitive cell lines are more efficient in translating DNA DSBs into chromosome breaks even before repair has taken place. This suggests that the mode of organization of DNA into chromatin may have an influence on the translation of DNA DSBs into chromosome damage and that one component of cellular sensitivity may include chromatin organization.

J 127 COMPUTERIZED IMAGING OF CARCINOGEN-DNA ADDUCTS ON HUMAN METAPHASE CHROMOSOMES, Natalie B. Parker[1], Loris McGavran[1], Miriam C. Poirier[2], Ofelia A. Olivero[2], Zeljko Cericevic[3], Louis C. Smith[1], and David K. Manchester[1], [1]The Children's Hospital Kempe Research Center, University of Colorado
School of Medicine, Denver, CO 80218, [2]The Laboratory of Cellular Carcinogenesis and Tumor Promotion, NCI, Bethesda, MD 20892, and [3]Department of Experimental Medicine, Cell Biology and Biochemistry, Baylor College of Medicine, Houston, TX 77030

Formation of carcinogen-DNA adducts is assumed to be a critical step in the mutagenic process. Previous investigations in Chinese hamster ovary cells indicate that adduct formation is not random when analyzed at the level of the chromosome (Olivero et al. Molec. Carcinogenesis 3: 37-43, 1990). We are studying the localization of chrysene-DNA adducts on human metaphase lymphocyte chromosomes using immunocytochemical techniques and computerized image analysis. Mitogen-stimulated methotrexate-arrested human peripheral blood lymphocytes were briefly exposed to chrysene in the presence of a human placental microsomal activation system. When released and allowed to divide, exposed cells demonstrated mitoses with increased antibody binding to chromosomes compared to unexposed controls. Adducts appear as staining aggregates on the surface of the chromosome after immunostaining with a polyclonal antibody that recognizes a variety of polycyclic aromatic hydrocarbon-DNA adducts followed by a secondary biotinylated antibody and a peroxidase-diaminobenzidine detection system. Digitalized computer images of chrysene-adducted chromosomes have been produced that permit quantitation by densitometry and will allow factor analysis of patterns of adduct formation and their relationship to chromosome banding patterns. These methods provide a useful model for the study of the formation of carcinogen-DNA adducts and their possible role in mutagenesis and chromosome damage.

J 128 **DIRECT CORRELATION BETWEEN METHYLATION STATUS AND EXPRESSION OF THE HUMAN O-6-METHYLGUANINE DNA METHYLTRANSFERASE (MGMT) GENE.** Russell O. Pieper, Roger A. Kroes, Bernard W. Futscher, and Leonard C. Erickson. Section of Hematology/Oncology, Loyola University Medical Center, Maywood, IL 60153.
MGMT is a DNA repair enzyme which stoichiometrically and irreversibly transfers mutageneic and potentially cytotoxic O-6 methyl/alkylguanine lesions from DNA to its active site. To assess the role of DNA cytosine methylation in the regulation of MGMT expression, the methylation status of the MGMT gene was determined in 13 human cell lines characterized by a wide range of MGMT expression. Southern blot methylation analysis using an MGMT cDNA probe and the methylation sensitive restriction enzymes Msp I, Hpa II, and Hha I revealed a direct, rather than inverse, correlation between the methylation status of the regions of the gene recognized by the probe and MGMT expression. Chronic exposure of cells expressing high levels of MGMT to the DNA hypomethylating agent 5-azacytidine decreased MGMT gene methylation, and reduced both MGMT activity and steady state MGMT mRNA levels by 60% relative to controls. These results suggest that expression of the MGMT gene is associated with DNA cytosine methylation, but in a unique and paradoxically direct fashion.

J 129 SPONTANEOUS OR MUTAGEN-MEDIATED EXPRESSION OF A PROMOTERLESS *NEO* GENE INTEGRATED AT DIFFERENT GENOMIC SITES OF RAT2 FIBROBLASTS, Ileana Quinto, Giuseppe Scala, Massimo Mallardo, Maria R. Ruocco and Francesco De Lorenzo, Department of Biochemistry and Biotechnology, 2nd Medical School of Naples, University of Naples, Naples, Italy.
The aim of the present study was to develop a cell system to analyse the frequencies and the type of gene rearrangements occurring either spontaneously or following damage to DNA. For this purpose a promoterless *neo* gene was randomly integrated in Rat 2 fibroblasts to act as a reporter gene of rearrangements resulting in its expression. Nine G418-sensitive clones differing in number of copies or in integration sites of *neo* were examined. These clones were all able to generate G418-resistant subclones at frequencies ranging from $1x10^{-8}$ to $6x\ 10^{-5}$. Only one clone produced a dose-related increase in G418-resistant subclones when treated with the mutagens mitomycin C or methylmethanesulfonate. Amplification and enhanced transcription of the *neo* gene were observed in both the spontaneous and mutagen-induced G418-resistant subclones. These results indicate that DNA structures, which are able to amplify spontaneously, show different susceptibility to mutagen-mediated amplification. This is relevant in view of the major role played by mutagen-mediated amplification in carcinogenesis.
This study was supported by grants from A.I.R.C. (Milan), C.N.R. Target Project on Biotechnology and Bioinstrumentation (C.N.R., Rome), and Third Project on AIDS (I.S.S., Rome).

J 130 DIMETHYLSULPHATE INDUCED DOUBLE STRAND DNA BREAKS: DEFECTIVE REPAIR AND ITS CORRECTION BY GENE CLONING IN A MUTANT MUNTJAC CELL LINE

Anderson J. Ryan, Simon Bouffler, Amanda Evans and Robert T. Johnson, C.R.C. Mammalian DNA Repair Group, Dept. of Zoology, University of Cambridge, U.K.

We have previously provided evidence for a decreased rate of double strand DNA break rejoining after high doses of dimethylsulphate in an Indian muntjac cell line sensitive to alkylating agents. We have extended these earlier studies by using alternating field inversion gel electrophoresis (AFIGE) to quantitate more exactly the extent of dimethylsulphate, methylnitrosourea and X-ray induced DNA double strand breaks. Using alkylating agent resistant (DM) and sensitive (SVM) muntjac cells, we find that dimethylsulphate (but not methylnitrosourea) is a very potent inducer of DNA double strand breaks in both lines, even at sublethal doses. Moreover, the sensitivity of SVM to dimethylsulphate seems to be due to a problem in the repair of the particular type of double strand break this agent produces, since no clear differences in cytotoxicity are observed between SM and DM when X-ray induced DNA double strand breaks are produced. In order to understand the mechanisms/genes responsible for this sensitive phenotype, we have transfected SVM cells with human DNA and selected transfectants with increased resistance to dimethylsulphate. The levels of dimethylsulphate induced single-strand and double strand DNA breaks, together with their subsequent repair, are related to cytotoxicity and chromosome aberrations in DM, SVM and transfectant cell lines.

J 131 REDUCTIVE METHYLATION OF THE N-TERMINUS OF ENDONUCLEASE V ERADICATES CATALYTIC ACTIVITIES—EVIDENCE FOR AN ESSENTIAL ROLE OF THE N-TERMINUS IN THE CHEMICAL MECHANISMS OF CATALYSIS, Robert D. Schrock, III and R. Stephen Lloyd, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

The pyrimidine dimer-specific DNA repair enzyme, endonuclease V, was chemically modified by reductive methylation, a technique which specifically methylates primary amino groups. Upon reaction of endonuclease V with [^{14}C]-formaldehyde in the presence of the reducing agent $NaCNBH_4$, it was discovered that < 1 methylation per endonuclease V molecule was required to reduce both the glycosylase and the phosphodiesterase activities by 80%. Pyrimidine dimer-specific binding was not eradicated at a level of methylation equivalent to 0.8 CH_3/endonuclease V molecules, but was eradicated at higher levels of methylation. Endonuclease V which had been modified with an average of 0.8 CH_3/molecule was digested with *Staphylococcus aureus* strain V-8 protease and the peptides subsequently separated by HPLC. Radiolabel was exclusively found on peptides including the N-terminus, as determined by percent amino acid composition. Neither intact 0.8 CH_3-endonuclease V nor radiolabeled peptides were able to be sequenced by Edman degradation, indicating blockage of the amino terminus by methylation. This study shows strong evidence for the unprecedented involvement of the amino terminus in the chemical mechanisms of endonuclease V.

J 132 MUTAGENIC POTENTIAL OF 8-OXOdG. Shibutani, S., Moriya, M., Takeshita, M. and Grollman, A.P., Department of Pharmacology, State University of New York at Stony Brook, Stony Brook, NY 11794

Oxidative damage to DNA may be manifested by the presence of 8-oxo-7-hydroxydeoxyguanosine (8-oxodG); we examined the miscoding properties of this lesion *in vitro* and its mutagenic properties in *E. coli*. Kinetics of base insertion and extension were measured during translesional synthesis on oligodeoxynucleotide templates containing 8-oxodG. In contrast to an early report (Kuchino *et al., Nature* **327**, 77, 1987), only dCMP and dAMP are incorporated opposite 8-oxodG. Transient inhibition of chain extension occurred 3' to the modified base. DNA polymerases α and δ preferentially insert dAMP opposite the modified base while polymerases β and pol I preferentially insert dCMP. DNA polymerases with proofreading functions fail to remove dA and dC from the 3' terminus when paired with 8-oxodG. Efficient chain extension from the dA:8-oxodG pair was observed with all polymerases tested. G→T transversions predicted by these *in vitro* experiments was confirmed using a gapped duplex plasmid containing a single 8-oxodG residue located in the single-strand region of the gap. The mutational frequency was 0.5%. These experiments suggest that relatively mild oxidative damage to DNA leads to mutagenic changes which ultimately may result in genomic instability.

J 133 ALKYLATION-INDUCED DNA DAMAGE IN PROLIFERATING CELLS IN RELATIONSHIP TO STUCTURAL CHANGE CAUSED BY INHIBITORS OF REPLICATION-ASSOCIATED ENZYMES, Bernard W. Stewart and Daniel R. Catchpoole, Children's Leukaemia and Cancer Research Unit, Prince of Wales Children's Hospital, Sydney, NSW 2031, Australia.
Measurement by benzoylated-DEAE cellulose chromatography of single stranded (ss) regions in preparations of deproteinized sheared DNA from proliferating human lymphoblastoid cells (CCRF CEM) indicates two types of structural change associated with DNA replication: stretches of unpaired nucleotides extending for 200 residues ('short') and also for 1-4 kilobases ('long') (Stewart B.W. *et al*, Exp. Cell Res., in press). This complex patten of structural change is perturbed by exposure of cells to methyl methanesulfonate (maximum concentration, 5mM). Immediately after such treatment, increase in the amount of DNA exhibiting ss character is attributable to a greater population of DNA having 'short' ss regions; more complex structural change is evident over the next 1-2 hours. Comparison has been made of this effect with that following exposure of cells to inhibitors of polymerases, topoisomerases, ligase and other activities associated with DNA replication. Change in the amount of both 'short' and 'long' ss regions was apparent, suggesting involvement of particular enzyme activities with certain lesions and hence their association with aspects of post-replication repair.

J 134 DIFFERENT EXPANDED CHROMOSOME REGIONS IN A SINGLE CELL LINEAGE SELECTED FOR AMPLIFICATION AND DEAMPLIFICATION OF THE UMP SYNTHASE GENE, D. Parker Suttle[1,2] , Sarita Goorha[1] , and Mazin B. Qumsiyeh[3]. [1]Veterans Administration Medical Center; [2]Dept. of Pharmacology, St. Jude Children's Research Hospital; [3]Division of Genetics, University of Tennessee, Memphis, TN.
The UMP synthase gene is stably amplified within expanded chromosome regions (ECRs) in Chinese hamster lung cells selected for resistance to pyrazofurin and 6-azauridine, inhibitors of the bifunctional enzyme UMP synthase. Because the metabolism of 5-fluorouracil (FU) to its cytotoxic form is initiated by UMP synthase, growth of cells containing UMP synthase amplification in FU enables selection of cell that have undergone deamplification, rapid loss of the amplified UMP synthase gene copies. Acquisition and deletion of ECRs correlated with the selection for amplification or deamplification of the UMP synthase gene. Detailed cytogenetic analyses and fluorescent *in situ* hybridization on three consecutive cycles of amplification in a single cell lineage showed unique structure and/or position for the ECR. In the first cycle, the ECR formed a homogeneously staining region on a small marker chromosome M3. In the second amplification step, a chromosomal break occurred at the site of the endogenous UMP synthase gene on chromosome M2, however amplification occurred on a altered M3 chromosome as an abnormally banded region. The third cycle of amplification resulted in an variable HSR located on the derivative chromosome M2. The variation in position, size and structure of the consecutive ECRs can be explained by differences in coamplified DNA sequences or by complex chromosome rearrangements. The ability to cycle cells of a single lineage through states of amplification and deamplification will facilitate study of the gene amplification process and the factors that effect the composition and stability of amplified regions. Supported by VA Merit Review Award and in part by CA-21765 from NCI and by ALSAC.

J 135 INHIBITORY EFFECTS OF TRANSCRIPTION ON INTRAMOLECULAR HOMOLOGOUS RECOMBINATION. Takeshita, M., Chang, C.-N., Peden, K., and Grollman, A.P., Department of Pharmacology, State University of New York at Stony Brook, Stony Brook, NY 11794
Transcription plays a significant role in processes which affect genomic stability. The effects of gene expression on intramolecular homologous recombination were studied in plasmids in which a transcriptionally-active gene was placed in the vicinity of tandem copies of the kanamycin/neomycin (*neo*) resistance gene. Two copies of the *neo* gene, derived from pSV2*neo* and arranged in tandem, were inactivated by introducing non-overlapping internal deletions. Conversion to kanamycin (Kan) resistance, determined in $RecA^+$ and $RecA^-$ strains, was found to involve a double reciprocal crossover between the *neo* genes. In $RecA^-$ cells, the 5' copy was converted to the functional *neo* gene in the majority of cases, whereas in $RecA^+$ cells, the 3' *neo* gene was corrected. When the tetracycline resistance gene (*tet*) was placed 3', 5' or between the *neo* genes, the recombinants were found with 2-30 fold less frequency. This inhibitory effect occurred in both $RecA^+$ and $RecA^-$ strains and was dependent on the direction of transcription of the *tet* gene. Mutation of the promoter eliminated the inhibition. The mechanism involved in this phenomenon may be significant for understanding the homologous recombination in eukaryotic as well as prokaryotic cells.

J 136 THE FUNCTIONAL ROLES OF THE ATPase SITES OF *ESCHERICHIA COLI* UvrAB IN NUCLEOTIDE EXCISION REPAIR. Sambasivamoorthy Thiagalingam and Lawrence Grossman, Department of Biochemistry, The Johns Hopkins University, 615 North Wolfe Street, Baltimore, MD21205

Site directed mutagenesis and biochemical characterization of the representative mutant proteins of *Escherichia coli* UvrA suggest that it has two functional ATPase sites which allows in distinguishing the roles of ATP hydrolysis and binding in repair reactions. The individual ATPase sites can independently hydrolyse ATP. Both the single and double mutants of UvrA, surprisingly, bind strongly to DNA. The ability to form a UvrAB nucleoprotein complex is, likewise, unaffected by mutations in the ATPase sites of UvrA. UvrA, with bound ATP, forms the most favored conformation for DNA binding. In contrast to the wild type UvrA, the ATPase site mutants lack the ability to exhibit enhanced binding to damaged DNA. Dissociation of tightly bound nucleoprotein complexes from the undamaged sites requires hydrolysis of ATP by the C-terminal ATPase site of UvrA. UvrAB helicase activity requires ATP hydrolysis by both UvrA and UvrB. Evidence is presented that ATP binding as well as hydrolysis are required for the damage recognition step enabling UvrAB to discriminate between damaged and undamaged sites on DNA.

J 137 FORMATION OF 7-(2-HYDROXYETHYL)GUANINE IN VARIOUS TISSUES FOLLOWING MULTIPLE EXPOSURES OF MICE TO ETHYLENE OXIDE BY INHALATION, Vernon E. Walker, Patricia B. Upton*, Thomas R. Skopek*, and James A. Swenberg*, Chemical Industry Institute of Toxicology, Research Triangle Park, NC 27709 and *Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC 27599.

Ethylene oxide (EO), a widely used chemical with potential for human exposure, is thought to initiate its carcinogenic effects in rodents by electrophilic attack at critical sites in DNA. The relationship between chronic exposure to EO and the formation of 7-(2-hydroxyethyl)guanine (7-HEG) in DNA of target (lung and spleen) and nontarget tissues (brain, kidney, liver and testis) was investigated in B6C3F1 male mice exposed by inhalation to 100 ppm EO for 6 hr/day for 1, 3, or 5 days, or 2 or 4 weeks (5 days/wk). DNA samples from control and treated mice were analyzed for 7-HEG using selective enrichment and separation by RP-SCX HPLC with fluorescence detection. In exposed mice, the adduct was detectable in all tissues analyzed and increased in concentration approximately linearly for 3-5 days before the rate of increase began to level off with further exposures. The extent of formation was similar in all tissues except testis in which formation was about 60% lower than in brain and lung (32.8 +/- 0.6 and 31.5 +/- 1.3 (SEM; n=4) pmol 7-HEG/mg DNA, respectively) after 4 weeks of EO exposure. Similar relationships between chronic exposure to EO and formation of 7-HEG were recently demonstrated in exposed rats (Walker et al., Mutation Res., in press). The similarity in 7-HEG formation in target and nontarget tissues suggests that the tissue specificity for tumor induction is due to factors in addition to DNA adduct formation.

J 138 MODULATION OF CELLULAR ONCOGENE EXPRESSION FOLLOWING EXPOSURE OF MICE TO IONIZING RADIATIONS, Gayle E. Woloschak, Aaron Anderson and John Panozzo, Biological and Medical Research Division, Argonne National Laboratory, 9700 S. Cass Ave., Argonne, IL 60439-4833

Many studies have documented the importance of altered cellular oncogene expression in contributing to the development of neoplasia. We studied BCF1 mice for the effects of total body exposure to γ-rays (300 cGy) or JANUS fission-spectrum neutrons (50 cGy) in modulating expression of cellular oncogenes in gut and liver tissues. We selected specific cellular oncogenes (c-fos, c-myc, c-src, and c-H-ras) based on expression in liver and gut tissues from untreated mice. As early as 5 min following whole body exposure of BCF1 mice to γ-rays, we detected increased accumulation of mRNA specific for c-src and c-H-ras in both liver and gut tissues. The accumulation of c-fos-RNA was slightly decreased in gut but was unaffected in liver tissue from γ-ray-irradiated mice relative to untreated controls; c-myc mRNA accumulation was unaffected in all tissues examined. Exposure to JANUS neutrons, on the other hand, caused a decrease in accumulation of mRNA specific for c-H-ras, c-fos, and c-src in gut and liver tissues within 5 min following exposure. By 60 min following exposure, amounts of each mRNA began to return to control levels. These experiments document that modulation of cellular oncogene expression can occur as an early event resulting from radiation exposure and suggest that this modulation may play a role in radiation-induced carcinogenesis. In addition, they document differential in vivo effects of different qualities of radiation on gene modulation. (Supported by U.S. Department of Energy, Office of Health and Environmental Research, under contract W-31-109-ENG-38.)

J 139 EFFECT OF ULTRAVIOLET RADIATION B ON HUMAN PRIMARY NEONATAL MELANOCYTES AND METASTATIC MELANOMAS. Douglas T. Yamanishi, Malabika De, Barna De, Julie A. Buckmeier, Mark J. Graham, and Frank L. Meyskens Jr., Clinical Cancer Center and Dept. of Med., UC Irvine, Irvine, CA 92717.
Epidemiological evidence suggests that excess intermittent exposure to ultraviolet (UV) radiation has been associated with the transformation of human melanocytes. Human melanocytes and melanomas were irradiated with UV radiation B using a Stratalinker with UVP bulbs. Cell survivals obtained from UVP bulbs display a proliferative response with UV treatments of melanocytes and melanomas less than 10 mJ/cm^2. Optimal UV induced proliferative response appears to be around 5-7.5 mJ/cm^2. With UV radiation B doses greater than 10 mJ/cm^2, a plateau response is observed. This plateau effect may be due to the reduction in the range of 280-300 nm UV radiation B delivered by these bulbs. Cultivation of melanocytes in complete medium minus serum and TPA following UV irradiation is observed to induce a cell proliferative response. The proliferative response is observed with UV radiation B doses less than 10 mJ/cm^2 in 2 days. After five days, this UV induced response is lost. The effect of UV radiation B on gene expression are being pursued.

J 140 EFFECT OF UV-INDUCED PYRIMIDINE DIMERS ON THE CATALYTIC REACTION OF EUKARYOTIC TOPOISOMERASE II, E. Lynn Zechiedrich, Richard W. Hamilton, R. Stephen Lloyd, and Neil Osheroff, Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN 37232-0146
Topoisomerase II is essential for the viability of eukaryotic cells. This enzyme is important for the maintenance of chromosome structure and also plays critical roles in many aspects of DNA physiology. Since pyrimidine dimers are the primary short wave UV-photoproduct found within DNA, the effect of these lesions on the catalytic activity of topoisomerase II was investigated. The catalytic relaxation of pBR322 DNA by topoisomerase II was inhibited by the presence of pyrimidine dimers. When ~20 dimers were present per plasmid molecule, the type II enzyme was inhibited ~50%. Although dimers affected overall catalysis, they did not impair the enzyme's DNA binding, cleavage/religation, double-stranded DNA strand passage, or ATP hydrolysis reactions. Therefore, we conclude that UV-induced lesions inhibit enzyme function by decreasing the ability of topoisomerase II to undergo enzyme turnover, the process by which it reinitiates a new round of catalysis. These results indicate that repair of UV photoproducts are important for the efficient function of topoisomerase II in the eukaryotic cell. Supported by NIH Grants GM-33944, ES-04091, ES-00267, and ACS Faculty Research Award FRA-370.

Recombination and Mutagenesis

J 200 **CHARACTERISATION OF KIN17, A PROTEIN EXPRESSED IN MOUSE EMBRYOS AND TRANFORMED CELLS**, Jaime F. Angulo, Evelyne Rouer†, Agnès Tissier, Philippe Horellou*, Richard Benarous† and Raymond Devoret, Groupe d'Etude "Mutagénèse et Cancérogénèse", Laboratoire d'Enzymologie, and *Neurobiologie Cellulaire et Moléculaire, CNRS, F-91198 Gif-sur-Yvette, France, and † Institut Cochin de Génétique Moléculaire, 22 rue Méchain, F-75014 Paris, France.

Kin proteins are defined as a group of mammalian proteins that cross-react with monospecific antibodies raised against *E. coli* recA protein (Angulo *et al.*, 1989, Mutation Res., 217, 123-134). We have cloned 5 cDNA fragments encoding parts of kin proteins. *KIN17* cDNA codes for the most immunoreactive protein. *KIN17* mRNA, expressed at a low level in normal embryonic cells, is revealed by RT-PCR on embryonic total RNA, while *KIN17* mRNA is not detected by northern blot in normal mouse tissues such as muscle, spleen and liver. Yet, *KIN17* gene is efficiently transcribed in AtT-20 - a transformed cell line derived from a pituitary adenoma - as well as in RIN - a cell line derived from a rat insulinoma. These findings suggest that *KIN17* gene expression may be related to the rate of cell division. The fact that *KIN17* homologous sequences are present in rat and human genomic DNAs may indicate that this gene is highly conserved. The deduced amino acid sequence revealed the presence of a motif of 15 amino acids homologous to the C-terminal region of recA protein. A ten amino acid motif is reminiscent of a sequence present in the C-terminal region of N-myc oncoprotein. We entertain the idea that kin17 protein may be involved in DNA transactions.

J 201 DEREPRESSION OF THE E.COLI SOS RESPONSE ENHANCES DELETIONS IN A DERIVATIVE OF PLASMID pBR325, Elias Balbinder, Dept. Biochemistry,Biophysics and Genetics, University of Colorado Med. School, Denver, CO 80262

The possibility that genetic rearrangements could result from the participation of the SOS response was suggested by Echols (Cell,25:1,1981) and has received experimental support in reports that duplications were enhanced by SOS induction (Dimpfl & Echols,Genetics 123:255,1989 and refs. therein). We have tested the possibility that deletions may also be stimulated by the SOS response by monitoring the deletion frequency of a 60 bp palidromic fragment cloned into the EcoRI site of the chloramphenicol acetyl transferase (cat) gene of pBR325. The derived plasmid (pOCE15) was introduced into isogenic strains containing different combinations of recA$^+$, lexA$^+$and umuC$^+$ and their mutant alleles recA730, ΔrecA306 (a null allele of recA), lexA71::Tn5 (a null allele of lexA) and umuC122::Tn5(a null allele of umuC, which decreases the mutagenic response). Deletion frequency was quantitated as the reversion from chloramphenicol sensitivity to resistance ($Cm^S \rightarrow Cm^r$). We observed that (1) deletion frequency was increased in lexA71::Tn5 and recA730 strains and was highest in double mutants containing both alleles, and (2) introduction of ΔrecA306 and/or umuC122::Tn5 into lexA71::Tn5- derepressed strains lowered deletion frequency.These results support the idea that some deletions can be brought about by SOS processing. The data also suggest an additional role for RecA protein in deletion formation which may be similar to that recently reported by Witkin's lab. (Sweasy et al,J.Bact. 172:3030,1990) for point mutations.

J 202 SEQUENCE, METHYLATION, AND CHROMATIN ANALYSIS OF CARCINOGEN-INDUCED REARRANGEMENT BREAKPOINTS, Frederic G. Barr[1,2] and Beverly S. Emanuel[1], [1] Division of Human Genetics and Molecular Biology, Children's Hospital of Philadelphia and [2] Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA 19104

A cell culture system in which gene rearrangements are induced by chemical carcinogen treatment has been previously developed by one of us (Barr et al., Mol. Cell. Biol. 6:3023-3033, 1986 and Barr et al., Nucleic Acids Res. 18:129-135, 1990). The quiescent endogenous thymidine kinase (TK) gene in RJK92 Chinese hamster cells can be activated following exposure to N-methyl-N'-nitro-N-nitrosoguanidine. TK gene rearrangements occurred in 20% of the TK-expressing variants; the breakpoints were localized to a 1.6 kb region 6 kb 5' to the TK gene. The wild-type version of this breakpoint region has been cloned and sequenced. Using this sequence information, the carcinogen-induced breakpoints are being cloned from the rearranged TK genes by the inverse polymerase chain reaction (PCR) procedure, a variation of PCR which amplifies an unknown DNA sequence lying adjacent to a known sequence. The wild-type rearrangement partner is then isolated by a second inverse PCR of unrearranged genomic DNA. Sequence analysis of the first carcinogen-induced rearrangement breakpoint and partner (RP15) and comparison with the corresponding region of the TK gene has revealed short repeats directly at the breakpoint and a nearby AT-rich region in each rearrangement partner. There is also a type B1 Alu element in the RP15 region but not in the corresponding wild-type TK region. Southern blot analysis of the RP15 region showed a demethylated CG-island, indicating the presence of an active gene, located 1-2 kb from the breakpoint in the region presumably deleted by the rearrangement. DNase I-hypersensitive sites have also been detected and support the presence of an active chromatin structure in this region. Finally, pulsed-field gel electrophoresis and low agarose concentration conventional gel electrophoresis analyses suggest that this rearrangement is an interstitial deletion measuring less than 50 kb.

J 203 INDUCTION OF SIGNAL TRANSDUCTION AND DECREASE IN REPLICATION FIDELITY BY THE TUMOR PROMOTER 12-O-TETRADECANOYL-PHORBOL-13-ACETATE (TPA), Jan J.B. Boesen, Paul H.M. Lohman, and Jo W.I.M. Simons, MGC-Department of Radiation Genetics, State University of leiden, Wassenaarseweg 72, 2333 AL Leiden, The Netherlands.

Mouse T-lymphoma cells, GRSL13, were treated with the tumor promoter TPA. The induction of transcription of c-*fos*, *fosB*, c-*jun*, *junB* and collagenase was studied as well as the mutation rate in the progeny of treated cells. It was found that mRNA-levels of *fosB*, *junB* and collagenase, all known to be involved in the growth factor signal transduction pathway were enhanced which was dependent on culture conditions of the treated cells, growing cells having less response than stationary cells. No transcription of c-*fos* and c-*jun* was observed in control and TPA treated cells. These results suggests that transcription of c-*fos* is not a prerequisite for the induction of transcription of collagenase. The mutation rate was significantly enhanced in the progeny of TPA treated cells from 4.2×10^{-7}/cell/generation to 9.8×10^{-7}/c/g. Fluctuation analysis showed that TPA leads to a temporary enhancement of the mutation rate up to the eighth generation after treatment. The enhancement of the mutation rate is less apparent in growing cells than in stationary cells (1.8-fold and 2.9-fold respectively) which is in agreement with the hypothesis that induction of the signal transduction pathway leads to genetic instability.

J 204 FIDELITY OF DNA REPLICATION BY CELL EXTRACTS OF DIPLOID HUMAN FIBROBLASTS AND HELA CELLS. Jayne C. Boyer, David C. Thomas, and Thomas A. Kunkel, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park., NC 27709.

Cytosolic extracts derived from diploid human fibroblasts and HeLa cells were used to replicate double-stranded phage M13mp2SV DNA in the presence of SV40 large T antigen. Mutation frequencies were measured using the *lacZ* alpha-complementation gene as the target DNA. Unreplicated M13mp2SV DNA was completely digested by *Dpn* I, an enzyme that cuts only fully methylated sequences. DNA replication products generated by both fibroblast and HeLa extracts were primarily monomer-length circles and were resistant to *Dpn* I digestion. This suggests that the DNA replication products resulted from semiconservative replication. Most of the products of replication were resistant to digestion by *Mbo* I, an enzyme that cuts only fully unmethylated DNA, and thus, resulted from a single round of replication. Mutation frequencies were measured by transfecting mismatch repair-defective cells with *Dpn* I-digested DNA and scoring mutant light blue or white plaques. The forward mutation frequency in M13mp2SV DNA replicated by extracts of normal diploid fibroblasts (12.3×10^{-4}) was similar to that of the HeLa cells (11.9×10^{-4}) and about twice the background frequency. Thus, the replication complex of HeLa cells was not appreciably error-prone in comparison to that of normal diploid fibroblasts. Experiments using altered replication conditions, such as dNTP pool imbalances and sensitive reversion assays are underway to more accurately determine the relative base substitution and frameshift error rates during bidirectional replication in various human cell extracts.

J 205 PREFERENTIAL STRAND SPECIFICITY OF MUTATION IN THE HAMSTER dhfr GENE INDEPENDENT OF PHENOTYPIC SELECTION,
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We showed that among forward mutations in the CHO dihydrofolate reductase (dhfr) gene induced with (±)-3a,4b-dihydroxy-1a,2a-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene (BcPH), the preferred induced mutation type was a purine to T transversion, 92% of the targeted purines were on the nontranscribed strand of the DNA, and all induced mutations occurred at the sequence 5'RR 3' (R is a purine and the mutated base is underlined) (Carothers et al. (1990) PNAS 87:5464-5468). It has been shown by others (Mellon et al.(1987) Cell 51:241-249) that efficient DNA repair of structure-distorting lesions is selective for the transcribed strand of this gene. To test if phenotypic selection plays a role in influencing this mutational strand preference, we designed a reversion assay. This study used a dhfr$^-$ nonsense mutant (DU6) in which the relevant sequence on the nontranscribed coding strand is 5'TTC TAA AGA 3', with the 3'AAG ATT TCT 5' complementary sequence on the transcribed template strand. TAA in the coding strand replaced CAA (Gln_{35}) at the 5' end of the dhfr$^+$ gene. Use of a mutant in which the preferred sequence context is present on both strands for BcPH-induced reversion was expected to allow similar frequencies for mutation at all 3 As (underlined). Previously, isolation of DU6 revertants induced with ethylmethanesulfonate showed that mutation of the A on the transcribed strand would yield selectable substitutions of Lys (AAA) or Glu (GAA) by transversion (Urlaub et al.(1989) Mol.Cell.Biol. 9:2860-2880). We have isolated and sequenced 34 independent DU6 BcPH-induced revertants. By A to T transversion, 100% of base changes in these revertants occurred at the As on the nontranscribed strand yielding Tyr (TAT), Leu (TTA), and in a single case Phe (TTT) replacements. A similar analysis is underway using a mutant in which the termination codon is at the 3' end of the gene to learn if the mutational strand preference is influenced by position. The results thus far indicate that in the dhfr gene this strand preference is not attributable to the selection. The data is strikingly consistent with evidence for preferential DNA repair of the transcribed strand, although it does not rule out the unlikely possibility of in vivo strand specific BcPH modification. Supported by NIH Grants CA21111 & CA39547.

J 206 IN VIVO DNA REPLICATION ACROSS FROM 8-HYDROXYGUANINE AFTER POLYMERASE-MEDIATED SITE-SPECIFIC INSERTION YIELDS G:C TO T·A TRANSVERSIONS.

Keith C. Cheng[1], David S. Cahill[1], Susumu Nishimura[2], Hiroshi Kasai[2] and Lawrence A. Loeb[1]. [1]Department of Pathology SM-30, University of Washington, Seattle, WA 98195 [2]Biology Division, National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan.

Oxidative damage to DNA causes mutations that may contribute significantly to spontaneously occurring cancers. 8-OH-guanine is one of the major forms of such damage. To determine the mutagenic potency and spectrum of this adduct, we have developed a system for DNA polymerase-mediated site-specific insertion of guanine analogues into a specific site using a uracil-containing single-stranded DNA M13mp2 derivative, M13G*1. Any single base substitution at this site is readily detected as a blue plaque against a background of white plaques.

8OH-G is at least 8-fold more error-prone than normal G. Among 54,000 plaques from an 8-OH-guanine insertion experiment, we found 40 blue plaques with G·C to T·A transversions at the target site. No such mutations were found among 37,000 control plaques screened. The rates of other mutations were indistinguishable from control rates. Adenine rather than cytosine was inserted by the cell opposite template 8-OH guanine at an approximate frequency of one in 500. Such a rate would allow viability despite the reported high spontaneous frequency of this lesion in the genome.

J 207 KINETIC ANALYSIS OF NI(II) EFFECTS ON DNA REPLICATION BY POLYMERASE α, Nelwyn T. Christie, Yue G. Chin, Elizabeth T. Snow, and Mitchell Cohen, Department of Environmental Medicine, New York University Medical Center, New York, NY 10016

Prior studies have shown that Ni(II) stimulates DNA synthesis by cell extracts and by purified DNA polymerases. Our current studies indicate that Ni(II) binds more tightly to the polymerase than Mg(II) and prevents or alters the binding by Mg(II). In this study we also show two apparently "anomalous" effects of Ni(II) on DNA synthesis in vitro, (1) stimulation of synthesis in the absence of Mg(II), and (2) competitive inhibition of Mg(II)-stimulated synthesis. Ni(II)-stimulated (0.5 mM $NiSO_4$) synthesis by pol α in dialyzed HeLa cell extracts is approximately 25% of the extent of the synthesis catalyzed by 2 mM Mg(II) after a 1 h incubation; and, the initial velocity of the reaction is proportionally increased. In reactions containing 2 mM Mg(II) combined with suboptimal concentrations of Ni(II), the overall synthesis is reduced in a concentration-dependent manner and is intermediate between the maximal level with Mg(II) alone and the level with Ni(II) alone. The inability of Mg(II) to completely prevent the Ni(II) inhibition of DNA synthesis in HeLa cell extracts is substantiated by experiments in which preincubation of the extracts with Mg(II) did not prevent the inhibitory effects of Ni(II) on DNA synthesis. Lineweaver-Burke plots of the kinetics of nucleotide (dTTP) incorporation by purified pol α in the presence of increasing amounts of Ni(II) indicate that Ni(II) is a competitive inhibitor of Mg(II). Substitution of Ni(II) for Mg(II) also promotes increases in the polymerase-dependent incorporation of modified nucleotides. In reactions using increasing ratios of ddCTP/dCTP, the ddCTP incorporation in the presence of 0.5 mM Ni(II) was two-fold higher then the incorporation in the presence of 2 mM Mg(II). These studies are consistent with previously observed decreases in replication fidelity in the presence of Ni(II) and with our prior hypothesis that Ni(II) may produce varied effects on the microsteps of DNA replication normally regulated by Mg(II). This work has been supported by grants ES-00260 and ES-04895 from the NIEHS, by grant R-184751 from the USEPA, and by grant CA-45664 from the NCI.

J 208 PURINE STARVATION, ERROR-PRONE DNA REPAIR AND CHROMOSOME REARRANGEMENT IN CHO CELLS, Andrew Collins and Diane Black, Department of Molecular and Cell Biology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland.

A phenotypic revertant of the purine auxotroph CHO cell line $Ade^{-}C$ was obtained by a combination of UV irradiation and purine starvation, which results in error-prone DNA repair. Revertant cells have a measurable, though still low activity of glycinamide ribonucleotide synthetase (GARS, the enzyme defective in $Ade^{-}C$ cells), and can survive without exogenous hypoxanthine. The restored enzyme activity correlates with the presence of a large telomeric chromosome, apparently derived by rearrangement of normal chromosomes. Sublines of the revertant grown with hypoxanthine lose the marker chromosome, detectable GARS activity and the ability to survive without exogenous purines. We are investigating the possibility that translocation and/or gene amplification leads to over-expression of the defective GARS gene and enough enzyme activity to supply purines needed for survival.

J 209 A THIRD ESSENTIAL ROLE FOR RecA PROTEIN IN UV-MUTAGENESIS IN PROKARYOTES,
Raymond Devoret, Adriana Bailone and Marie Dutreix, Groupe d'Etude "Mutagénèse et Cancérogénèse", Laboratoire d'Enzymologie, CNRS, F-91198 Gif-sur-Yvette, France.

Mutagenesis following UV-irradiation is a convenient experimental model for the study of mutagenesis by chemical carcinogens. UV-mutagenesis involves two processes: (i) the production by UV-light of specific DNA lesions, (ii) the induction of SOS genes that will express proteins permitting the replication apparatus to by-pass UV-lesions. Two RecA-directed reactions are known to be involved in the mechanism of mutation formation: (i) the cleavage of LexA repressor that induces the expression of SOS genes, (ii) the cleavage of UmuD protein that activates UmuD protein to UmuD'. The question arises as to whether RecA protein has another (a third) essential function in UV-mutagenesis.

We have characterized a dominant mutation called recA1730 that inhibits mutagenesis (Dutreix et al 1989). The RecA1730 protein is dominant over wild type RecA protein. A recA1730/recA^{+} heterogenote is resistant to UV-light and fully recombinogenic but no mutations can occur after DNA damage. This deficiency is not alleviated by increasing the amount of UmuDC proteins or UmuD'. Interestingly, the presence of MucA and MucB proteins (plasmid Umu analogs) restore mutagenesis. RecA1730 protein may trap UmuD' protein but not MucA'.

A possible third essential role of RecA protein in mutagenesis might be that RecA protein will deliver UmuD' protein at the site of lesions to facilitate replication over DNA damage.

[1]Dutreix et al, 1989, J. Bacteriol. 171, 2415-2423.

J 210 MUTATIONS AFFECTING SPONTANEOUS DNA DUPLICATION, Elizabeth G. Gurney and Theodore Gurney, Jr., Department of Biology, University of Utah, Salt Lake City, UT 84112

We are studying spontaneous DNA duplications in SV40-transformed mouse cell lines to determine the molecular mechanism(s) involved. SV40-transformed mouse cells frequently have spontaneous duplications within or near integrated SV40 DNA. Excision or transposition of integrated SV40 DNA is not detected in non-permissive mouse cells. Our cell line X1 has an unusually high rate of duplication at this locus, over 10^{-2} events per cell per division. The rate is so high that every monolayer clone of X1 has rearrangements, mainly direct tandem single duplications of 1-2 kb. Six independent mutant cell lines with lower rates of duplication were isolated from X1 using two screening strategies. Four had duplications within the T antigen coding sequence and produced larger mutant T antigens. The other two produced wild-type T antigen, as did X1, but had deleted a 1.1 kb duplication present in X1. The 1.1 kb duplication contained a mouse-SV40 junction and a $(CA)_{16}$ repeat in mouse DNA. The features of the mutants suggest two phenomena that contributed to the high rate of duplication in X1: 1) Since T antigen apparently affects duplication in the mouse cell mutants and has a known role in SV40 DNA replication in primate cells, occasional T antigen-dependent overreplication of integrated SV40 DNA appears likely. 2) Since wild-type T antigen alone was not sufficient to produce the high rate of duplication in X1, recombinogenic DNA sequences, as in the 1.1 kb duplication, are probably also required to resolve overreplicated DNA into tandem duplications.

J 211 LIKELY ROLE OF REPLICATION ORIGIN IN SPONTANEOUS DNA DUPLICATIONS, Theodore Gurney, Jr. and Timothy Beagley, Biology Department, Un. of Utah, Salt Lake City, UT 84112

It is not yet known how the first duplication of an unduplicated DNA sequence occurs; perhaps there is more than one molecular mechanism. SV40-transformed mouse cells offer a model system for studying this phenomenon because of frequent rearrangements of integrated SV40 DNA. We are studying the SV40-transformed mouse line X1 which has the highest rate of spontaneous SV40 DNA duplication yet described, well over 10^{-2} events per cell per division. The SV40 DNA in X1 is integrated at a single site in one chromosome. Genomic DNA from the region around the integrated viral DNA of X1 was cloned into lambda and then subcloned into M13. M13 probes, representing short sequences at different distances in both directions from the integrated SV40 DNA, were used to find how far rearrangements extended into flanking mouse DNA. The probes incidentally tested the possibility of spontaneous rearrangements at similar mouse sites in the other chromosome, the one without integrated SV40 DNA. We found no detectable duplications except within or very near integrated SV40 DNA. Even within integrated SV40 DNA, rearrangements fell off rapidly with distance from the integrated SV40 origin of DNA replication. The results suggest a role for the SV40 origin in duplication of mouse DNA, possibly through re-replication or over-replication. If so, spontaneous re-replication must extend only a short distance from the origin or else subsequent recombination must also occur close to the origin.

J 212 TELOMERE LENGTH IN NORMAL AND SV40 TRANSFORMED HUMAN FIBROBLASTS,
Karen Hubbard-Smith, Jose R. Pardinas, Harvey L. Ozer,
and Michael B. Small, Department of Microbiology and Molecular Genetics,
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Telomeres are specialized structures found at the ends of eucaryotic chromosomes, whose length reduction has been implicated in cellular senescence in vitro and perhaps in vivo. In the study presented, we have measured telomere lengths in DNAs isolated from a normal human fibroblast cell line and its pre-immortal and immortalized derivatives generated by SV40 transformation. Telomere length was determined at various passages during the lifespan of these cells by Southern analysis using probes for telomeric and sub-telomeric repeat sequences. Normal diploid fibroblasts displayed shortened telomeres upon serial passage. Telomeric repeats in the pre-immortal cells were also reduced, with the most dramatic reduction for transformed cells which had survived "crisis". Interestingly, telomere lengths slightly increased upon extended passage of such immortalized cells. These results confirm previous studies which showed a reduction of telomere length as a function of age of cells in culture. They demonstrate that transformation by SV40 does not present a bias for increased cell proliferation by offering a selective advantage for cells containing large telomeres. The results also suggest that telomere length may be stabilized upon immortalization.

J 213 DNA DAMAGE AND MUTAGENESIS IN A SINGLE STRANDED MAMMALIAN SHUTTLE VECTOR INDUCED BY SINGLET-OXYGEN.
Carlos F.M. Menck[1] and Denise T. Ribeiro[2]. 1. Dept. of Biology, Inst. of Biosciences, Cx.P. 11461, Univ. of São Paulo, 05499 SP, 2. Dept. of Cellular Biology, Univ. of Brasilia, DF, Brazil.

The effects of singlet oxygen ($^1O_2$), generated by thermal decomposition of the endoperoxide of the water-soluble $NDPO_2$ (disodium 3,3'-{1,4-naphthylidene} dipropionate), on a single stranded (ss) shuttle vector were analysed. Our observations of DNA mobility in agarose gel eletrophoresis demonstrate that $^1O_2$ induces a much higher level of phosphodiester chain breaks in ss DNA than in double stranded (ds) DNA. This may be due to a higher accessibility of the guanine residue, which may be primarily damaged by $^1O_2$. The damaged vector was transfected into monkey COS7 cells where ss DNA is converted to ds replicative form DNA. After three days, the extrachromossomal DNA was extracted and the plasmids rescued in E. coli to study mutagenesis. There is a significative increase in the mutation frequency of damaged ss DNA, in comparison with untreated DNA. Our conclusions are that $^1O_2$ induces breaks in the backbone of ss DNA and that the $^1O_2$-damaged molecules are mutated after passage through mammalian cells. Furthermore, preliminary results indicate that, at sequence level, both damages and mutations occur preferentially on guanine residues.

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J 214 MUTATOR GENES AND GENOME INSTABILITY IN MAMMALIAN CELLS
Mark Meuth, Masatake Yamauchi, Jeremy Whelan, Maria Caligo, and Geraldine Phear
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We are analysing the mechanisms maintaining genome stability in mammalian cells through the selection of mutant cell strains having increased rates of mutation at independent genetic loci. By various approaches three such mutator strains of CHO cells have been isolated and characterized and in one case the mutator gene responsible for the increased mutation rate has been cloned and sequenced. The types of mutations induced by all three have been determined. One induces base substitutions (predominantly T->C and A->C) driven by the dCTP pool imbalance produced by a regulatory mutation of CTP synthetase. The second strain generates a wide range of base substitutions at a substantially (~100x) higher rate suggesting a more general defect in DNA replication or mismatch repair while the final mutator strain has a significant increase in the rate of deletion. The CTP synthetase structural gene has recently been cloned using chromosome mediated gene transfer techniques (Yamauchi, et al., EMBO J. 9, 2095-2099, 1990) and the domain responsible for the mutator phenotype has been identified. Current work in this laboratory focusses on the analysis of the potential role of this mutant CTP synthetase in drug resistance developing during cancer chemotherapy and in tumour progression and metastasis.

J 215 INCREASED SPONTANEOUS INTRACHROMOSOMAL MITOTIC RECOMBINATION: A COMMON FEATURE OF THE CHROMOSOME INSTABILITY SYNDROMES. M. Stephen Meyn, James Bainton, and Laura Herzing. Departments of Human Genetics and Pediatrics, Yale University, New Haven, CT 06510.

We recently developed two sets of vectors designed to study mitotic genetic recombination in human fibroblast lines. The first is a group of retroviral vectors that, upon infection, integrate tandem, non-complementary, mutant copies of a dominant selectable marker gene *(neo)* into chromosomal DNA. Recombination between the two integrated *neo* genes is detected when it results in the reconstitution of a wild-type gene and expression of drug resistance. The second set of vectors use copies of a reporter gene, *E. coli lacZ*, as their recombination substrate. Recombination between the *lacZ* genes is detected by expression of β-galactosidase. After transfering these vectors into fibroblast lines, clones containing single-copy integrants were identified and used in our studies. Frequencies of recombination between integrated copies of the *neo* genes or *lacZ* genes in these cells were measured and spontaneous recombination rates determined by fluctuation analysis.

Using the *neo*-based retroviral vectors, rates of spontaneous intrachromosomal recombination for a normal cell line (GM637D), a Lesch-Nyhan line (GM847) and a xeroderma pigmentosa line (GM4429C) were similar to each other and averaged 6.29×10^{-6} events/cell/generation. In contrast, rates of intrachromosomal recombination for 4 chromosome instability cell lines were markedly elevated: 8.58×10^{-4} events/cell/generation for two ataxia telangiectasia lines (GM5849 and GM9607), 8.25×10^{-5} for a Fanconi anemia line (GM6914) and 5.6×10^{-5} for a Bloom syndrome line (GM8585). We are analyzing DNA from the resultant $G418^R$ colonies to determine the types of recombination that have taken place as well as using the non-retroviral *lacZ* vector system to confirm our findings.

Our results provide *in vitro* molecular evidence for aberrant mitotic recombination in the chromosome instability syndromes and identify a new class of hyperrec mutants. Abnormal mitotic recombination *in vivo* in these patients may explain many aspects of their diseases: *e.g.* high cancer risk, elevated frequency of chromosomal aberrations, mutagen sensitivity and immune defects. (Supported by NIH R01 GM38588).

J 216 INCREASED PRODUCTION OF ABORTED TRANSCRIPTS FROM DNA CODING STRANDS CONTAINING CYTOSINE ARABINOSIDE. Thomas Mikita(1) and G. Peter Beardsley(2). Departments of Molecular Biophysics and Biochemistry(1), and Pediatrics(2) and Pharmacology(2). Yale University School of Medicine. New Haven, Ct. 06510.

Utilizing a chemical synthetic approach to introduce the nucleoside analog, cytosine arabinoside(araC) into DNA oligomers, we have continued our studies into how the misincorporation of this antileukemic agent into DNA creates sites of DNA dysfunction. Our previous work has focused on chemically and physically characterizing DNA duplexes containing araC sites(1988, Nucleic Acids Res. 16, 9165), as well as investigating the mechanistic details of DNA polymerase inhibition at these sites(1988, Biochemistry, 27, 4698). Model building studies have suggested that araC-G base pairs could not be introduced into a type A helix without steric clashes, whereas a B type structure of duplex DNA can readily accomodate an araC-G base pair. Since DNA-RNA hybrids are of the A type, we wanted to see if the DNA-RNA hybrid that is generated during transcription would be destabilized at araC sites in a DNA coding strand, thereby increasing the rate of aborted transcripts. To test this we have chemically introduced araC at various positions in a 38mer DNA duplex which contains the T7 promoter and a 16 nucleotide coding sequence. This was used as a substrate for T7 RNA polymerase in vitro. Compared to controls, few full length transcripts were observed if the araC residue was situated at sites within the first 6 positions of the coding strand. At sites past position 10, full length transcripts were decreased only by a factor of 2-3 relative to controls. Results of spectroscopic studies of DNA-RNA hybrids containing araC-G base pairs will also be reported.

J 217 THE ROLE OF RECOMBINATIONAL HOTSPOTS IN GENOME INSTABILITY IN MAMMALIAN CELLS, John P. Murnane and Barbara R. Young, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143-0750

Transfected DNA commonly demonstrates elevated rates of recombination for a transient period following integration, however, at rare sites this instability can continue indefinitely. We have been investigating this process to determine how recombinational hotspots are formed, and the types of DNA rearrangements they promote. Continuous instability at an integration site in cell line LM205 resulted in amplification by a two-step process: an intial tandem duplication involving random nonhomologous recombination, followed by further amplification by high rates of homologous recombination (10^{-2} events/cell/generation) between the duplicated regions. The involvement of cell DNA adjacent to the integration site is indicated by the rarity of continuously unstable integration sites (2 of 157), and the fact that instability can be transferred to other locations along with the integrated sequences and surrounding cell DNA. The importance of cell sequences is also demonstrated by a second unstable integration site in cell line KB319 where the rearrangements are confined to the adjacent cell DNA. However, the integrated sequences or events which occur at the time of integration are also required for instability, because high rates of recombination are not apparent in the cell DNA at the KB319 site prior to integration, and the cell sequences alone do not appear to demonstrate high rates of recombination when integrated at other locations. Interestingly, the cell sequences where recombination occurs in KB319 are unclonable in bacteria, similar to recombinational hotspots identified in the Cystic Fibrosis gene. Current research involves further attempts to identify the cell sequences within the unclonable region in cell line KB319, as well as establishing the importance of other interesting cell sequences cloned from the surrounding region, which include telomeric repeat sequences and a previously unidentified repetitive sequence with homology to a recombination site in the BCR gene involved in Ph translocation.

J 218 EVIDENCE FOR GAP REPAIR DURING EXTRACHROMOSOMAL RECOMBINATION IN MAMMALIAN CELLS, Jac A. Nickoloff[1] and Richard J. Reynolds[2],

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To investigate mechanisms of homologous recombination in Chinese hamster ovary cells, we have employed electroporation to transfect cells with heteroallelic pairs of pSV2neo derivatives. In previous studies employing calcium phosphate coprecipitation (CPP), recombination between two linear substrates (L × L crosses) or between linear and circular substrates (L × C crosses) yielded primarily crossover products by a nonconservative mechanism (e.g. Lin et al. Mol. Cell. Biol. 10, 103-112; 1990). With electroporation, we have found similar results with L × L crosses. Electroporation-mediated L × C crosses, however, yielded crossover and noncrossover products at equal frequencies. Furthermore, crossover products of L × C crosses were apparently formed by a conservative process. The products of L × C crosses are consistent with events occurring by a gap repair mechanism. In related studies we have found that linearizing pSV2neo stimulates transfection 30-fold with electroporation but only 2- to 3-fold with CPP. Differences in the results obtained with CPP and electroporation may be due to more extensive degradation during gene transfer by CPP. The degradation of circular molecules to linear molecules would reduce differences in transfection efficiencies for linear and circular molecules. During homologous recombination, degradation of circular molecules would cause C × L crosses to mimic L × L crosses. Our results suggest that mammalian cells are capable of mediating extrachromosomal recombination by two mechanisms, one requiring breaks in both molecules, producing primarily crossover products, and a second mechanism that requires only a single break, producing both crossover and noncrossover products. This research was supported by Public Health Service awards CA 42390 and CA 51871 from the National Institutes of Health and by Department of Energy contract #W-7405-ENG-36.

J 219 ADENOVIRUS AS A PROBE TO STUDY HOMOLOGOUS RECOMBINATION IN MAMMALIAN CELLS, Andrew J. Rainbow and Jose E. Castillo, Department of Biology and Radiology, McMaster University, Hamilton, Ontario, L8S 4K1, Canada.

We are using adenovirus as a probe to study the nature and extent of homologous recombination in mammalian cells. The technique gives a quantitative measure of homologous recombination between adenovirus type 2 (Ad2) and Ad5PyMTR3 and allows an examination of the viral and host factors which affect recombination in various mammalian cell types. Ad5PyMTR3 is an insertion mutant of Ad5 containing polyoma virus (Py) DNA inserted into a deleted E1 region of the Ad5 genome. Although Ad2 and Ad5 have more than 99% DNA homology, they differ sufficiently in their restriction endonuclease patterns, such that following simultaneous infection, recombinant viral DNA molecules containing the Py insert can be detected and quantified by Southern blotting and hybridization to a radioactive Py DNA probe. Using this technique we are able to quantitate the recombinant molecules produced at frequencies down to at least 1 in 100. Recombination was detected in Chinese Hamster Ovary cells, Monkey Kidney cells, human HeLa cells, normal human fibroblasts and SV40 transformed human fibroblasts. In experiments using HeLa cells, we were able to show that recombination between the Py insert on Ad5PyMTR3 and a number of unique restriction enzyme sites on Ad2 increased with the distance from the Py insert to the restriction site. Also in HeLa cells, the amount of recombination detected increased with increasing amounts of viral DNA synthesis, with an increase in the ratio of Ad 2 to Ad5PyMTR3, with an increase in the multiplicity of infection and with increasing UV dose to the infecting virus.

J 220 PREFERENTIAL INTEGRATION OF MARKER DNA INTO THE CHROMOSOMAL FRAGILE SITE AT 3p14: A NOVEL APPROACH TO CLONING FRAGILE SITES. FV Rassool, ME Neilly, E van Melle, R Espinosa, TW McKeithan and MM Le Beau. Section of Hematology/Oncology, Univ. of Chicago, Chicago, IL 60637.

Fragile sites are specific regions of chromosomes that are prone to breakage. In cells cultured under conditions that induce fragile site expression, high levels of inter- and intra-chromosomal recombination have been observed involving chromosomal bands containing fragile sites. To determine whether expression of specific fragile sites would facilitate preferential integration at these recombination hotspots, we transfected the vector SV2Neo into a Chinese hamster X human somatic cell hybrid containing a derivative chromosome 3 as its only human component. Chromosome 3 contains a common fragile site at 3p14.2 (FRA3B) which is induced by aphidicolin. Both cells induced to express FRA3B and the uninduced control cells were transfected with the vector SV2Neo. Clones containing stably integrated SV2Neo were isolated for further analysis. In situ hybridization of biotin-labeled SV2Neo to metaphase chromosomes revealed 2 or 3 integration sites in each clone, and 4 of 13 clones transfected under conditions of FRA3B induction showed integration of SV2Neo at 3p14. Eight of these clones also showed specific integration into hamster chromosome 1. The 6 control clones, however, showed an apparently random pattern of SV2Neo integration. To determine the relative distance between the integration sites, we are currently performing pulse field analysis of the clones showing integration at 3p14. These results suggest that expression of FRA3B allows preferential integration of marker DNA into that site. A corresponding site of integration has also been identified on chromosome 1 of the Chinese hamster. Bacteriophage libraries have been prepared from clones showing specific integration into FRA3B, and SV2Neo is being used to isolate the flanking sequences. The nature of the DNA sequences at fragile sites is unknown, and these sequences have been difficult to isolate; our procedure may represent a novel approach to the cloning of fragile sites.

J 221 SPONTANEOUS MUTATION RATES IN MAMMALIAN CELLS: EFFECT OF DIFFERENTIAL GROWTH RATES AND PHENOTYPIC LAG.

Colette J. Rudd[1], Diane Daston[2], and William J. Caspary[2], [1]SRI International, Menlo Park, CA 94025 and [2]NIEHS/NIH, Research Triangle Park, NC 27709.

Analysis of mutation rates in mammalian cells is complicated by the time required for expression of the mutant phenotype after the occurrence of a new mutation in a particular gene. This factor is especially important when the growth rates of a mutant cell and an average wild-type cell are different. Two methods have been commonly used to estimate mutation rates of mammalian cells in culture. The first measures the change in mutant frequency over time; it requires a large cell population and mutants that grow at the same rate as wild-type cells. The second method, the fluctuation test of Luria and Delbrück, is insensitive to growth rates but sensitive to phenotypic lag. We have developed a method that more accurately measures the spontaneous rate of formation of mutations at the thymidine kinase locus ($tk^{+/-} \rightarrow tk^{-/-}$). L5178Y mouse lymphoma cells ($tk^{+/-}$) were suspended in semisolid medium for up to three days in the absence of the selective agent, trifluorothymidine (TFT). This prevented cells with new mutations from mixing with wild-type cells during the expression of the TFT-resistant phenotype. Mutant colonies were then selected *in situ* at various times by adding an overlay of TFT. A spontaneous mutation rate of at least 37 mutations per 10^6 cell divisions was measured at the *tk* locus. This rate was 50 times higher than found when conventional methods for calculation of mutation rates were used. We suggest that procedures that ignore differential growth rates and phenotypic expression times can lead to qualitatively erroneous estimates of mutation rates.

J 222 DISLOCATION OF CHROMATIN ELEMENTS IN PROPHASE INDUCED BY 3,3'-DIETHYLSTILBESTROL: A NOVEL MECHANISM BY WHICH MICRONUCLEI CAN ARISE, Dietmar Schiffmann, Helga Stopper and Umberto De Boni*, Inst. of Toxicology, University of Würzburg, F.R.G., * Dept. of Physiology, University of Toronto, Canada.

The mechanisms of estrogen-mediated carcinogenesis are currently under much debate. The synthetic estrogen diethylstilbestrol (DES) transforms Syrian hamster embryo (SHE) fibroblasts neoplastically in vitro without detectable DNA damage. Instead, it is known that DES induces micronuclei (MN) and near-diploid aneuploidy in these cells. In order to clarify the origin of these MN and their possible role as an intermediate step in aneuploidy induction, we have now studied the time course of their formation in living SHE cells during mitosis. The analog 3,3'-DES was chosen to induce MN since it proved to be more efficient than DES. The cells were labelled with DAPI (DNA fluorescence) and monitored live using UV-light and phase contrast microscopy. A S.I.T. (silicon intensifier target) camera was used to allow low UV-dosage in order to avoid cellular damage. Lagging chromosome elements were detected as early as prophase, persisting throughout metaphase/anaphase. These structures formed MN after karyokinesis. Visualization of kinetochores in 3,3'-DES-induced MN by CREST-antibodies yielded a considerable number of CREST-reactive MN (> 60 %) most likely indicating the presence of whole chromosomes/chromatids. Furthermore, these results suggest a causal relationship between the interference of estrogens with the function of the mitotic apparatus, the formation of MN and aneuploidy induction.

J 223 EXPRESSION OF A *lacZ* TRANSGENE INTEGRATED AT THE *hprt* LOCUS OF MURINE EMBRYONIC STEM CELLS, Jessica Shaw-White and James R. Stringer, Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati School of Medicine, Cincinnati, OH 45220-0524

Transgenes in mice typically exhibit different expression patterns in different transgenic lines. While the basis for this phenomenon is not understood, it is widely believed that the site at which the transgene becomes integrated into the mouse genome is the primary factor responsible for determining the pattern of expression. Another factor that could influence transgene expression is the structure of tandem arrays produced when transgenesis derives from microinjection of DNA into the male pronucleus. We are using gene targeting in embryonic stem cells to investigate the role of chromosomal position-effects on transgene expression. A plasmid was constructed in which *lacZ* gene was placed downstream of the herpes simplex virus thymidine kinase gene promoter. The *tklacZ* gene was linked to a *neo* gene and to segments of the mouse *hprt* gene. This reporter gene was transfected into ES cells by electroporation and cell lines were isolated that contained *tklacZneo* integrated either in the *hprt* gene or at random chromosomal loci. Expression of *lacZ* was assayed by histochemical staining for *E.coli* beta-galactosidase activity in ES cells and in differentiated derivatives obtained by *in vitro* differentiation. Targeted cell lines expressed *lacZ* in most but not all cell types. Nontargeted cell lines expressed the transgene in a similar fashion. Experiments with differentiated cells present in tumors induced by injection of ES cells into syngeneic mice and by production of chimeric embryos by injection of ES cells into blastocysts are in progress.

J 224 A NOVEL ASSAY USED TO MEASURE THE RECOMBINATION FREQUENCY OF ALU SEQUENCES, M. Richard Shen and Prescott L. Deininger, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, New Orleans, LA 70112

Alu sequences are present in excess of 500,000 copies per haploid human genome. Alu sequences have been shown to be involved in illegitimate and homologous recombination events in the LDL receptor, the gamma, delta, beta globin gene complex and the insulin receptor. Alu sequences have also been shown to be involved in some chromosomal translocation events of the Philadelphia chromosome as well as translocation of the short arms of the X and Y chromosomes to produce an XX male. Many of the Alu recombination junctions characterized thus far lie within the left arm of the Alu sequence. In order to delineate whether Alu sequences actually promote recombination or are found in recombination junctions because of their ubiquity, a recombination assay has been developed that takes advantage of positive thymidine kinase selection. This assay involves two vectors containing segments of the thymidine kinase gene. Illegitimate or homologous recombination between these two vectors via the test DNA sequences would reconstitute a functional thymidine kinase gene. We are presently measuring the recombination frequency of Alu sequences BLUR 8 and BLUR 11 in the Ltk- mouse fibroblast cell line.

J 225 DNA DAMAGE AND DELAYED MUTATIONS. Jo W.I.M. Simons[1], Ada G.A.C. Knaap[2], Harry Vrieling[1,3], Albert A. van Zeeland[1,3], Malgorzata Z. Zdzienicka[1,3], Matthieu J. Niericker and Sandrine Stuivenberg[1]. 1. Department of Radiation Genetics and Chemical Mutagenesis, State University of Leiden, 2. National Institute of Public Health, Bilthoven, 3. J. A. Cohen Institute, Interuniversitary Institute for Radiopathology and Radiation Protection, Leiden; The Netherlands.

Fluctuation analysis has been performed with ENU-treated mouse lymphoma cells. This approach allows to discriminate between directly induced mutations and delayed mutations. The treatment leads to a large number of delayed mutations. Sequence analysis of the mutations shows that the delayed mutations are for 90% AT-transversions in which the A was always in the transcribed strand. Two hypotheses could explain the delayed mutations: one ascribes the delayed mutation to the induction of infidelity of DNA synthesis the other assumes that there are persistent DNA-lesions which have a low probability of giving mutations.
As explanation for the observed delayed mutation the hypothesis of induction of infidelity of DNA synthesis is preferred to an explanation based on persistent DNA-lesions (kinetics of the delayed response; number of ethyl adducts available for the number of observed mutations; modulation of the delayed mutational response by 3-aminobenzamide; correspondence with the spontaneous mutation spectrum).

J 226 HPRT SEQUENCE ALTERATIONS IN MALE PATIENTS RECEIVING CISPLATIN-BASED TREATMENT, T.R. Skopek, N.F. Cariello, T.R. Craft, J.E. Cochrane, J. Nicklas[a], J.P. O'Neill[a], and R. Albertini[a]. University of North Carolina, Chapel Hill, NC 27599 and [a]University of Vermont, Burlington, VT 05401.

We are determining the DNA sequence specificity of mutagenesis occurring in vivo in the peripheral T-lymphocytes of male patients receiving cisplatin-based therapy. For comparison, the mutational specificity of cisplatin is also being determined in vitro under controlled culture conditions. The genetic target in both the in vivo and cell culture studies is the X-linked hypoxanthine-guanine phosphoribosyl transferse (hprt) gene. In vivo mutational specificity is determined primarily by automated DNA sequence analysis of PCR-amplified hprt cDNA from 6-thioguanine-resistant mutant colonies. Mutants displaying aberrant mRNA splicing (primarily exon loss) are further characterized by sequence analysis of the appropriate exon/intron regions of hprt following PCR amplification from genomic DNA. Complex mutant populations generated in vitro with cisplatin are being analyzed using denaturing gradient gel electrophoresis (DGGE). The in vivo spectrum collected to date is a complex mixture of base substitutions, frameshifts, and deletions in the hprt coding region at both AT and GC base pairs, as well as mutations leading to aberrant hprt mRNA splicing. Many of the mutations observed were associated with Pu-Pu or Pu-X-Pu sequences, regions known to be adducted by cisplatin. Only 6/32 of the mutant sites observed in the coding region of cisplatin-treated patients have been seen previously in vivo in unexposed individuals. Preliminary work in vitro has revealed four mutant sites in exon 3, one of which has been observed in vivo in a cisplatin patient.

J 227 MISMATCH REPAIR IN EXTRACTS OF HUMAN CELLS, David C. Thomas, John D. Roberts and Thomas A. Kunkel, Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

We have detected a general repair process for DNA heteroduplexes in HeLa cell extracts. Using a variety of M13mp2 DNA substrates containing single base mismatches and extrahelical bases, extensive repair is observed after incubation with HeLa cell cytoplasmic extracts and subsequent transfection of bacterial cells with the treated DNA. Most, but not all, mispairs as well as two frameshift heteroduplexes are repaired efficiently. Parallel measurements of repair in HeLa extracts and in *E. coli* suggest that repair specificities are similar for the two systems. The presence of a nick in the molecule is required for efficient repair in HeLa cell extracts, and the strand containing the nick is the predominantly repaired strand. Mismatch-dependent DNA synthesis is observed when comparing radiolabeled restriction fragments, produced by reaction of the extract with heteroduplex and homoduplex molecules. Specific labeling of fragments, representing a region of approximately 1000 base pairs and containing the nick and the mismatch, is detected for the heteroduplex substrate but not the homoduplex. The repair reaction is complete after 20 minutes and requires added Mg++, ATP and an ATP-regenerating system, but not dNTPs, which are present at sufficient levels in the extract. An inhibitor of DNA polymerase β, ddTTP, does not inhibit mismatch-specific DNA synthesis. Aphidicolin, an inhibitor of DNA polymerases α, δ and ε, inhibits both semiconservative replication and repair synthesis in the extract. Butyl-phenyl dGTP also inhibits both replicative and repair synthesis, but at a concentration known to preferentially inhibit DNA polymerase α rather than δ or ε. This suggests that DNA polymerase α may function in mismatch repair. Overall, this general repair system is very similar to the methyl-directed mismatch repair pathway in *E. coli*, but may recognize nicks instead of undermethylation as the signal to direct repair to the newly synthesized strand at the replication fork.

Oncogenes and Tumor Suppressors

J 300 SCRUTINY OF THE HUMAN K-ras-2 GENE FOR MUTATIONS USING DENATURING GRADIENT GEL ELECTROPHORESIS, Ezra S. Abrams and Leonard Lerman, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139. Denaturing Gradient Gel Electrophoresis (dgge) can be used to detect single base changes in DNA fragments of up to several hundred bp in length. We have used the PCR to amplify the five coding exons of the K-ras-2 gene, so that mutations in each exon can be detected. However, maximal sensitivity is obtained only by careful choice of PCR oligonucleotides, and by addition of a GC clamp to each fragment. Computer programs which predict the melting properties of any DNA sequence were used to select PCR amplimers for each of the coding exons of the K-ras-2 gene. Up to three sets of PCR amplimers can be used simultaneously for both the PCR and subsequent analysis on a DGG. We have found 3 regions of the K-ras-2 gene which are polymorphic in the population; these DGG polymorphisms are two or three allele systems, and appear to be distinct from the known RFLPs in this gene. We are currently screening DNA from individuals with different tumors; DNA from both tumor and non-tumor tissue is analyzed to distinguish mutations from polymorphisms.

J 301 LACK OF INVOLVEMENT OF BCL-2 AND C-MYC ONCOGENES IN SAUDI NON-HODGKIN'S LYMPHOMA, Chaker N. Adra, Magid Amer, M. Ashraf Ali, Mohammed Akhtar, Abdulrazzaq Haider and Mohammed Hannan. Department of Biological & Medical Research (MBC-03), King Faisal Specialist Hospital & Research Centre, P.O. Box 3354, Riyadh 11211, Saudi Arabia

Malignant lymphoma appears to be (relatively) the most common neoplasm in the Kingdom of Saudi Arabia constituting approximately 11% of all malignancies. We have studied 17 cases of non-Hodgkin's lymphomas (NHLs) at the immunohistochemical and molecular level. The selected cases included 3 low grade, 11 intermediate grade and 3 high grade NHLs (classified according to the international Working Formulation). An overwhelming majority of these lymphomas had a diffuse growth pattern. Immunophenotyping with a panel of monoclonal antibodies stained the neoplastic cells from all cases with HLA-DR, with the B-cell markers, CD19, CD22, but did not stain these cells with the T-cell markers CD2, CD3, CD4 and CD5. Monoclonal immunoglobulin κ and λ light chains were demonstrated in 10 cases (6κ, 4λ). By Southern blot analysis, clonal rearrangement of the immunoglobulin heavy chain gene was detected in 7 cases (2 low grade, 5 intermediate grade). These data suggested that the neoplastic cells of all the selected cases have a B-cell phenotype and have a monoclonal origin. The oncogene bcl-2 was in the germ line configuration in all cases except one. A non-germ line c-myc-related fragment was detected in two high grade tumors, the β and γ chain genes of the T-cell receptor were in the germ line configuration in all. None of the cases, however, showed an enhanced level of bcl-2 or c-myc RNA by Northern blot analysis. The lack of overexpression of bcl-2 and c-myc in the tumor cells suggested that these two oncogenes may not play an important role in the Saudi NHLs studied. A similar observation was made in cutaneous B-cell lymphoma (Delia *et al.*, Cancer Research 49: 4901, 1989). The relevance of these findings in two different types of lymphomas needs further investigation.

J 302 IDENTIFICATION OF HUMAN DNA SEQUENCES WHICH ARE INVOLVED IN THE MACHINERY OF TUMOR SUPPRESSION, Sergei Arsenian, Eva Uzvölgyi, George Klein, and Janos Sumegi. Department of Tumor Biology, Karolinska Institutet, Box 60400, S-104 01, Stockholm, Sweden.

Retinoblastoma (Rb) is a prototype model for the study of recessive oncogenes. The loss of activity of both normal alleles of this cancer supressor function confers susceptibility to hereditary retinoblastoma. Recent studies indicate that homozygous inactivation of both Rb alleles has been observed in different types of tumors including osteosarcoma, synovival sarcoma and other softtissue sarcomas, small-cell lung carcinoma, and breast carcinoma. This suggest a broader role for the retinoblastoma proteingene in cancer supression. With the help of retrovirus mediated gene transfer we could introduce the intact Rb gene into retinoblastoma and osteosarcoma cells that had inactivated endogenous Rb genes. We could demonstrate that expression of the exogenous Rb gene supressed the neoplastic phenotype of the target cells. Using subtractive cDNA techniques we are going to isolate DNA sequences which show different level expression in retinoblastoma cells compared to reconstituted cells. We wish to characterize these DNA sequences and understand their role in the tumor suppression.

J 303 MALIGNANT TRANSFORMATION OF HUMAN FIBROBLASTS WITH A MUTANT FORM OF BETA-ACTIN TOGETHER WITH A *RAS* ONCOGENE OR C-*MYC*, Lucy R. Bahn, Steven G. Bacsi, Bert N. Fukunaga and Winston A. Salser, Department of Biology, University of California, Los Angeles, CA 90024

Transformation to the tumorigenic state is more difficult to accomplish *in vitro* with human cells than with rodent or other mammalian cells. We have been able to induce fully malignant tumors in Hut12 human fibroblasts (a non-tumorigenic fibrosarcoma line) by transfection with a mutant form of the human *ß-actin* gene (sm *ß-actin* gly-244 -> asp-244, Leavitt 1987: MCB 7(7)) together with *ras* (EJ bladder carcinoma, EJ-*ras*) or c-*myc* (R. Weinberg). We have also induced tumorigenicity in KMST-6 cells, a non-tumorigenic cell line derived by mutagenesis of normal human fibroblasts (M. Namba), using sm *ß-actin* (3 tumors/8 mice injected).

DNA was transfected into cells with the *neo* gene used as a G418 selectable marker. Transfected cells were injected s.c. into nude mice (5x106 cells/mouse) and resultant tumors were allowed to grow to at least 1 cm in diameter. Cells from excised tumors were cultured in the presence of G418. Genomic integration of transfected DNA in the tumor cells was confirmed by Southern blotting. Presence of the mutant form of *ß-actin* was verified using PCR. When the oncogenes were transfected singly, 18 of 96 mice developed tumors (c-*myc* 0%, EJ-*ras* 30% and sm *ß-actin* 19%). Of these tumors none were accompanied by metastases or invasion. However, when combinations of the oncogenes were transfected, 31 of 70 mice developed tumors and of those tumors 5 presented with metastases or invasion. In marked contrasts to results reported by others with rodent cells we found that use of super coiled (sc) plasmid was much more effective than linearized DNA for the transfection of these human cells. The transfection efficiency using linearized DNA was <.01% while the efficiency for sc DNA ranged from 1 to 10%. The tumorigenicity of the cells transfected with linear DNA was also much less than cells transfected with sc DNA. Only 10% of linear DNA transfected cells formed tumors while 64% of cells transfected with sc DNA formed tumors in the case of *ß-actin* alone. Furthermore, the latency period to tumor formation was reduced from one month or more with linear DNA to less than two weeks with sc DNA. Growth rate of tumors with linear DNA was about 1 mm (diameter) per week. In contrast, tumors induced with sc DNA grew by at least 5 mm per week.

We have converted non-tumorigenic cells to the fully malignant state using a combination of a mutant *B-actin* gene and c-*myc* or EJ-*ras*. One of the non-tumorigenic cell lines used was derived from a fibrosarcoma, however, *ß-actin* used alone was also sufficient to induce tumorigenic behavior in KMST-6 cells, derived from normal human fibroblasts. Interestingly, the transfection efficiency and incidence, growth rate and latency of tumor formation were all greatly improved by the use of sc DNA.

J 304 MOLECULAR GENETIC ALTERATIONS IN HUMAN ENDOMETRIAL CARCINOMA, Jeff Boyd*, John I. Risinger*, David G. Walmer¶, Bert Vogelstein§, and J. Carl Barrett*, *Laboratory of Molecular Carcinogenesis, NIH/NIEHS, Research Triangle Park, NC 27709, ¶Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC 27710, §Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, MD 21231.

Mutations and reduced expression of the DCC gene were recently demonstrated to occur in human colorectal carcinomas, implying a tumor suppressive function for the DCC gene product. In order to assess the potential relevance of DCC mutations to endometrial carcinoma, we examined cell lines from 11 human tumors. Structural alterations in the DCC gene were observed in 5 of 11 (45%) endometrial carcinomas. These represented insertional mutations similar to that previously observed in colorectal carcinoma. In addition, DCC expression was greatly reduced or absent in 7 of 11 tumors, but expressed in normal human endometrium. Such abnormalities in DCC structure or expression were not observed in cell lines from human carcinomas of the uterine cervix, ovary, or breast. In addition, point mutations in a ras allele were found in 6/11 endometrial tumor lines, including four at K-ras/12 (GGT→GAT in two and GGT→GTT in two), and three at H-ras/61 (CAG→CAT). One tumor contained a mutation at K-ras/12 and H-ras/61. These data suggest that mutations in the DCC and ras genes occur with high frequency in human endometrial carcinoma.

J 305 INTRODUCTION OF CHROMOSOMES 11 AND 3 MODIFY THE GROWTH AND TUMORIGENICITY OF A HUMAN EPIDERMOID SQUAMOUS CELL CARCINOMA CELL LINE, Kathleen Conway and Bernard E. Weissman, Lineberger Cancer Research Center, University of North Carolina Medical School, Chapel Hill, NC 27599

The deletion or inactivation of tumor suppressor genes is implicated in the etiology of most human cancers. Karyotypic deletions and loss of heterozygosity for specific chromosomal loci have been reported in squamous cell carcinomas (SCC) of the lung, however, few studies have examined the genetic defects associated with the development of skin SCCs. Introduction of single human chromosomes into tumorigenic recipient cell lines by microcell fusion provides a method of identifying chromosomes that carry suppressor genes. In this study, introduction of a normal human chromosome 11 into an HPRT-deficient variant of the near-diploid human skin SCC cell line, A388, resulted in a partial suppression of its tumorigenicity in athymic nude mice without an alteration of in vitro growth characteristics. We observed a delayed latency (>75 days) and a slightly reduced tumor take in the chromosome 11 microcell hybrids as compared with the A388 parent which forms tumors with a latency of 1 week. Introduction of a t(X;3p) chromosome resulted in the formation of microcell hybrids with altered morphology and reduced growth rate. The 3p hybrid colonies were extremely compact and stratified suggesting that 3p may promote cellular differentiation. As the 3p hybrids were passaged, they simultaneously lost both the altered morphology and the introduced 3p chromosome. We are currently evaluating the putative differentiation-promoting effect of chromosome 3p using differentiation markers as well as localizing the site on chromosome 11 that produces the suppression of in vivo growth in the A388 SCC line. This work was supported by grant number CA39602 from the National Cancer Institute.

J 306 IDENTIFICATION OF A MUTATION IN THE RB1 GENE WHICH RESULTS IN REDUCED PENETRANCE IN FAMILIAL RETINOBLASTOMA. Jean Couture[1], Karen Peterson[2], Janet Gruhn[1], Carmen Sapienza[2], Louise C. Strong[1] and Marc F. Hansen[1], [1]Department of Molecular Genetics, University of Texas M.D. Anderson Cancer Center, Houston, TX 77030 and [2]The Ludwig Institute for Cancer Research, Montreal, Canada H3A 1A1

Familial retinoblastoma is characterized by autosomal dominant inheritance with a high penetrance and multiple tumor clones per gene carrier. We have observed a rare family with 5 retinoblastoma cases in 3 sibships with at least 4 unaffected carriers. Karyotypic analysis excluded translocation and linkage analysis, using several polymorphic loci within and flanking the RB1 gene, revealed a common chromosome 13 in all affected and several unaffected members. We hypothesized that this family carried a mutation in the RB1 gene that coded for an altered, but partially functioning protein. To test this, we examined RNA from a lymphoblastoid cell line from an affected family member. Reverse transcription and PCR analysis were done using 3 overlapping primer sets spanning the entire exon sequence of the RB1 gene. We consistently obtained one product corresponding to the normal RNA and one that was approximately 600 bp smaller.The smaller RNA product was sequenced and shown to have a deletion of exons 16 - 20 due to an inframe mutation that eliminated a splice acceptor site. We conclude that the mutant RB1 gene most likely results in a partially functional protein, accounting for the reduced incidence of tumors in the individuals who carry it.

J 307 PROTO-ONCOGENE ACTIVATION IN LIVER AND LUNG TUMORS FROM B6C3F1 MICE EXPOSED CHRONICALLY TO METHYLENE CHLORIDE. T.R. Devereux, J.F. Foley, R.R. Maronpot, F. Kari and M.W. Anderson, Nat. Inst. Env. Hlth. Sci., Research Triangle Park, NC 27709. Methylene chloride has been the subject of recent toxicological and carcinogenesis studies because of significant human exposure and widespread use in industrial processing, food preparation and agriculture. In this study, liver and lung tumors, induced in female B6C3F1 mice by inhalation of 2000 ppm methylene chloride (5 hours/day, 5 days/week continuous exposure), were examined for the presence of activated proto-oncogenes. DNA from 50 liver tumors was screened by sequencing and oligonucleotide hybridization of PCR amplified ras gene fragments for H-ras second exon mutations. 35 mutations were detected in the 61st codon, 16 C to A transversions in base 1, 15 A to G transitions in base 2 and 4 A to T transversions in base 2. Since this mutation profile appears to be similar to that identified for the H-ras gene in spontaneous liver tumors, methylene chloride may act by promoting cells with spontaneous lesions. DNA from 22 lung tumors was screened for K-ras first and second exon mutations, and 6 mutations were identified, 2 in exon one and 4 in exon 2. The exon one mutations included a G to T transversion in the 2nd base of codon 12 and a G to C transversion in the first base of codon 13, while the 4 mutations identified in exon two were all in the third base of codon 61; three were A to C transversions and one was an A to T transversion. This incidence of K-ras gene activation is greater than that observed with spontaneous lung tumors from this resistant mouse strain. The nude mouse transfection assay is being used to examine for the presence of other activated proto-oncogenes in the tumors which were negative in the PCR analysis for ras mutations.

J 308 FLUCTUATION ANALYSIS OF v-Ha-ras TRANSFORMED HUMAN BRONCHIAL EPITHELIAL CELLS. Kathleen Forrester[1], Jennifer Kispert[1], Andres Klein-Szanto[2], Kaija Linnainmaa[3], Brenda I. Gerwin[1] and Curtis C. Harris[1], [1]Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892, [2]Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111, [3]Institute of Occupational Health, Topeliuksenkatu 41aA, SF00250 Helsinki, Finland.

Infection of nontumorigenic SV40 T "immortalized" human bronchial epithelial cells (BEAS-2B) with a Zip-v-Ha-ras vector led to a mass culture (BZR) which was tumorigenic in nude mice. Tumor cell lines (BZR-T33, BZR-T35) derived from passage of the mass culture in vivo exhibit increased tumorigenicity, v-Ha-ras protein level and gene copy number. In order to determine whether this resulted from selection of more tumorigenic cells from a heterogeneous population or a progression in tumorigenicity during passage in vivo, individual BZR clones were isolated. These clones exhibited varying degrees of tumorigenicity. The most tumorigenic clone (clone 10) had an increased v-Ha-ras copy number and protein level, while a nontumorigenic clone did not express the exogenous gene. In addition, the integration sites of the v-Ha-ras gene were similar in clone 10 and BZR-T33. These data suggest that selection of a pre-existing cell from the BZR culture occurred upon injection into nude mice. However, one clone with an intermediate level of tumorigenicity did not express the v-Ha-ras gene suggesting that additional mechanisms are involved in this retrovirally induced tumorigenic conversion.

J 309 POLYMERASE CHAIN REACTION ANALYSIS OF LOSS OF HETEROZYGOSITY IN OVARIAN CANCER, Maurizio Genuardi, Paola Tanci and Giovanni Neri, Istituto di Genetica Umana, Università Cattolica, Rome, Italy.

The identification of regions of the human genome containing tumor suppressor genes is based upon detection of specific deletions at high frequency in tumor samples. This goal has been achieved so far by both cytogenetic analysis and allele dosage studies of polymorphic loci. In order to speed up the search for loss of heterozygosity, we have devised a method which relies upon PCR amplification of polymorphic DNA sequences, followed by quantitative assessment of the amplified fragments by densitometry. A highly polymorphic VNTR locus from chromosome 17p, D17S5, which maps close to the P53 gene, was amplified starting from target DNAs extracted from matched pairs of ovarian carcinomas and normal tissues from the same patients. Two amplification products of different lenght, corresponding to the two alleles, were observed in all samples, both tumor and normal. However, in 4/12 (33.3%) cases, the yield of one allele was much lower (<50%) in tumor relative to constitutional DNA, as expected when the target sequence is underrepresented consequent to a deletion present in a relevant fraction of the cells examined. These results were independently confirmed by Southern blot analysis performed on 10 /ug of genomic DNA from each sample.

J 310 PCR AMPLIFICATION AND DIRECT SEQUENCING OF THE p53 GENE IN SPONTANEOUS AND CHEMICALLY-INDUCED CD-1 MOUSE LIVER AND LUNG TUMORS, Tamra L. Goodrow, Karen R. Leander, Richard D. Storer, and Matthews O. Bradley, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

Inactivating point mutations and/or small deletions in the p53 gene have been found in human liver and lung tumor cell lines and in human lung tumors. The purpose of this work is to evaluate spontaneous and chemically-induced CD-1 mouse liver and lung tumors for mutations in critical regions of the p53 gene. We have devised a simple sequencing strategy for analysis of exons 5-8 of the mouse p53 gene. In order to prevent amplification of the processed pseudogene, we sequenced introns 5, 6, 7 and 8 and then amplified a 1.45 Kb fragment of the gene from genomic tumor DNA samples using specific primers internal to introns 4 and 8. Sequencing primers located 20-35 bases inside the intron-exon junction of each exon were used with standard dideoxy sequencing methods. Nine spontaneous hepatocellular carcinomas (HCC), eight N-nitrosodiethylamine-induced HCC, and eight 7,12 dimethylbenzanthracene-induced HCC were analyzed for mutations using this strategy. Preliminary evidence indicates that there are no mutations in exons 5-8 present in any of the tumors examined. Analysis of murine lung tumors is currently in progress.

J 311 ALTERED EXPRESSION OF PUTATIVE TUMOR SUPPRESSOR GENE p53 ASSOCIATED WITH MALIGNANT CONVERSION IN A MURINE MULTISTEP CELL TRANSFORMATION MODEL, Kyung-An Han and Molly F. Kulesz-Martin, Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, NY 14263.

In order to understand the genetic basis of multistage carcinogenesis, an epidermal cell model is used in which initiated, benign tumor-producing and carcinoma stages were derived from a cloned parental cell strain. The normal parental cells were cultured under conditions favoring either proliferation or differentiation to control for differences due to normal functional states. An increased steady-state level of p53 RNA was detected in moderately- and poorly-differentiated carcinoma cells compared to papilloma and normal epidermal cells. However, abnormal expression of p53 was not detectable in the nontumorigenic initiated cell precursors of each carcinoma cell lineage, suggesting that altered p53 expression occurred during malignant conversion. Immunoprecipitation and western immunoblot analyses have shown that the level of p53 protein in moderately-differentiated carcinoma cells was elevated, while p53 protein in the poorly-differentiated carcinoma cells was negligible compared to normal parental cells. Attempts to identify p53 mutations associated with these malignant changes are in progress by means of the polymerase chain reaction and sequencing. Supported by NCI Grants CA31101, CA24538, CA16056, and a Biomedical Research Support Grant S07 RR05648.

J 312 EXPRESSION OF PROTO-ONCOGENES IN TROPHOBLAST FROM ANEMBRYONIC PREGNANCY. Deborah J Henderson and Gudrun E Moore. Action Research Laboratory, Institute of Obstetrics and Gynaecology, Queen Charlotte's and Chelsea Hospital, RPMS, London, W6 0XG. U.K.

Up to 16% of pregnancies are anembryonic - a condition where the fetus is absent but the trophoblast remains viable for up to 8 to 14 weeks. The causes of anembryonic pregnancy are unknown, although there are similarities with complete hydatidiform mole. Complete moles have only a paternal contribution to their genome. Our studies have revealed that trophoblast from anembryonic pregnancy has a genetic contribution from both parents and therefore does not appear to arise by the same genetic process as complete hydatidiform mole.

Proto-oncogenes are thought to have important roles in growth and development. Expression of certain cellular oncogenes may control rapid cell growth in the early placenta. Placenta has been described as pseudo-malignant tissue because of the way it proliferates rapidly and invades the myometrium, but how this differs from malignant transformation of trophoblast is unclear.

Complete hydatidiform mole is associated with an increased risk of choriocarcinoma. Complete mole has been shown to have abnormal expression of certain proto-oncogenes, in a pattern similar to that in choriocarcinoma cell lines. Studies have suggested that there may be higher rates of hydatidiform mole and choriocarcinoma in women with a history of spontaneous abortion, many of which may have been anembryonic.

We are studying the expression of various proto-oncogenes in trophoblast from anembryonic pregnancy and in normal age matched controls.

J 313 GROWTH FACTOR EXPRESSION IN HUMAN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK. Carol Bova Hill, Tim Lansing and Tona Gilmer. Department of Chemotherapy, Glaxo Research Labs., RTP, NC 27709.

Tumor progression is often marked by an increase in growth factor expression as compared to normal tissue, providing a possible growth advantage for the aberrant cells. The growth factor expression may or may not be a reflection of increased genomic copy number. Our laboratory and others have demonstrated DNA amplification in human squamous cell carcinoma of the head and neck for two members of the heparin-binding growth factor family (HBGF), int-2 (HBGF-3) and hst (HBGF-4), colocalized to chromosome 11q13. We were interested in the expression of these growth factors in addition to other growth factors, TGF-β1, TGF-α, bFGF (HBGF-2), and FGF-5 (HBGF-5). We have analyzed 31 tumors and regional lymph node metastases for expression by Northern blot analysis as compared to 6 normal tissue samples. Only 2 samples showed expression of int-2 while no hst mRNA was detected. Approximately 30% of the samples showed detectable levels of FGF-5 and bFGF. Varying levels of TGF-β1 were also found in the tumor tissue with very low expression found in normal tissue. Approximately 50% of the samples expressed TGF-α, and TGF-α was coordinately expressed with EGF-receptor mRNA. Similar expression patterns were seen in eight independent cell lines derived from human head and neck squamous cell carcinomas. We conclude that the expression of these growth factors may be important in the growth of these tumors.

J 314 IDENTIFICATION OF DNA SEQUENCES SPECIFICALLY BOUND TO HUMAN RETINOBLASTOMA GENE PRODUCT, pp110RB, Frank Hong, Pascale Rio, Yuewei Qian, Chi-Yao Chang, Nan Ping Wang, Eva Y.-H. P. Lee and Wen-Hwa Lee, Department of Pathology, University of California at San Diego, La Jolla, CA 92093

Complete inactivation of the human retinoblastoma gene (RB) is considered essential in genesis of retinoblastoma and several other tumors. Following the cloning of RB gene, its encoded product was identified as a nuclear phosphoprotein associated with a DNA binding activity. A detailed analysis using various fusion RB proteins has subsequently revealed that its DNA binding activity may reside in its C-terminal part. To answer whether RB-DNA interaction is of a specfic nature, several approaches were undertaken to characterize DNAs that are bound to RB protein. This required us to prepare RB protein in a highly purified form and engineering DNAs to be PCR amplifiable. Here we show evidence that RB protein can indeed exist in a complexed form with DNA. Sequences of the DNAs specifically bound to RB protein are described.

J 315 IDENTIFICATION OF A CELLULAR PROTEIN THAT COMPETES WITH SV40 LARGE T ANTIGEN FOR BINDING TO THE RETINOBLASTOMA GENE PRODUCT, Shi Huang, Wen-Hwa Lee and Eva Y.-H. P. Lee, Department of Pathology, School of Medicine, University of California, San Diego, La Jolla, CA 92093-0612

Tumor suppressor genes, such as the human retinoblastoma susceptibility gene (Rb), have become widely recognized recently as vital elements in control of cell growth and tumor formation. This role is indicated, in part, by the suppression of tumorigenicity of human tumor cells after retrovirus-mediated Rb replacement. How Rb acts to bring about this suppression is not clear, but one clue is that Rb forms complexes with the transforming oncoproteins of several DNA tumor viruses, and that two regions of Rb essential for such binding are frequently mutated in tumor cells. These observations suggested that endogenous cellular proteins might exist that bind to the same regions of Rb and thereby mediate its function. We report here the identification of one such Rb-associated protein of M_r 46,000 (RbAP46) that is present in several human cell types. Two lines of evidence support the notion that RbAP46 and SV40 T antigen have homologous Rb-binding properties: first, several mutated Rb proteins that failed to bind to T also did not associate with RbAP46; and second, both T antigen and T peptide (aa 99-120) were able to compete with RbAP46 for binding to Rb. The apparent targeting of the RbAP46-Rb interaction by oncoproteins of DNA tumor viruses strongly suggests that formation of this complex is functionally significant.

J 316 Transcriptional repression of the Her-2/*neu* protooncogene by transforming oncogenes from DNA tumor virus. Mien-Chie Hung, Dihua Yu, Duen-Hwa Yan, Rong-lang Yen, and Anngabin Matin, Department of Tumor Biology, The University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030 The adenovirus E1A,SV40 large T antigen and E7 of human papilloma virus 16 are known to regulate gene expression at the transcriptional level. In this report, we demonstrate that transcription of HER-2/*neu* protooncogene can be strongly repressed by these three genes in Rat-1 cells. The transcriptional repression induced by E1A is attributed to the conserved region 2 of the E1A proteins, that is required for binding to the retinoblastoma (RB) gene product. The target for E1A repression was localized within a 139-bp DNA fragment in the upstream region of Her-2/*neu* promoter. These results indicate that E1A negatively regulates Her-2/*neu* gene expression at the transcriptional level via a specific DNA element. The DNA region responding to large T and E7 gene products and the effect of RB protein binding to these three DNA tumor virus gene products on *neu* gene expression are currently under investigation.

J 317 CHROMOSOMAL DELETIONS IN OSTEOSARCOMA . Kanji Ishizaki, Toshikazu Yamaguchi (Radiation Biology Center, Kyoto University), Junya Toguchida, Yoshihiko Kotoura, Takao Ymamuro (Dept. Orthopedic Surgery, Fac. Med., Kyoto University)

We have analyzed allelic deletions at polymorphic loci located on each chromosome in osteosarcomas by using polymorphic DNA markers(obtained from Dr. Y. Nakamura, Cancer Institute, Tokyo). So far, we have analyzed chromosomes 1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22. All chromosomes analyzed showed allelic deletions with frequencies of 4 to 80 %. Among them chromosome 13 and 17 , both of which harbors tumor suppressor genes, showed significantly high frequencies of allele loss, 70 % for #13 and 74 % for #17. We have analyzed structural anomalies of the Rb gene on #13 and the p53 gene on #17 by Southern hybridization and found structural changes of the Rb gene in 43 % of tumors and those of the p53 gene in 28%. Cases with increased rate of chromosomal deletions in whole genome of tumor showed tendency to be worse prognosis. On the contrary, retaining of both copies of chromosome 17 was significantly correlated with good prognosis.

J 318 PURIFICATION OF XERODERMA PIGMENTOSUM GROUP A PROTEIN BY COMPLEMENTATION OF REPAIR-DEFECTIVE CELL EXTRACTS, Christopher J. Jones, Peter Robins, Maureen Biggerstaff, Tomas Lindahl and Richard D. Wood, Clare Hall Laboratories, Imperial Cancer Research Fund, South Mimms, Herts EN6 3LD, U.K.
Patients with the inherited disorder xeroderma pigmentosum (XP) have a high incidence of sunlight-induced skin cancers, and cell lines from these patients show a defect in DNA excision repair. We are studying this defect *in vitro* by incubating damaged circular plasmid DNA with extracts ("transcription/splicing-type") from normal and XP-derived human cell lines (Wood *et al.*, 1988, Cell, 53:97). A protein has been purified >5000 fold from calf thymus that corrects the repair defect in XP complementation group A cell extracts. The protein restores normal repair synthesis to extracts from three independently derived XP-A lymphoid cell lines, but does not complement extracts from XP-B, XP-C, or XP-D cell lines. The XP-A complementing activity copurifies with a DNA-binding polypeptide that migrates with a relative molecular weight of 42 kDa on SDS-polyacrylamide gels. The relationship of this protein to that encoded by a recently isolated XP-A complementing gene (Tanaka *et al.*, 1989. Proc. Natl. Acad. Sci. USA, 86:5512) is being investigated by peptide sequencing and immunological studies.

J 319 **EXPRESSION OF p53 ONCOPROTEIN CORRELATES WITH CESSATION OF PROLIFERATION IN HUMAN HEMATOPOIETIC CELLS, Michael B. Kastan and Ruth W. Craig, The Johns Hopkins Oncology Center, Baltimore, Md. 21205.**
Although recent data strongly suggests that the p53 gene product is a "tumor suppressor", the mechanism of action of p53 protein has been difficult to elucidate. Both the very short half-life of the wild-type form of the protein in normal cells and the inability to clone out cell lines expressing this inhibitory protein contribute to this difficulty. We utilized a recently developed two-color flow cytometric assay to measure relative levels of p53 protein in normal human hematopoietic cells (fresh bone marrow from normal volunteers). We found that p53 protein is undetectable in the proliferative progenitor cell populations, but is expressed in the non-proliferative, terminally-differentiated cell populations. This is the first demonstration that p53 protein expression is correlated with cessation of proliferation in a normal tissue in vivo. We also found that the majority of primary acute myeloid and lymphoid leukemias express low (suggestive of wild-type) levels of p53 protein, which demonstrates a significant difference between leukemia cells and the phenotypically similar normal hematopoietic progenitors and suggests that some leukemia cells can proliferate despite the expression of p53. Further, the myeloblastic leukemia cell line, ML-1, expresses p53 mRNA, but not p53 protein during log phase growth. However, TPA treatment of ML-1 cells, which results in a cessation of proliferation and differentiation to monocyte/macrophages, induces expression of p53 protein (despite decreases in p53 mRNA) in temporal association with cessation of proliferation. The low levels of p53 protein in ML-1 cells are strongly suggestive of the presence of the wild-type p53 gene. Lastly, we find that treatment of hematopoietic cells with low (non-lethal) doses of DNA damaging agents, such as gamma or UV irradiation or actinomycin D, causes an inhibition of DNA synthesis and a temporally associated transient increase in p53 protein levels. These results demonstrate that expression of p53 protein, but not mRNA, is closely linked to cessation of proliferation of human hematopoietic cells and that it may play a role in the cellular response to DNA damage, probably by contributing to the inhibition of DNA synthesis. The ML-1 cells should provide a useful model for studies of the biochemical mechanisms of action of p53 protein.

J 320 ORIGIN OF THE INITIAL MUTATION OF RETINOBLASTOMA GENE IN THE GENESIS OF NON-HEREDITARY RETINOBLASTOMA, Mitsuo Kato[1], Kanji Ishizaki[1], Yosuke Ejima[1], Akihiro Kaneko[2], Hiroshi Tanooka[3], Masao S. Sasaki[1], [1]Radiation Biology Center, Kyoto University, Kyoto 606, Japan, National Cancer Center, [2]Hospital and [3]Research Institute, Tokyo 104, Japan,
We studied the frequency of loss of heterozygosity (LOH) on chromosome 13 in the genesis of retinoblastoma (RB) and determined the parental origin of retained chromosome in tumors. In 30 (67%) of 45 cases, LOH was detected in at least one locus on chromosome 13. When these patients were classified into two groups, hereditary and non-hereditary forms, the LOH was detected in 14 (52%) of 27 hereditary cases and in 16 (84%) of 19 non-hereditary cases. Comparison with the results of hereditary cases indicates that the excess of LOH in non-hereditary tumors may be due to the involvement of the gross deletions as the initial somatic mutations. When this different pattern of initial mutations between hereditary and non-hereditary tumors is considered, approximately 70% of the initial somatic mutations are estimated to be gross deletions of the region involving the RB gene. In 7 non-hereditary tumors, 5 (71%) lost paternal allele and 2 (29%) lost maternal allele. Combining these data, we assume that, like sporadic osteosarcoma, the initial somatic mutations to non-hereditary retinoblastomas also preferentially occur on paternally derived chromosome.

J 321 p53 MUTATIONS, ras MUTATIONS AND p53-HEAT SHOCK 70 PROTEIN COMPLEXES IN HUMAN LUNG CARCINOMA CELL LINES, Teresa A. Lehman, William P. Bennett, Robert A. Metcalf, Judith A. Welsh, Jill Ecker, Brenda I. Gerwin and Curtis C. Harris, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892.
The K-ras oncogene and the p53 tumor suppressor gene are frequently mutated in human lung carcinomas. These mutated genes also cooperate in the immortalization and neoplastic transformation of rodent cells. To test the hypothesis that mutations in K-ras and p53 are necessary for maintenance of the immortalized and/or neoplastically transformed states of human bronchial epithelial cells, frequently mutated regions of the ras (K-, H- and N-) as well as the p53 gene were sequenced in an SV40 immortalized human bronchial epithelial cell line (BEAS-2B) and nine human lung carcinoma cell lines. Both ras oncogenes and the p53 genes were wild type in the immortalized BEAS-2B cells, and were frequently, but not always, mutated in the carcinoma cell lines. Detection of p53 mutations by polymerase chain amplification and direct DNA sequencing was corroborated by p53 immunocytochemistry and coimmunoprecipitation of p53 with heat shock protein 70. In conclusion, lung carcinomas are a genetically heterogenous group of tumors and the results suggest that ras and p53 mutations are frequently involved in lung carcinogenesis.

J 322 **A t(4;22) IN A MENINGIOMA POINTS TO THE LOCALIZATION OF A PUTATIVE TUMOR SUPPRESSOR GENE,** Ronald H. Lekanne Deprez, Nicole A. Groen, Nick A. van Biezen and Ellen C. Zwarthoff, Department of Pathology, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, the Netherlands.
Meningioma presumably develops when both alleles of a suppressor gene on chromosome 22 are inactivated. Cytogenetic analysis of meningioma cells from one particular patient (MN32) displayed the following stem line karyotype: 45, XY, -1, 4p+, 22q-, 22 pter->q11::1p11->qter, thus with rearrangements of both chromosomes 22. Using hybrid cell lines in which the 22q- and 4p+ markers are segregated we showed that these markers were the product of a reciprocal translocation t(4;22)(p16;q11). Based on these results we presume that in this tumor a tumor suppressor gene is deleted in the case of the dicentric marker and that the t(4;22) disrupts the second allele of this gene. We are trying to map the breakpoint in the latter translocation using CHEF electrophoresis. To aid our search for the gene 25 single copy probes for chromosome 22 were isolated and assigned to different regions of the chromosome. These probes were tested for their ability to detect polymorphisms in human DNA (RFLP's) and were used for the construction of a long-range physical map. Chromosome 22 contains about 55 Mb of DNA, so far we have identified about 15 distinct Not I fragments with a total length of 10 Mb.

J 323 ONCOGENE EXPRESSION LEVELS IN NITROFURAN TRANSFORMATION OF RAT BLADDER EPITHELIAL CELLS, Angela M. Mann, Tsuneo Masui, Timothy L. Macatee, Samuel M.Cohen, Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-3135.
A rat bladder carcinogenesis model using the nitrofuran, N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT), and sodium saccharin (NaS) has been established by our laboratory. An *in vitro* model, utilizing 2-amino-4-(5-nitro-2-furyl)thiazole (ANFT), the water soluble metabolite of FANFT, followed by NaS or NaS/urea, has allowed further investigations into the effects of these chemicals on primary rat bladder epithelial cells at the molecular level. Initially, we examined the expression levels of the *ras* product (p21) and the mutations in the coding region of *ras* genes from cell lines established from FANFT-induced bladder carcinomas as well as from bladder epithelial cells transformed by ANFT *in vitro*. In addition, since it has been shown that more than one event is necessary for full transformation, we have compared the transcript levels of several other oncogenes (neu, src, mos, myb, myc, fos, jun) to clarify what role they play in chemical transformation in this system.

J 324 H-ras MUTATIONS IN RAT URINARY BLADDER CARCINOMAS INDUCED WITH N-[4-(5-NITRO-2-FURYL)-2-THIAZOLYL]FORMAMIDE (FANFT) AND SODIUM SACCHARIN, SODIUM ASCORBATE, OR OTHER SALTS. Tsuneo Masui, Angela M. Mann, Timothy L. Macatee, and Samuel M. Cohen Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198-3135.
Male F344 rats were given 0.2% FANFT for 6 wk, then given 3% or 5% sodium saccharin, 5% sodium ascorbate, 3.12% calcium saccharin, 1.34% sodium chloride, or 5.2% calcium saccarin + 1.34% sodium chloride for 72 wk. Protein and DNA were extracted from 80 bladder transitional cell carcinomas (TCCs) out of 78 rats. P21 expression was examined by Western blotting using monoclonal antibody against p21 (NCC-RAS 004). H-ras mutation was examined by direct sequencing of amplified DNA including H-ras exons 1 and 2. Sequencing results obtained presently showed mutations at codon 61 (CAA to CGA in 11 TCCs; to CTA in 1 TCC) and at codon 13 (GGC to GTC in 3 TCCs). Mutations at codon 61 were confirmed by faster mobility of p21 band in Western blots. Codon 13 mutants did not show change in mobility of p21 band. There was no remarkable difference in the level of p21 expression among tumor samples. Overall incidence of H-ras mutation among bladder carcinomas was 18.75% (15/80) and there appears to be no difference in the incidence and pattern of H-ras mutation among different treatment groups.

J 325 LOSS OF THE TUMOR SUPPRESSOR PHENOTYPE IS ACCOMPANIED BY ALTERATIONS IN THE NUCLEAR MORPHOLOGY OF SYRIAN HAMSTER OVARY CELLS, *Kenneth J. Pienta, Jeffery Boyd, J. Carl Barrett, and *Donald S. Coffey, *Johns Hopkins Oncology Center, Baltimore, MD 21205 and the National Institute of Environmental Health Sciences, Res. Triangle Park, NC 27709
A central and early event in chemical, viral, and spontaneous carcinogenesis is an alteration in nuclear morphology. These alterations include changes and variations in nuclear size and nuclear shape. Such changes in nuclear morphology have been a hallmark in the diagnosis of cancer. It is unknown as to what causes the nuclear changes and here we study this problem in relation to suppressor gene function. The Syrian Hamster Embryo (SHE) cell line is a useful model to study changes in cellular structure and function because it can be transformed in discrete stages. Using this model we demonstrate that immortalization of SHE control cells with either asbestos or the synthetic estrogen diethylstilbesterol (DES) does not alter nuclear shape. However, as these cells are progressively passaged, subclones are generated which have lost the ability to suppress tumor growth when hybridized to fully transformed tumor cells. These subclones, which have lost the suppressor phenotype, demonstrate concurrent alterations in their nuclear shape as demonstrated by electron microscopy and a quantitative microscopic assay. Loss of the suppressor phenotype may be the early paraneoplastic event which causes the alterations in nuclear morphology which are seen in virtually all types of tumors.

J 326 Ha-ras GENE MUTATIONS IN PRIMARY HUMAN SKIN CANCERS AND IN NIH 3T3 CELLS TRANSFORMED BY IN VITRO UV-IRRADIATED Ha-ras PROTO-ONCOGENE DNA. William E. Pierceall and Honnavara N. Ananthaswamy, Department of Immunology, University of Texas M.D. Anderson Cancer Center, Houston, TX 77054.
Our previous studies have shown that human skin cancers occurring on sun-exposed body sites frequently contain activated Ha-ras oncogenes capable of inducing morphologic and tumorigenic transformation of NIH 3T3 cells. In this study, we analyzed human primary squamous cell carcinomas (SCC) and basal cell carcinomas (BCC) occurring on sun-exposed body sites for mutations in codons 12/13 and 61 of Ha-, K- and N-ras oncogenes by amplification of genomic tumor DNAs by polymerase chain reaction followed by dot blot hybridization to synthetic oligonucleotide probes designed to detect single base pair mutations. The results indicated that 11 of 24 (46%) SCC and 5 of 16 (31%) BCC contained mutations exclusively at the second position of Ha-ras 12th codon (GGC to GTC) resulting in a glycine to valine amino acid substitution. In addition to the mutations in the Ha-ras oncogene, three of the SCC also contained highly amplified copies of the N-ras gene in their genomic DNA. To examine whether UV radiation can induce similar mutations in the Ha-ras proto-oncogene, we irradiated the naked pEC plasmid DNA containing the normal human Ha-ras proto-oncogene with an FS-40 sunlamp (UV-B, 280-400 nm) and then transfected into NIH/3T3 cells using focus formation and cotransfection and tumorigenicity assays. Analyses of secondary or tertiary foci or nude mouse tumors revealed that most of the foci displayed GGC-GTC mutations in the second position of codon 12 (gly-val), whereas most of the nude mouse tumors displayed GGC-AGC mutations at the first position of codon 12 (gly-ser). Taken together, these studies indicate that Ha-ras is activated frequently in human non-melanoma skin cancers and that the activating mutations may have been induced directly by UV radiation present in sunlight. Supported by NIH grant R01 CA 46523.

J 327 EXPRESSION OF WT1, WILMS' TUMOUR GENE, IN FOETAL HUMAN KIDNEY AND WILMS' TUMOUR, Veronique Poirier, Sue J Tyler, Keith W Brown and Norman J Maitland, CLIC Research Unit, Department of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, UK

It is now well established that chromosome 11 is playing a major role in the development of Wilms' tumour, a childhood nephroblastic tumour. The cDNA of putative Wilms' gene (WT1) located at locus 11p13 has been recently isolated by D. Housman et al. Using in situ mRNA hybridisation, we confirmed the expression of the WT1 in developing tubules and blastema cells in human fetal kidney biopsies and mainly in blastema cells in Wilms' tumour biopsies (K. Pritchard-Jones et al 1990). In order to extend our research and make identification of the protein easier, divers WT1 restriction fragments from the 3'end of the cDNA have been cloned in bacterial expression vectors PEX1-3 (K K Stanley et al 1984). The latter is derived from a crolac Z gene fusion under a Pr promoter of bacteriophage , allowing any open reading frame DNA to be expressed as a hybrid β galactosidase protein. After injection into rabbit, polyclonal antibodies have been tested for immunofluorescence on cells in culture and tissue biopsies, and immunoprecipitation techniques. Data will be presented.

J 328 MAMMALIAN DNA TOPOISOMERASE II CLEAVAGE SITES IN THE HUMAN c-MYC GENE PROMOTER, Yves Pommier[1], Jean-François Riou[2], Ann Orr[1], and Kurt W. Kohn[1], [1] Laboratory of Molecular Pharmacology, NIH, Bethesda, MD 20892, USA, and [2] Rhône-Poulenc Santé, 94403 Vitry-sur-Seine CEDEX, FRANCE

1) Using plasmid DNA (5'-flank of the human c-myc gene: exon 1 and its flanking regions) and purified mouse leukemia (L1210) topoisomerase II (topo II), we found a cluster of strong cleavage sites induced by amsacrine in a 100 bp regions encompassing the P2 promoter. The major site was at position 2499 on the coding strand and 2502 on the transcribed strand. This 4 base pair stagger is consistent with previously reported double-strand cleavage by topoisomerase II. DNase I footprinting showed a protected region of 15-20 bp around the cleavage site. Weaker sites also occurred inside the P1 promoter as well as 200 base pairs upstream from exon 1 and in the first intron.
2) A strong in vivo amsacrine-induced cleavage site was also found in the P2 promoter from human small cell lung carcinoma cells (NCI N417). These results show that topo II may play an active role in regulating c-myc gene expression, possibly by modulating the torsional wave generated by transcription at its initiation site.

J 329 SUPPRESSION OF TUMORIGENICITY OF MOUSE L CELLS BY A NORMAL COPY OF HUMAN CHROMOSOME 9, B.W. Porterfield, J.D. Rowley and M.O. Diaz, Department of Medicine, Section of Hematology/Oncology, The University of Chicago, The Pritzker School of Medicine, 5841 S. Maryland Ave., Box 420, Chicago, IL, 60637.

Somatic cell hybrids were prepared by fusing normal human fibroblasts to tumorigenic mouse L cells. A normal human chromosome 9 was selected for by using methylthioadenosine phosphorylase activity, an enzyme involved in purine metabolism whose function was previously mapped to the short arm of chromosome 9 (9p). The hybrids were analyzed for changes in the transformed phenotype, including growth rate, saturation density, contact inhibition, serum requirement, anchorage independent growth in soft agar and tumorigenicity in nude mice. Cloning efficiency in soft agar was reduced from 100% for L cells to less than 25% for the hybrids. The L cells formed tumors in nude mice with a frequency of 100%, when 1 X 10^6 cells were injected, while the hybrid cells rarely formed tumors . Experiments with hybrid cells that contain deletions in chromosome 9 are in progress. We previously showed that loss of 9p, detected by loss of interferon genes on 9p, is common in some human cancers; these data suggest that a putative tumor suppressor gene(s) is located on chromosome 9 (9p22).

J 330 P53 MUTATIONS AND CHROMOSOME 17P DELETIONS IN COLORECTAL CANCER, Colin A. Purdie[1], John R. Jenkins[2], Andrew H. Wyllie[1] and Colin C. Bird[1]. 1. CRC Laboratories, Department of Pathology, University of Edinburgh Medical School, Teviot Place, Edinburgh, UK. 2. Marie Curie Research Institute, The Chart, Oxted, Surrey, UK.

The tumour suppressor gene p53 lies within a region of chromosome 17p which is frequently deleted in colorectal cancer. To test the hypothesis that the carcinogenic effect of 17p deletion relates exclusively to alterations at the p53 locus, we sequenced p53 DNA from 12 cancers, 6 with and 6 wihtout 17p deletions. DNA for dideoxy sequencing was obtained from 100 pooled clones derived from subcloned, PCR amplified genomic DNA. Allelic losses were determined in genomic Southern blots, probed with YNZ 22.1 and MCT 53.1 (RFLP probes on 17p). 7 cases had point mutations all but one of these in regions highly conserved through evolution. Cases with and without mutation were found in both 17p retained and deleted groups. In particular, 2 cases which had lost one 17p allele contained only wild-type (WT) p53 sequence. Immunohistochemistry of the same 12 cancers using an antibody specific for mutant p53 (PAb240) confirmed that all positively staining cases had mutations and that negative cases contained only WT DNA. Using antibody positivity as a marker of gene mutation, 37 further cancers were analysed. Of 19 cases which were antibody negative (and therefore presumed WT) 8 had 17p deletions. These results suggest that p53 is not the sole target for 17p deletions; another locus on 17p may also be involved in colorectal carcinogenesis.

J 331 A PUTATIVE TUMOR SUPPRESSOR GENE ON MURINE CHROMOSOME 7 AFFECTS N-METHYL-N-NITROSOUREA-INDUCED LYMPHOMA DEVELOPMENT, Ellen R. Richie, Don C. Morizot and Joe M. Angel, Science Park-Research Division, University of Texas M. D. Anderson Cancer Center, Smithville, TX 78957

Inbred mouse strains vary in their susceptibility to development of N-methyl-N-nitro-sourea-induced (MNU) induced thymic lymphomas. The AKR strain is highly susceptible, developing a high (80%) incidence of thymic lymphomas by 4-6 months of age, while C57L mice have a low incidence (40%) of lymphomas that do not appear until >7 months of age. We recently reported (Angel et al, **J. Natl. Cancer Inst.** 81:1652, 1989) that albino F_2 progeny developed a higher incidence MNU induced lymphomas than nonalbino progeny suggesting that a gene tightly linked to the albino (c) locus on chromosome 7 influences tumor development. These studies have now been expanded to include backcross as well as additional F_2 mice. The data confirm an association between lymphoma development and homozygous inheritance of AKR alleles at a putative tumor suppressor locus on chromosome 7. Analyses of RFLP at the Hbb, Fes and other polymorphic chromosome 7 loci are underway to map the location of the tumor suppressor locus. Recent data also demonstrate that female mice are significantly more susceptible to lymphoma development than males. Tumor incidence in reciprocal backcross mice suggests that this is not a sex-linked phenomenon, but due perhaps to hormonal influences.

J 332 CLONING OF A ZINC FINGER PROTEIN WHICH BINDS TO AN ORIGIN-LIKE SEQUENCE OF DNA REPLICATION IN ALU REPETITIVE SEQUENCES, Kazuichi Sakamoto, Claire Driscoll and Bruce Howard, Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892

We have been interested in the hypothesis that interspersed repetitive sequences which can be transcribed by RNA polymerase III may have regulatory functions in mammalian cell growth. Our previous studies showed that members of the Alu family as well as 7SL RNA genes cause temporary suppression of DNA replication in HeLa cells when these genes are introduced by the DEAE-mediated transfection method. This inhibitory activity depends in part on the presence of an undecameric sequence, GAGGCNGAGGY, which is homologous to the T-antigen binding region in the SV40 core DNA replication origin. A partially purified HeLa nuclear extract showed binding activity to oligonucleotides containing this GAGGCNGAGGY motif. In addition, this protein fraction exhibited specific binding to the sequence CTG(G/T)AAT, which occurs adjacent to the GAGGCNGAGGY motif in both the Alu consensus and the SV40 DNA replication origin. We isolated a cDNA clone which encodes binding activity for GAGGC-containing oligonucleotides from a HeLa λgt11 cDNA library. This cloned protein, when expressed in *E. coli*, exhibited a similar binding specificity to the partially purified protein. DNA sequence analysis revealed two potential Cys_2-His_2 zinc finger binding domains. The possible role of this Alu binding protein in cell growth regulation will be discussed.

J 333 COMPARISON OF ACTIVATED ONCOGENES IN HUMAN SKIN CANCERS FROM NORMAL INDIVIDUALS AND XERODERMA PIGMENTOSUM PATIENTS, Leela DAYA-GROSJEAN, Caroline ROBERT, Christiane DROUGARD and Alain SARASIN, Laboratory of Molecular Genetics, Institut de Recherches Scientifiques sur le Cancer, B.P. n° 8 - 94801 - VILLEJUIF (France)

Clinical and epidemiological evidence has clearly shown that the principle factor involved in the development of skin cancers in man is ultraviolet (UV) irradiation. Patients suffering from the highly cancer-prone skin disorder, xeroderma pigmentosum (XP) are known to have a molecular defect in their ability to repair UV damaged cellular DNA.

We have shown the presence of activated ras oncogenes (N-ras and Ki-ras) due to point mutations within specific codons together with modifications of other oncogenes (amplification and/or rearrangement) in XP skin tumors. The types of mutations observed on ras oncogenes in XP tumors can be explained by the presence of non repaired Py-Py DNA lesions. Similarly, the high level of Ha-ras amplification observed in XP tumors may also be attributed to unrepaired lesions. Activated oncogenes are known to play an important role in the process of human malignancy and therefore it was interesting to compare ras gene mutations in skin tumors of normal individuals with those from XP patients. DNA from basal cell carcinomas and squamous cell carcinomas from both types of patients were amplified using the polymerase chain reaction and ras mutations detected by differential hybridisation. Point mutations were searched for in all the ras codons tested, and the results showed a frequency of 25 % in ras mutations in skin carcinomas from the normal population compared to 70 % in XP patients. This significantly different level of ras mutation confirms the importance of unrepaired lesions in DNA which result in point mutations and play a significant role in tumor progression.

J 334 NON-RADIOACTIVE DETECTION OF LOSS OF HETEROZYGOSITY IN THE p53 GENE IN RENAL CELL CARCINOMA AND BLADDER CANCER USING THE POLYMERASE CHAIN REACTION, Hideyuki Saya[1], Koji Oka[1], Toru Nishi[1], Jiro Ishikawa[3], Janet M. Bruner[2] and Rei Takahashi[3], Department of Neuro-Oncology[1] and Department of Pathology[2], The University of Texas M. D. Anderson Cancer Center, Houston, TX 77030; and The Center for Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381[3]

The human p53 gene, a putative tumor suppressor gene, has a polymorphism in amino acid residue 72. We recently developed a method of detecting codon 72 polymorphism in this gene by digestion of polymerase chain reaction-amplified DNA using allele-specific restriction endonuclease. This polymorphism allows the identification of loss of heterozygosity for the coding region of the p53 gene in limited tissue samples in a short time without using radioactive materials. We examined 33 patients with renal cell carcinoma and 29 with bladder cancer; heterozygosity in the p53 gene was lost in 60% (6 of 10 informative cases) and 73% (8 of 11 informative cases) of the renal and bladder tumors, respectively. Additionally, the assay's sensitivity could be improved by using DNA extracted from frozen sections of the tumors. Because the proportions of tumor cells and nontumor cells could be assessed by microscopic evaluation of the frozen sections, we were able to minimize contamination from nontumor cells, which occasionally causes false readings of retained heterozygosity. This simple and sensitive method for detecting loss of heterozygosity in the p53 gene makes it possible to rapidly screen a large number of tissue samples and has the potential to be a useful diagnostic tool for a wide variety of human neoplasms.

J 335 INHIBITION OF CELL PROLIFERATION BY TGFß1 IS BLOCKED IN EPSTEIN-BARR VIRUS (EBV) POSITIVE TRANSFORMED OR MALIGNANT B CELLS BUT NOT IN EBV NEGATIVE NORMAL OR MALIGNANT B CELLS. P.J. Smith, S. Jones, I. Dunn and D. Moss. Queensland Cancer Fund Research Unit, Queensland Institute of Medical Research and Department of Pathology, University of Queensland Medical School, Herston, Queensland, Australia, 4006.

Transforming Growth Factor ß1 (TGFß1) is a potent inhibitor of lymphoid cell proliferation. The proliferation inhibitory effect of TGFß1 was studied using endemic EBV-associated Burkitt's lymphoma cell lines (eBL), sporadic non EBV-associated BL cell lines (sBL), normal B cells transformed with EBV (LCLs), and IL-4 dependent normal B cell cultures. All BL lines studied had t(8:14) characteristic of this tumour where the c-myc locus is translocated on to chromosome 14 and consequently deregulated. Proliferation of sBL and normal B cells, but not eBL and LCLs were inhibited by TGFß1. Growth inhibition correlated with presence of the EBV genome and not with translocated c-myc. Pietenpol et al (1990) suggested that TGFß1 promoted synthesis or modification of a protein that regulates c-myc transcription and this effect is abrogated by viral transforming proteins associated with HPV or SV40. From the data presented here we propose that the presence of EBV also results in production of a factor capable of blocking the action of this regulatory protein, which may provide a basis for the role of EBV in BL.

Pietenpol, J.A. et al. PNAS 87: 3758-3762, 1990 and Cell 61: 777-785, 1990.

J 336 A NOVEL EWING SARCOMA TRANSLOCATION VARIANT IDENTIFIED IN A DYSMORPHIC CHILD, Jeremy Squire, K. Mohan Pai, Paul Thorner, Herman Yeger, Kwan Ng, and Rosanna Weksberg. Departments of Genetics and Pathology, Hospital for Sick Children and University of Toronto, Toronto, and Departments of Pediatrics and Pathology, Chedoke-McMaster Hospital and McMaster University, Hamilton, Ontario, Canada.

Ewing sarcoma is characterized by the presence of a reciprocal chromosome translocation t(11;22)(q24;q12). We have recently identified a novel variant translocation in a 14 year old girl with unusual dysmorphic features and Ewing sarcoma. The translocation involves both the accepted Ewing sarcoma breakpoints and chromosome 4 [4q-, t(4;11)(q21;q24), 22q-]. The constitutional karyotype of stromal fibroblasts present within the tumor cultures was normal. It is possible that the Ewing sarcoma tumor translocations originated as a result of a complex three-way rearrangement involving chromosomes 4, 11, and 22. Further molecular analysis of genes that map to the vicinity of 4q21 such as the Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF), interleukin 2 (IL2) and the oncogene c-*kit* might provide clues to explain the association with congenital abnormalities and the etiology of this tumour.

J 337 HIGH EXPRESSION OF P53 RNA IS ASSOCIATED WITH LOW STAGES OF OSTEOGENIC DIFFERENTIATION IN RADIATION- INDUCED BONE TUMORS.
P. Günter Strauss[1], Karin Müller[2], Horst Zitzelsberger[3], and V. Erfle[1]. [1]GSF-Abteilung für Molekulare Zellpathologie, [2]GSF-Institut für Pathologie, [3]GSF-Institut für Strahlenbiologie, D-8042 Neuherberg, FRG.
Enhanced expression of p53 has been found in a wide variety of tumor types and cell lines. It has been reported by other authors that gross genomic alterations of the p53 gene region in humans correlated with the development of osteogenic tumors, and a high incidence of osteosarcomas had been observed in p53 transgenic mice.
We have investigated radiation-induced osteogenic sarcomas of BALB/c mice, which exhibited various stages of osteogenic differentiation, for alterations in structure and expression of p53. The tumors were classified for their stage of osteogenic differentiation acccording to a model based on the temporal activation of bone cell marker genes during osteogenic differentiation (Strauss et al., J. Cell Biol., 110, 1369, 1990).
Our data indicate that high expression of p53 RNA is not compatible with advanced osteogenic differentiation of the tumors. As bone tumors can reach a high level of differentiation, the data indicate that p53 in addition to its role in DNA replication, may have a distinct function in the control of the osteogenic differentiation.

J 338 DEVELOPMENTAL POTENTIAL OF PARTHENOGENETIC EMBRYOS FROM LTXBO MICE MAY REFLECT CHANGES IN GAMETIC IMPRINTING PATTERNS RELATED TO SPONTANEOUS OVARIAN TUMOUR FORMATION, Susannah Varmuza and Ian Rogers, Department of Zoology, University of Toronto, 25 Harbord St., Toronto, Ontario, Canada, M5S 1A1.

LTXBO mice develop spontaneous ovarian tumours at a very high frequency (penetrance approx. 90%). The phenotype of the tumours is unexpected given their parthenogenetic origin. Most tumours contain trophoblastic elements, and these are frequently the only phenotype. Parthenogenetic embryos do not produce trophoblast well. We investigated the developmental potential of experimentally induced parthenogenetic embryos from these mice, and found that they appear to be more robust than parthenotes from (C57Bl/6XCBA) F1 females, suggesting that a change in gametic imprinting pattern may have occurred in LTXBO mice. F1 mice derived from reciprocal crosses between LTXBO and TgMBG (outbred) mice develop ovarian tumours and produce robust parthenotes, indicating that the trait(s) are dominant. Whether tumour formation relates to imprinting anomalies remains to be demonstrated.

J 339 PREFERENTIALLY EXPRESSED cDNA IN NONTUMORIGENIC ENDOMETRIAL CARCINOMA MICROCELL HYBRIDS, Hideto Yamada and J. Carl Barrett, Laboratory of Molecular Carcinogenesis, NIEHS, Research Triangle Park, NC 27709
We have shown that a single human chromosome 1, 6, or 9 can suppress the tumorigenicity of a uterine endometrial carcinoma cell line, HHUA (Yamada et al., Oncogene 5: 1141-1147, 1990). In order to understand the molecular changes responsible for this suppression of tumorigenicity, we made a λgt10 cDNA library from mRNA of nontumorigenic microcell hybrids with an introduced chromosome 6 and screened 10^5 cDNAs by the method of +/- differentially screening. After two cycles, we isolated five cDNA clones that were preferentially expressed in microcell hybrids. One of these was expressed at relatively high level in all the nontumorigenic microcell hybrids (including those suppressed by chromosome 1, 6, or 9) when compared with the parental cells or tumorigenic microcell hybrids. Sequence analysis revealed that this cDNA was homologous to human mitochondrion 14 mRNA. Down regulation of this gene may be associated with tumorigenic conversion.

J 340 SUPPRESSION OF *NEU* ONCOGENE INDUCED TRANSFORMATION BY THE ADENOVIRUS-5 E1A GENE PRODUCTS, Dihua Yu, Kathleen Scorsonene, Mien-Chie Hung, Department of Tumor Biology, The University of Texas, M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, Texas 77030 We have previously found that transcription of the *neu* oncogene can be strongly repressed by the E1A gene products. To further study if this transcriptional repression of *neu* by E1A can inhibit the transforming ability and tumorigenicity of the *neu* transformed cells, we have introduced the E1A gene into the *neu* transformed B104-1-1 cells and developed B104-E1A cell lines that express E1A proteins.These B104-E1A stable transfectants have reduced transforming activity compared to the parental B104-1-1 cell line: The E1A transfectants showed flat morphology , reduced DNA synthesis and cell growth rate, lower efficiency to grow in soft agar and reduced ability to induce tumors in Nu/Nu mice. From these results, we conclude that E1A can suppress the transforming phenotype of the *neu* oncogene transformed cells. To study if E1A could effect metastatic potential, we first tested whether tail vein injection of *neu* transformed B104-1-1 cells can induce experimental metastasis in nude mice. 21 days after injection, we observed metastatic nodules in the lungs of nude mice. The brain, heart, liver, kidneys, spleen and ovaries were also examined and no metastasis were found. This is the first experimental evidence that *neu* oncogene can lead to increased metastatic potential. We then further investigated if expression of E1A in these *neu* transformed cells can reduce its metastatic potential by injecting B104-E1A transfectants into the tail vein of nude mice. No metastatic nodules were found. We conclude that E1A gene products also have inhibitory effect on the metastatic phenotype of the *neu* oncogene transformed cells.

J 341 Analysis of the t(14;18) translocation by pulsed-field gel electrophoresis (PFGE) reveals breakpoints distal to the minor cluster region, Andrew D. Zelenetz and Ronald Levy, Department of Medicine, Division of Oncology, Stanford Medical School, Stanford, CA 94305-5306
The translocation of the heavy chain J_H locus to the *bcl*-2 proto-oncogene is a common cytogenetic abnormality in human lymphoma. In particular, it is seen in about 85% of follicular small cleaved cell lymphoma. The chromosome 18 breakpoints are clustered into two regions; the major breakpoint region (mbr) within the 3' untranslated region of the *bcl*-2 proto-oncogene accounts for approximately 60% of the cases and the minor cluster region (mcr) >20 kb 3' of *bcl*-2 accounts for approximately 25% of the breakpoints. Because of variability in the position of the breakpoint, detection of the t(14;18) by Southern blot analysis provides an important clonal marker for the tumor. However, conventional gel electrophoresis will fail to detect the translocation in 15-25% of cases. We have applied pulsed-field gel electrophoresis to the analysis of the t(14;18) and found that it is more sensitive than conventional electrophoresis. Cases of follicular lymphoma where selected because conventional electrophoresis and Southern blot analysis had failed to reveal an abnormality; PFGE was able to detect the t(14;18) in 6/8 cases. In 7 matched tissue specimens from cases of tumor progression of FL to diffuse lymphoma a clonal t(14;18) could be detected confirming their common clonal origin in 7/7 cases. In all cases (5/5) where a t(14;18) translocation was observed by cytogenetics co-migration of a band detected with probes for *bcl*-2 and J_H was demonstrated. Even in 1/2 cases where the karyotype was normal a translocation could be detected by PFGE. By sequential application of probes for the mbr and mcr, two cases were identified with chromosomal breakpoints distal to the mcr. The position and significance of these remote breakpoints is being investigated.

Carcinogenesis and Tumor Progression

J 400 VL30-MEDIATED MALIGNANT CONVERSION: A PLEIOTROPIC EVENT PRODUCING GENETIC INSTABILITY. Garth R. Anderson, Daniel L. Stoler, Cheryl A. Russo and Cathy A. Roberts. Department of Molecular and Cellular Biology, Roswell Park, Buffalo, NY 14263.

As neoplastic transformation represents uncontrolled expression of signal-responsive cell proliferation, one form of malignant conversion appears to originate in loss of control of a signal-responsive fibroblast program utilized during wound healing. This response is evidently normally induced by the anoxic wound environment activating a retrotransposable element, which in turn induces expression of a number of cellular genes.

The normal fibroblast response to anoxia occurs in stages and results in expression of several features also characteristic of malignant tumor cells: induction of retrotransposon VL30 RNA, induction of three glycolysis-related proteins including cancer associated p34/LDH-k, and secretion of the malignancy-associated proteases cathepsins D and L. At sixteen hours a sequestered endonuclease is induced; a similar endonuclease is found in malignant cells. Full cell viability continues for at least 72 hours of anoxia, and all phenomena are reversible. This response matches the needs of fibroblasts during the anoxic phase of wound healing, where they migrate into the wound, obtain their energy by glycolysis, and must secrete proteases and nucleases to facilitate removal of wound debris.

Endonuclease action is believed to initiate gene amplification, and obviously can cause deletions/translocations. Schimke has shown anoxia is uniquely effective in triggering gene amplification. Along with those features of the anoxic fibroblast response associated with invasiveness, the anoxia inducible endonuclease may have an important role in malignancy by creating random chromosomal breaks leading to genetic instability.

J 401 PREFERENTIAL METHYLATION OF THE Ha-ras GENE BY METHYLNITROSOUREA IN RAT MAMMARY GLANDS, Michael C. Archer, Shi-Jiang Lu and Jamie R. Milligan. Ontario Cancer Institute, Toronto, Canada M4X 1K9

Exposure of female rats during sexual development to a single dose of 30 mg/kg of methylnitrosourea (MNU) results in a 90% incidence of mammary tumors. Approximately 85% of such tumors contain a transforming Ha-ras gene activated by the same G → A transition at codon 12. Since MNU is known to induce specifically G → A transitions and to be chemically labile under physiological conditions, it seems likely that the Ha-ras gene is activated during the initiation of carcinogenesis. Although Ki- and N-ras genes may also be activated by MNU by G → A transitions at codon 12, these genes are not activated in the rat mammary tumor model described above. Indeed, in various animal tumors induced by a variety of chemical carcinogens, activation of one particular oncogene predominates. By inducing strand breaks at 7-methylguanine residues followed by gel electrophoresis under denaturing conditions and Southern analysis, we show in the present study, that a 10kb restriction fragment containing the Ha-ras gene is extensively methylated by MNU in DNA isolated from mammary glands of female rats 4h following carcinogen treatment. In contrast, fragments of similar size containing both the Ki- and N-ras genes are methylated less extensively. Moreover, the extent of methylation of the three ras genes by MNU correlates with their transcriptional activity. These results suggest that the extent of interaction of a carcinogen with an oncogene, which depends on its transcriptional activity, is a factor in determining whether the gene is mutated during the initiation of carcinogenesis.

J 402 POTENTIAL ROLE OF NUCLEASES IN CARCINOGENESIS: CLONING OF DNase I, Vera W. Byrnes and Matthews O. Bradley, Merck Sharp & Dohme Research Laboratories, West Point, PA 19486

Certain weak carcinogens produce DNA double-strand breaks (DSB) only at cytotoxic dose levels. If deoxyribonucleases (DNases) are released from their intracellular storage vesicles in cells undergoing a reversible (sublethal) cytotoxic insult, then DNases may be responsible for the DNA DSB we see in neutral elution assays with cultured cells at cytotoxic dose levels. Such DNA DSB could cause mutations and chromosome aberrations.

To investigate the potential role of nucleases in the induction of DNA damage, neoplastic transformation and tumorigenesis, we have cloned bovine pancreatic DNase I and are examining its expression. Bovine pancreatic mRNA was reverse transcribed, and the cDNA was amplified by mixed oligonucleotide primed PCR. These degenerate PCR primers were designed based on bovine DNase I amino acid sequence, and the resulting PCR product encoded an almost full length DNase I protein coding sequence. This PCR product was purified and amplified with another set of primers which had 5' nucleotides encoding both restriction enzyme sites and"best guess" codons for the amino and carboxy terminal amino acids of DNase I. This resulted in a PCR product encoding full length DNase I. However, sequencing showed that the nucleotide sequence differed from the published bovine protein sequence (Paudet, H.K. and T.-H. Liao, J. Biol. Chem. 261:16012-16017, 1986), at amino acids 38 and 39, resulting in an inversion of Gln and Glu. We are currently subcloning DNase I into an inducible expression vector to directly examine the effects of inappropriate DNase I expression in mammalian cells.

J 403 ANTIONCOGENES IN LUNG CARCINOMA CELL LINES OF DIFFERENT INVASIVE AND METASTATIC POTENTIAL, J. Caamano, B. Ruggeri and A.J.P. Klein-Szanto, Department of Pathology, Fox Chase Cancer Center, Philadelphia, PA 19111

There is little information regarding loss of antioncogenes function during tumor progression. In an attempt to study the association between the acquisition of metastatic invasive phenotype and changes in tumor suppressor genes, we characterized the *in vivo* and *in vitro* behavior of eight non small cell lung carcinoma cell lines, and studied several antioncogenes at the DNA, RNA and protein level.

No differences were found in the Rb and K-*rev*-1 genes. Allelotype analyses of chromosome 17p showed that 3 of 8 cell lines had lost heterozygosity and another two gained an extra allele. Southern analysis revealed that the p53 gene was partially deleted in one case. p53 transcripts were undetectable in the cell line carrying this deletion and extremely low expression was found in one other cell line, both of them were among the most malignant examined. Immunoprecipitation studies detected p53 protein in 6 of 8 cell lines. The p53 protein in these 6 lines had an extended half-life and co-immunoprecipitated with hsp 70 antiserum.

Collectively these results suggest that different mutations or deletions in the p53 gene are present in many human lung carcinoma lines and that the cell lines exhibiting the most advanced invasive-metastatic phenotypes were characterized by the most notable changes in p53.

J 404 **Evidence for altered regulation of a mammalian gene induced by DNA-damaging agents and growth arrest signals in tumor cell lines . Albert J. Fornace, Jr., Mathilda A. Papathanasiou, M. Christine Hollander, Edward Farhangi*, Isaac Alamo, Jr., Michael A. Beckett*, and Ralph R. Weichselbaum*, N.C.I., N.I.H., Bethesda, MD, and *Univ. of Chicago, Chicago, IL.**

In both bacteria and eukaryotes, one effect of DNA damage is the transient inhibition of DNA synthesis and cell growth; such delays can have a protective effect since mutants lacking growth arrest responses are hypersensitive to certain DNA-damaging agents. We have recently isolated cDNA clones for 5 hamster genes that are coordinately induced either by DNA-damaging agents or by other cell treatments that induce growth arrest such as serum reduction, medium depletion, or contact inhibition; these genes have been designated *gadd* (growth arrest- and DNA damage-inducible)[1]. One these genes, *gadd45*, has been found to be induced x rays in a variety of cell types including human fibroblasts and EBV-transformed lymphoblasts. Induction in cells from patients with the radiosensitive disorder ataxia telangiectasia was found to be significantly reduced compared to that in normal cells. In most tumor cell lines tested, this response was lost; e.g., x-ray induction was seen in only 1 of 8 cell lines derived from human head and neck tumors. cDNA and genomic clones for both the human and hamster *gadd45* gene have been isolated and sequenced. Studies with the promoter of the A45 *gadd* gene are underway and a region (-350 to +150 relative to transcription start site) containing a DNA-damage-inducible element has been identified. Various results, which will be presented, indicate that this DNA-damage response is not mediated by protein kinase C (PKC), but may involve another kinase since it is blocked by the kinase inhibitor H7. Our studies indicate that DNA damage and growth arrest can activate a PKC-independent signal transduction pathway which is altered in many tumor cell lines.

[1] Fornace, A.J. Jr., Nebert, D., Hollander, M.C., Papathanasiou, M., Fargnoli, J., and Holbrook, N.: Molec. Cell. Biol. 9:4196-4203, 1989.

J 405 CYTOGENETIC CHARACTERIZATION OF TWO METASTATIC MURINE T-CELL DERIVED HYBRIDOMAS WITH DIFFERENT ORGAN COLONIZING ABILITY, Dumitru-Dorel Ciupercescu, Corina Schmidt, Hendrik Verschueren*, Dominique Van Hecke and Patrick De Baetselier, Dept. Cellular Immunology, Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, 1640 St-Genesius Rode, Belgium; *Pasteur Instituut, 1180 Brussel.

Two metastatic murine T-cell hybridomas, derived from two common fusion partners (the noninvasive, nonmetastatic BW5147 lymphoma and the Il-2 dependent T-cell line CTL-D) were selected for the present cytogenetic study. Despite of their common genomic origin and similar tumorigenic and invasive properties, the 19SpSp cell line colonised extensively the spleen and liver, while the 14KiKi cell line metastasized more readily the kidneys. In order to relate genomic structure with the organ specific pattern of metastasis, the two cell lines were cytogenetically investigated using GTG-, CBG- and AgNOR-banded chromosomes. The 19SpSp cell line had a modal number of 52 chromosomes. The most frequent karyotype was: 52,X,-1,+2,-3,-4,-4,-6,+13,+14,+17, -18, +t(1;19)(qA1.3;qA1.3), +t(3;3)(qA1.1;qA1.1qF2.1), +der(4), +t(4;15;?)(qA1.1;qA1.1qF1.3;?), +del(5)(qG1.1), +t(5;11)(qG1.1qG3.3;qE2.3), +t(6;6)(qA1;qA1), +t(8;16)(qA1.1;qA1.1), +7mar. The 14KiKi cell line had a modal number of 60 chromosomes. The most frequent karyotype was: 60,X,+2,-4,+8,+8,+9,+10,+12,+12,+13,+13,+14,+15,-17,-18,-18, +t(1;19)(qA1.3;qA1.3), +t(3;3)(qA1.1; qA1.1qB1), +der(4), +t(4;18)(qD1;qA2?), +t(4;15;?) (qA1.1;qA1.1qF1.3;?), +del(5)(qG1.1), +t(5;11)(qG1.1qG3.3;qE2.3), +t(6;17)(qF3;qB1.1), +6mar. This analysis revealed the following main facts: (1) Despite of their extensive karyotypic diversity, the two cell lines shared 7 identical structural abnormalities (SA). (2) There were 24.7 *vs* 26.4% numerical abnormalities and 30.3 *vs* 25.0% SA for 19SpSp and 14KiKi, respectively, indicating that a higher proportion of SA might be associated with a shorter survival rate after i.v. inoculation (20 *vs* 35 days for 19SpSp and 14KiKi, respectively). (3) Some of the frequently observed breakpoints were coincidental with known murine fragile sites: c-fra(3)(qB), c-fra(4)(qD1) and c-fra(5)(qG1). This suggests that certain breaks and rearrangements preferentially occured within these regions. Further analyses of other available clones with identical origin and similar tumorigenic properties would offer a better understanding of the relationship between genomic structure and organ specific metastasizing ability of these cell lines.

J 406 GENETIC VARIABILITY OF HUMAN SKIN TUMOR CELLS DURING PROPAGATION IN VITRO AND IN VIVO AND ITS IMPLICATION FOR TUMOR PROGRESSION. N.E. Fusenig, U. Pascheberg, S. Altmeyer, P. Boukamp
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One of the most prominent characteristics of tumor cells is their genetic instability with inherent tendency to progredient changes (visible at the chromosomal level) concomitant with further propagation.
In squamous skin carcinoma (SCC) derived cell lines we identified numerical and structural chromosomal aberrations which progressed with further passages, concomitant with improved growth behavior in culture without, however, resulting in tumor progression in vivo.
In contrast, melanoma-derived human cell lines were cytogenetically rather stable with prolonged cultivation. In vivo passages (as heterotransplants in nude mice), on the other hand, resulted in additional structural aberrations and most of these alterations were maintained in vitro.
Immortalization of human skin keratinocytes in vitro (by SV40 or HPV16 DNA or occurring spontaneously), was always associated with numerical and structural chromosomal alterations, although no marker chromosomes common to the different cell lines were noticed. Similarly to the SCC lines, also these cells revealed a tendency to a continuous increase in structural chromosomal alterations, as analyzed in detail during 200 passages of the spontaneous HaCaT cell line. The increasing alterations in karyotype led to a heterogenous cell population which, nevertheless, remained nontumorigenic.
Transfection of the c-Ha-ras oncogene into HaCaT cells yielded tumorigenic (benign and malignant) clones with an individual set of marker chromosomes and a tendency for a loss or underrepresentation of chromosome 1p.
Integration of the ras oncogene had occurred in different chromosomes, which after transfer into early HaCaT passages caused tumorigenicity though mostly of the benign phenotype.
From these data, we conclude that genetic lability of tumor cells is associated with earliest transformation stages (immortality). Numerical changes can be correlated with growth behavior in vitro, but even massive chromosomal alterations are not sufficient for tumorigenic conversion. The altered karyotype with inherent tendency to increased aberrations, however, seems to be a prerequisite for tumorigenic conversion (rendering cells more vulnerable) by foreign genes and subsequent selection.

J 407 TOPOISOMERASE II ACTIVITY AS RELATED TO PROLIFERATIVE AND TUMORIGENIC POTENTIAL AND GENE AMPLIFICATION IN RAT LIVER EPITHELIAL CELLS, Ingrid J. Hall[1], E. Lynn Zechiedrich[2], Neil Osheroff[2], and Thea D. Tlsty[1]
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The activity of topoisomerase II was assessed in a panel of rat liver epithelial cells. While these cells show similar rates of proliferation, they exhibit levels of neoplastic transformation which range from 0 to 66% and levels of spontaneous gene amplification which differ by up to two orders of magnitude. Enzyme activity was determined by monitoring ATP-dependent DNA catenation and relaxation in nuclear extracts and the sensitivity of cells to etoposide, a potent topoisomerase II-targeted antineoplastic drug. Three conclusions were drawn from this study. First, enzyme levels better reflect the proliferative index of cells rather than their tumorigenicity. Second, the sensitivity of cells to etoposide correlates with their topoisomerase II content rather than with their tumorigenic potential. Third, neither increased nor decreased topoisomerase II activity is required for gene amplification.

J 408 THE EFFECT OF RADICAL OXYGEN TO THE CELLS DERIVED FROM PATIENTS WITH FANCONI ANEMIA.
Tomoko Hashimoto[1)], Rei Takahashi[2)], Atsuko Ogawa[1)], Hiromi Sakamoto[1)], and Jun-ichi Furuyama[1)]
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Fanconi anemia (FA) is one of the chromosomal instability diseases with high incidence of cancer. The peripheral blood lymphocytes and fibroblasts obtained from the patients cultured *in vitro* show spontaneous chromosomal breakage. The lymphocytes are also reported to be hypersensitive to high concentration of oxygen. Thus, we examined chromosomal breakage in EB-virus transformed lymphoblastoid cell lines (LCLs) and a fibroblast obtained from patients with FA after treatment with H_2O_2 or paraquat (PQ). FA-LCLs showed 5-10 times higher frequency of chromosomal aberrations under the normal culture condition than normal control LCLs, and the frequency of the chromosome aberrations was significantly induced when these cells were treated with PQ. When cysteine was added to the culture medium, the frequency of chromosomal aberrations was significantly reduced. Thus, chromosomal breakage in FA cells could be induced with radical oxygen in culture media. H_2O_2 has been reported to induce the expression of *jun* and *fos* gene in mouse cells (Shibanuma *et al.*, 1989). The FA fibroblast was treated with H_2O_2 and the expression of genes known to be expressed in a cell-cycle dependent manner was examined. After the treatment of H_2O_2, expression of *fos, myc,* and RB genes was induced within 60-120 min. The level of expression of these genes in FA cells was higher than in normal control fibroblasts. The potential roles of these genes in hypersensitivity of FA cells to radical oxygen is being investigated.

J 409 A CASE OF EGFR EPISOMAL AMPLIFICATION IN THE DIFI CELL LINE, Roger R. Hewitt, Maureen Goode, Debbie Brock, Norman Prather, Randy Legerski, and Michael Siciliano, Department of Molecular Genetics, M.D. Anderson Cancer Center, Houston, TX 77030

The DiFi cell line was established from a colorectal cancer patient. Cytological and in situ hybridization analysis utilizing biotinylated probe indicates that EGFR amplification is associated with double minute chromosomes. Pulsed-field gel electrophoresis of gamma irradiated DiFi DNA demonstrates that EGFR is present in covalent circular episomes, two major species of 650 and 1300 kb, and a minor species of 2000 kb. The genetic structure within amplified EGFR appears to retain a nearly normal restriction enzyme map, which has persisted unchanged during approximately three years in culture. The approximately 50-fold amplification of EGFR is reflected in comparably increased levels of EGFR on the cell surface and increased mRNA, which includes two species of normal size. Macrorestriction maps of the DiFi episomes suggest that one copy of EGFR is included in each monomer episome of 650 kb. Enriched preparations of episomes can be isolated by methods that selectively denature genomic DNA and by preparative pulsed-field gel electrophoresis. While the initial molecular events that initiated EGFR amplification are unknown, the limited number of homogeneous episomal forms that persist in the cell line is consistent with a model of episome excision from the genome. Subsequent replication and nonrandom segregation could then lead to their accumulation. In this model, growth advantage is assumed to be conferred on cells with the largest number of episomes, because of the transforming activity of EGFR or some other gene(s) resident in the episomes.

J 410 AGE-ASSOCIATED FREQUENCY OF CHROMOSOMAL FRAGILE SITE EXPRESSION IN DOGS OF THE BOXER BREED, A BREED AT INCREASED CANCER RISK. Diana Stone, Peter B. Jacky1,and David J. Prieur. Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA and Kaiser Permanente Medical Program (PBJ), Portland, OR 97015.

Peripheral blood lymphocytes from 4 clinically normal Doberman Pinscher and 4 clinically normal Boxer dogs and from 9 Boxer dogs with a history of cutaneous mast cell tumors were cultured for folate-sensitive fragile site expression. Whole blood cultures were established in RPMI 1640 medium and lymphocytes were stimulated with pokeweed mitogen. Bactrim (Roche), containing trimethoprim and sulfamethoxazole was added for the last 24 hours of culture and 100 cells from each culture were examined. Cells from Boxer dogs with mast cell tumors had a greater frequency of both spontaneous chromosome damage and folate-sensitive fragile site expression than cells from clinically normal dogs, but this observation was attributed to an unintended selection bias for younger Boxer dogs without mast cell tumors and older Boxer dogs with mast cells tumors and an increased frequency of spontaneous chromosome breakage and folate-sensitive freagile site expression with increasing age in dogs of the Boxer breed. Thus age of Boxer dog and not tumor status was the critical factor associated with these indicators of chromosome fragility. The results of this study lend support to the hypothesis of an association between chromosome instability and a genetic predisposition to develop cancer, as Boxer dogs are a phenotypically identifiable, highly inbred group of animals at increased risk of developing cancer and show features of increased chromosome fragility with advancing age. These animals provide a useful model with which to investigate the relationships of fragile sites, aging, genome instability, mutagenesis, and carcinogenesis. (Supported by NIH grant RR00515 and grants from the American Cancer Society and Morris Animal Foundation).

J 411 NEU AMPLIFICATION AND OVEREXPRESSION IN OVARIAN ADENOCARCINOMA-DERIVED CELL LINES. Bonnie L. King, Darryl Carter and Barry M. Kacinski, Dept. of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT, 06510

Amplification and overexpression of the neu proto-oncogene have been observed in a variety of human adenocarcinomas, including those of the ovary. Although many studies have found that high level neu DNA amplification and expression are associated with poor prognosis, the role of neu in neoplastic epithelial cell physiology is poorly understood. To study the role of neu in ovarian epithelial cells we have characterized eight ovarian adenocarcinoma-derived cell lines for neu amplification and expression using Southern, Northern and Western blotting techniques. Our preliminary analysis revealed: 1) neu DNA sequence amplification accompanied by overexpression of transcripts and neu protein in two of the eight lines (SKOV3 and YAOVBIX1). 2) Both of the independently isolated neu-positive cell lines expressed a novel 7.0 kb major transcript in addition to the 4.8 kb transcript commonly observed in most neu-positive tumor cell lines. 3) Two of the cell lines (YAOVBIX1 and YAOVBIX3), isolated as morphologically distinct clones from the ascites fluid of an ovarian cancer patient, differed dramatically in their level of neu amplification and expression. YAOVBIX1 exhibited both neu DNA amplification and overexpression of transcripts and protein, whereas YAOVBIX3 did not.

J 412 EPSTEIN-BARR VIRUS (EBV) INTEGRATES WHEN IT INFECTS B CELL LINES. Lori D. Klaman, Elizabeth A. Hurley, Jeanne B. Lawrence* and David A. Thorley-Lawson. Dept. of Path.,Tufts University School of Medicine, Boston, MA 02111. *Dept. of Cell Biology, University of Mass. Medical Center,Worcester, MA 01605.

We have used Gardella gel analysis of intact DNA, Southern blotting of digested DNA and fluorescence in situ hybridization to define the state of the EBV genome in persistently infected cells. The fluorescence in situ hybridization technique has allowed us to directly visualize both integrated and episomal EBV DNA at the single cell level. Circularization of the EBV genome is rarely detected upon infecting activated normal B cells. The virus can persist upon infection of a proliferating B cell target, EBV negative Burkitt lymphoma tumour cell lines. Here, the virus preferentially integrates into the host DNA (10/16 clones). The integrated virus is linear and usually intact although 3 isolates have deletions from the left hand end including the latent origin of replication. Preliminary analysis of one integrant shows that there is at least one intact terminal repeat on each end. This rules out the possibility that episomes fail to form because the terminal repeats are degraded before they can join and raises the question as to why such a genome, since it is intact, did not circularize.We have sequenced the first integration site and found that it has occurred adjacent to a G repeat in both the virus and the cell DNA that resembles the HSV and CMV *a* sequence that is thought to be the cleavage/packaging signal. Therefore, integration may be an aberration of a viral processing mechanism. Additional integration events are currently being cloned and sequenced.

J 413 NON-TUMORIGENIC HUMAN LIVER EPITHELIAL CELL CULTURES FOR CHEMICAL AND BIOLOGICAL CARCINOGENESIS INVESTIGATIONS, John F. Lechner[1], Duane T. Smoot[1], Andrea A. M. A. Pfeifer[2], Katharine E. Cole[1], Ainsley Weston[1], John D. Groopman[3], Takayoshi Tokiwa[1] and Curtis. C. Harris[1], [1] Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892, [2]Nestec Ltd. Research Center, Lausanne, Switzerland, & [3]The Johns Hopkins University School of Hygiene & Public Health, Baltimore, MD 21205

Normal human liver epithelial cells from 6 separate donors have been grown for 4 passages (12 population doublings) in a serum-free medium. Four of these cultures have been transformed with the SV40 large T antigen gene. The life span of two of these is indefinite, whereas the others is greatly extended. The transformed cells are initially positive for cytokeratin 18, SV40 large T-antigen, albumin, P450IA1 and detoxifying enzymes. However, with continued growth they loose the ability to utilize ornithine in leu of arginine and they cease expressing most hepato-specific proteins (cytochrome p450s, acute phase proteins & albumin). In addition, cytokeratin 19 can be detected. With modifications of culture conditions, however, the expressions of the above proteins can be modified. Specifically, when incubated as roller cultures, the cells reacquire the ability to metabolize benzo[a]pyrene and aflatoxin B1 to electrophilic derivatives that form carcinogen-DNA adducts. Further, when co-cultured with skin fibroblasts on membranes, the cells synthesize albumin and acute phase proteins. Therefore, by using appropriate culture conditions, the "immortalized" human liver epithelial cells exhibit the differentiated functions of hepatocytes in vivo. Accordingly, these cells may serve as a useful model for chemical and biological carcinogenesis studies of human liver epithelial cells.

J 414 GENETIC STABILITY OF HUMAN FIBROBLASTS MALIGNANTLY TRANSFORMED *IN VITRO*. J. Justin McCormick, Dajun Yang and Veronica M. Maher, Michigan State University, Carcinogenesis Laboratory, East Lansing, Michigan 48824. We have developed an indefinite lifespan human fibroblast cell strain (MSU-1.1) that can be transformed to malignancy by transfection of the H-, K-, or N-*ras* or the v-*fes* oncogene. We have also found rare spontaneous clonal variants of MSU-1.1 that are malignantly transformed. Such malignant variants also arise after carcinogen treatment. The parent MSU-1.1 cell strain has a stable, near-diploid karyotype, composed of 45 chromosomes including two marker chromosomes. All of the numerous H-, K-, or N-*ras* transfected derivatives examined have exhibited this same stable karyotype. However, five of five carcinogen-treated or spontaneous malignant transformants each exhibited unique chromosome changes in addition to the marker chromosomes of the parental strain. These data indicate that activated *ras* or *fes* oncogenes do not cause genetic instability of the parental strain. They further suggest that spontaneous and/or carcinogen-induced oncogene activation commonly takes place as a result of major chromosome alterations. We have no evidence from the MSU-1.1 lineage of cells that is compatible with the theory that human cancer cells are inherently genetically unstable. (Research supported by DOE Grant DE-FG02-87ER60524, NCI Grant CA21289, and NIEHS Contract NO1-ES-65152.)

J 415 QUANTITATIVE DETERMINATION OF CARCINOGEN INDUCED ras GENE MUTATIONS PRIOR TO CELL TRANSFORMATION; CHEMICAL-SPECIFIC ACTIVATION AND CELL TYPE-SPECIFIC RECRUITMENT OF ONCOGENES, Hisayoshi Nakazawa, Anne-Marie Aguelon and Hiroshi Yamasaki, International Agency for Research on Cancer, Lyon, France

BALB/c 3T3 cells were exposed to 7,12-dimethylbenz(a)anthracene (DMBA) and Ha- and Ki-ras mutations (A to T transversion at codon 61) were determined quantitatively with modified T7 polymerase-PCR RFLP method (T7PCR-RFLP) during the transformation period (Mol. Carcinogenesis, 3, 202-209, 1990). Mutations of both ras genes were detected from 2-3 days after exposure to DMBA. The percentage of cells with mutated Ki-ras but not of those with mutated Ha-ras continued to increase in DMBA-treated dishes. Transformed foci started to appear after 3-4 weeks of DMBA treatment and all of transformed foci analyzed had Ki-ras and not Ha-ras mutation. MCA, MNNG, TPA and UV light did not induce, and foci produced by these carcinogens did not contain A^{182} to T mutation in Ha- or Ki-ras genes. We concluded that DMBA efficiently induces Ki- and Ha-ras mutations in BALB/c 3T3 cells but only Ki-ras mutated cells are recruited in the process of cell transformation. In order to see whether Ha-ras mutation can, under certain circumstances, play a role in DMBA-induced BALB/c 3T3 cell transformation, BALB/c 3T3 cells were pretreated with 5-azacytidine (5AZC), inhibitors of topoisomerases or ADP-ribose polymerase, before DMBA treatment. In case of 5AZC pretreatment, 3 out of 7 foci contained Ha-ras, another 3 foci Ki-ras and one focus contained both Ha- and Ki-ras mutations. Foci produced with other pretreatments are now being analyzed. These results suggest that the pattern of oncogene recruitment can be modified, presumably through gene expression modification.

J 416 **CHANGES IN VIRAL AND CELLULAR GENE EXPRESSION CONTRIBUTE TO THE PROCESS OF TUMOR PROGRESSION OF ADENOVIRUS TRANSFORMED, CELLS, Ulrich Nielsch, C. Fognani, and L.E. Babiss, Department of Molecular Cell Biology, The Rockefeller University, N.Y., N.Y. 10021**

Using several Ad5-transformed rodent cell lines, we have established a panel of genetically related cell-types that have been selected at defined stages of tumor progression. An analysis of viral E1A, E1B and E4 gene expression from the integrated viral genomes in these cells revealed no changes in the rates of transcription or cytoplasmic mRNA accumulation, during the progression process. In contrast, we have observed a step-wise increase in the total amount of a transformation specific modified form of the E1A proteins, suggesting post-transcriptional and post-translational regulatory pathways are contributing to the progression process. No changes in cellular RB-E1A protein interactions or changes in Rb gene expression were observed. However, there was a 5-fold increase in cellular p53 gene expression in the metastatic tumor derived cell line, when compared to the non-transformed parental rodent cell line. By constructing cDNA libraries from these cell lines, and applying +/- plaque hybridization screening, we have selected for clones which are either induced or suppressed during tumor progression. We found that type 1 collagen gene expression is specifically suppressed in highly progressed tumor-derived cell lines. An analysis of how the viral E1A proteins regulate collagen gene expression, and the identification of several genes which are switched on during the tumor progression process will be presented.

J 417 CHROMOSOMAL CHANGES AND PROGRESSIVE TUMORIGENESIS OF HUMAN BRONCHIAL EPITHELIAL CELLS, Yasushi Ohnuki[1], Roger R. Reddel[2], Steven E. Bates[1], John F. Lechner[3] and Curtis C. Harris[3], 1. Huntington Medical Research Institutes, Pasadena, CA 91101; 2. Children's Medical Research Foundation, Camperdown, N.S.W. 2050, Australia; 3. Laboratory of Human Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, NIH, Bethesda, Md, 20892.

An SV40-transformed human bronchial epithelial cell line, BEAS-2B, has undergone progressive changes, including the development of tumorigenicity, during *in vitro* passaging. Cytogenetic studies of BEAS-2B and its derivative tumor cell lines were performed using various banding techniques, such as G-, Q-, C-, and AgNOR, to determine whether there is a relationship between the progressive changes and karyotypic abnormalities. Karyotypes were particularly analyzed for the development of specific common markers and the loss of specific chromosomes which may carry putative tumor suppressor genes. Tumorigenicity was assayed in athymic nude mice. The karyotypes evolved as follows: (1) apparently random changes associated with SV40-induced transformation were the only changes present up until passage 12; (2) clonal changes, i.e. three or four common markers, associated with immortalization occurred between passages 12 and 21; (3) progressive changes associated with the development of weak tumorigenicity were observed between passages 21 and 45, including an increased frequency of cells which have all four common markers and a higher incidence (more than 50%) of cells which lost one homologue of chromosomes 8, 15, 16, and 22; and (4) a specific change, del (3p) characterized by the specific marker t(3q15q), was seen in tumor cell lines with significantly increased tumorigenicity. Changes in the biological properties of this clonally derived cell line were thus clearly associated with progressive karyotypic changes. The cells with increased tumorigenicity have accumulated a higher number of chromosomal abnormalities. This cell line is being studied as an *in vitro* model of multistep carcinogenesis, and is expected to be extremely useful for further cytogenetic and molecular biological analyses of progressive tumorigenesis.

J 418 LINKAGE ANALYSIS IN THE FAMILY CANCER SYNDROME LYNCH TYPE II, **Pauline J. Parry*, Chris Harocopos+, Joan Slack***, *Department of Clinical Genetics, Royal Free Hospital, Hampstead, London, England, +St Marks Hospital, City Road, London, England.

The Lynch type II (Hereditary Non-Polyposis Coli) syndrome is characterised by autosomal dominant inherited predisposition to cancer. Colon cancer is the most common malignancy in the pedigrees but in addition ovarian, pancreatic, stomach, breast, brain, skin and other cancers are typical. The mean age of tumour development is low.

The autosomal dominant inherited mechanism of the syndrome has been formally demonstrated (1). The Lynch syndromes have been estimated to constitute 5 - 10% of the total colorectal cancer burden.

The study was established to identify, by linkage analysis, a candidate gene involved in Lynch Type II. 23 pedigrees fulfilled the criteria to be involved in the study.

On the basis of allele loss studies (2) and physiological studies (3) chromosomes 18, 17, and 5 were identified as candidates to be used in the linakge analysis. DNA probes were chosen which covered the length of each chromosome.

The LOD scores for the probes were calculated using Liped and Linkage programmes allowing for sex specific penetrance of the Lynch type II gene and will be presented.

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2 : Vogelstein et al : Science 244:207-211, 1989.
3 : Bowman et al : Ca Genet Cytogenet 34:153-154, 1988.

J 419 EXPRESSION OF pp35, A PROLIFERATION-ASSOCIATED NUCLEAR PHOSPHOPROTEIN, IS INCREASED IN NEOPLASIA, Gary R. Pasternack, Tseng-Hui T. Chen, Sami N. Malek, Mark E. Sherman, and Francis P. Kuhajda. Department of Pathology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

pp35 is a recently described proliferation-associated nuclear phosphoprotein (Malek *et al.*, J. Biol. Chem. **265**, 13400 1990). While pp35 shows a wide tissue distribution, expression appears confined to proliferating cell compartments. Small dense resting B cells undergo over a 10-fold induction of the protein when driven to proliferate with lipopolysaccharide; in intestinal epithelia, pp35 is immunohistochemically detectable only in crypt epithelia. Consistent with this, pp35 message is tightly regulated when quiescent, serum-starved BALB/c 3T3 cells are stimulated by serum to undergo semi-synchronous proliferation; the message is barely detectable except for a ~6 h period roughly corresponding to the G1-S boundary.

Antibodies to murine pp35 identify similar species in human tissues. Proliferative and neoplastic lesions show increased anti-pp35 staining both in frequency of positive cells and in staining intensity. While pp35 is exclusively nuclear in normal tissues and proliferative lesions, neoplastic tissues show both nuclear and cytoplasmic staining. Grading of malignant lymphomas solely on the basis of pp35 staining stratifies them according to malignant potential. These data suggest that pp35 expression may be related to the degree of malignant potential, and that human pp35-specific reagents may prove useful in establishing tumor prognosis as further studies unfold.

J 420 COINCIDENT HYPERAMPLIFICATION AND HYPERMUTATION IN THE SAME CELL LINE, Mary Ellen Perry, Mairead Commane, Mark Meuth, and George R. Stark, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, England

A Chinese hamster ovary cell line (pro$^-$MTX2), isolated by stepwise selection in methotrexate (MTX), is resistant to MTX by virtue of a stable amplification of the dihydrofolate reductase gene. This line has an increased rate of gene amplification at two loci distinct from DHFR. This amplificator line also has a high mutation rate, and the mutations are of every type tested (transitions, transversions, deletions). The defect in pro$^-$MTX2 appears to affect repair of mutations as well the control of gene copy number. A second Chinese hamster ovary mutator line, isolated in araC, has an increased rate of point mutations due to misincorporation from altered dCTP pools. This line was not selected via amplification of any gene and does not amplify at a high rate. Therefore, not all mutators are amplificators and it is not solely the increased number of mutations in the genome of the cell that propels it to amplify at high rates.

J 421 DEVELOPMENT OF SENSITIVE REVERSION ASSAYS TO MONITOR BASE-SUBSTITUTION MUTATIONS DURING TUMOR INITIATION IN TRANSGENIC MICE. Bradley D. Preston and Sandy Price, Laboratory for Cancer Research, Rutgers University College of Pharmacy, Piscataway, NJ 08855-0789.
The initiation of cancer is thought to occur via initial DNA damage and subsequent mutation of dividing somatic cells. Several DNA lesions are promutagenic in cells in culture, and it is assumed, but not proved, that these same lesions contribute to carcinogenesis *in vivo*. To better characterize potential carcinogenic DNA lesions *in vivo*, we are studying mutagenesis in transgenic mice during tumor initiation. Our approach is to study mutations in shuttle vectors inserted into the genomic DNA of transgenic mice. We have constructed three shuttle vectors in which mutations can be scored and sequenced following selection in *E. coli*. These vectors (øX174 *am*3, M13mp2 A_{89} & M13mp2 C_{141}) are based on reversion assays in bacteriophage øX174 and M13 that detect all 6 possible base-substitution mutations. The advantage of these vectors over forward mutation assays is their low spontaneous mutation frequency ($<10^{-6}$) and thus high sensitivity for scoring mutations. Three transgenic mouse lines (øX174 *am*3, øX174 wild-type & M13mp2 A_{89}) have been established by pronuclear microinjection of linearized shuttle vector DNA into fertilized F2 eggs from crosses of (C57BL/6 x CBA)F1 males and females. The number of transgenes per diploid genome are approximately 5, 15, and 50 for the øX174 *am*3, øX174 wild-type, and M13mp2 A_{89} transgenic mouse lines, respectively. These animals are currently being used to compare the frequencies and types of mutations induced in tissues with different carcinogen susceptibilities and in carcinogen-sensitive and -resistant mice. These studies will help to identify carcinogenic DNA lesions and to determine if organotropism and strain susceptibilities to carcinogenesis correlate with mutagenesis.

J 422 RETROVIRAL INDUCED LEUKEMIA IN BALB.K MICE, Michael B. Prystowsky, Kathleen Tumas, Beth Overmoyer, Steve Ksiazek, and Kenneth Blank, Dept of Pathology, Univ of Pennsylvania, Philadelphia, PA 19104-6142
The Gross virus (NB tropic) is a murine leukemia virus that causes thymic lymphoma and leukemia when injected into any strain of neonatal mice. A variant of the Gross virus called E55+, initially isolated in Dr. Ken Blank's laboratory, causes thymic lymphoma in neonatal and in some strains of adult mice. In our laboratory we have sequenced 98% of the env gene of E55+ virus. When E55+ env gene was compared to the Friend murine leukemia virus env gene 37 point mutations were found. Of those 37 base changes 17 resulted in amino acid substitutions of which 6 are considered non conserved changes. Using the sequence information, we constructed two oligonucleotides that span 984 bases of the gp70 and p15(E). The oligonucleotides were used as primers for the polymerase chain reaction assay to detect viral DNA and RNA in infected tissues. Balb.k mice were inoculated with E55+ virus and sacrificed at one week, two week and two week intervals thereafter to follow the localization of the virus during the development of the neoplasm. Preliminary results of the time course indicate that the bone marrow, spleen and lymph nodes have intergrated virus by one week. The thymus does not have integrated provirus until two weeks post inoculation. Both spleen and lymph node remain positive throughout the time course until leukemia develops at which time they are strongly positive for intergrated provirus. Bone marrow is strongly positive at week one with decreased intensity thereafter until the animal is leukemic at which time the bone marrow becomes strongly positive again.

J 423 IMMORTALIZATION AND TRANSFORMATION TO TUMORIGENICITY OF HUMAN DIPLOID FIBROBLASTS USING A MUTA**GENE**: THE SV40 T ANTIGEN, F. Andrew Ray and Paul M. Kraemer, Cellular and Molecular Biology Group, Los Alamos National Laboratory, Los Alamos, New Mexico, 87545
We define a mutagene as a gene encoding a mutagenic protein. Unlike a traditional mutagen, a mutagene, can be expressed continually, thus providing a source of continuing genomic instability and chromosome mutation. We have recently shown that the SV40 large T antigen caused structural and numerical chromosome aberrations when expressed in human diploid fibroblasts (J. Cell. Biochem. 42:13, 1990). Chromosome mutation was observed in all newly expanded, T antigen positive clones prior to the acquisition of neoplastic traits such as anchorage independence, colony formation in low serum or immortalization. We report here that we have used three different plasmids which have in common the presence of this mutagene and have transformed human diploid fibroblasts to tumorigenicity in nude mice. Ten of thirty-one T antigen positive clones survived crisis and are immortal. The frequency of immortalization was 4.6×10^{-8}. The immortal cells continued to acquire a progressively more neoplastic phenotype but did not become tumorigenic in nude mice. However, cells injected into collagen sponges, pre-implanted subcutaneously, formed sporadic tumors with long latencies. Cells from these tumors, formed tumors at high frequency, with short latency and without the requirement for pre-implanted sponges. This work was supported by US DOE.

J 424 UNUSUAL GENE AMPLIFICATION IN A HUMAN CELL LINE IS DETECTED DURING *IN VITRO* CULTURING IN NON-SELECTIVE MEDIUM, Helene Roelofs, Hans den Dulk, Judith Tasseron de Jong and Micheline Giphart-Gassler, Department of Molecular Genetics and Department of Radiation Genetics, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands

From a human osteosarcoma cell line carrying in its genome one copy of a plasmid on which a *tk* and a *gpt* gene are located a clone has been isolated in which the expression of the *tk* gene has been switched off by *de novo* DNA methylation in the promoter region of this gene. Upon prolonged growth in medium containing mycophenolic acid (*gpt* selection) cells accumulate in which the DNA region containing the plasmid sequences has been amplified 2-10 fold. Most of these "amplified" cells have an unusual amplification pattern i.e.: one amplicon remains present at its original location while the newly formed DNA copies are detected mainly on different chromosomes. This means that duplication, excision and transposition precedes amplification to high copy numbers. It is assumed that the accumulation of "amplified" cells is caused by some selective advantage, but we have several lines of evidence that the expression of *gpt* is not involved in this selection. Even after subcloning and prolonged growth in non-selective DMEM medium we can detect in the population individual "unusually amplified" cells using fluorescence *in situ* hybridization. We suggest that enhanced expression (maybe obtained only by transposition) of some unknown gene on the amplicon contributes to the growth advantage *in vitro*.

J 425 TUMORIGENESIS IN BEAGLE DOGS REVEALS AN EARLY DECISION FOR DEVELOPMENT TO ANEUPLOIDY IN CANCER CELLS, Roland Sennerstam and Gert Auer, Department of Pathology, Division of Cell analysis, Karolinska Hospital, 104 01 Stockholm, Sweden.

Tumorigenesis was studied in brochial epithelia of five Beagle dogs. 50mg of 20--methylcholantrene (20-MC) suspended in 1.5 ml of sterile water was injekted weekly in the submucosa of the bifurcation of the apical and cardiac bronchi by means of a special needle through the bronchofiberscope. Cell sampling for cytomorphologic and cytochemical analysis was performed by means of a bronchofiberscopic brushing technique at the beginning of each experiment and thereafter immediately prior to injecting the 20-MC. Two control dogs were similarly treated with sterile warter only. Histologic and cytologic grading were done after Papanicolaou-staining. The slides were then de-stained and re-stained with a combined DNA and protein staining method (Feulgen-Naphthol-yellow). The amount of DNA and protein (mass) of each cell were estimated in a rapid scanning and integrating microspectrophotometer. Tumor progression was followed until cancer in situ cells appeared. In three of the five experiments aneuploid tumors was developed with a G1 peak bar in the triploid region and in two experiments the G1 peak bar appeared in the near diploid region. The difference in the two types of DNA distributions was observed in moderate dysplasia and clear cut in cancer in situ. The protein/DNA ratio was estimated and revealed an evident difference already in the mild and moderate dysplasia with a higher coefficient of variation (CV) in protein/DNA ratio for tumor progressions ending up in aneuploid cancers. The results show that the decision towards either near diploid or aneuploid tumors is taken very early in tumorigenesis and can be observed already in mild dysplasia. The higher CV in protein/DNA ratio indicates an increased degree of protein/DNA dissociation in a tumor progression leading to aneuploidy as compared to development to near euploid tumors. In human breast cancer cell lines reflecting near euploid and aneuploid cancers the aneuploid line showed a marked disturbed mitotic distribution of both protein and DNA indicating mitotic error to play a role in the generation of aneuploid cells, a theory that can explain early and great changes in DNA contents of the cells.

J 426 CHROMOSOME INSTABILITY IN PERIPHERAL BLOOD LYMPHOCYTES (PBL) OF CHRONIC CARRIERS OF HEPATITIS B VIRUS (HBV). Daniela Simon, Thomas London[+], Hie-Won L. Hann [O+], Barbara B. Knowles, The Wistar Institute, Philadelphia, PA, [+]Fox Chase Cancer Center, Philadelphia, PA, [O]Thomas Jefferson University, Philadelphia, PA.

The chronic carrier state of HBV is a high risk factor associated with the development of primary hepatocellular carcinoma (PHC). Metaphase chromosome spreads from peripheral blood lymphocytes of forty individuals, which were either HBV chronic carriers or HBV negative cases of the same ethnic origin were investigated. Chromosome and chromotide gaps and breaks as well as other mitotic aberrations were far more frequent in the HBV chronic carrier population than in the HBV-negative population. Among all HBV carriers, individuals with circulating HBV in peripheral blood as evidenced by Southern blot analysis, were all serologically positive for HBeAg and exhibited significantly the highest incidence of chromatid and chromosome gaps and breaks. Within HBV carriers with family history of PHC a threnl toward chromosome instability was detected. These data suggest that chromosome instability in PBL, results from HBV infection. Individuals HBeAg positive and HBV-DNA positive manifest the highest degree of chromosome instability. These results suggest the possibility that HBV might be directly or indirectly involved in generating genetic lessions. Consequences of these lessions and their role in hepatocellular carcinogenesis will be discussed.

J 427 CYTOGENETIC ABNORMAL CLONES IN FIBROBLASTS FROM XERODERMA PIGMENTOSUM MUTATION CARRIERS, Miria Stefanini, Alessandra Casati, Rosa Giorgi* and Fiorella Nuzzo
Istituto di Genetica Biochimica ed Evoluzionistica C.N.R. 27100 Pavia Italy, *Dip. Genetica Microbiologia, Università Pavia
Chromosome analysis was carried out in cultured fibroblasts from unaffected skin of 13 xeroderma pigmentosum (XP) patients (7 XPC, 1 XPA, 3 XPD, 2 patients with the association of XPD and trichothiodystrophy -TTD-) and 21 family members. A low frequency of structural chromosome changes was detected in cultures from all but one individuals. Furthermore different abnormal clones, some of which present in the primary explant and in the early culture passages, were observed in 6 individuals carrying the XPC mutation (3 homozygotes and 3 heterozygotes), in 1 XPD patient and in 2 TTD/XPD heterozygotes. The anomalies were apparently balanced translocations, deletions and inversions. The analysis of rearrangements did not reveal preferential involvement of chromosome bands although some breakpoints were more frequent and common to different families. Since a similar cytogenetic pattern consisting of multiple unrelated clonal chromosome rearrangements is present in epithelial tumor as well as in premalignant lesions from XP and non XP patients, the clonal anomalies detected in normal cells carrying XP mutations may represent the initial step of the process leading to neoplastic transformation.

J 428 INTERRELATIONSHIP OF MICRONUCLEUS FORMATION, GENOMIC LOSS AND CELL TRANSFORMATION INDUCED BY DIETHYLSTILBESTROL (DES), Helga Stopper, Petra Lenz, Dietmar Schiffmann, Umberto De Boni** and Baldev K. Vig**, Inst. of Toxicology, University of Würzburg, Germany, Dept. of Physiology, University of Toronto, Canada**,. Dept. of Biology, University of Nevada Reno, USA.

Certain nongenotoxic carcinogens (e.g.some estrogens like DES) cause mitotic disturbances resulting in displacement, nondisjunction and subsequent loss of chromatin elements. DES induces micronuclei (MN) and neoplastic transformation in Syrian hamster embryo (SHE) fibroblasts. However, the key events at the early onset of this multistage process are still under debate. Our previous results show that DES induced MN contain kinetochores (K^+) suggesting the presence of whole chromosomes/chromatids. We now have analyzed the meta-anaphase ring arrangement of chromosomes in SHE cells. After DES treatment, a threefold increase in the number of chromosomes displaced from the ring was observed. In mouse LA 9 cells we found a similar increase, with a majority of K^+ chromosomes . Most likely, these displaced chromosomes can give rise to the observed K^+-MN. Furthermore, a 10% subpopulation of these MN exhibits chromatin compaction (electron microscopy). Since such MN will not be reintegrated, genomic loss will occur, possibly depending on the type of chromosome/chromatid enclosed. In addition, the frequency of DES-mediated SHE cell transformation also is 10% of the one for MN formation. Further investigations will show, whether these phenomena are related to the loss of tumor suppressor genes.

J 429 LINKAGE DISEQUILIBRIUM BETWEEN THY-1 AND ataxia telangiectasia (AT) IN A TURKISH POPULATION, Sharon Teraoka, Tod Bedilion, Glen Ferguson, Barbara Schaffer, Ozden Sanal, Nuran Salman, Asli Tolun, Richard A. Gatti, Lincoln McBride, and Winston A. Salser, Dept. of Biology, UCLA, Los Angeles, CA 90024
Ataxia telangiectasia (AT) is an autosomal recessive disorder with a myriad of symptoms including an increased level of spontaneous chromosomal translocations, predisposition to cancer (especially lymphoma and leukemia), and an increased risk of breast cancer in heterozygous females. AT is also characterized by progressive cerebellar ataxia, immunodeficiencies, and hypersensitivity to ionizing radiation. The fundamental defect in AT is thought to be in DNA processing or repair. AT has been localized to chromosome 11q22-23, a region containing many candidate genes. A LOD score of 3.63 at θ=0 was previously established by following a single RFLP in the thy-1 gene. We have used polymerase chain reaction techniques to create a more informative haplotype in the thy-1 region. In the Turkish population, we have found a significant linkage disequilibrium between AT and thy-1. Several regions of thy-1 were amplified by PCR from affected and unrelated control individuals. The products were sequenced either directly or from cloned material, using an Applied Biosystems automated sequenator in order to identify additional RFLPs and other sequence variations that do not affect known restriction sites. This is a rapid method of searching any marker gene to reveal polymorphisms and, thus, take full advantage of available pedigrees otherwise uninformative for that region. Nine polymorphisms were found in a total of 6859 bp of genomic DNA from the thy-1 region sequenced from both AT affected and unrelated control individuals. Two of the new RFLPs together with the previously characterized Msp polymorphism constitute an informative thy-1 haplotype. Comparison of thy-1 haplotype frequencies in AT affecteds (60 chromosomes from 41 families) versus controls in the Turkish population showed linkage disequilibrium between AT and thy-1 with a X^2=9.7 at the p=.0018 level of significance. This disequilibrium could be explained if a significant number of the Turkish AT cases arose from a gene defect mapping very close to thy-1 in a founder of considerable antiquity. That AT affecteds from scattered villages in Turkey should share the same thy-1 haplotype after many meioses suggests close linkage. This does not conflict with other evidence mapping AT complementation groups to an 8cM region defined by stromelysin and NCAM, approximately 20 cM centromeric to thy-1, since 3 AT complementation groups map to chromosome 11. Demonstration of linkage disequilibrium in a geographic region where a stable village structure has persisted for thousands of years is a powerful approach for precisely mapping a gene defect, especially where genetic heterogeneity or lack of large numbers of affecteds reduce the power of more conventional pedigree analysis.

J 430 GENOMIC ALTERATIONS IN HUMAN BREAST AND OVARIAN CANCERS.

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Due to potential associations with particular tumor stages or a negative outcome of the disease the incidence of genetic alterations in human cancers has drawn extensive attention. We studied series of ovarian and breast tumors for the incidence of proto-oncogene amplification and loss of alleles. Proto-oncogene amplification is a frequent event in breast cancer since five genes or genetic clusters were found amplified (*MYC, ERBB2-ERBA1, INT2-HST-BCL1, BEK, FLG*) and approximatly 50% of the 415 tumors surveyed presented at least one these genes amplified. Each amplification seemed representative of distinct, yet not exclusive, tumor subsets. In comparison the proto-oncogene amplification is not as frequent in ovarian as in breast cancer. As a matter of fact only *MYC* showed an incidence of amplification (25%) comparable to that found in breast tumors.

Chromosomal deletions, defined by loss of heterozygosity (LOH), are frequently encountered in breast cancer, as testified by numerous reports. We undertook a systematic study of LOH using markers located on chromosomes 1 (p and q arms), 3p, 11p, 13q, 17p. Every location studied presented LOH in a range of 20 to 40% of the informative cases. None of the defined LOH showed an association with the amplification of a particular gene. More detailed statistical analyses are underway in order to determine if the advent of gene amplification or LOH specifies a special cohort of tumors. If this would be the case then gene amplification or/and LOH could be considered as landmarks of the genomic destabilization which has taken place in breast tumor cells. Thus this leaves the question wether the introduction of genomic instability may not be considered as a driving force in cancerogenesis.

J 431 COINCIDENT DECREASE IN $p34^{cdc2}$ LEVELS AND RB DEPHOSPHORYLATION (RB-P^-) IN RETINOIC ACID(RA) DIFFERENTIATED NEUROBLASTOMA CELLS(NB).

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NB is a tumor, like retinoblastoma, in which loss of genetic material may be important in the development of malignancy. NB is a pediatric embryonal tumor in which several genetic alterations including amplification of MYCN, chromosome 1p deletion, loss of heterozygosity on chromosomes 14 and 11 and loss of a potential suppressor gene on chromosome 17 have been described. Despite these genetic alterations, RA treatment of some NB cell lines can arrest cell growth and induce differentiation. This suggests genes necessary to control growth and differentiation are not lost but that their regulation is altered. To study the mechanisms of growth control and differentiation in RA treated NB cells, we assessed the levels of $p34^{cdc2}$, a protein kinase whose activity is critical for cell cycle progression, and RB, the product of a tumor suppressor gene that is a substrate for $p34^{cdc2}$ kinase in vitro. RB is phosphorylated(RB-P^+) in cells progressing through the cell cycle while RB-P^- has been proposed to regulate cell differentiation. $p34^{cdc2}$ decreases 75-fold in RA treated KCNR NB cells which growth arrest and differentiate while levels decrease only 2-fold in RA treated AS NB cells which is only partially growth inhibited and fails to differentiate. cdc2mRNA is transiently decreased in RA treated KCNR at 4 days yet approximate control levels at 7 days. At 7 days transcription of cdc2mRNA decreases 2-fold although there is also an increase in mRNA stability at this time in RA treated KCNR cells. Coincident with the decrease in $p34^{cdc2}$, RB-P^- is predominantly detected in RA treated KCNR while in RA treated AS cells where $p34^{cdc2}$ levels are high, only RB-P^+ is found. This is the first report of a dramatic decrease in $p34^{cdc2}$ levels coinciding with changes in the phosphorylation of RB in tumor cells induced to differentiate and suggests a mechanism which links cessation of cell proliferation and induction of differentiation.

J 432 THE CONSENSUS SEQUENCE FOR THE HYPERMETHYLATED CpG ISLAND PROMOTER REGION OF THE HUMAN L1 TRANSPOSONS DERIVED USING *mcr*$^-$ HOSTS, David M. Woodcock, Penelope J. Crowther and Judith P. Doherty, Molecular Genetics, Peter MacCallum Cancer Institute, Melbourne, Victoria 3000, Australia.

Mammalian genomes typically contain up to 100,000 copies of dispersed repetitive elements which are evolutionarily related to know transposable elements of lower eukayotes. These sequences are the L1 or LINE-1 family which have been shown to be evolutionarily and somatically mobile. We are interested in understand the mechanism of control of transposition and whether their elements contribute to instability of the genomic under certain conditions. The 5' presumptive control region of the human L1 (L1Hs) contains a CpG island which is highly atypical in that it is normally very heavily methylated *in vivo*. We have used optimally methylation-tolerant (deletion *mcr*$^-$) host strains to clone this region in order to generate an improved consensus for this presumptive control region of the L1Hs transposon. The 100 base pairs of the 5' end of L1Hs contains a consensus RNA polymerase III internal promoter necessary for the generation of full length transcripts. Such a transcript can form a tRNA-like clover leaf structure which may be important in priming the second DNA strand after reverse transcription. The 5' end also encodes another potential short ORF within the CpG island. If this ORF is functional, the protein product should constitute a marker for actively transposing L1Hs elements.

Late Abstracts

p53 MUTATIONS IN LYMPHOID NEOPLASIA. Gianluca Gaidano, Paola Ballerini, Jerry Z. Gong, Giorgio Inghirami, Elizabeth W. Newcomb, Ian T. Magrath, Daniel M. Knowles and Riccardo Dalla-Favera, Department of Pathology and Cancer Center, Columbia University, New York, NY 10032; Department of Pathology and Cancer Center, New York University, New York, NY 10032; Pediatric Branch, National Cancer Institute, Bethesda, MD 20892

Mutations affecting the p53 "tumor suppressor" gene have been found associated with several types of human neoplasia, whereas no comprehensive data are available for lymphoid malignancies. We have investigated p53 gene mutations in 229 human tumors of B- or T- cell origin (202 fresh cases and 27 cell lines) including Acute Lymphoblastic Leukemia (ALL), non-Hodgkin Lymphoma (NHL) and Chronic Lymphocytic Leukemia (CLL). For each sample, PCR-amplified fragments corresponding to exons 5 through 9 were analyzed by the Single Strand Conformation Polymorphism (SSCP) analysis; positive cases were further analyzed by direct sequencing of PCR products. Mutations in p53 coding sequences were detected in 46/229 samples and are specifically associated with: i) Burkitt's lymphoma (14/36 biopsies; 17/27 cell lines); ii) B-CLL (6/40) and, in particular, with its late progression stage known as Richter's syndrome (3/7). In the vast majority (43/46) of cases, p53 mutations were represented by point mutations (43 events), frequently (46%) involving base transitions at CpG dinucleotides. 74 % of the observed mutations fell into highly conserved domains of the protein as previously described for other tumor types. Although there is no substantial evidence for the loss of 17p locus in lymphoid malignancies at the cytogenetic level, many samples examined are homozygous or hemizygous for the mutated allele, suggesting that the normal allele has been lost. Our data show that, in addition to EBV infection and c-myc oncogene activation, p53 mutations are a common genetic lesion in Burkitt 's lymphoma; in addition, mutated p53 genes may play a role in tumor progression in B-CLL.

MECHANISM OF GENE AMPLIFICATION: GENERATION OF INVERTED REPEAT DUPLICATIONS. S. Lavi, D. Hassin, S. Karby, S. Cohen, Z. Ophir and E. Seroussi. Dept. of Cell Biology, Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel.

To elucidate the molecular mechanism of DNA amplification an in-vitro system was developed (Berko et al., MCB 10, 75-83, 1990). This system consists of cytosolic extracts from carcinogen treated CO60 cells, T antigen and a plasmid (8.2 Kb) containing the viral origin of replication. Extracts from the treated cells catalyze the overreplication of the 2.4 Kb origin region, while extracts from untreated cells are incapable of supporting replication. Though the template molecules are not fully replicated, a substantial fraction of the amplified DNA molecules underwent two rounds of replication yielding MboI sensitive fragments originating from the origin region (MboI cleaves only rereplicated molecules). The rereplicated molecules are released from the template plasmid and are of heterogeneous sizes. Denaturation followed by fast renaturation and S_1 nuclease digestion revealed that these molecules harbor hairpin structures. Hairpin structures were not detected amongst the replication products from reactions catalyzed by HeLa extracts. Analysis of the in-vivo amplified DNA extracted from carcinogen treated CO60 cells demonstrated that a large fraction of the de novo amplified sequences have IR structures.

We propose that arrest in the advance of the replication forks leads to template switch and to aberrant "U turn replication" yielding hairpin structures which are released from the template. These structures might serve as precursors for the generation of inverted repeat duplications. Experiments supporting this model will be presented.

ABNORMALITIES OF CHROMOSOME 9p IN NON-SMALL CELL LUNG CANCER TISSUE (NSCLC) AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) CELL LINES, Robyn Lukeis*, Ursula Kees[x], Louis Irving[o], Sue Hasthorpe[+] and Margaret Garson*, *Department of Cytogenetics, St. Vincent's Hospital, Melbourne, Victoria, [x]W.A. Research Institute for Child Health, Princess Margaret Hospital, Perth, Western Australia, [o]Department of Thoracic Medicine, Repatriation General Hospital, Heidelberg, Victoria, [+]Cell Biology Research Unit, Peter MacCallum Cancer Institute, Melbourne, Victoria, Australia.

Abnormalities of chromosome 9p leading to deletion at bands 9p21-p22 are a non-random finding in lymphoid malignancies (10% of patients), but are also being reported at a high frequency in some solid tumors (e.g. mesothelioma, glioma). We have observed a high frequency of abnormalities of 9p in a cytogenetic study of fresh tissue from patients with NSCLC (18/23), confirming our original observation, and also in cell lines derived from patients with ALL (5/6), a much higher incidence than occurs in ALL in general. In NSCLC patients, in complex karyotypes, various structural or numerical abnormalities led to common regional 'loss' distal to 9p22 and were seen in all histological subtypes (8/10 squamous, 7/10 adenocarcinoma, 3/3 large cell). Breakpoints were variable between 9q13-p22. In the ALL cell lines, 4 lines had breakpoints at 9p13 or p22, 1 had monosomy 9, with 3 lines having cytogenetic evidence of loss distal to 9p22. The region of 9p lost in these two tumor types may overlap and involve loss of common genes significant in tumor suppression for a variety of cell types. Such abnormalities appear to give proliferative advantage to ALL cells in long-term culture.

ROLE OF ADHESION/INTEGRIN MOLECULES AND CYTOKINES IN THE RECRUITMENT, MIGRATION AND LOCALIZATION OF INFLAMMATORY LEUKOCYTES TO THE DERMIS AND EPIDERMIS DURING TUMOR PROMOTION.

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Following topical application of the tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) to the epidermis of SENCAR mice, circulating peripheral blood PMN collected by orbital sinus puncture were increased in numbers and oxidized greater amounts of 2', 7' dichlorofluorescin diacetate (DCFH) than cells isolated from acetone control mice, a measure of hydroperoxide production. These leukocytes also displayed enhanced chemoattractive responsiveness to Interleukin-8 (IL-8) that was comparable to the chemotactic peptide f-MLP (10^{-7} M) and greater than that to IL-1 at equimolar doses. Tail vein injection of antibodies directed against either Intercellular Adhesion Molecule (ICAM-1) or Leukocyte Function Associated Antigen (LFA-1) [25, 50, 100 ug] significantly diminished TPA-induced epidermal inflammation and inhibited migration of leukocytes into the upper dermis and epidermis. These observations suggest that inflammatory mediators may recruit PMN from the marginal pools into the circulation and subsequently direct migration of these activated cells into the dermis and epidermis. Furthermore, these studies suggest that adhesion/integrin molecules may be important in modulating cutaneous localization of leukocytes during the tumor promotion process.