IONIZING RADIATION DAMAGE TO DNA: MOLECULAR ASPECTS

Organizers: Susan Wallace and Robert Painter

January 16-21, 1990

Plenary Sessions Page

Poster Sessions

Keynote Address

CB 001 IONIZING RADIATION AND OTHER ENVIRONMENTAL CARCINOGENS, Richard B. Setlow, Biology Department, Brookhaven National Laboratory, Upton, NY 11973

There are three environmental agents for which there are reasonable, but obviously inadequate, carcinogenic dose response data for populations--cigarette smoking, sunlight exposure, and ionizing radiation. The data for chronic smoking usually relate mortality to total cigarettes smoked, age at which smoking begins and age at which it stopped. Lung cancer involves more than initiating events in cellular DNA because the carcinogenic risk decreases when smoking ceases. The initiating agents could be derived from benzo(a)pyrene or from tobacco specific nitrosamines. The heterogeneity in the response of the smoking population is presumed to be explicable in terms of variations in metabolic activation, detoxification, and DNA repair. Non melanoma skin cancer is a result of chronic sunlight exposure and is estimated by incidence rates because mortality is very low. Judging from animal studies and from xeroderma pigmentosum, DNA repair deficit people, DNA is the most important target and hence, wavelengths between 290 and 320 nm are presumed to be the effective ones because of the high yield of photoproducts in this wavelength range. There are no good individual exposure data. The epidemiological data usually used relate incidence to the average annual exposure at ground level weighted by an appropriate action spectrum. The wavelengths effective in melanoma mortality are not well known. The best available data for evaluating the carcinogenic risks of ionizing radiation come from studies of the mortality of the Japanese population who survived exposure to the acute gamma irradiation at the time of the atomic bombings. The BEIR V Report gives the best estimates of risk as a function of exposure taking a linear response for all cancers except leukemia for which a linear-quadratic response was used. The report estimates that a lifetime exposure to one mGy/yr (approximate background radiation excluding exposure to radon and medical procedures) would account for about 3% out of the normally expected 18% lifetime cancer mortality. The role of DNA repair of ionizing radiation damage in the susceptibility to radiation induced cancers is not clear. We should look for innovative ways to extrapolate from high doses and dose rates to low doses and low dose rates. We need better ways to unravel the molecular changes and, if they are in DNA, their locations in the genome as well as learning the roles of radiation in inducing oncogenes or in activating antioncogenes. (This work was supported by the Office of Health and Environmental Research of the U.S. Department of Energy.)

Excision Repair of Radiation Damage

CB 002 BASE EXCISION REPAIR ENZYMES FROM EUKARYOTIC SOURCES, Paul W. Doetsch, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA 30322.

A wide variety of chemically distinct base modifications are produced by ionizing radiation and oxidizing agents. These base damages include products of pyrimidine ring saturation (eg. thymine glycol), ring fragmentation (eg. urea), ring contraction, as well as exocyclic ring modifications (eg. uracil and hydroxymethyluracil). Damaged purines such as the imidazole ring-opened formamidopyrimidine derivatives of adenine and guanine and other oxidation products including hypoxanthine are also produced by such agents. Eukaryotes possess a number of enzymes that recognize and remove many of these damaged base products and such enzymes have been isolated and characterized from various sources ranging from yeast to human cells. These enzymes mediate the critical first steps in the excision repair of oxidative and radiation-induced DNA base damage, namely the recognition and removal of the base lesion. Virtually all of the eukaryotic base excision repair enzymes characterized to date eliminate the damaged base via an N-glycosylase activity to produce an abasic site in the substrate DNA. Some of these enzymes such as the uracil- and hydroxymethyluracil-DNA glycosylases recognize specific, individual chemical products whereas other enzymes such as the redoxyendonucleases possess broad substrate specificities directed against a variety of chemically diverse products that have lost ring aromaticity and planarity. In addition to possessing N-glycosylase activities, a subset of the eukaryotic base excision repair enzymes also possess apurinic/apyrimidinic (AP) endonuclease activities.

The application of DNA sequencing and HPLC methodologies as well as other techniques has greatly increased our knowledge of eukaryotic base excision repair enzymes with respect to their identification, substrate specificities, and mechanisms of action. Recent molecular modeling studies employing computer-generated oligonucleotide structures containing specific radiation damage products have also provided insights into elucidating the molecular signals responsible for repair enzyme recognition of substrates. The biological roles of these enzymes with respect to maintaining genetic stability in eukaryotes in response to radiation as well as to endogenously-produced DNA damages will also be discussed.

This work was supported by PHS grants CA 42607 and CA 01441 from the National Cancer Institute, DHHS.

CB 003 PRODUCTION OF BASE-FREE SITES IN DNA BY IONIZING RADIATION AND CLEAVAGE BY MAMMALIAN REPAIR ENZYMES, W. David Henner, and Barbara J.S. Sanderson, Division of Hematology/Oncology, Oregon Health Sciences University, Portland, OR 97201. Ionizing radiation and some antineoplastic drugs such as bleomycin and neocarzinostatin produce apurinic and apyrimidinic (AP) sites in DNA, as well as other base-free lesions such as 4-hydroxyapurinic sites. AP sites are also frequently produced spontaneously in cellular DNA by depurination and as a consequence of the action of repair glycosylases. Mammalian cells contain at least two distinct classes of endonucleases that cleave DNA at base-free sites. The major AP endonuclease activity, cleaves immediately 5' to the base-free site leaving a 3' hydroxyl terminus and a 5'phosphate deoxyribose moiety (Class II). Studies using oligonucleotides containing synthetic abasic sites indicate that this enzyme recognizes the absence of a base and does not require the presence of a deoxyribose sugar for DNA cleavage to occur. This activity requires Mg^{2+} and has been purified to homogeneity from several mammalian tissues.

The other form of AP activity cleaves AP sites 3' to the base-free site, leaving a 5' phosphate and a 3'phosphate-sugar residue (Class I). This form of AP endonuclease is Mg^{2+}-independent and can not be resolved from the redoxyendonuclease active on gamma-irradiated, oxidized or heavily UV-irradiated DNA. Neither Class I or Class II AP endonuclease alone can remove the deoxyribose residue from DNA after cleavage and the involvement of 3' diesterase or 5'dRpase activities (respectively) are necessary steps prior to polymerase action. It is likely that there exist additional, as yet undiscovered, forms of mammalian AP endonucleases, if the diversity parallels that of the E. coli system.

CB 004 MECHANISM OF ACTION OF *ESCHERICHIA COLI* BASE EXCISION REPAIR ENZYMES. Yoke W. Kow, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405. Ionizing radiation produces a wide spectrum of DNA damages which include DNA breaks, abasic sites and base damages. In phage systems, base damages have been estimated to constitute the bulk of the lethal events caused by ionizing radiation. In *Escherichia coli*, base damages are repaired predominantly through the base excision repair pathway which involves two major classes of repair enzymes, namely, DNA N-glycosylases and AP endonucleases. There are a number of DNA N-glycosylases in *E. coli* including uracil N-glycosylase, hypoxanthine N-glycosylase, 3-methyladenine N-glycosylase, formamidopyrimidine N-glycosylase, endononucleases III, VIII and IX and others. Based on the biochemical properties of these enzymes, they can be divided into two categories. Simple glycosylases such as uracil, hypoxanthine and 3-methyladenine N-glycosylases release the modified base, leaving behind an AP site with intact DNA backbone; whereas endonucleases III, VIII, IX and formamidopyrimidine N-glycosylase have an associated 3' AP endonuclease activity.

Endonulcease III is the best studied N-glycoslyase of the latter category and recognizes a wide spectrum of thymine radiolysis products. The N-glycosylase activity of endonuclease III acts through a transiminization reaction between the enzyme lysinyl residue and the imine linkage of the ring-opened sugar moiety of the damaged base. The damaged base is then released, with the concomitant formation of an imine-enzyme cmplex. The complex then catalyzes the elimination of the 3' phosphodiester bond by a β-elimination reaction, giving rise to the observed associated 3' AP endonuclease activity. Other enzymes in this category, N-glycosylases with an associated 3' AP endonuclease activity may operate through a similar mechanism.

In order to generate a good primer terminus for repair polymerase, the AP site that is left after the action of a N-glycosylase, be it nicked or intact, is further acted upon by a 5' AP endonuclease, which hydrolyzes the phosphodiester bond 5' to the abasic site. The major 5' AP endonuclease activities in *E. coli* are exonuclease III and endonuclease IV. Exonuclease III accounts for over 85% of the AP endonuclease activity in *E. coli*, and is thought to recognize the damage by virtue of its ability to recognize the inter-DNA space 5' to the AP site. However, in addition to recognizing AP sites, the enzyme incises 5' to lesions that are capable of ring opening, relieving the steric hinderance that it might impart to the incoming nucleophile attacking the 5' phosphodiester bond. Endonuclease IV has a substrate specificity that overlaps that of exonuclease III to a large extent, and might therefore have a similar mode of action. After the AP site is processed by the phosphodiesterase activity of a 5' AP endonuclease, repair polymerization and ligation of the nick complete the base excision repair pathway. This work is supported by a NIH grant GM37216.

CB 005 EXCISION REPAIR OF RADIATION DAMAGE, Jacques Laval, Timothy O'Connor and Serge Boiteux, Groupe Réparation des Lésions Radio et Chimioinduites, UA 147 CNRS, U 140 INSERM, Institut Gustave Roussy, 94805 Villejuif Cedex, France.

Ionizing radiation induces strand breaks in DNA and a large variety of base damage, including pyrimidine hydrates and imidazole ring-opened purines i.e. the formamidopyrimidine (Fapy) derivatives. These lesions are actively repaired in eukaryotes and prokaryotes by a specific DNA glycosylase. In Escherichia coli, Fapy residues are excised by the product of the fpg gene, the FPG protein. This gene maps at 81.7 min on the E. coli chromosome and has been cloned, sequenced and overexpressed (1).

Besides its DNA glycosylase activity the FPG protein nicks DNA containing AP-sites (2) by a mechanism of βδ-elimination and is therefore an AP-lyase. This enzyme exhibits a wide substrate specificity including the imidazole ring-opened form of 7-methylguanine, chloroethylguanine, and N-2 aminofluorene C(8)-guanine. Therefore, this enzyme apparently removes purine derivatives modified either at the C8 or N7 provided that the imidazole ring is ruptured.

The FPG protein contains one zinc atom per protein molecule. The protein sequence has a consensus sequence for a Zn-finger.

A mutant defective in the FPG protein has been isolated, which did not exhibit unusual sensitivity to γ-radiations. The quadruple mutant defective in all the known AP-endonucleases is under construction.

(1) Boiteux et al., EMBO J. 6, 3177, 1987.
(2) O'Connor and Laval, PNAS, 86, 5222, 1989.

Genetic Recombination and Radiation Repair

CB 006 INTRAGENIC RECOMBINATION BETWEEN HOMOLOGOUS CHROMOSOMES IN CHO CELLS, Alan R. Godwin and R. Michael Liskay, Departments of Human Genetics and Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06510

A system to study intragenic recombination between homologous chromosomes has been devised. The tk- CHO cell line, AT32 2E-5, was transfected to generate a cell line containing two Xho I linker insertion mutant Herpes Simplex Virus type 1 thymidine kinase (tk) genes in direct orientation. Forward and subsequent back selection for TK generated cell lines each carrying a single mutant tk allele or a direct duplication of the same allele at the same chromosomal insertion site. Cell fusion was used to generate tetraploid lines carrying the tk alleles at homologous positions on homologous chromosomes. The frequency of spontaneous interchromosomal recombination is less than 4×10^{-9}. The frequency of intrachromosomal recombination between the same tk alleles at the same chromosomal insertion site is 6.7×10^{-5}. Thus, the frequency of interchromosomal recombination is at least four orders of magnitude lower than intrachromosomal recombination in CHO cells. Due to the finding that homologous recombination leading to homozygosity of recessive oncogenes can be a mechanism for tumor formation, agents known to induce recombination in other organisms are being tested.

CB 007 REINITIATION OF STRAND EXCHANGE ON A THREE-STRANDED DNA INTERMEDIATE FORMED BY RECA PROTEIN, Charles M. Radding, B. Jagadeeshwar Rao and Biru Jwang, Departments of Human Genetics and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510

Efficient homologous pairing de novo of duplex DNA with a single strand coated with RecA protein requires saturation and full extension of the single strand by the protein. However, strand exchange, the transfer of a strand from duplex DNA to the nucleoprotein filament, which follows homologous pairing, does not require the stable binding of RecA protein to single-stranded DNA. When RecA protein was added back to isolated protein-free DNA intermediates in the presence of sufficient ADP to inhibit strongly the binding of RecA protein to single-stranded DNA, strand exchange nonetheless resumed at the original rate and went to completion. Characterization of the protein-free DNA intermediate suggested that it has a three-stranded region to which RecA protein binds. Part of the nascent displaced plus strand of the deproteinized intermediate was unavailable as a cofactor for the ATPase activity of RecA protein, and about 30% resisted digestion by P1 endonuclease, which acts preferentially on single-stranded DNA. At the completion of strand exchange, when the distal 5' end of the linear plus strand had been fully incorporated into heteroduplex DNA, a three-stranded nucleoprotein complex remained that dissociated only over the next 50-60 min. Deproteinization of this complex also yielded a three-stranded DNA complex in which the nascent displaced strand was partially resistant to both E. coli exonuclease I and P1 endonuclease. This three-stranded DNA complex showed a broad melting transition between 37°C and temperatures high enough to melt duplex DNA. We conclude that RecA protein promotes strand exchange via long three-stranded intermediates. Even after deproteinization, the nascent displaced third strand appears to remain intimately associated with the new heteroduplex DNA over a region of 1.5 - 2.0 kb. Functionally, strand exchange can be subdivided into two stages: one in which the three-stranded intermediate is formed and one in which the intermediate is reduced to a new heteroduplex DNA molecule plus a separated parental strand. The nature of the three-stranded intermediate is under further study.

CB 008 CHROMOSOMAL DIVERSITY AND RECOMBINATIONAL REPAIR OF DOUBLE-STRAND BREAKS, Michael A. Resnick* and Torston Nilsson-Tillgren**. Yeast Genetics/Molecular Biology Group, National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709 USA and **Institute of Genetics, University of Copenhagen, Denmark

Chromosomal interactions and recombination provide opportunities for repair, expression of recessive markers and rearrangements between nonhomologous chromosomes. Using the yeast Saccharomyces cerevisae we are exploring mechanisms of interactions, barriers and genetic controls on recombination when chromosomes are DNA divergent. We have used ionizing radiation to induce double-strand breaks (DSBs) which are normally repaired via recombination between homologues in diploid G-1 cells. Strains were constructed with one chromosome pair (among 16) being homoeologous (20% DNA sequence divergence). The genetic and molecular consequences of DSBs can be examined in these functionally equivalent homoeologues. The homoeologous chromosome is derived from the related yeast Saccharomyces carlsbergensis.

At low nonlethal doses, DSBs in the homologous regions of the genome (i.e., 15 pairs) appeared to have no genetic consequence. However, they had a profound effect on the divergent chromosomes (III or V). At nonlethal doses, nearly 10 to 15% of the cells lost one of the homoeologues (1); the efficiency was 0.3-0.4 per DSB in the divergent chromosomes. Irradiation of a yeast strain carrying a single artificial chromosome containing human DNA (i.e., completely divergent) increased the loss efficiency 2-3 times. We conclude that 1. most if not all DSB repair occurs via recombination; 2. chromosomes containing unrepaired DSBs have a high likelihood of being lost; 3. unrepaired DSBs are not lethal and 4. even divergent chromosomes can undergo limited recombinational repair and also translocation. We have examined further the interactions between divergent chromosomes and shown that ionizing radiation can induce intragenic recombination between divergent genes. Such recombination, whose genetic control we have identified, is likely to lead to new and novel versions of the original genes.

(1) Resnick, M.A., Skaanild, M., & Nilsson-Tillgren, T. PNAS 86(1989)2276

CB 009 THE FORMATION AND RESOLUTION OF RECOMBINATION INTERMEDIATES
Stephen C. West, Berndt Müller, Christine Jones, Carol Parsons and Börries Kemper*, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts. EN6 3LD, U.K. and * Institüt fur Genetik, Universität Köln, F.R.G.
Recombining DNA molecules are connected at the point of strand-exchange by a crossover known as a Holliday junction. Such Holliday junctions may be formed *in vitro* by the enzymatic action of the *E. coli* RecA protein. RecA gains access to duplex DNA by nucleation from a short single-stranded gap, forming helical nucleoprotein filaments which are capable of interaction with homologous duplex DNA molecules. Holliday junctions made by RecA protein are resolved by the phage-encoded T4 endonuclease VII or T7 endonuclease I. Cleavage occurs in either of the two possible orientations to form recombinant DNA molecules. The interaction of these nucleases with Holliday junctions has also been studied using synthetic DNA substrates which are bound with high affinity, as observed by gel retardation assays. Footprinting experiments reveal that only two of the four DNA strands that comprise the Holliday junction are protected by T4 endonuclease VII from hydroxyradical attack. The binding site occupies no more than six bases at the site of the junction.

Molecular Basis of Ionizing Radiation Mutagenesis

CB 010 ANALYSES OF GENE MUTATIONS ARISING *in vivo* IN HUMAN T-LYMPHOCYTES, Richard J. Albertini, Janice A. Nicklas and J. Patrick O'Neill, Genetics Laboratory, University of Vermont School of Medicine, Burlington, VT 05401
Mutations arising *in vivo* in the X-chromosomal hypoxanthine-guanine phosphoribosyltransferase (*hprt*) gene can be characterized at the DNA level in T-lymphocytes isolated from human peripheral blood. "Background" mutants from normal young adults (mutant frequency values ~ 10^{-6} - 10^{-5}) show approximately 15% with structural alterations of the gene, i.e. deletions and complex changes, detected on Southern blots. Breakpoints distribute evenly along the length of the gene, with a frequency of 0.0024 breaks/kb/mutation. Intra- versus extragenic breakpoint frequencies suggest that deletions up to ~ 90 kb can be tolerated. The remaining 85% of normal adult mutant T-cell isolates, studied by sequencing, show a variety of changes including single base changes (transitions and transversions), single base additions and losses, microdeletions, frameshifts and apparent splice site changes. No type of "point-mutation" predominates. Analyses of T-cell receptor (TCR) gene rearrangement patterns among mutants reveal occasional replicate recovery of mutants deriving from the same *in vivo* mutational event, which in adults, occurs in post-thymic cells. Studies of *hprt* T-cell mutants from human placental cord blood, reflecting fetal mutational events, show quite a different pattern. Here, ~ 85% of the mutants show structural changes of *hprt*, with most involving deletions of exons 2 and 3. Mutant frequency values are ~ 10-fold lower in newborns, and TCR gene patterns suggest pre-thymic *in vivo* mutations. Adult human cancer patients receiving total body ionizing irradiation from internal emitters (Radioimmunoglobulin Therapy) have elevated mutant frequencies, and show the "adult" pattern of *hprt* mutations, except that 33% have structural alterations of the gene. Again, breaks are linearly distributed, yielding a frequency of 0.0043 breaks/kb/mutation with a calculated maximum tolerated deletion size of 143 kb. This "spectrum" is intermediate between the adult "background" spectrum, and one generated by 300 rad external beam γ irradiation of G_0 human T-cells *in vitro*. This research was supported by NCI CA30688, NCI 5 PO1 CA 43791-02 and DOE FG0287ER60502.

CB 011 IONIZING RADIATION MUTAGENESIS IN MAMMALIAN CELLS, Helen H. Evans, Department of Radiology, Case Western Reserve University, Cleveland, OH 44122

In 1927 Muller identified ionizing radiation as the first mutagen and originated the study of mutagenesis. Muller observed that exposure of D. melanogaster to X rays caused both single gene and multigene effects, and he raised the question of the nature of the genetic damage responsible for these effects. The proportion of the two types of mutation and the nature of the genetic damage are still subjects of investigation more than 60 years later. Base change mutations induced by ionizing radiation are caused by changes in or loss of the coding properties of the purine or pyrimidine bases due to proton migration, saturation of double bonds, ring fragmentation or production of abasic sites. Radiation-induced changes in the levels of deoxynucleotide triphosphate precursors also have been shown to result in base-change mutations. Radiation-induced double strand breaks in the deoxyribose phosphate backbone can yield frame shift mutations and both small and large deletions as well as chromosome inversions and translocations. Conversion of double-strand breaks to chromosome aberrations is thought to involve recombinational mechanisms and has been found to increase in the absence of repair (i.e., in repair deficient cells, in the presence of repair inhibitors, or in the G2 phase of the cell cycle).

The proportion of radiation-induced single gene vs. multigene mutations is influenced by many factors, one of the most important of which is the genetic system employed. Mutations affecting multiple genes are likely to be lethal in haploid systems as well as in hemizygous regions in diploid systems. Thus, in the case of haploid and hemizygous target genes, recovered mutants exhibit primarily base change and frame shift mutations as well as intragenic deletions. In contrast, in diploid systems at heterozygous loci, the vast majority of recovered mutants harbor multilocus lesions. The mode of radiation administration also affects the ratio of single gene vs. multigene mutations. The proportion of mutants with multilocus lesions, thought to result from the interaction of single damaged sites, increases with dose and with ion density and decreases with dose rate in repair-competent strains of N. crassa but not in strains of L5178Y mouse lymphoma. The proportion has also been found to change with the cell cycle stage at the time of irradiation, and with the presence of radiation protectors. The proportion of mutants with multilocus lesions has been found to increase in repair-deficient cell strains. Although much has been learned in the years since Muller first observed the mutagenic effects of ionizing radiation, much remains to be elucidated concerning the mechanism of mutation induction.

CB 012 USE OF MUTATIONAL SPECTRA AND RETROVIRUS INSERTIONAL MUTAGENESIS IN ELUCIDATING MAMMALIAN CELL RADIATION RESPONSE, ANDREW J. GROSOVSKY, University of California, Riverside, California 92521

Our laboratory is utilizing two complementary strategies in order to develop a more comprehensive understanding of mammalian cell response to radiation exposure. In one approach, we have developed a system for the comparison of mutational specificity at a single endogenous cellular locus available in either heterozygous or hemizygous form. Our other approach is to use insertional mutagenesis of human cells with retrovirus shuttle vectors in order to ultimately identify novel genes involved in human cell radiation response.

Loss of heterozygosity at previously heterozygous loci may occur by one of several possible mechanisms and account for a large fraction of all mutations occurring at such loci. In order to investigate loss of heterozygosity events, we have chosen the *aprt* locus of Chinese hamster ovary (CHO) cells as our model since it is readily available in either heterozygous or hemizygous form. Cloning and sequencing of the two heterozygous *aprt* alleles from the CHO derivative D423 identified a single polymorphic site, which does not create a restriction fragment length polymorphism. In order to evaluate loss of heterozygosity events at this locus, we devised a method which creates an artificial restriction fragment length polymorphism (A-RFLP) in one of these two alleles as a direct consequence of enzymatic amplification. Restriction enzyme digestion of the amplified sequences followed by polyacrylamide gel electrophoresis conveniently identifies the genotype of the DNA sample. Using this method, we have determined that 43% of spontaneous APRT$^-$ mutants derived from D423 have been lost heterozygosity at the *aprt* locus.

In order to characterize parameters of insertional mutagenesis in human cells, the human B lymphoblastoid cell line TK6 was exposed to infection with the retrovirus shuttle vector pZipNeo for periods of 12 to 72 hours. The infection efficiency varied with infection period, ranging up to 3% in cells exposed for 72 hours. The provirus copy number was surprisingly low (1 to 3 proviruses per cell) and unlike infection efficiency, did not increase with longer infection periods. Under these conditions, mutation induction ranged from approximately 5 fold at *hprt* and *tk*, to 30 fold at *aprt*. In 1/2 of the APRT$^-$ mutants, the *aprt* locus was shown to be disrupted by a retroviral shuttle vector insertion. However, no intragenic proviral integrations were observed for the other APRT$^-$ mutants despite the 30 fold increase in mutation frequency. Similarly, no proviral integrations were recovered within the *tk* or *hprt* loci. Nevertheless, the mutational spectra at both *hprt* and *tk* are clearly distinguished from spontaneous mutagenesis as reflected in a significant under-representation of genomic rearrangements and loss of heterozygosity events. Therefore, retroviral insertional mutagenesis in this system may be largely attributable to extinction of gene expression created by a retroviral insertion outside of the locus at risk.

CB 013 MUTAGENESIS OF Escherichia coli BY IONIZING RADIATION, Franklin Hutchinson, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven CT 06511.

The processes involved in mutagenesis by ionizing radiation are better understood in Escherichia coli than in any other cell. Most of the data are for doses between 3 and 20 kilorads, for which the number of mutations/colony-forming unit is linear in dose D. About 10-15% of these mutations are independent of induction of the SOS response, and there is no evidence that the damage sites require processing to form mutations. These mutations are mostly G:C to A:T transitions, which may be induced by a deaminated cytosine radiation product that is mistaken for thymine by the DNA polymerase. Most, 85-90%, of the mutations need induction of the SOS response and therefore require processing of the damage to form a mutation. The most frequent such mutation is a base change in which the existing base pair is replaced by an A:T pair; this may be caused by preferential insertion of an A or a T opposite a site at which the base has been lost, either by hydrolysis of a damaged base or by the action of a glycosylase. Large deletions induced by ionizing radiation are being studied in genes integrated into a λ prophage in the bacterial genome. This construction permits viable deletions of tens of kilobases, deletions that in a bacterial genome will usually form lethal mutations by eliminating one or more essential genes. No good data exist at present for the incidence of mutations at low dose D, but arguments will be given that it has the form $\alpha D + \beta D[1 - \exp(-D/D_0)]$, where D_0 is the dose at which the SOS response is induced to 63% of its total level. Experiments will be described that could show this, and verify the prediction that at the lowest doses the radiation-induced mutations should be mainly the SOS-independent G:C to A:T transitions. At doses above 20 kilorads, the induced mutation rate appears to be approximately quadratic in D. Suitable experiments will be described to explore the reasons for this behavior.

CB 014 MOLECULAR NATURE OF IONISING RADIATION-INDUCED MUTATIONS IN THE hprt GENE OF HAMSTER AND HUMAN CELLS. John Thacker, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 ORD, United Kingdom.

To understand the process of mutagenesis in mammalian cells it is important to analyse the molecular nature of spontaneous and induced mutations in representative mammalian genes. It is now apparent that, even with genes coding for inessential products, the spectrum of mutation types detected after irradiation depends upon the target gene used. In this study a large series of independently-isolated mutants lacking activity of the HPRT enzyme was isolated in V79 hamster cells after irradiation with gamma-rays or alpha-particles, and compared to those induced by ethyl methanesulphonate (EMS) or spontaneous mutants. Characterization by biochemical, genetic, and molecular techniques showed that the radiation and EMS-induced series differed substantially in the proportions of different types of mutation isolated. The majority of radiation-induced mutations were large genetic changes (predominantly deletions of all or most of the hprt gene), while all of the EMS-induced mutants and about 80% of spontaneous mutants had the characteristics of point mutations. Blot analysis of total RNA isolated from 59 mutants with presumptive point mutations showed that the majority expressed some hprt mRNA, without change in the size of the molecule. Additionally almost all of the EMS-induced mutations revert to HPRT-proficiency at measurable frequencies, suggesting that these mutations are base-pair substitutions in structural or control regions of the gene.

The finding of sizeable deletions induced by radiation in the hprt gene, despite its hemizygous state, has led us to consider these as important for further study. The breakpoints of partial hprt gene deletions, induced by ionising radiation, were mapped by restriction analysis and found to show some evidence of an increased frequency around the centre of the gene (exons 4-5). The sizes of deletions which appear to extend beyond the boundaries of the hprt gene are being measured by pulsed-field gel electrophoresis. Similar types of radiation-induced mutants are being found in a study using freshly isolated human fibroblasts, and we are at present attempting to map and clone the breakpoints of partial deletions from these human mutants with a view to understanding the mechanisms of mutation induction.

Biological Consequences of Specific DNA Lesions

C B015 GENETIC EFFECTS OF MODIFIED BASES FORMED IN DNA BY IONIZING RADIATION, John M. Essigmann, Ashis K. Basu and Michael L. Wood. Department of Chemistry and Whitaker College of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139

The mutational specificity and genetic requirements for mutagenesis by 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) and 8-hydroxyguanine have been investigated in Escherichia coli. Both are DNA lesions induced by ionizing radiation. Thymine glycol was positioned at a unique site in the single-stranded genome of a bacteriophage M13mp19 derivative. Replication of the genome in E. coli yielded targeted mutations at a frequency of 0.3%; the mutations were exclusively T→C. Mutagenesis was SOS- and nth-independent (nth encodes endonuclease III, a thymine glycol repair enzyme). The adduct was not detectably mutagenic in duplex DNA. A chemical rationalization for the mutation observed for thymine glycol was developed by applying molecular modeling and molecular mechanical calculations to the same DNA sequence studied in vivo. Modeling suggested that the 5R,6S-isomer of cis-thymine glycol, when not base paired, was displaced laterally by ~0.5 Å toward the major groove in comparison to the position that thymine would otherwise occupy. This perturbation of DNA structure should increase the likelihood of a guanine:thymine glycol wobble base pair during replication, which would explain the mutational specificity of the base observed in the genetic experiments. The mutagenic effects of 8-hydroxyguanine in the same experimental system will be described.

CB 016 NUCLEOTIDE INSERTION AND BYPASS OF ABASIC SITES BY DNA POLYMERASES INCLUDING AN SOS-INDUCIBLE POLYMERASE FROM *ESCHERICHIA COLI*, Myron F. Goodman, Cynthia A. Bonner, Sharada K. Devulapalle, Sharon Hays, James Penny, Sandra K. Randall, John Petruska and Kevin McEntee+, Department of Biological Sciences, University of Southern California, Los Angeles CA 90089-1340, +Department of Biological Chemistry and the Molecular Biology Institute, UCLA School of Medicine, Los Angeles, CA 90024 Abasic (apurinic and apyrimidinic) lesions are a common form of DNA damage resulting from exposure of cells to radiation and other mutagens. If, during DNA replication, an abasic lesion is present in the template at a replication fork, then DNA polymerase is obliged to either pause and possibly dissociate at the lesion or to insert a nucleotide opposite the lesion which could lead to a point mutation. It has been well documented that polymerases insert nucleotides opposite abasic lesions in a nonrandom manner, with purines being inserted much more efficiently than pyrimidines, and with A inserted with at least a 5-fold preference over G. While insertion opposite the lesion is a highly inefficient reaction, similar to the insertion of a mispaired nucleotide opposite a normal template base, extension beyond the lesion appears to be even far less efficient than insertion. We present data for these two reactions, nucleotide insertion opposite the abasic lesion and extension beyond the lesion with emphasis on the effects of nearest-neighbor 3'- and 5'-tempate bases on the reaction rates. Different polymerases show significant differences in their ability to bypass abasic lesions. In particular, we will compare *E. coli* polymerases I, II, and III from uninduced cells and Pol II and III from cells induced for SOS. We will also discuss our current attempts to look for proteins that stimulate lesion bypass by using *in vitro* complementation assays to compare extracts from induced and uninduced cells.

CB 017 REPAIR AND SITE SPECIFIC MUTAGENESIS AT ABASIC SITES IN DNA, Arthur P. Grollman, Department of Pharmacological Sciences, State University of New York at Stony Brook, Stony Brook, NY 11794. Chemical methods have been developed by which lesions produced by ionizing radiation may be incorporated at defined positions in DNA. Novel plasmid vectors have been constructed which allow mutagenic spectra to be determined, site-specifically, in bacteria and mammalian cells. We have used this experimental approach to establish mutagenic specificities of abasic sites and 8-oxodeoxyguanosine.

Duplex oligodeoxynucleotides containing adducts or other lesions, ligated into an SV40-based shuttle plasmid vector, are used to transform bacteria or transfect simian kidney (COS) cells. Mutations, fixed during replication, are identified by oligodeoxynucleotide hybridization and DNA sequencing techniques. The advantages of this experimental system include homogeneity of input DNA, lack of bias in the procedure used for detection and the potential for systematically altering base sequence in the vicinity of the adduct.

Alkali-stable abasic sites were introduced at the desired position of the Tc^R gene in one or both strands of the plasmid vector. Using this approach, we can show that synthetic abasic sites are mutagenic and that "readthrough" occurs in bacteria and mammalian cells. The same system was used to study mutagenesis induced by 8-oxodG. The structural and other biological effects of this base modification, when imbedded within a DNA duplex, have also been determined.

These studies were supported by Grants ES04068 and CA17395 from the National Institutes of Health.

CB 018 STRUCTURE/FUNCTION RELATIONSHIPS INVOLVED IN THE BIOLOGICAL CONSEQUENCES OF PYRIMIDINE RING SATURATION AND FRAGMENTATION IN PRODUCTS, Susan S. Wallace and Hiroshi Ide, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405. We have used the thymine ring saturation products, thymine glycol and dihydrothymine, and their alkali cleavage products, urea and β-ureidoisobutyric acid, as models for studying the intraction of DNA polymerases with free radical-induced thymine lesions in DNA. Thymine glycol is introduced into single-stranded M13 or f1 hybrid phage DNA by osmium tetroxide oxidation and the number of thymine glycols present in the DNA is quantitated by an antibody specific to the lesion. Dihydrothymine is introduced into phage DNA by incorporation of dihydrothymidine from supplemented culture medium and the number present in isolated phage DNA is quantitated by an antibody specific to this lesion. Thymine glycol and dihydrothymine are quantitatively converted by alkali hydrolysis to their cleavage products, urea and β-ureidoisobutyric acid respectively.

Both ring saturation products, thymine glycol and dihydrothymine, lack planarity and aromaticity, assume a half chain conformation and presumably would produce distortion in the DNA chain due to the alteration of base stacking and pairing interactions. Interestingly, although sequencing gel analysis of the newly synthesized products from lesion-containing templates showed thymine glycol in the template chair to be a strong block to DNA polymerases in vitro, dihydrothymine was at best an extremely weak block. The same is true in vivo, that is, it takes about 1-2 thymine glycol lesions to constitute a lethal hit in single-stranded phage transfecting DNA but a minimum of 18 dihydrothymines to produce an inactivating event using the same system.

Both urea and β-ureidoisobutyric acid present in the template strand are strong, probably absolute, blocks to DNA synthesis in vitro. Although β-ureidoisobutyric acid is devoid of pyrimidine ring structure and is apparently non-instructive, a nucleotide was incorporated opposite the putative site by Pol I and AMV reverse transcriptase. With T4 DNA polymerase that contains a highly active 3'-5' exonuclease, DNA synthesis was arrested almost exclusively one base prior to (3' to) the putative β-ureidoisobutyric acid site in the template strand. With urea, DNA synthesis was arrested 1 base prior to the lesion with all polymerases tested. In keeping with the results obtained in vitro, it takes about 1 urea or β-ureidoisobutyric acid residue to inactivate single stranded phage DNA, suggesting that both lesions are absolute replicative blocks in vivo.

This work was supported by grants NIH CA33657 and DOE DE-FG02-87-ER60510

Global Responses to Free Radical-Induced Damage

CB 019 **THE *soxR* REGULON: INDUCIBLE DNA REPAIR AND OTHER FUNCTIONS,** Bruce Demple, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138.

Most aerobic organisms can respond to superoxide-generating agents by switching on the production of the scavenging enzyme superoxide dismutase. In *E. coli*, this induction is part of a complex regulon whose activation depends on the newly discovered *soxR* gene. A DNA repair enzyme for radical-induced deoxyribose damage (endonuclease IV) is also a key member of this defense regulon, and we are identifying the specific damages that are targetted by this enzyme. We have also purified and characterized the yeast and human counterparts of endonuclease IV, and have cloned the structural gene for the yeast endonuclease. Several additional biochemical functions have been found to be controlled by *soxR*. The mechanism of *soxR*-mediated gene activation, the functions of inducible proteins, and the properties of the corresponding eucaryotic repair enzymes will be discussed.

CB 020 HYDROGEN PEROXIDE TOXICITY, Stuart Linn, Sherman M. Chin, Ernst S. Henle, and Rajagopal Chattopadhyaya, Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720.

Killing of *Escherichia coli* by hydrogen peroxide proceeds *via* two modes. Mode-one killing appears to be due to DNA damage, has a maximum near 1-3 mM H_2O_2, requires active metabolism during exposure, and is inhibited by iron chelators. Strains deficient in recombination DNA repair or excision repair of oxyradical damage, those lacking NADH dehydrogenase, or all strains grown anaerobically are sensitized to this mode. Mode-two killing is due to uncharacterized damage, occurs in the absence of metabolism and exhibits a classical multiple-order dose-response curve up to at least 50 mM H_2O_2. It is not inhibited by iron chelators.

Utilizing a mutant approach, we have concluded that the SOS response is utilized for enhanced levels of recA protein which in turn enhance recombinational repair capability. DNA polymerase I is especially important both for its polymerase and its 5' → 3' exonuclease activities and exonuclease III and endonuclease IV participate in removal of oxyradical damage. The induction of several operons by oxyradicals also leads to the enhancement of numerous metabolic protective functions. The most important of these for survival appears at this time to be SOD and NADH dehydrogenase.

The peculiar shape of the dose-response curve for mode-one toxicity can be reproduced *in vitro* for the iron-mediated, H_2O_2-dependent inactivation of phage lambda or nicking of DNA. Kinetic studies with free radical scavengers indicate that this Fenton reaction produces an active oxidant species that is not a free hydroxyl radical. Instead we suggest that it is an iron-complexed oxygen radical (ferryl radical) which is quenched by higher concentrations of H_2O_2. The Fenton reaction can be effectively driven by NADH but not by NADPH. This observation, coupled with genetic analyses of sensitivity to hydrogen peroxide, leads us to propose that NADH drives an intracellular Fenton reaction that is responsible for the generation of toxic DNA damage by H_2O_2. Depletion of NAD(H) pools following DNA damage might then be a stress response invoked to minimize free radical DNA damage, while NADPH pools can remain intact or be enhanced to provide for free radical scavenger activity.

We are currently studying the effects of free radicals generated variously by Fenton-type reactions upon nucleosides, nucleotides and polynucleotides and comparing damage products to those reported for gamma radiation. Ultimately, these products will be compared to those formed *in vivo* under various stresses.

CB 021 MNSOD INDUCTION IN E.COLI IN RESPONSE TO THE SUPEROXIDE MEDIATED STRESS OR TO CELLULAR POTENTIAL REDOX STATE CHANGES. SOD PROTECTION AGAINST SUPEROXIDE MEDIATED MUTAGENESIS. Danièle Touati and Brigitte Tardat, Génétique et Membranes, Institut Jacques Monod, CNRS, Université Paris VII, 2 Place Jussieu, 75251 Paris cedex 05, France.

The expression of manganese superoxide dismutase (MnSOD) through which the global level of SOD is modulated in E.coli is usually oxygen dependent. It is overproduced upon an increase of superoxide ($O_2^{\cdot}$) intracellular flux. Induction is controlled at transcriptional level, as shown by studies using protein and operon fusions with lactose operon genes. Regulation mutants in which MnSOD is expressed anaerobically have been isolated and characterized. Products of two regulatory genes, including Fur protein, act synergistically to negatively control expression of MnSOD when cells are in a reduced state, iron appearing to play the role of a sensor of the redox state of the cells.

Mutants totally lacking SOD show an increased spontaneous mutagenesis, oxygen dependent, recA independent. Factors possibly implied in the mutagenic process (repair enzymes, polymerases, oxidative stress conditions..) are tested and discussed. An increase of hydroxyl radical formation in absence of SOD is proposed to be directly responsible for the DNA lesions leading to mutagenesis.

CB 022 A SUPEROXIDE RESPONSE REGULON IN *ESCHERICHIA COLI*. Irina R. Tsaneva* and Bernard Weiss, Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109.

In *E. coli*, the genes for the DNA repair enzyme endonuclease IV (*nfo*), Mn^{2+}-superoxide dismutase (*sodA*), and glucose-6-phosphate dehydrogenase (*zwf*) are known to be induced by paraquat and similar redox reagents that generate superoxide radical anions in vivo. A regulon containing these genes was defined by the discovery of a controlling locus, *soxR*, which is located clockwise of *uvrA* and *ssb* at 92 min on the linkage map of *E. coli*. *soxR* mutants were isolated via their effects on the expression of β-galactosidase by an *nfo-lac* operon fusion. Because the fusion contained the translational signals of its intact *lacZ* gene, *soxR* is probably a transcriptional regulator. Two types of *soxR* mutants were isolated: ones that constitutively overexpress genes of the regulon and ones in which the regulon is no longer inducible by paraquat. By altering the cloned region *in vitro* we have produced additional mutations, some of which were transferred to the chromosome. A large deletion resulted in a non-inducible phenotype, indicating that the gene product is not a direct repressor of *nfo* and that it may be a positive regulator. An insertion or deletion near one end of the *soxR* region altered a 17K polypeptide and caused constitutive overexpression of the target genes. Mutations that were over 600 bp distant resulted in the non-inducible phenotype, suggesting that *soxR* might belong to an operon. In a *soxR* mutant that constitutively overexpresses *nfo* and *sodA*, only the latter was turned off by anaerobiosis, indicating a parallel mechanism for its control. *soxR* null mutants demonstrated an increased sensitivity to killing by superoxide generators, whereas the constitutive mutants were about as resistant as wild type cells.

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Ionizing Radiation and Human Disease

CB 023 PHENOTYPIC COMPLEMENTATION OF ATAXIA TELANGIECTASIA (AT) GROUP D CELLS BY A SINGLE HUMAN CHROMOSOME, Clare Lambert[1], Roger A. Schultz[2], David C. Johns[2], and Errol C. Friedberg[1], Department of Pathology[1], Stanford University, Stanford, CA 94305 and Division of Human Genetics, University of Maryland[2], Baltimore, MD 21201.

The human hereditary disease AT is characterized by mutiple clinical phenotypes, notably severe cerebellar ataxia, abnormal dilatation of small blood vessels (telangiectasia) and immunodeficiency. At the cellular level the disease is characterized by hypersensitivity to ionizing radiation or X ray-mimetic chemicals, abnormal DNA synthesis in both unirradiated and γ-irradiated cells, and abnormal cell cycle kinetics in γ-irradiated cells. The disease is genetically complex and 4 distinct genetic complementation groups (A, C, D and E) have been identified thus far. We have introduced genetically tagged (with the *E. coli neo* gene) human chromosomes into AT group D cells by microcell-mediated chromosome transfer from human-mouse hybrids. AT cells showing resistance to geneticin (G418) were screened for enhanced resistance to streptonigrin. We isolated a hybrid line carrying a single human chromosome which confers increased resistance to streptonigrin, bleomycin and γ rays. The complemented AT cells also show normal patterns of DNA synthesis and normal cell cycle kinetics after exposure to γ radiation. The complementing chromosome has no effect on the streptonigrin sensitivity of AT group A or C cells. Detailed cytogenetic characterization of the complementing hybrid by G banding shows the presence a single small rearranged human chromosome which cannot be positively identified. Molecular studies using human chromosome-specific probes are in progress in an attempt to identify the chromosome carrying the AT group D gene. AT group D cells are not complemented following microcell-mediated transfer of chromosome 11 (which carries the AT group A gene[1]). This suggests that the AT-A and AT-D genes are on distinct chromosomes.

1. Gatti, R. A. et al. (1988) *Nature* **336**:577-580.

CB 024 GENETIC FINE MAPPING OF A GENE FOR ATAXIA-TELANGIECTASIA (AT), Richard A. Gatti, UCLA School of Medicine, Los Angeles, CA 90024.

We are fine mapping the gene for ataxia-telangiectasia Group A (ATA) with the intention to eventually clone it. We have previously localized this gene to chromosome 11q22-23 (Nature 336: 577, 1988). The region was identified by probe/enzyme markers THY1/MspI and pYNB3.12/MspI. These markers are approximately 10 cM apart. Preliminary data suggested that the ATA gene did not localize within the interval defined by these markers. Additional analyses confirm this and further indicate that the ATA gene is not telomeric to this interval. We are now fine mapping a region of approximately 31 cM, centromeric to pYNB3.12 (now called D11S144). Preliminary data indicate that the markers STMY/TaqI and D11S144/MspI flank the ATA gene. Further evidence suggests that the gene for AT Group C (ATC) also localizes to this region. Together the AT complementation groups A and C comprise 80-85 percent of AT homozygotes. Isolating the AT gene(s) promises to elucidate mechanisms of radiosensitivity, DNA repair/processing, cancer susceptibility (particularly breast cancer and leukemia/lymphoma), cerebellar degeneration, immunodeficiency, chromosomal breakage and premature aging.

CB 025 APPROACHES TO THE STUDY OF GERM-LINE DNA DAMAGE CAUSED BY EXPOSURE TO THE ATOMIC BOMBS, James V. Neel and Chiyoko Satoh, Department of Human Genetics, The University of Michigan, Ann Arbor, MI 48109-0618, and Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-Ku, Hiroshima 732, Japan

The past studies on the genetic effects of the atomic bombs will be summarized, and it will be suggested that the genetic doubling dose for acute radiation in this specific situation is of the order of 1.0 Sv. The emerging and rapidly evolving techniques for carrying this study to the DNA level will then be presented. Based on the currently estimated doubling dose, calculations will be made of the magnitude of any study which was projected to yield a statistically significant difference between the children of exposed and controls. In addition, we will discuss how, on the assumption that the exposures were mutagenic, bodies of data which fail to demonstrate significant differences between the children of exposed and controls can still be used to set, at specified probability levels, lower limits to the doubling dose.

CB 026 TREATMENT RELATED ACUTE MYELOID LEUKEMIA: NEW INSIGHTS IN LEUKEMOGENESIS by Janet D. Rowley and Michelle M. Le Beau, Department of Medicine, University of Chicago, Chicago, IL 60637

Five-15% of patients who received cytotoxic therapy for a malignant disease develop a myelodysplastic syndrome (t-MDS) or acute myeloid leukemia (t-AML). Among 104 patients with t-MDS or t-AML, 50 had received only chemotherapy, 33 only radiotherapy and 62 had received both. The mean time to bone marrow dysfunction was 50 months. Two frequent chromosome changes in t-AML and t-MDS are loss of chromosome 7 or part of the long arm [-7/del(7q)] or loss of the long arm, or all of chromosome 5 [-5/(del(5q)]. 93% of our patients have a clonal abnormality, which involves chromosomes 5 and/or 7 in 79% of patients. Band 5q31 (called the critical region) is deleted in every patient with a 5q- chromosome regardless of whether they have t-AML/t-MDS, or AML or MDS de novo. There are fewer patients with a del(7q) chromosome and it appears that there may be two regions that are deleted in different patients, either 7q22 to q31 or 7q32 to q34. We have identified genes on the long arm of chromosomes 5 and 7 for use in mapping these deleted regions. The genes encoding a number of hematopoietic growth factors are localized on chromosome 5 in bands q23-q33, including GMCSF, IL3, IL4, IL5, and CSF1 as well as other genes related to growth and differentiation CD14, EGR1, FGFA, ADRB2R and the protooncogene FMS, which encodes the receptor for CSF1. GMCSF and IL3 are linked, as are IL4 and IL5. These gene clusters are not linked to each other. These genes are deleted in most patients with a 5q- chromosome. These data suggest that Knudson's hypothesis regarding loss of growth regulating genes is also applicable to leukemia. It is our assumption that "anti-leukemia" genes are located in the critical regions of chromosomes 5 and 7, and that loss of function through chromosome deletion or mutation, of one or several of these genes is associated with leukemogenesis. The challenge is to use our mapping data to identify these critical genes.

Cloning Radiation Repair Genes

CB 027 CLONING RADIATION REPAIR GENES IN E. coli, Richard P. Cunningham, Susan M. Saporito, Peter M. Wistort and Hitomi Asahara, Department of Biological Sciences, SUNY Albany, Albany, NY 12222.
We have cloned the genes for endonucleases III and IV from E. coli and we have sequenced these genes as well as the gene for exonuclease III from E. coli. These studies have revealed interesting features about the genetic structure and regulation of these genes. Exonuclease III is transcribed from a promoter lacking a consensus -35 site and most probably requires a positive regulatory protein for transcription. Endonuclease IV is the proximal gene in an operon that also is transcribed from a promoter with a poor -35 site, suggesting that some regulatory mechanism might be required for efficient transcription. Quite surprisingly, the primary structures of exonuclease III and endonuclease IV are unrelated despite their very similar enzymatic activities. Endonuclease III appears to be the distal gene in an operon containing several genes. The promoter has been sequenced and is similar to a consensus promoter.

We have used the cloned genes to create disrupted genes in vitro. These mutant genes have been transferred to the E. coli chromosome for genetic studies. Our studies suggest that exonuclease III and endonuclease IV play a major role in the repair of AP sites. The role of endonuclease III in the repair of AP sites is not known; its major function may be the repair of damaged bases by its glycosylase activity.

We have subcloned the gene for endonuclease III onto an expression vector and purified the protein for biochemical and biophysical studies. Our biochemical studies have revealed the mechanism of action of endoIII and our biophysical studies show that endonuclease III is an iron-sulfur protein.

CB 028 GENETIC AND MOLECULAR STUDIES ON RECOMBINATIONAL REPAIR GENES IN YEAST

R.K. Mortimer, J. Game, D. Schild, J. Kans, K. Sitney and G. Cole
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The yeast *Saccharomyces cerevisiae* has three well-characterized pathways of DNA repair and approximately 40 genes are known to be associated with these pathways. For ultraviolet-induced lesions, excision repair and post-replication repair (error-prone) repair are the major routes of dealing with this type of damage although the recombinational repair pathway can effect repair of UV lesions in the absence of one or both these other pathways. The recombinational repair genes appear to be primarily responsible for dealing with strand breaks caused by ionizing radiations and chemicals. Our laboratory has concentrated on this group of genes as well as on some related genes. We have cloned and sequenced several of them (*RAD51, RAD54, RAD55, RAD57, RAD24*) and have studied their inducibility by DNA damage. *RAD51* and *RAD54* are inducible while *RAD52* is noninducible. We have defined the sequences necessary for constitutive expression and induction of *RAD54* and, very interestingly, have shown that lack of induction fails to change the radiation sensitivity of cells. By gene disruption experiments, we have shown that *RAD24* is an essential gene while none of the others tested is essential.

We have developed a sensitive procedure for studying the role of these recombinational repair genes in meiosis and in strand break repair. This procedure involves the use of circular chromosomes and these studies will be described.

CB 029 CLONING MAMMALIAN RADIATION REPAIR GENES, Christine A. Weber, Kerry W. Brookman, Nigel J. Jones* and Larry H. Thompson, Biomedical Sciences Division, Lawrence Livermore National Laboratory, P.O. Box 5507, Livermore, CA 94551, U.S.A. and *School of Biological Sciences, University College of Swansea, Singleton Park, Swansea SA2 8PP, United Kingdom

Ionizing-radiation sensitive mutants have been isolated from rodent cells and are being used to identify and clone the corresponding human genes. Our approach to this genetic analysis has been to classify the mutants into complementation groups, to identify the human chromosomal location of the complementing gene, and to clone and characterize the human genes. Six complementation groups of ionizing-radiation sensitive rodent mutants have been identified (1). The human genes that correct mutants representing two of these complementation groups, EM9 and *irs1*, have been assigned to human chromosomes 19 and 7, respectively (2). The V79 mutant *irs1* is ~2-fold hypersensitive to killing by ionizing radiation and has a greatly elevated level of radiation-induced chromosomal aberrations. However, the repair of strand breaks is normal in bulk DNA, and DNA synthesis shows a normal inhibition response. *Irs1* has a complex phenotype, being ~100-fold hypersensitive to the cross-linking agent mitomycin C and ~2-fold sensitive to UV-radiation. Analysis of a human-hamster hybrid cell line with a single chromosome 7 as the only detectable human material suggests that a single-gene defect is responsible for the multiple sensitivities. The complementing human gene, *XRCC2* (*X*-ray *R*epair *C*ross *C*omplementing), has not yet been cloned. The CHO mutant EM9 is 2-fold hypersensitive to killing by ionizing radiation and has a reduced rate of strand-break repair, a 10-fold elevated level of sister chromatid exchange (SCE), and a reduced efficiency of homologous recombination. Chlorodeoxyuridine is a highly effective selective agent in complementation studies. The complementing human gene, *XRCC1*, was isolated from a cosmid library of an EM9 tertiary transformant and is ~33 kb in size. In transformants of EM9, the human *XRCC1* gene restores the resistance to γ-rays, the rate of strand break rejoining, and the number of SCEs to wild-type. A near full-length *XRCC1* cDNA was isolated from a human fibroblast cDNA library in a mammalian expression vector, pcD2 (3). This cDNA clone partially corrects EM9, as reflected by the intermediate number of SCEs observed in cDNA transformants. Nucleotide sequence analysis of the cDNA insert and the genomic 5'-flanking region indicates *XRCC1* encodes a 633 amino acid protein. A putative promoter region containing two GC boxes and a CAAT box was identified. (Work performed under the auspices of the U.S. Department of Energy by LLNL under Contract W-7405-ENG-48.)

(1) Jones, N.J., Cox, R., and Thacker, J. (1988) Mutat. Res., 193:139-144.
(2) Siciliano, M.J., Carrano, A.V., and Thompson, L.H. (1986) Mutat. Res. 174:303-308; Siciliano, M.J. and Thompson, L.H., unpublished data.
(3) Chen, C. and Okayama, H. (1987) Mol. Cell. Biol. 7:2745-2752.

Radiation Damage and Repair in Chromosomes

CB 030 DIFFERENTIAL REPAIR OF DNA DAMAGE IN HUMAN GENES: INVOLVEMENT OF RNA POLYMERASE II, Steven A. Leadon and David A. Lawrence, Division of Cell and Molecular Biology, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

We have examined repair of UV- and aflatoxin B1 (AFB1)-induced DNA damage in the human metallothionein (hMT) gene family. We found that the initial rates of repair of both types of damage in three different transcribed hMT genes were more rapid than in the genome overall. Repair of UV damage in a nontranscribed hMT gene which shows cell type-specific expression and in a hMT processed pseudogene were about the same as in the genome overall, while repair of AFB1-induced damage was deficient in these genes. Inducing higher levels of transcription of the hMT genes increased the initial rate of repair in the transcribed hMT genes compared to that observed when they are transcribed at a basal level. The rates of repair in the nontranscribed hMT genes were not changed by the inducing treatments. These results suggest that repair efficiency depends on the level of transcription associated with a gene and on the type of damage introduced into a DNA sequence. We have extended these studies by comparing the rate of repair on the transcribed strands of the hMT genes to the nontranscribed strands. We found that the initial rate of repair on the transcribed strand of a gene was at least 3-fold faster than on the nontranscribed strand. The rate of repair on the nontranscribed strand of an active gene, on both strands of an inactive gene, and on both strands of a regulatory region 5' to an active gene were similar to that of the genome overall. Inducing higher levels of transcription selectively increased the rate of repair on only the transcribed strand. Our findings suggest that these differences in repair involve the transcriptional complex, either by having repair enzymes associated with it or by the arrest of transcription at a lesion in the DNA being a critical signal that selectively directs repair to the transcribed strand of a gene. In order to examine what role the transcriptional apparatus itself plays in directing repair on the transcribed strand of a gene, we treated cell cultures with an in vitro inhibitor of RNA polymerase II, alpha-amanitin. We found that the preferential repair on the transcribed strand was eliminated by treatment with alpha-amanitin and that the rate of repair was now similar to that found for the genome overall. In addition, the increased repair rate on the transcribed strand of a gene when transcription is induced was also abolished with alpha-amanitin. The initial rates of repair on the nontranscribed strand of the gene and overall initial repair levels were not reduced by this inhibitor. Taken together, it appears that the initiation of repair on the transcribed strand of a gene is independent of repair on the nontranscribed strand and may directly involve the transcriptional apparatus.

CB 031 THE USE OF RESTRICTION ENDONUCLEASES TO MIMIC CHROMOSOME DAMAGE INDUCED BY IONIZING RADIATION, W.F. Morgan,[1] D. Ager,[2] J.W. Phillips,[1] and R.A. Winegar,[1] [1]Laboratory of Radiobiology and Environmental Health, and [2]Department of Radiation Oncology, University of California, San Francisco, CA 94143.

Restriction endonucleases bind to specific DNA sequences and cleave the DNA helix to produce double strand breaks. We have introduced restriction endonucleases into exponentially growing cells by electroporation, enabling us to examine the cytogenetic lesions induced by endonucleases at different stages of the cell cycle, and to investigate how intercellular DNA repair processes modulate enzyme-induced chromosome damage. Since the cytogenetically important lesion induced by ionizing radiation is thought to be the double strand break, we have used a variety of enzymes with different recognition sequences and evaluated the role of cohesive-ended and blunt-ended double strand breaks in chromosome damage and repair. DNA double strand breaks were analyzed by pulsed field gel electrophoresis and breakage related to chromosome aberration yields and cell survival. Restriction endonuclease induced breaks are probably much "cleaner" than those induced by ionizing radiation. So in an attempt to mimic radiation induced breaks we have modified enzyme induced breaks utilizing T_4 ligase, exonuclease III, alkaline phosphatase, or mung bean nuclease, then examined their effect on the generation of chromosome damage. These data will be discussed in relation to current concepts on the mechanisms of cytogenetic damage induction. This work was supported by the Office of Health and Environmental Research of the U.S. Department of Energy, contract no. DE-AC03-76-SF01012.

CB 032 SEQUENCE SPECIFIC REPAIR OF ADDUCTS IN DNA: POSSIBLE EFFECTS OF CHROMATIN STRUCTURE AND GENE EXPRESSION, David A. Scicchitano[1], Isabel Mellon[2] and Philip C. Hanawalt[3], [1]Div. of Pathology and Toxicology, American Health Foundation, Valhalla, NY 10595-1548; [2]Dept. of Pathology, Univ. of Kentucky, Lexington, KY 40536-0093; [3]Dept. of Biological Sciences, Stanford Univ., Stanford, CA 94305-5020. Methylating agents such as dimethyl sulfate produce a variety of deleterious lesions in DNA. The aberrant bases 7-methylguanine (7mG) and 3-methyladenine (3mA) constitute approximately 74% and 18% of the total genomic damage, respectively. The repair of N-methylpurines in the DHFR gene of CHO-B11 cells occurs at a slightly faster rate when compared to their removal from total cellular DNA. Furthermore, essentially no differences exist among removal rates of these lesions for the active gene, an unexpressed region located downstream, or between the individual strands of the DHFR gene. These findings probably reflect the repair of the most abundant lesion 7mG and are notably different from the repair data for ultraviolet induced cyclobutane pyrimidine dimer photoproducts. Dimers are cleared poorly from the total genome of CHO-B11 cells but are well repaired in the transcribed strand of the DHFR gene. Experiments with E. coli have demonstrated that the removal rates for dimers from the transcribed and non-transcribed strands of the lac gene are the same when the gene is uninduced. In contrast, the repair rate in the transcribed strand of the E. coli lac gene increases by an order of magnitude when transcription of the locus is activated by the addition of IPTG to the medium. The observed differences for the DNA sequence specific removal of 7mG and dimers can be accounted for by at least two hypotheses. First, differences in processing of the lesions could be explained by the existence of two distinct excision pathways. 7mG is removed from DNA either spontaneously or by a glycosylase, whereas dimers are removed by nucleotide excision. Second, these findings support the notion that lesions in DNA that do not halt the progression of nucleic acid synthesis, such as 7mG, are not removed selectively from active genes, but lesions that block transcription (i. e. dimers) are removed at a significantly more rapid rate from expressed genetic loci. The mechanism for targeting repair to an active region may involve enhanced distortion of the DNA helix by the interaction of the lesion and the stalled RNA polymerase, thus causing initiation of nucleotide excision repair.

Late Addition

CB 033 **MODIFICATION OF DEOXYRIBOSE-PHOSPHATE RESIDUES BY ATAXIA TELANGIECTASIA CELL EXTRACTS.** LR Karam, P Calsou, WA Franklin, RB Painter, T Lindahl. ICRF Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, U.K.

We have been investigating the release of DNA 5'-terminal deoxyribose-phosphate residues from enzymatically incised apurinic/apyrimidinic sites by human cell extracts. During the course of these studies, the unexpected observation was made that ataxia-telangiectasia cell extracts modify deoxyribose-phosphate (dRp) residues by converting them to an adduct form, dRp-X, which shows altered chromatographic properties on HPLC analysis. The chemical nature of the adduct is as yet unknown, but dRp-X is stable to both heat and acid. The modification requires an enzymatic activity and a low-molecular weight co-factor. Extracts of normal cells contain a dialyzable inhibitor that suppresses the reaction occurring with ataxia telangiectasia cell extracts. Formation of dRp-X has been observed in 7 out of 7 ataxia telangiectasia lymphoblastoid lines tested, which represent at least 3 different genetic complementation groups. Similar modification of dRp did not occur with extracts of cells of normal origin, nor those representing Fanconi's anaemia, xeroderma pigmentosum, Bloom's syndrome, or Werner's syndrome.

DNA Repair Enzymology; Recombination

CB 100 EFFECTS OF DIMERS OR PHOTOLYASE-DIMER COMPLEXES IN E. COLI ON TRANSCRIPTION AND SOS INDUCTION, R. Bockrath, M. Kwasniewski and B. Li, Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202
In UV-mutagenesis of E. coli, bulky lesions like pyrimidine dimers in DNA initiate induction and activation of SOS functions for mutagenesis and target the site of a DNA alteration. When cells in the dark contain high levels of DNA photolyase, which binds to dimers, the production of mutants targeted at dimers is greatly reduced. This was attributed to disruption, by the photolyase-dimer complex, of the transdimer DNA synthesis that normally fixes a mutant sequence (J Bact 170, 5371; Mut R 226, 259). We now describe contrasting effects of dimers or photolyase-dimer complexes (in dark) on transcription. Cells (uvrA) exposed to UV were returned to growth medium containing IPTG to derepress synthesis of beta-galactosidase. The levels of this activity were measured at 10-40 min post-UV to ascertain the rates of enzyme synthesis. These rates decreased exponentially with UV (single hit) 2.6 times more rapidly when the cells contained amplified photolyase. This approxitmate 2-fold difference may indicate that photolyase-dimer complexes in either DNA strand block transcription but that dimers alone, only in one strand, can block transcription. Amplified photolyase did not alter the initial induction kinetics for SOS (using a sfiA::lacZ fusion) but did increase inactivation of expression after larger exposures. Thus, converting dimers to the very bulky lesion of a photolyase-dimer complex seems to dramatically stop transdimer DNA synthesis, at least double the probability of stopping transcription but add nothing to the character of events initiating SOS induction. Research supported by N.I.H. grant GM21788.

CB 101 ISOLATION OF A FORMAMIDOPYRIMIDINE-DNA GLYCOSYLASE (fpg) MUTANT OF ESCHERICHIA COLI K12, Serge Boiteux[1], Olivier Huisman[2], Timothy O'Connor[1] and Jacques Laval[1], [1]Groupe "Réparation des Lésions Radio et Chimioinduites" UA 147 CNRS, U 140 INSERM, Institut Gustave Roussy, 94805 Villejuif Cedex, France. [2]Unité Génie Microbiologique, Institut Pasteur, rue du Docteur E. Roux, 75015 Paris Cedex, France.
The fpg gene of E. coli coding for the FPG protein, a DNA glycosylase which excises imidazole ring-opened purines and nicks DNA at apurinic sites (O'Connor and Laval (1989) PNAS 86, 5222) was cloned on a multicopy plasmid (Boiteux et al. (1987) EMBO J., 3177). The plasmid copy of the fpg^+ gene was inactivated by cloning a kanamycin resistance gene into the open reading frame, yielding the fpg-1::Kn^R mutation. This mutation was transferred to the chromosome. The resulting fpg mutant exhibited no detectable Fapy-DNA glycosylase activity in crude lysates. The insertion mutation was localized by means of genetic crosses between mtl and pyrE, at 81.7 min on the E. coli linkage map (Boiteux and Huisman (1989) Mol. Gen. Genet. 215, 300).
The formamidopyrimidine-DNA glycosylase defective strain does not show unusual sensitivity to the following DNA damaging treatments: (i) methylmethanesulfonate, (ii) N-methyl-N'-nitro-N-nitrosoguanidine, (iii) ultraviolet light, (iv) γ-radiation. The fpg gene is neither part of the SOS regulon, nor the adaptive response to alkylating agents.
The fpg-1 mutation was transduced into E. coli strains defective in DNA repair genes such as recA, uvrA, nth, nfo, xth, tagA or alkA. The sensitivity to alkylating agents and γ-radiations of these mutants is under study.

CB 102 COVALENT COMPLEX FORMATION BY E. coli DNA ALKYLTRANSFERASE WITH BCNU-TREATED OLIGODEOXYNUCLEOTIDES, Thomas P. Brent[1], Prescilla E. Gonzaga[1], and Geoffrey P. Margison[2], [1]Department of Biochemical and Clinical Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38101, [2]Paterson Institute for Cancer Research, Manchester, UK.
DNA alkyltransferases have been isolated from a wide variety of organisms, ranging from prokaryotes to higher eukaryotes. These enzymes all repair O^6-alkylguanine, a highly mutagenic lesion in DNA. Although the enzyme mechanism for O^6-alkylguanine repair appears essentially the same for bacterial and mammalian alkyltransferases, their substrate specificities are different. The *ada* gene protein from E. coli also repairs O^4-alkylthymine and alkylphosphotriesters in DNA, whereas the mammalian transferase lacks these functions. Precursors of cytotoxic DNA crosslinks, induced by antitumor chloroethylnitrosoureas (e.g. BCNU) also are removed by both E. coli and human transferases. Our previous studies have shown that the human transferase forms a covalent complex with these crosslink precursors. We report here that the 37,000 dalton *ada* protein from E. coli and a 20,000 dalton C-terminal fragment of this protein which contains the O^6-alkylguanine transferase function, also form such complexes. However, an N-terminal construct, which has only the alkylphosphotriester transferase activity, does not form a complex with BCNU-treated oligodeoxynucleotide. These results are consistent with our earlier proposal that covalent complex formation involves the cyclic adduct O^6,N^1-ethanoguanine. Supported by Grants CA 14799, CA 36888 and CA 21765 from the NIH and by ALSAC.

CB 103 MODIFICATION OF DEOXYRIBOSE-PHOSPHATE RESIDUES BY ATAXIA-TELANGIECTASIA CELL EXTRACTS. P Calsou, LR Karam, WA Franklin, RB Painter, T. Lindahl. ICRF Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, U.K.

We have been investigating the release of DNA 5'-terminal deoxyribose-phosphate residues from enzymatically incised apurinic/apyrimidinic sites by human cell extracts. During the course of these studies, the unexpected observation was made that ataxia-telangiectasia (AT) cell extracts modify deoxyribose-phosphate (dRp) residues by converting them to an adduct form, dRp-X, with altered HPLC chromatographic properties. The chemical nature of the adduct is as yet unknown, but dRp-X is stable to heat and acid. The modification requires an enzymatic activity and a low-molecular weight co-factor. Extracts of normal cells suppress the reaction occurring with AT cell extracts. Formation of dRp-X has been observed with 5 out of 5 AT lymphoblastoid lines tested, representative of at least 3 different genetic complementation groups. Similar modification of dRp did not occur with extracts of cells of normal origin, or representing Fanconi's anaemia, xeroderma pigmentosum, Bloom's syndrome, or Werner's syndrome.

CB 104 A NOVEL 3'-DNA DIESTERASE AND APURINIC/APYRIMIDINC ENDONUCLEASE FROM HUMAN CELLS, Davis S. Chen, Tory Herman and Bruce Demple, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138 A synthesized DNA fragment containing 3'-phosphoglycolaldehyde (3'-PGA), an analogue of the 3'-phosphoglycolate produced by oxidative DNA damage, was used to analyze the enzymes from HeLa cells that hydrolyze these esters to produce 3'-OH termini. Two activities have been resolved during conventional liquid chromatography. We examined these two activities using polyclonal antibodies directed against the major HeLa AP endonuclease, and by gel filtration chromatography. One of the activities corresponds to a 37-kd protein that was precipitated by the anti-AP endonuclease antibodies. The other activity did not cross-react with the antibodies and eluted in the position of a 48-kd globular protein on gel filtration. Both proteins have Type II (hydrolytic) AP endonuclease activity as well as 3'-diesterase, but in different ratios. Two such enzymes were also found in other human cell lines and in CHO cells. The characteristics of these proteins and work toward cloning of their structural genes will be discussed.

CB 105 ANALYSIS OF THE STRUCTURE OF URACIL-DNA-GLYCOSYLASE FROM E. coli. Devchand, P.R. , Varshney, U. and van de Sande, J.H. , Department of Medical Biochemistry, University of Calgary, 3330 Hospital Dr., Calgary, Alberta, Canada. T2N 4N1.

Uracil-DNA-Glycosylase (UNG), a base-excision repair enzyme, catalyses the excision of uracil from DNA. Our laboratory has identified the first complete nucleotide sequence of UNG from E. coli (Varshney, U. et al J.Biol.Chem.(1988) 263: 7776). A bacteriophage T7 RNA polymerase/promoter expression system (Tabor, S. and Richardson, C.C. Proc.Natl.Acad.Sci.(1985) 82: 1074) is being used to overexpress UNG. Purification of UNG will facilitate structural studies of the protein. Deletion mutant analyses have indicated that the N-terminal region is essential for enzyme activity. Heteroduplexes of plasmids exposing this region (a.a. 48 to 156) were constructed. These were subjected to sodium bisulphite mutagenesis, to induce cytosine to uracil base conversions. E. coli RZ1032 (dut^-, ung^-) cells were transformed with the mutated plasmids and assayed for enzyme activity. Two assay systems were used to identify the mutants: a biological assay using uracil-containing lamda phage; and a fluorometric assay. Missense mutants were differentiated from nonsense mutants by size , on protein transblots probed for UNG using antibodies. These missense mutants were sequenced via the dideoxy-sequencing method. The protein sequences of the mutants were analysed to reveal key amino acid residues which are essential for the activity of UNG: either by maintaining the tertiary structure or by direct involvement in the active/binding site.

CB 106 CLONING OF NOVEL GENES FOR THE REPAIR OF OXIDATIVE DNA DAMAGE IN *ESCHERICHIA COLI*, Janet S. Evans, Robert J. Melamede and Susan S. Wallace, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405. A variety of external stresses exert deleterious effects on bacterial and eukaryotic cells through the generation of intracellular active oxygen species. The existence of cellular systems for the repair of oxidized DNA bases demonstrates the biological significance of oxidative damages.

E. coli cells appear to contain multiple endonuclease activities for the excision repair of oxidative DNA base damages and alternative repair pathways may exist that can initiate the repair of damages normally repaired by endonuclease III. One of the endonucleases, endonuclease VIII, has been purified and characaterized.

An endonuclease III-deficient mutant of *E. coli* has been used as the host strain for a wild type gene bank constructed in a multicopy vector (pBR322). Several screening methods are being used to detect those clones with elevated activities of endonucleases of interest, including endonuclease VIII, and so thereby localize the genes for these novel repair enzymes to discrete DNA fragments. These clones will be used to determine to what extent these individual enzymes function uniquely in specific repair pathways. This work is supported by NIH Grant CA33657.

CB 107 CHARACTERIZATION OF DNA SUGAR PRODUCTS RELEASED AT BOTH 5' AND 3' INCISED AP SITES BY DNA DEOXYRIBOPHOSPHODEISTERASE OF E. COLI
William A. Franklin and Margarita E. Sandigursky, Department of Radiation Oncology, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY 10467
The dRpase activity of E. coli catalyzes the release of 2-deoxyribose-5-phosphate (2-dR-5-p) from incised AP sites following the cleavage of DNA by a 5' AP endonuclease such as endonuclease IV. The enzyme will also release a deoxyribose-phosphate product following cleavage with an AP lyase such as endonuclease III, which cleaves the DNA via a β-elimination reaction. The base-free sugar product remaining at the 3' incised AP site has been identified as 2-3,-didehydro-2,3-dideoxyribose-5-phosphate (ddR-5-p). It is difficult to resolve ddR-5-P and 2-dR-5-p on chromatographic systems; however, the product ddR-5-p is partially unstable, and will undergo a rearrangement following heat or treatment with alkali to the closed ring product 2-oxocyclopent-1-enyl phosphate. Following treatment with phosphatase, the product cyclopentane-1,2-dione is formed. We have synthesized the compound cyclopentane-1-2-dione, and have demonstrated that the product is obtained following heat and phosphatase treatment of the sugar phosphate product released by either dRpase or endonuclease IV on DNA containing 3' incised AP sites produced by cleavage with endonuclease III.

CB 108 MECHANISTIC STUDIES OF THE β-ELIMINATION REACTIONS CATALYZED BY UV ENDONUCLEASE V FROM BACTERIOPHAGE T_4 AND ENDONUCLEASE III FROM E. COLI, John A. Gerlt,[1] Abhijit Mazumder,[1] Michael J. Absalon,[2] JoAnne Stubbe,[2] and Richard P. Cunningham,[3] Department of Chemistry and Biochemistry,[1] University of Maryland, College Park, MD 20742, Department of Chemistry,[2] Massachusetts Institute of Technology, Cambridge, MA 02139, and Department of Biological Sciences,[3] SUNY, Albany, NY 12222.

UV Endonuclease V from bacteriophage T_4 catalyzes both a glycosylase reaction in which the 5'-glycosidic bond of a pyrimidine photodimer is hydrolyzed to yield an aldehydic abasic site and a β-elimination reaction in which the 3'-phosphodiester bond of the abasic site is cleaved. Endonuclease III from *E. coli* catalyzes both a glycosylase reaction in which the glycosidic bond to a damaged pyrimidine base is labilized and a β-elimination reaction in which the 3'-phosphodiester bond is cleaved. Using substrates that are stereospecifically labeled with ^3H in the 2-position of the abasic site, we have determined that the stereochemical course of the b-elimination reaction catalyzed by each enzyme is *syn*, the result of abstraction of the 2-pro*S* proton and formation of the *trans* geometric isomer of the α,β-unsaturated aldehyde reaction product. The same stereochemical course is observed using either single- or double-stranded substrates containing aldehydic abasic sites. The stereochemical courses of these β-elimination reactions require that the reactions proceed from either the free aldehyde or an activated derivative and not from the mixture of cyclic hemiacetals which predominates. Further mechanistic investigations of the reactions catalyzed by these enzymes are in progress.

CB 109 EXPRESSION OF FUNCTIONAL POLY(ADP-RIBOSE) POLYMERASE IN *ESHERICHIA COLI* AND SIGNIFICANCE OF ITS PUTATIVE ZINC-FINGER STRUCTURES. Miyoko Ikejima, Shigeru Noguchi, Rumiko Yamashita, Tsutomu Ogura, Hiroyasu Esumi, Takashi Sugimura, D. Michael Gill and Masanao Miwa, National Cancer Center Research Institute, Tokyo 104 (JAPAN) and Tufts University School of Medicine, Boston, MA 02111(MI, DMG).

Poly(ADP-ribose) polymerase is a eukaryotic DNA binding protein which synthesizes poly(ADP-ribose) when cellular DNA is damaged. The N-terminal DNA-binding domain contains several potential DNA-binding motifs such as two putative Zn fingers, two helix-turn-helixes and two clusters of basic amino acids. The enzyme may have a role in DNA repair.

We have developed a cDNA expression system for this enzyme in *E. coli*. The expressed polymerase after partial purification showed the same electrophoretic mobility on SDS-gel, turnover number and DNA-dependency as native polymerase.

Deletion of the region spanning both Zn fingers resulted in a 99% loss of activity. The residual 1% activity was not DNA-dependent. Point mutants, in which two crucial cysteines were both converted to tyrosine and the second Zn binding structure could not form, had wild-type activity. Similar experiments for the first finger are in progress. Thus the zinc-finger region seems to be necessary for efficient enzyme activity but it is not yet clear that zinc-fingers themselves are necessary.

CB 110 DETECTION OF FACTORS IN MAMMALIAN NUCLEAR EXTRACTS AND ISOLATION OF HUMAN cDNAS THAT ENCODE PROTEINS THAT RECOGNIZE DNA CONTAINING APURINIC SITES, Jack Lenz, Sharon Okenquist, Joseph LoSardo, Krista Hamilton[1] and Paul Doetsch[1], Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY and [1]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA. Repair of damaged DNA depends on the ability of cellular factors to distinguish the damaged sites and to execute the steps involved in removal or reversal of the damage. One step at which factors might be able to distinguish damaged from undamaged DNA is at the level of binding. Using electrophoretic mobility shift assays, we identified at least one factor from the nuclei of mammalian cells that bound preferentially to DNA containing apurinic (AP) sites, a lesion that can be induced by ionizing radiation or other agents. Specificity for AP DNA was verified by competition experients and by comparing DNA probes of different sequences. These results demonstrate that proteins exist in mammalian cells that preferentially bind to DNA containing AP sites and that they can be detected using binding assays. A binding assay based on the use of B-galactosidase-cDNA fusion proteins was subsequently used to isolate recombinant clones of human cDNAs that encoded proteins that preferentially bound apurinic DNA to undamaged, methylated ,or UV-irradiated DNA.

CB 111 BLEOMYCIN-INDUCED DAMAGES IN Escherichia coli DNA THAT REQUIRE ENDONUCLEASE IV FOR THEIR REPAIR, Joshua D. Levin and Bruce Demple, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138. E. coli mutants that lack the gene for endonuclease IV (nfo) are hypersensitive to killing by bleomycin. Chromosomal DNA was isolated from bleomycin-treated E. coli cells and assayed for its ability to support repair synthesis by DNA polymerase I in vitro. Purified endonuclease IV consistenly activated repair synthesis 2-3-fold on the DNA isolated from either wild type or nfo$^-$ cells that had been treated with bleomycin. However, the DNA isolated from bleomycin-treated E. coli cells expressing a level of endonuclease IV similar to that induced by paraquat was activated $\leq$ 1.5-fold for repair synthesis by the purified enzyme. Endonuclease IV, normalized to its 3'-phosphoglycolaldehyde DNA diesterase activity, was 3-5 times more efficient in activating these primers than was purified exonuclease III. Endonuclease IV is apparently necessary for the repair of certain bleomycin-induced damages that are refractory to exonuclease III. This special feature may account for the induction of endonuclease IV as part of the response to superoxide stress.

CB 112 MECHANISM OF POLY(ADP-RIBOSE) SYNTHESIS. Gerald Marsischky and D. Michael Gill, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111. The nuclear enzyme poly(ADP-ribose) polymerase is a 113 Kd protein which is stimulated by DNA breaks to make poly(ADP-ribose) from NAD. It is thought to play some early role in DNA repair and possibly DNA recombination. The first product of polymer synthesis is poly(ADP-ribose) which is covalently attached to the polymerase. We have shown previously that the residue on the enzyme-distal end of the growing chain is not covered over by new polymer synthesis, indicating that new residues are added at the enzyme-proximal end of the chain (as on a ribosome).[1987. JBC 262, 17641-17650] However, only a small fraction of the enzyme molecules carried pulse labelled chains. We now show new evidence supporting proximal addition, in which the bulk of the polymerase molecules are followed. We have used phosphodiesterase to modify the enzyme-distal ends of [^{32}P] labeled polymer. This treatment produces a mixture of the complete digestion product, enz-ribose-P, and the partial digestion product, enz-(ADP-ribose)$_n$-ribose-P, neither of which can be extended by a distal addition mechanism. These modified labeled chains are elongated when they are incubated with fresh, unlabeled NAD along with a phoshodiesterase inhibitor. We have shown this in two ways. 1) Chains released from the enzyme by treatment with alkali are much longer after the second NAD incubation than after phosphodiesterase treatment. 2) Synthesis of polymer on naive polymerase causes a large shift in its electrophoretic mobility in an SDS polyacrylamide gel. This is also true for enzyme molecules which carry phosphodiesterase modified polymer stubs, indicating that all such modified chains can be extended.

CB 113 NOVEL DNA REPAIR ENDONUCLEASES IN *E. COLI*: ENDONUCLEASES VIII and IX, Robert J. Melamede, Yoke Wah Kow and Susan S. Wallace, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vt 05405. Endonucleases VIII and IX appear to be novel *E. coli* endonucleases. Endonuclease VIII is very similar to endonuclease III with respect to its size (25,000), glycosylic activity and substrate range, preferring DNA that contains urea residues and thymine glycol over other substrates tested. 5 mM Mg++ can substitute for 100 mM NaCl in reactions with either endonuclease III or endonuclease VIII. Endonuclease VIII, unlike endonuclease III, prefers thymine glycol-containing DNA in the relaxed form over the supercoiled form. Endonuclease VIII can be isolated from nth mutants that lack endonuclease III.

Endonuclease IX appears to be a urea glycosylase that releases radiolabeled urea from DNA containing ^{3}H urea residues. Endonuclease IX has an apparent native molecular weight of about 25,000 daltons and can be isolated from nfo and xth mutants that lack endonuclease IV and exonuclease III respectively. This work is supported by the National Institues of Health Grant CA33657.

CB 114 LESION LOCATION BY THE UvrABC ENDONUCLEASE INVOLVES 5' TO 3' TRANSLOCATION ALONG THE DAMAGED STRAND, Maureen M. Munn and W. Dean Rupp, Departments of Therapeutic Radiology and of Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, CT 06510.

The UvrABC endonuclease of *Escherichia coli* recognizes a wide variety of DNA lesions and incises the damaged DNA strand on both sides of the modified nucleotide. The first step in this reaction is location of the lesion by UvrA or the UvrAB subcomplex. We have used a duplex M13 circle with a unique, specifically-positioned, damaged site to test whether lesion recognition involves directional translocation along the DNA. The DNA substrate was cleaved with a restriction endonuclease on either side of the damaged site approximately 300 nucleotides away, and these linear DNA molecules were used as substrates in the UvrABC nicking assay. UvrABC nicking was significantly higher on the linear molecule with the long arm of the damaged strand extending 5' from the lesion. This result suggests that location of the lesion involves 5' to 3' translocation along the damaged DNA strand.

CB 115 MOLECULAR CLONING OF THE GENE (*APE*) FOR THE MAJOR APURINIC/APYRIMIDINIC ENDONUCLEASE OF *SACCHAROMYCES CEREVISIAE*, Sonya C. Popoff and Bruce Demple, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

A DNA repair protein (M_r=40,500) that is the major DNA 3'-diesterase for radical induced nucleotide fragments, as well as the major apurinic/apyrimidinic (AP) endonuclease in yeast, was purified and used to raise polyclonal antisera. Antisera obtained from two rabbits cross reacted specifically with the purified diesterase and with a M_r 40,500 protein in yeast cell-free extracts, as observed on immunoblots. The antisera also immunoprecipitated 95-98% of both the 3'-diesterase and the AP endonuclease activities from yeast extracts. These data provide independent confirmation of previous biochemical experiments from this laboratory showing that the M_r 40,500 protein is the major DNA repair 3'-diesterase and AP endonuclease in yeast.

The specific antisera were immunopurified and used to identify the structural gene from a yeast genomic expression library in λgt11. The identity of the AP endonuclease (APE) gene was confirmed by the following criteria: 1) synthetic oligonucleotide probes, corresponding to amino acid sequence of the N-terminus and an internal region of the protein, hybridized specifically to the cloned DNA on Southern blots; and 2) the DNA sequence of a region of the cloned gene matches the known partial amino acid sequence exactly. A DNA fragment corresponding to the endonuclease coding region was used to probe total yeast RNA. This DNA fragment hybridized specifically to a 1.6 kb RNA on Northern blots, a size consistent with a transcript encoding a 40.5-kilodalton polypeptide. Enzyme-deficient strains are being constructed using targeted gene disruptions.

CB 116 MECHANISMS OF EXCISION OF DEOXYRIBOSE-5-PHOSPHATE RESIDUES FROM 5'-ABASIC SITES IN DNA BY MAMMALIAN CELL EXTRACTS, Allan Price and Tomas Lindahl, Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, EN6 3LD, U.K. The major mammalian AP endonuclease catalyzes hydrolytic cleavage of the phosphodiester bond 5' to the abasic residue in DNA. We have investigated the subsequent repair step of excision of the terminal sugar-phosphate residue using fractionated calf thymus cell extracts. The major 5'->3' exonuclease acting on 5'-^{32}P-labelled poly(dA)·oligo(dT), the 42-44 kDa protein DNase IV, appears unable to release deoxyribose-phosphate residues from 5'-termini. This enzyme exhibits many of the properties of the 5'->3' exonuclease function of E.coli DNA polymerase I. The active release of 5'-terminal base-free sugar-phosphate residues instead occurs by two different mechanisms: (i) Hydrolytic release catalyzed by a higher molecular weight activity. This enzyme may be the mammalian equivalent of the E.coli dRpase (Franklin and Lindahl, EMBO J. 7,3617;1988) and it is being further purified and characterised. (ii) Release by β-elimination promoted by small protein(s) and low-molecular weight compounds such as polyamines. The physiological relevance of the latter reaction is uncertain.

CB 117 DAMAGE RECOGNITION KINETICS OF THE E. COLI UVRABC NUCLEASE COMPLEX FOR MODIFIED APURINIC (AP) SITES , Amanda Snowden[1], Yoke Wah Kow[2], and Bennett Van Houten[1], [1]Department of Pathology, [2]Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405. The principle enzyme activity in *E.coli* nucleotide excision repair is the UvrABC excision nuclease (ABC). This enzyme complex recognizes a variety of different DNA lesions, presumably due to the helical distortion caused by the lesion. Recently it has been shown to act on DNA containing minor distortions such as O^6-methyl guanine. It was of interest to determine if this enzyme acts on AP sites, a common radiolysis product. While AP sites did not appear to be substrate, modified AP sites were substrate for ABC. These AP sites were modified using a series of O-alkylhydroxylamines of increasing bulkiness (O-methyl, O-ethyl, O-allyl, and O-benzyl). Using a fluorometric incision assay, it was shown that the rate of incisions by the UvrABC complex increased with the bulkiness of the lesion. This suggests that the bulkier lesion (O-benzyl) may cause a more significant distortion of the helix and, therefore, be a better substrate for the enzyme complex. Using PM2 DNA containing pyrimidine dimers as substrate, kinetic studies were performed to examine the UvrAB complex formation, the rate of UvrC binding and incision, and the optimum ratios of the three subunits. This data suggests that the rate-limiting step occurs prior to the binding of the UvrC subunit.

CB 118 MECHANISM OF ACTION OF THE N-GLYCOSYLASE ACTIVITY OF ENDONUCLEASE III FROM *ESCHERICHIA COLI*, Yoke W. Kow and Grace Sterling, Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405. Endonuclease III from *Escherichia coli* is an N-glycosylase with an associated 3' AP endonuclease activity. The 3' endonuclease activity has been shown to act by β-elimination, leading to the formation of an α,β-unsaturated sugar at the 3' terminus. The N-glycosylase activity of endonuclease III recognizes a wide spectrum of thymine radiolysis products. We proposed earlier that the N-glycosylase activity of endonuclease III occurs by way of a transiminization reaction between the lysinyl group of endonuclease III and the iminium group of the ring-opened sugar moiety of the base lesion. By using different reagents that specifically modify different amino residues in endonuclease III, we provide evidence that in addition to lysinyl residue, histidine residues are involved in the N-glycosylase reaction. The role of histidine residue in the N-glycosylase activity will be discussed. This work is supported by National Institutes of Health Grant GM37216.

CB 119 RECOGNITION OF OXIDATION DAMAGE BY THE *E.COLI* UVRABC NUCLEASE COMPLEX, Ben Van Houten[1], Sharon Illenye[1], Gustavo Fondez[2], Susan Wallace[2], and Yoke W. Kow[2], Departments of [1]Pathology and, [2]Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405. While several enzymes have been isolated from *Escherichia coli* which act on oxidation damage, the exact mode of repair which occurs *in vivo* is not clearly understood. The nucleotide excision repair enzyme, UvrABC nuclease (ABC) displays a remarkable diversity in its ability to act on many structurally unique classes of DNA damage. The substrate specificity of ABC for thymine glycol (TG) residues was examined by treating PM2 DNA with the oxidizing agent, osmium tetroxide. Using a fluorometric incision assay which monitors the conversion of supercoiled DNA to nicked-open circular DNA, it was found that UvrABC nuclease efficiently recognizes and incises PM2 DNA containing TG adducts, but not the structurally related dihydrothymine residue. TG residues in supercoiled DNA were incised more efficiently than TG residues in covalently closed relaxed DNA. Using terminally labeled DNA fragments which were randomly damaged with osmium tetroxide it was also found that ABC's incision sites for TG residues are consistent with the cleavage pattern for other types of DNA damage, such as UV-induced pyrimidine dimers. Transfection experiments with OX 174 DNA containing 1 - 25 TG adducts/ molecule were performed in host strains with various repair capacities. It was found that while a *uvrA* mutant showed little or no difference from wild type cells, the *nth* (endoIII) *uvrA* double mutant, shows one log greater sensitivity than the single *nth* mutant alone. The results of these experiments demonstrate a role for the UvrABC nuclease complex in the repair of thymine glycol adducts, a common form of DNA damage resulting from oxidation damage.

CB 200 PARTIAL CLONING OF KIN GENE THAT CODES FOR A MOUSE PROTEIN REACTING WITH ANTI-RECA ANTIBODIES. Jaime F. Angulo*, Evelyne Rouer**, Richard Benarous** and Raymond Devoret*, *Study Group of Mutagenesis, Enzymology, CNRS, 91198 Gif-sur-Yvette, France and **Institut de Pathologie Moleculaire, 24 rue du Fg St-Jacques, 75014 Paris, France.

A polypeptide of about 120 kDa, called KIN, has been identified in rat FR 3T3 cells by immunoblotting using affinity-purified antibodies against the RecA protein of *Escherichia coli* (38 kDa). The KIN protein as shown by fluorescent light microscopy and electron microscopy is essentially concentrated in the nucleus. Its level is higher in proliferating than in quiescent cells. Cell tratment with mitomycin C increases the level of the KIN protein. Proteins with the same electrophoretic mobility were detected in mouse, monkey and human cell lines as well as in rat and mouse embryos (Angulo JF, Moreau PL, Maunoury R, Laporte J, Hill AM, Bertolotti R, Devoret R, 1989, Mutation Res. 217:123-134)

We have constructed a lambda gt11 cDNA library from 12-day mouse embryo mRNA. Five clones each expressing a different polypeptide reacting with anti-RecA antibodies were isolated. One clone lambda KIN2 is 660 bp long. A fragment of 440 bp shows 66% homology with the amino-terminal part of RecA protein of *Pseudomonas aeruginosa* and *E. coli*.

We believe that some domains of prokaryotic RecA proteins are highly conserved during evolution.

CB 201 The Isolation Of **D. radiodurans** DNA Fragments That Convey Radiation Resistance To recA⁻ **E. coli**, Louis R. Barrows, Department of Pharmacology and Toxicology, and the Utah Regional Cancer Center, University of Utah, Salt Lake City, UT 84112

Deinococcus radiodurans (previously called **Micrococcus radiodurans**) are renowned for their exceptional resistance to killing by ionizing radiation. In this work, a cosmid library of **D. radiodurans** DNA was grown in ED8767 **E. coli** (recA56) and exceptionally radiation resistant **E. coli** were isolated (survivors of 1,000 Gy gamma irradiation). Cosmid DNA was isolated from the radiation resistant **E. coli** and fragments of the cosmid were subcloned into pBR322 and grown again in recA⁻ **E. coli** (either **ED8767** or **NM554**). Again radiation resistant **E. coli** were isolated. **E. coli** containing the subcloned fragments of **D. radiodurans** DNA, and treated with 1,000 Gy radiation, exhibited a survival of approximately 10^{-1} instead of the 10^{-11} exhibited by the non-transfected **E. coli.** This is interpreted as evidence for the cloning of a **D. radiodurans** for the repair of radiation-induced DNA damage. It is hypothesized that this **D. radiodurans** gene functions in a fashion similar to the recA gene of **E. coli.**

CB 202 THE NUCLEOTIDE SEQUENCE OF THE *RECA* GENE FROM *STAPHYLOCOCCUS AUREUS*. Ken W. Bayles[1], Ronald E. Yasbin[2] and John J. Iandolo[1]. [1]Division of Biology, Kansas State University, Manhattan, KS 66506. [2]Department of Biological Sciences, UMBC, 5401 Wilkens Ave., Baltimore, MD, 21228.

Repair of DNA damage caused by ionizing radiation has predominantly been studied in Gram-negative bacteria. These studies have shown that the repair systems in general and the SOS response in particular have been highly conserved in a number of different species. However, recent studies in the Gram-positive, spore forming bacterium *Bacillus subtilis*, has revealed that there may be considerable differences in the regulation and/or function of DNA repair systems including the SOS-like or SOB regulon. These differences include the induction of DNA-damage inducible genes (*din*) in response to the presence of hydrogen peroxide, and the involvement of developmental cycles and differentiation processes in the activation of DNA repair mechanisms. Furthermore, the *recE*$^+$ gene from *B. subtilis* (the *Escherichia coli recA*$^+$ analogue) is unstable in *E. coli* suggestion that the *recE* gene has diverged substantially from *recA*. The study of these differences will be valuable in elucidating the mechanisms of the DNA repair processes. It is unknown whether the differences seen between the repair processes of *B. subtilis* and *E. coli* can be attributed solely to the fact that the former has distinct developmental stages. Alternatively, real differences may exist between the repair systems of Gram-positive and Gram-negative organisms. In order to investigate these possibilities, the gene that codes for the RecA protein of the Gram-positive bacterium *Staphylococcus aureus* was cloned and sequenced. Comparison of this gene to its analogues in *E. coli* and *B. subtilis* reveal that their sequences are highly similar. However, the inability to clone an intact copy of the *S. aureus recA*$^+$ gene in *E. coli* again suggests that their has been significant divergence. A comparative analysis of the DNA sequences and the operator/promoter regions of these genes will be presented.

CB 203 **ASSAYING GENETIC RECOMBINATION BETWEEN ALLELES AT THE ENDOGENOUS HUMAN THYMIDINE KINASE LOCUS,** M.B.Benjamin, D.W.Yandell and J.B.Little. Harvard Univ. School of Public Health. Boston, MA 02115.

RFLP and sequence analysis suggests that somatic recombination and gene conversion may be involved in the reduction to homozygosity for mutations at tumor-suppressor loci. For many retinoblastomas, Wilm's tumors, meningiomas and osteosarcomas, such recombination may play a fundamental role in tumorigenesis. If human somatic recombination can be initiated as part of the cellular response to DNA damage, much as it is in yeast, this reduction to homozygosity may be an untargeted result of damage elsewhere in the genome. We are currently developing a plasmid-free system for studying inter-allelic recombination events at the human tk locus *in situ* on chromosome 17. *Hetero*allelic TK deficient (tk-/-) lymphoblastoid lines were generated from the parent tk+/+ homozygote by exposure to the frameshift mutagen ICR-191. Frameshift mutations were located and characterized by PCR amplification and direct sequencing. Since the tk-/- mutants result from a different frameshift in each allele, mechanisms for their reversion to the selectable TK+ phenotype are restricted to inter-allelic recombination, gene conversion and some frame-restoring base-pair deletions and additions. Spontaneous reversion frequencies for these lines differ widely: a line carrying two frameshifts displaced 7.7kb apart in its heteroalleles reverts to TK+ 50 times more frequently than a line carrying a homozygous frameshift. This is consistent with the involvement of a recombination or gene conversion event in reversion at this locus. The availability of chromosome 17 DNA probes, and the presence of an RFLP at the downstream terminus of the tk gene, in addition to tk sequence information will allow for the genetic dissection of revertants. We are currently investigating the fate of each frameshift in the revertant lines derived from these tk-/- mutants. We hope to be able to use this system to study the effects of ionizing radiation damage on genetic recombination in human somatic cells.

CB 204 **DNA SEQUENCE AND TRANSCRIPTIONAL ANALYSIS OF *mei-41*, A DROSOPHILA GENE REQUIRED FOR REPAIR OF DAMAGED DNA**, David M. Binninger, Douglas R. Oliveri, Satnam S. Banga and James B. Boyd, Department of Genetics, University of California, Davis, CA 95616

The *mei-41* gene is implicated in DNA repair because mutants at that locus are sensitive to a broad spectrum of mutagens including X-rays and γ-irradiation. The structural gene was previously cloned by chromosome walking and verified by utilizing a *mei-41* mutant arising from integration of a P-element transposon (Banga *et al.*, submitted). Here we describe the developmental and tissue-specific expression of the *mei-41* gene and DNA sequence analysis of a cDNA. The major transcript is 2.2 kb although RNAs of altered lengths were found in two ethyl methanesulfonate induced alleles and in a mutant harboring a P-element. This transcript is most abundant in embryos and female meiotic tissue, but is not detectable in larvae and pupae. Conceptual translation of the 1,152 nucleotide open reading frame predicts a 43 kD protein product. The predicted protein structure suggests the presence of two potential DNA binding sites. However, searches of the NBRF protein, Swiss protein, EMBL and Genebank databases failed to identify any previously cloned sequences with significant homology.

This work was supported by grants from DOE (EV70210), NIH (GM 32040) and the Energy, Science and Technology Program of UERG.

CB 205 COMPLEMENTATION OF THE RADIATION SENSITIVITY OF AN X-RAY SENSITIVE CHINESE HAMSTER MUTANT BY A HUMAN cDNA LIBRARY, David J. Chen, Francesca Chavez-Giles, and Paige Pardington, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

We report here a novel transfection and selection strategy for complementation of an X-ray sensitive mutant line, xrs-6, using cloned human cDNA. Xrs-6 cells are deficient in repair of radiation-induced DNA double-strand breaks and highly sensitive to X-rays and the radio-mimetic compound bleomycin (BL). A human cDNA library constructed in vector pCD2 containing the neo transcriptional unit was obtained from Dr. H. Okayama [Mole. & Cell. Biol. 7:2745-2752, (1987)]. Human cDNA library was introduced into the mutant cells by the calcium phosphate precipitation technique. Following selection to G418 resistance, transformant colonies were dispersed into individual cells and selected for bleomycin resistance. Four independent $G418^r$ and BL^r transformants were identified from 20 transfection experiments. For secondary transfection, genomic DNA isolated from a primary repair-proficient transformant line was sheared to approximately 50 kb and co-transferred with plasmid pSV2-gpt DNA to the mutant cells. The selection procedure in the secondary transfection was based on mycophenolic acid and xanthine resistance (MX^r) for selection of stable gpt transformants. To date, we have isolated three MX^r and BL^r transformants in 20 independent transfection experiments. All three secondary transformants contain the neo gene and the plating efficiency in G418-containing medium is about 10%. Since the neo gene was linked to the human cDNA, the $G418^r$ phenotype observed in the secondary transformants was inherited from the cDNA containing primary transformant. This double transfection procedure provides a mechanism that eliminates the possibility of selection of spontaneous repair-proficient revertants from the population. Currently, we are in the process of isolating the repair gene from the repair-proficient primary transformant DNA. (This research was supported by the USDOE under contract W-7405-ENG-36 and NIH grant CA50519-01.)

CB 206 ISOLATION OF AN ENDO-EXONUCLEASE GENE, yNucR, THAT REQUIRES A FUNCTIONAL RAD52 GENE. Terry Y.-K. Chow, MRC group in Radiation Biology, U. of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.

We have previously identified and characterized an endo-exonuclease (yNucR) in Saccharomyces cerevisiae whose levels are influenced by the RAD52 gene (1,2). Using the antibody to yNucR, the gene encoding this nuclease has been isolated from a lambda gtll yeast genome expression library. Tn10 insertion mutagenesis into the fragment eliminates the CRM. When the yNucR gene is cloned into YEp213 plasmid and is transformed into Rad^+ cells, we observed an increase of 3-10 times more CRM and 1.5-2.0 times more of immuno-precitable nuclease activity. Furthermore, the transformants exhibits higher resistance to MMS than corresponding Rad^+ cells. The yNucR gene is mapped to chromosome XI and disruption of the gene with Leu2 insertion is lethal to the cell, suggesting the yNucR is essential. Preliminary genetic results with ts mutant though meiosis suggest a role of the nuclease in recombination. (Supported by NCIC and FRSQ).

(1) T.Y.-K. Chow and M.A. Resnick (1987). J. Biol. Chem. 262: 17659-17667.
(2) T.Y.-K. Chow and M.A. Resnick (1988). Mol. Gen. Genet. 211: 41-48.

CB 207 REPAIR OF DNA DOUBLE-STRAND-BREAK (DSB) BY THE RecE RECOMBINATION PATHWAY OF Escherichia coli, Amikam Cohen, Moshe Shalit and Anat Nussbaum, Department of Molecular Genetics, The Hebrew University-Hadassah Medical School, Jerusalem, Israel 91010. An experimental system that facilitates delivery of linearized substrates of the bioluminescence recombination and repair assay into E. coli cells was used to investigate DSB repair by the RecE recombination pathway. Plasmids carrying Vibrio fisherii bioluminescence genes were cleaved within the luxA gene and ligated to λ phage arms. Infection of cells that synthesize EcoRI endonuclease releases a substrate that may be repaired by recombination with cloned endogenous luxA sequence. Kinetics of DSB repair is determined by measuring light intensity. Repair mechanism is investigated by manipulating the repair substrate and analyzing the reaction products. Genetic analysis of the recombination-repair reaction indicates its dependence on exonuclease VIII, independence of recA activity and sensitivity to RecBCD enzyme. Repair of the DSB by recombination with the endogenous luxA sequence involves loss of markers adjacent to the break, and conversion of markers flanking the break from the homologous sequence on the endogenous plasmid. The applicability of the DSB-repair models to recombination to the RecE and by related pathways will be discussed.

CB 208 INTERACTION OF LESIONS FORM THE DOMINANT MECHANISM OF RADIATION-INDUCED CHROMOSOMAL EXCHANGE, *Michael N. Cornforth, Cell Biology Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545*
Following exposure to ionizing radiations, many of the chromosomal aberrations that appear in eukaryotic cells at metaphase result from exchanges that occur between different chromosomes. The fact that these exchanges increase roughly as the square of the dose for low LET radiations prompted early investigators to conclude that they were produced through the interaction of radiogenic lesions present on both the chromosomes involved. More recently, this concept of "lesion-lesion" interaction has been challenged on biophysical and molecular grounds. From biophysical considerations, the concept of saturable repair has emerged as an alternative explanation of this, and other, radiobiological phenomena. Saturable repair models normally assume linear dose dependence of critical lesions. Implicit in this assumption is the notion that damage responsible for cell killing (and therefore chromosomal aberration production) is produced through a "lesion-*non*lesion" mode of interaction; e.g., in this context, only one of the two chromosomes involved in an exchange needs to be altered by radiation. To the extent that vector-based systems of homologous recombination represent fundamental molecular processes manifest at the chromosomal level, molecular studies have lent support to this idea. In the present study, the specific case of lesion-nonlesion interaction, via a telomere-break rejoining mechanism was examined through the use of *in situ* hybridization of a telomere-specific DNA probe. [Such a mechanism has been proposed within the Molecular Theory of radiation action (Chadwick and Leenhouts).] In this case no evidence was found to support dicentric formation by this process in γ-irradiated normal human fibroblasts. To test the plausibility of lesion-nonlesion interaction for the more general case (i.e., without regard to any particular mechanism), mitotic Hela cells were fused together to determine whether interchanges would occur between the chromosomes of previously separate genomes, after the resulting cell syncytia reached mitosis. A high frequency of intergenomic exchanges were observed when γ-irradiated cells were fused with each other, while very few such exchanges occurred when irradiated cells were fused with unirradiated cells. These results suggest that most aberrations result from the interaction of radiation-induced lesions present at both sites involved in the exchange, and raise questions regarding the validity of homologous recombination as a mechanism underlying radiation-induced chromosomal exchange.

CB 209 INTERCHROMOSOMAL DNA REARRANGEMENTS ARE STIMULATED BY CHEMICAL MUTAGENS AND BY DIRECTED DNA DOUBLE-STRAND BREAKS IN *S. CEREVISIAE*, Michael T. Fasullo, Jill Stark, and Rodney Rothstein, Department of Genetics and Development, College of Physicians and Surgeons of Columbia Unversity, New York, N.Y. 10032. We have constructed a variety of yeast strains to study the process by which DNA damage stimulates chromosomal rearrangements. DNA rearrangements can be generated directly by use of *his3* recombinational substrates as described previously [Fasullo & Davis (1987) Proc. Natl. Acad. Sci. USA *84*, 6215-6219]. A variety of chemical carcinogens stimulate both interchromosomal and intrachromosomal rearrangements. For example, when the two recombination substrates are on chromosomes II and IV respectively, reciprocal translocations are stimulated by 4-nitrosoquinoline oxide (NQO).These reciprocal translocations are also stimulated 100-fold by DNA double-strand breaks generated by the HO endonuclease in a strain in which the 110bp HO recognition sequence from MATα was subcloned into the middle of the substrate positioned on chromosome IV. We screened for mutants that are defective in the NQO stimulation of mitotic, ectopic recombination. Two of these mutants are X-ray sensitive (5% and 0.9% survival at 23krads). Other criteria being used to characterize these and other mutants include expression of the *DIN* genes, UV sensitivity, and sensitivity to other chemical carcinogens.

CB 210 CARCINOGEN-INDUCED HOMOLOGOUS RECOMBINATION BETWEEN DUPLICATED GENES STABLY INTEGRATED WITHIN THE GENOME OF MAMMALIAN CELLS, INCLUDING NORMALLY-REPAIRING AND REPAIR-DEFICIENT HUMAN CELLS, Veronica M. Maher, Nitai P. Bhattacharyya, Tohru Tsujimura, Yenyun Wang, and J. Justin McCormick, Carcinogenesis Laboratory, Michigan State University, East Lansing, MI 48824 We have been studying carcinogen-induced homologous recombination in a tk$^-$ mouse L cell line which contains a single integrated plasmid which duplicate copies of the Herpes tk gene, each containing an 8bp XhoI site inserted in a different place and with the neo gene located between them. Only by undergoing a productive recombinational event between the two non-functional tk genes can a functional gene product be made and the recombinant be selected with HAT medium. With this system, we determined that the frequency of recombination induced by UV radiation or a series of mutagens that form multi-ringed DNA adducts is linearly related to the number of DNA adducts or photoproducts. The majority of the events are consistent with non-reciprocal transfer of genetic material, i.e., gene conversion. To examine the mechanisms involved and the role of DNA repair, we transfected a series of human cells which have normal rates of nucleotide excision repair or which are deficient in this process with the plasmid carrying the Htk genes or a related plasmid carrying duplicated copies of the gene for hygromycin resistance. Using these two assay systems, we have found that there is no significant difference in the rate of spontaneous recombination among the human cell lines, but nucleotide repair-proficient cells have a much lower frequency of recombination induced by UV and multi-ringed adducts than repair-deficient cells. (Supported by Grant CA48066 from the NCI and by Contract 87-2 from the HEI.)

CB 211 DETECTION OF MARKED DIFFERENCES IN INTRACHROMOSOMAL RECOMBINATION RATES IN XERODERMA PIGMENTOSA AND ATAXIA TELANGIECTASIA CELL LINES BY A RETROVIRAL-BASED VECTOR SYSTEM. M. Stephen Meyn, Yale University, New Haven, CT 06510.

I have developed a series of retroviral vectors that can be used to measure intrachromosomal recombination events in normal human fibroblast lines and lines with altered DNA repair abilities. These vectors, upon infection, integrate tandem, complementary, mutant *neo* genes into chromosomal DNA. The integrated *neo* genes can then undergo recombination, resulting in reconstitution of a wild-type gene and expression of G418 resistance. The vectors were created from the pB2d self-inactivating retroviral vector, the *neo* gene from pCM1polA, and the *hph* gene from pHyg. The two *neo* genes are in direct repeat orientation surrounding the *hph* gene. The *neo* mutants used in these vector constructs were created by insertion of BamH1 linkers at their EcoR52 and NaeI sites respectively. The retroviral vectors were used to transfect a retroviral-packaging cell line (PA317) and virus-producing subclones isolated. Packaged vector RNA from these lines was then used to infect normal human cell lines and lines established from patients with the cancer-prone genetic diseases xeroderma pigmentosa, Bloom syndrome, Fanconi anemia and ataxia telangiectasia. Infected clones were isolated, single-copy integrants identified by Southern blotting and spontaneous rates of recombination between *neo* genes in the infected human lines measured by fluctuation analysis.

In initial experiments the xeroderma pigmentosa cell line GM4429C was found to have an average rate of 4.91×10^{-6} G418^R colonies/cell/generation for 4 independently-infected clones. This rate of intrachromosomal recombination is similar to that reported for other mammalian fibroblast lines. In contrast, the average rate of intrachromosomal recombination for 4 independently-infected clones of the ataxia telangiectasia cell line GM5849 was over a hundred-fold higher: 8.06×10^{-4} G418^R colonies/cell/generation. DNA from G418^R colonies arising during these fluctuation tests is now being analyzed to determine the nature of the recombinational events. These studies are also being extended to include normal cell lines and additional mutant lines. (Supported by NIH R01 GM38588).

CB 212 STABILITY OF RECOMBINATION SITES FOLLOWING DNA REARRANGEMENTS IN HUMAN CELLS, **John P. Murnane and Barbara R. Young, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143**

Integration of plasmids into cellular DNA is commonly followed by additional recombination events in the region of the integration site, analysis of which may provide information on secondary recombination events following spontaneous or ionizing radiation-induced chromosomal rearrangements. Both homologous and nonhomologous recombination occurred subsequent to plasmid integration, producing duplications, deletions and amplification of both plasmid and surrounding cell sequences. Recombination occurred in some clones at extremely high rates of up to 10^{-2} events/cell/generation as evident by restriction fragment length polymorphism. Recombination was most commonly observed in the first few cell generations following integration, however, in rare instances high rates of recombination continued indefinitely. Because these continuously unstable integration sites were rare (2 of 127), and because recombination in one clone occurred only within the cellular DNA, the plasmid sequences alone did not appear to be the cause of the instability. Large regions of DNA cloned from an unstable integration site were capable of transmitting high rates of recombination to other integration sites. However, attempts to identify the specific sequences responsible have thus far been unsuccessful, indicating that multiple sequences in different locations may be involved. These results suggest the possibility that many recombination events which occur following ionizing radiation may be secondary events brought about by unstable regions of chromatin created by the initial DNA rearrangements, and that this instability may persist for many cell generations in a minority of cells in the population.

CB 213 "COOPERATIVE" VS "NONCOOPERATIVE" MECHANISM FOR DNA DOUBLE-STRAND BREAK REPAIR, J.Y. Ostashevsky, SUNY-HSCB, Brooklyn, New York 11203

Two possible mechanisms of double-strand break (DSB) repair were considered in the framework of the DSB-model (Ostashevsky 1989). In "cooperative" mechanism, DSBs occurring in the same DNA molecule are repaired on an "all-or-none" principle. In "noncooperative" mechanism, each DSB is repaired independently. "Cooperative" mechanism describes the experimental data much better than "noncooperative" one. Thus, it predicts the DNA molecular weight for V79 cells equal to 3.6×10^{10} Da, which is within the range of the viscoelastometry estimate of $(2\text{-}4) \times 10^{10}$ Da obtained for the same cells (Ostashevsky et al 1988). In contrast, the "noncooperative" mechanism predicts the time constants of DSB repair for V79 and human fibroblast cells, which are 1.5-2 times higher than their experimental values.

A possible suggestion how the "cooperative" mechanism works is the following. Its limiting step may be the recognition and/or binding of the repair enzyme to the first DSB site on the DNA molecule. After binding, the repair molecule can slide along the DNA molecule, eliminating the suggested limiting step for remaining DSBs. Sliding of a protein molecule along a DNA molecule is a known phenomenon for the DNA binding regulatory proteins (Berg and von Hippel 1985).

This work was supported by grants from the G. Harold and Leila Y. Mathers Charitable Foundation and NIH/NCI (R01-CA-39045).

CB 214 **A NOVEL PATHWAY OF DNA DOUBLE-STRAND BREAK REPAIR**, Petra Pfeiffer, Silke Thode, Antje Schäfer, Walter Vielmetter and Institute of Genetics, University of Cologne Weyertal 121, D-5000 Cologne 41, F.R.G.

Repair mechanisms of illegitimate recombination can join nonhomologous DNA double-strand breaks irrespective of their structure and sequence. Extracts from *Xenopus laevis* eggs efficiently join such nonhomologous DNA termini. This is assayed by conversion of linear DNA substrates with defined nonhomologous restriction ends into covalently closed circular monomers. Termini, either blunt ended or with 4 nucleotides long 3' or 5' protruding single strands (PSS), are successfully joined *in vitro* in any tested combination (Pfeiffer & Vielmetter, N.A.R. 16, 907; (*)). Here we analyse the joining mechanism of "nonpolar" substrates α(blunt/3'PSS or 5'PSS; 5'PSS/3'PSS) in the *in vitro* system. During joining, the sequences of the involved PSS are preserved by fill-in DNA synthesis. This is feasible for 5'PSS ends but not for 3'PSS ends which fail to prime fill-in DNA synthesis at their recessed 5'phosphates. Since no unusual DNA-ligases are available in the extract system which are able to ligate DNA single-strands, a primer cannot be provided by ligation of the 3'PSS to the partner terminus. Nevertheless, we show that fill-in DNA synthesis of 3'PSS ends not only can be primed at the 3'OH of the partner terminus in the absence of ligation and thus must precede ligation but also is a necessary prerequisite for ligation. Therefore, priming at the unlinked partner terminus requires prealignment of terminus pairs by a novel mechanism. We postulate the existence of stabilizing terminal DNA binding proteins that prealign nonpolar and polar ends (5'PSS/5'PSS; 3'PSS/3'PSS; (*)) and thus render the priming of fill-in DNA synthesis and further repair reactions possible.

CB 215 γ-RAY-INDUCED RECOMBINATION IN CHINESE HAMSTER OVARY CELLS, Richard J. Reynolds and Jac A. Nickoloff, Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

Both the transformation of mammalian cells by exogenous vector DNAs and homologous recombination between vector DNAs in mammalian cells are stimulated by cleavage with restriction endonucleases. It has been assumed that the double-strand breaks introduced by restriction endonucleases serve as good models for breaks induced by ionizing radiations. The induction of vector recombination by ionizing radiation, however, has been difficult to demonstrate in mammalian cells. By using electroporation to transform CHO cells we have been able to reduce background transformation and homologous recombination frequencies and to demonstrate dose dependent stimulation of both cell transformation and homologous recombination by ^{60}Co γ-rays. Similar dose responses were observed with both normal and radiation-sensitive xrs-5 cells although frequencies were lower with xrs-5 cells. If corrected for the reduced transformation efficiency, homologous recombination in xrs-5 cells appears to be normal. These results are consistent with results obtained in protocols employing restriction endonucleases. This research was conducted under contract from the U.S. Department of Energy.

CB 216 INTERSTRAND CROSSLINKS AND DOUBLE-STRAND BREAKS INDUCE INDIRECT PLASMID RECOMBINATION IN YEAST, Wilma A. Saffran and Elsworth Smith, Department of Chemistry, Queens College-CUNY, Flushing, NY 11367

Unmodified yeast integrating plasmids, lacking replication origins, transform yeast cells with low efficiency, but double strand breaks or psoralen crosslinks stimulate stable transformation by inducing homologous recombination between plasmid and chromosome. We have examined the cotransformation frequency of undamaged plasmid DNA paired with partially homologous plasmids bearing either psoralen crosslinks or double-strand breaks. Psoralen photoreaction of pUC18-HIS3 induced chromosomal integration of unmodified pUC18-URA3 in a dose-dependent manner. Similarly, cleavage at restriction sites within (pUC18) or outside (HIS3 or URA3) the region of homology stimulated the co-integration of intact circular plasmid. Broken ends at distances of over 700 base pairs from homologous sequences were able to induce cotransformation. Damaged non-homologous DNA did not stimulate transformation by unmodified plasmids. The damaged and undamaged plasmids were found to be integrated together, either at the chromosomal locus homologous to the damaged plasmid or, less frequently, at the site homologous to the undamaged plasmid.

CB 217 DEFECTIVE DNA DOUBLE-STRAND BREAK REJOINING IN xrs-5 CELLS IS ASSOCIATED WITH AN ALTERATION IN CHROMOSOME STRUCTURE, Jeffrey L. Schwartz, Jeff D. Shadley, Janet M. Cowan, Janet Stephens, and Andrew T.M. Vaughan, Department of Radiation and Cellular Oncology, University of Chicago, Chicago, IL 60637, and Department of Immunology, Birmingham University, Birmingham, (UK). The Chinese hamster ovary (CHO) cell line xrs-5 was isolated from CHO-K1 cells as a radiation-sensitive mutant. The radiation sensitivity is associated with a defect in DNA double-strand break rejoining. The xrs-5 phenotype is also associated with an alteration in chromatin structure. Metaphase chromosomes from xrs-5 are shorter and thicker than those from CHO-K1 cells. Electron microscopy studies suggest that the increased thickness is due to large looped structures coming out from the chromosome core. Nucleoid studies suggest that xrs-5 cells do not differ in replicon loop size or supercoiling, however, there are differences between K1 and xrs-5 in their binding of ethidium bromide as nucleoids. We hypothesize that the radiation sensitivity and defective repair ability in xrs-5 cells is due to an alteration in chromosome organization, most likely at the level of the nuclear matrix which affects the cells ability to keep breaks aligned and in close proximity for accurate repair.

CB 218 METHYLATION OF REPAIR AND RECOMBINATION INTERMEDIATES, Steven S. Smith, Lori A. Jardines and David J. Baker, Division of Surgery, City of Hope National Medical Center, Duarte, CA 91010. Oligodeoxynucleotides spanning codon 12 of the human c-Ha-*ras* gene were found to be exceptionally good substrates for *de novo* methylation by human DNA(cytosine-5)methyltransferase. In the complex formed by two complementary 30mers, only the C-rich strand was methylated by the enzyme in initial velocity experiments. Guanines at the 3' end of the G-rich strand of the complex could not be completely modified by dimethyl sulfate suggesting tetrameric bonding at these G-residues. An eight-strands structure, composed of four duplex DNAs at one end, joined to a G4-DNA segment at the other with the junction between the two DNA forms at codon 12, can account for our results. G4-DNA structures may form the basis for synaptic pairing between homologous bivalents. Our results raise the possibility that cytosine methylation applied to DNA may play a role in the control of chromosome pairing in a variety of normal and repair related processes.

CB 219 ANALYSIS OF TRANSFECTION EFFICIENCY IN XR-1: A GAMMA RAY SENSITIVE AND DNA DOUBLE-STRAND BREAK REPAIR DEFICIENT MUTANT, T.D. Stamato, N. Denko, C. Waldren, R.A. MacLaren and A.J. Giaccia, The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA.

In G1 to early S-phase, the XR-1 Chinese hamster cell is extremely sensitive to killing by gamma-rays and deficient in DNA double-strand break repair (DSB), while in late S-phase, the mutant has nearly normal gamma-ray resistance and repair capacity. The efficiency of DNA transfection was examined in exponentially growing XR-1 cells and its parent using plasmid DNA containing the neomycin resistance gene. Using both the calcium phosphate precipitation and electroporation methods, the efficiency of transfection for the XR-1 cell was consistently 5 to 7-fold less than its parent cell. Also, the transfection efficiency of linear DNA into exponentially growing XR-1 cells is 14-fold less than circular DNA, while in the parental cell linear DNA is more efficiently transfected than circular DNA. Although XR-1's transfection efficiency is lower than its parent cell, the amount of plasmid DNA incorporated into genomic DNA is similar to the parental cell. A XR-1:human hybrid cell which has wild-type gamma-ray resistance and DNA repair capacity also had wild-type transfection efficiency suggesting that the biochemical defect in the XR-1 cell is also responsible for its reduced transfection efficiency. When the transfection efficiency in density inhibited cultures (70% G1 cells) were compared, XR-1 had a 50-fold lower transfection efficiency than the parental cell. The above results are consistent with the DSB repair deficiency in the XR-1 cell, since stable integration of foreign DNA presumably requires that double-strand DNA break be created and repaired. Also, this suggests that, in S-phase, duplicated genetic material allows repair by homologus recombination.

CB 220 X-IRRADIATED PLASMIDS ARE REPAIRED AND RECOMBINED IN XENOPUS LAEVIS OOCYTES. S.E. Sweigert[1] and D. Carroll[2], Depts. of [1]Radiol. and [2]Biochem., Univ. of Utah Health Sci. Ctr., Salt Lake City, UT 84132. We have been studying repair and homologous recombination of X-irradiated pBR322-based plasmid DNA injected into Xenopus oocytes. One of the plasmids is deleted for part of the ampicillin resistance gene (pNA1, 3.6kb), and one is deleted for part of the tetracycline gene (pBRF11, 3.3kb). pNA1 irradiated in phosphate buffer with 0-70 Gy of 250 kvp X-rays was co-injected with unirradiated pBRF11 which had been given a restriction cut in the region of homologous overlap. X-irradiation of pNA1 converted it from supercoiled form to a mixture of nicked and linear molecules, and reduced its ability to transform E. coli to tet resistance; both effects were dose-dependent. Most or all of the pNA1 recovered from oocytes was in supercoiled form, indicating that strand breaks were repaired. Its transforming efficiency (TE) was increased by up to 15-fold, demonstrating repair at the functional level. In an E. coli strain deficient in the repair of oxidative base damage (nfo-xth double mutant), the TE of irradiated relative to unirradiated DNA prior to injection was lower than in the repair-proficient parent (for example 0.9% and 9.4%, respectively, at a dose of 25 Gy). Following incubation in the oocyte, the relative TE was similar in the two strains (21% and 20% for 25 Gy). Thus, oocytes also repair X-ray-induced base damage. Recombinant molecules (pBR322-sized, 4.3kb) were seen in Southern blots of irradiated, injected DNA, but not in control samples. Recombination was also detected as the ability to transform bacteria to both ampicillin and tetracycline resistance. Increases of 2.9-fold, 8.5-fold, and 24-fold (as compared to unirradiated injected controls) were observed for doses of 7.5, 25, and 55 Gy, respectively.

CB 221 DOUBLE STRAND BREAK REPAIR IN VITRO CATALYZED BY CELL-FREE EXTRACTS FROM YEAST. Lorraine S. Symington and Alison Rattray, Institute for Cancer Research and Department of Microbiology, College of Physicians and Surgeons of Columbia University, New York, NY 10032.

In *S. cerevisiae* DNA double strand breaks are efficiently repaired by a mechanism that involves recombination. We have constructed plasmid substrates to examine this process in a cell-free system. The substrates share 1.6kb of homology, including the Tc^r gene; the remainder of the plasmid sequences are non-homologous and contain different drug resistance markers. The plasmids contain different deletion mutations within the Tc gene, thus are Tc^s. Recombination between these plasmids can be assayed by several methods. The formation of Tc^r recombinants can be measured by transforming product DNA into an *E. coli recA* strain. The other assays measure recombination directly by Southern analysis of the reaction products. Repair of one of the deletion mutations is monitored using an oligonucleotide probe that has the same sequence as that deletion mutation; the formation of crossovers can be detected by novel restriction fragments. We have also been able to detect the formation of X-form recombination intermediates by 2D agarose gel electrophoresis. Recombination is stimulated by double strand breaks or gaps within homology. We have constructed isogenic strains containing *rad* null mutations to determine whether any have defects in recombination using these assays. The results from this analysis will be presented.

CB 222 REJOINING OF DNA DOUBLE-STRAND BREAKS BY EXTRACTS FROM NORMAL AND ATAXIA-TELANGIECTASIA CELL LINES John Thacker, Phillip North and Anil Ganesh, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 ORD, U.K.
The ability to repair DNA strand breakage is considered to be important for cells to survive exposure to ionising radiation. The radiosensitive human disorder ataxia-telangiectasia (A-T) has some characteristics which may be explained by a DNA break-repair defect, but cells from A-T patients do not generally show reduced break rejoining in conventional assays (gradient sedimentation; elution). However DNA transfer studies from our laboratory, using recombinant molecules broken enzymatically at specific sites, showed that an A-T cell line rejoined breaks with poor fidelity compared to normal human cells. We have now developed an in vitro method using cell extracts to show the same response. Protein extracts are prepared from cell nuclei and used to treat a recombinant plasmid broken at a specific site in a marker gene. The amount of rejoining of the plasmid molecules is assessed by gel electrophoresis, and the fidelity of rejoining by transformation of suitable bacterial cells. With EcoRI-cut plasmid we found that extracts from normal cells (MRC5V1) and an A-T line (AT5BIVA) give similar amounts of rejoining but that the fidelity of rejoining by the A-T extract is 30-fold lower than that of the normal cells. We are now assessing extracts from other A-T lines and from untransformed human cells, as well as the rejoining of different types of broken ends by cell extracts.

CB 223 MOLECULAR GENETIC STUDY OF recA MUTATIONS WHICH PARTIALLY SUPPRESS recF MUTATIONS IN E. coli K-12, Tzu-chien V. Wang and Kendric C. Smith*, Department of Molecular Biology, Chang Gung Medical College, Kwei-San, Tao-Yuan, Taiwan and *Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305.
The nucleotide change of three recA(Srf) mutations (i.e., recA801, recA803 and recA2020), which partially suppress recF mutation, has been determined. The recA803 produces a change of codon 37 from GTG(Val) to ATG(Met) (Madiraju et al., 1988. Proc. Natl. Acad. Sci.USA 85, 6592-6596). The recA801 produces a change of codon 257 from CAG(Gln) to CCG(Pro) (Clark, personal communication), and the recA2020 produces a change of codon 121 from ACC(Thr) to ATC(Ile). Heterozygotes containing any combination of two recA(Srf) alleles do not produce a synergistic suppression of the recF deficiency in DNA repair. The presence of double recA(Srf) mutations in the same recA gene also fails to produce a greater suppression of recF mutation. These results suggest that the mutant RecA protein produced by the three recA(Srf) mutants probably acts through the same mechanism in the suppression of recF deficiency in DNA repair.

CB 224 Molecular characterization of chromosome breakpoints in Drosophila melanogaster. Darrel R. Falk, Mohri Barizo, and Amie Briones, Dept. of Biology, Point Loma Nazarene College, San Diego, CA 92106. The premise on which this work is based is that considerable information about the events associated with rejoining two ends of a broken chromosome, can be gleaned by studying the resulting alteration to DNA at the resealed junction point. We have used radiation to induce a set of deficiencies of the paralytic gene of Drosophila. This study reports on the isolation and characterization of the junction point in two of the strains. Data is presented which indicates that in one strain the segment of DNA immediately adjacent to the junction point is not derived from the homolog in which the breakage occured, but rather is believed to be derived from the other non-broken, homolog. A model is presented which proposes that the damaged chromosome in this strain was repaired by using the intact homologous chromosome as a template for the sealing together of the two broken ends.

Mutagenesis; Oxidant Damage and Inducible Responses; Cellular Damage and Repair

CB 300 EFFECTS OF LESION SIZES ON MUTAGENESIS IN LAMBDA PHAGE INDUCED BY 60-COBALT-GAMMA RAYS, Heidi Bertram and Ulrich Hagen, Institut für Strahlenbiologie, Gesellschaft für Strahlen- und Umweltforschung, D-8042 Neuherberg, Federal Republic of Germany.

This study was undertaken to explore the effects of radiation-induced DNA lesions on the induction of mutations in the cI gene which is the repressor of lambda phage. DNA lesions such as strand breaks, defect bases and sugars, alkali-labile and S1 nuclease-sensitive sites can be found with most different numbers, distributions and associations depending from the mode of irradiation and the sensitivity of the DNA. Therefore the kinetics of mutation may vary with the pattern of lesions in the phage and the state of the host cells. Two ways of irradiation with 60-cobalt-gamma were used: i) bacteria-free phage suspensions were irradiated in the presence of a radical scavenger to amplify direct absorption of energy in the DNA target, ii) lambda phage DNA was irradiated in buffer, favourizing water radicals nearby the DNA to react with its moieties (indirect effect on DNA). Having measured the amount of DNA strand breaks and alkali-labile sites, the DNA was packaged in-vitro to infectious phage particles. In both cases (i, ii) the amount of cI mutations was determined using SOS-induced hosts. The in-vitro packaged phage were found to have second-order kinetics for mutagenesis indicating that two premutagenic events were necessary to produce one mutation. In contrast, bacteria-free phage suspensions showed single hit kinetics for gamma-ray mutagenesis. The increase of the mutation rate was mainly due to minor lesions, f.e. single strand breaks, alkali-labile sites and unidentified base and sugar damage. Clustured DNA damage - found only in irradiated phage suspensions - enhance mutagenesis in the cI gene.

CB 301 A SYSTEM TO STUDY MUTAGENESIS IN TRANSGENIC MICE USING A LAMBDA PHAGE SHUTTLE VECTOR.

Peter M. Glazer, Jerome M. Eisenstadt, Wilma P. Summers, Carolyn Barrett, and William C. Summers. Departments of Therapeutic Radiology, and Molecular Biophysics and Biochemistry, and Human Genetics, Yale Medical School, New Haven CT 06510.

In order to study mutagenesis by ionizing radiation in whole animals, transgenic mice containing, in their genomes, multiple copies of lambda phage shuttle vector DNA were constructed. This work is an extension of our studies of UV mutagenesis using a lambda phage shuttle vector in mouse L cells in culture (Glazer et al. PNAS 83:1041-1044. 1986). To produce transgenic mice, mouse embryonic stem (ES) cells were transfected with λsupFneo DNA containing the supF gene of E. coli as a target for mutagenesis. The transfected, antibiotic resistant ES cells were inserted into developing mouse blastocysts, which were placed into foster mothers to allow development into transgenic mice. Rescue of viable phage for analysis of the supF gene is accomplished by the addition of mouse DNA to lambda in vitro packaging extracts. We are currently modifying the method of in vitro packaging to yield increased efficiency of lambda rescue.

CB 302 MOLECULAR ANALYSIS OF IONIZING RADIATION-INDUCED MUTATIONS IN A HUMAN SHUTTLE PLASMID, Armin Jaberaboansari, Sankar Mitra, R. Julian Preston and Larry C. Waters, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831

The *E. coli supF*-tRNA gene in a human shuttle plasmid, pZ189 (Seidman *et al., Gene* 38:233, 1985), was used as a target for molecular analysis of mutations in human cells induced by low (X-rays) and high (α-particles) LET radiation. Following replication of the *in vitro*-irradiated plasmid in human cells, the mutant *supF*-containing molecules were cloned by phenotypic screening in *E. coli* and the mutations were identified by sequencing. The frequency of mutations in the *supF*, after irradiation with 40 Gy (≈0.5 strand break/molecule), was 2-3 times that in the unirradiated control (3×10^{-4}). The distribution of mutations was highly non-random and remarkably similar in both irradiated and control DNAs. Most of the mutations were transitions involving G•C pairs and occurred selectively at most but not all 5'-TC (3'-AG) sequences. These mutations (at C's) were preferentially located in the non-transcribed strand. The control plasmid showed about 20-fold higher frequency of mutations after transfection and growth in human cells than when grown directly in *E. coli*. We propose that mutations in the control plasmid result from oxidative damages that occur during its incorporation into human cells and that similar lesions are also induced by X-irradiation. The hot spots of mutation suggest a critical role for neighboring sequences and tertiary structure of the target DNA in the production and/or processing of these damages during repair and replication. (Supported by the Office of Health and Environmental Research, U. S. Department of Energy under contract DE-AC05-840R21400 with the Martin Marietta Energy Systems, Inc.)

CB 303 MOLECULAR ANALYSIS OF IN VIVO, SOMATIC MUTATIONS OF THE HPRT GENE IN MICE, **Irene M. Jones, Karolyn Burkhart-Schultz, and Cheryl L. Strout, Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550**

To analyze the effect that ionizing radiation has on the spectrum of mutations in mammalian cells in vivo, we are studying mutations of the hypoxanthine phosphoribosyltransferase gene (*hprt*) in lymphocytes of the mouse. By using the mouse, we are able to manipulate factors that may affect the mutations recovered in vivo, including dose, time since exposure, age, dose rate. Using restriction fragment length analyses, we have detected a high proportion of deletions among spontaneous mutations of *hprt*. Studies of mutants isolated from irradiated mice are in progress. Polymerase chain reaction screening for retention of exons 1 and 9 has been used to prescreen the lesions of thioguanine resistant lymphocyte clones recovered from irradiated mice. These studies have revealed that the proportion of mutants with presumptive loss of the entire locus is increased 3 weeks after exposure to 400 cGy (Cesium-137), whereas 18 months after exposure to 344cGy there are fewer complete locus deletions than in age matched controls. Southern analysis of these mutations recovered from irradiated mice is in progress. This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract number W-7405-ENG-48.

CB 304 MOLECULAR ANALYSIS OF RADON-INDUCED MUTANTS, R. F. Jostes[1], T. L. Morgan[1], R. A. Gies[1]. E. Fleck[2], K. Gasper[2], and F. T. Cross[1], [1]Biology & Chemistry Department, Pacific Northwest Laboratory, Richland, WA 99352 and [2]Division of Basic Sciences and Mathematics, Whitman College, Walla Walla, WA
An in vitro system for exposing mammalian cells to radon gas and its daughters has been developed in our laboratory. Survival data using the CHO C-18 cell line indicates that the survival response after radon irradiation is an exponential function with a D37 of 62 cGy. Radon-induced mutations at the HGPRT locus in Chinese hamster ovary cells show a linear induction response with an induced frequency of 1.3 x 10^{-6} mutations per viable cell per centigray. To date, we have isolated 26 individual HGPRT$^-$ mutants from cell cultures irradiated with 90 cGy of Rn administered in vitro. Induced frequencies (two experiments) were both 19X the spontaneous frequency. Southern blot analysis of these cell lines indicate that 13 of the mutations (50%) were caused by a complete deletion of the locus. Seven (27%) mutations resulted from alterations within the gene and the remaining 6 (23%) were caused by changes undetectable by this analysis. This differs from the limited spontaneous data (8 mutants) in that one deletion was noted in the spontaneous (12%) while 4 mutants showed alterations (50%) and 3 mutants exhibited no change (38%). (Work supported by the U.S. Department of Energy under Contract DE-AC06-76RLO 1830).

CB 305 TRACK STRUCTURE EFFECTS AND MUTATIONAL YIELDS AT AN AUTOSOMAL AND AN X-LINKED LOCUS IN HUMAN CELLS. Amy Kronenberg, Department of Biophysics, UC Berkeley, and Division of Cell and Molecular Biology, Lawrence Berkeley Laboratory, Berkeley, CA 94720
The mutagenic effectiveness of densely ionizing particle beams has been examined for two genetic loci in the human B-lymphoblastoid cell line, TK6 as a function of track structure. Cells were exposed to two ^{28}Si ion beams differing in initial energy (456 MeV/u or 670 MeV/u) LET (61 keV/um for 456 MeV/u; 50 keV/um for 670 MeV/u), and overall track dimensions. The yield of mutants was determined for two loci: the X-linked hypoxanthine phosphoribosyltransferase locus (hprt) and the autosomal thymidine kinase locus (tk) which is heterozygous in the TK6 cell line. For each of the silicon ion beams, the overall mutant yield as a function of dose or incident particle fluence was greater for the autosomal locus. For each endpoint examined (cell killing, mutation to tk^-, or mutation to $hprt^-$) the 456 MeV/u beam was more effective than was the 670 MeV/u beam. The effect was most dramatic for the induction of mutants at the tk locus (16.1 x 10^{-7} mutants/cGy for 456 MeV/u beam vs. 4.9 x 10^{-7} mutants/cGy for 670 MeV/u beam. For the induction of hprt-deficient mutants, the yield was reduced from 4.2 x 10^{-7} for the 456 MeV/u beam to 2.1 x 10^{-7}/cGy for the 670 MeV/u beam. The reduced effectiveness of the more energetic beam is also reflected in a shift in the D_0 from 28 cGy for the less energetic beam to 42 cGy for the 670 MeV/u beam. Chatterjee and Magee have shown that the track dimensions for heavy ion beams are independent of particle atomic number and dependent on energy. The preliminary data presented here indicate that small variations in track dimensions and LET may have a large impact on the likelihood of causing heritable somatic alterations in specific genetic loci in human cells.

CB 306 SITE DIRECTED MUTAGENESIS BY 5-HYDROXYMETHYLURACIL FOLLOWING IN VIVO REPLICATION OF ØX-174*am3*DNA, Dan D. Levy and George W. Teebor, Departments of Environmental Medicine and Pathology, NYU School of Medicine, New York, NY 10016

5-hydroxymethyluracil (HmUra) is formed in DNA as a product of oxidative attack on the methyl group of Thy. It is removed from DNA through the action of the repair enzyme, HmUra-DNA glycosylase. To determine whether the conversion of Thy to HmUra is mutagenic, HmdUTP was synthesized and inserted into a target codon in place of Thy by *in vitro* DNA polymerase mediated extension of an oligonucleotide primer annealed to ØX-174*am3* virion DNA. *E. coli* spheroplasts were transfected with the HmUra containing DNA and the yield of revertant phage determined. The sensitivity of the assay for detection of point mutations was 1 error/ 10^4 bases. The introduction of HmUra in place of Thy did not result in an increase in revertant phage. These data indicate that the oxidation of Thy to HmUra does not result in substantial mutagenesis. This result supports the hypothesis that HmUra-DNA glycosylase functions to protect against mutations arising from the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine followed by deamination to HmUra. This reaction sequence results in the formation of an HmUra residue opposite Gua which, if unrepaired, would lead to G:C->A:T transitions.

CB 307 DENATURING GRADIENT GEL ELECTROPHORETIC ANALYSIS OF X-RAY-INDUCED MUTATIONS IN HUMAN CELLS, R. T. Okinaka[1], G. F. Strniste[1], N. F. Cariello[2], P. Keohavong[2], A. Oller[2], and W. G. Thilly[2], Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545[1], and Center for Environmental Health Sciences, MIT, Cambridge, MA 02139[2].

The polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) techniques have been utilized to amplify DNA fragments and to separate mutant from wildtype sequences from genomic DNAs isolated from large populations of cells [Science (1989) 243:737]. These methods are currently being employed to determine ionizing radiation-induced mutational spectra in exon 3 of the human HPRT locus. Cultures of the lymphoblastoid TK-6 cell line (1 x 10^9 cells) exposed to two, 200 rad split doses of X-rays yielded between 2700 and 4100 initial surviving 6-thioguanine resistant (6-TG^r) mutants in four independent experiments. HPRT exon 3 fragments were amplified from DNA extracted from these mixed 6-TG^r cell populations by employing a high fidelity PCR technique. These fragments were then analyzed by DGGE. Initial observations indicate at least one mutant signal occurs at a significant level (0.5-1% of the total $HPRT^-$ mutants) in three of four X-ray induced cultures. This result suggests a potential radiation induced mutational "hotspot". Direct-sequencing of these fragments indicate that the $HPRT^-$ phenotype results from a thymidine (T) deletion in a region containing four successive T's at base-pair positions 379-382 in exon 3. Additional comparisons indicate that the X-rayed populations also contain 10-15 unique signals (non-hotspots) in the earliest melting regions. Sequencing data on four of these fragments indicate that they are randomly distributed small deletion events (8-22 bases). The isolation and characterization of the cells harboring these mutations is the subject of current investigations. This work was supported by the USDOE under contract W-7405-ENG-36; DOE Grant DE-FG02-86-ER60448, and US NIEHS Grants 1-P42-ES04675, 5-P01-ES00597, 1-P50-ES03926-05.

CB 308 DETERMINATION OF GAMMA RAY INDUCED MUTATIONS IN NORMAL AND REPAIR DEFICIENT CELLS USING A SHUTTLE VECTOR SYSTEM. Sikpi M.O., Denette E.R. and Lurie A.G., Division of Oral and Maxillofacial Radiology, University of Connecticut Health Center, Farmington, CT 06032.

The SV-40-based shuttle plasmid, pZ189, was used to study the nature of gamma-ray-induced DNA mutations in normal and ataxia telangiectasia (AT) human cells. pZ189 was introduced into the human repair proficient (GM606) or AT (GM2783) lymphoblast cells and the cells containing the plamsid were irradiated 24 hrs later. Following another 24 hr incubation to allow mutation fixation, the progeny plasmid molecules were rescued and introduced into E. coli MBM7070 to select for plasmid molecules with mutations. Base alterations were determined by sequencing. Plasmid survival decreased as the gamma-ray dose increased in both GM606 and GM2783 cells. At 10Gy, the percentage of viable progeny molecules from GM606 cells was approximately 50, while that from GM2783 cells was only about 18. Mutation frequencies were also dose dependent, ranging from 7.7x10-5 at 1Gy to 2.2x10-4 at 20Gy for the GM606 cells. Background mutation frequency was 4.8x10-5. Preliminary results suggest greater mutation frequencies for the GM2783 cells. The majority (37/48) of the independent mutants from GM606 cells contain base substitutions exclusively, with 83 of 84 changes involving G-C base pairs. Mutants from GM2783 are being sequenced to identify the changes.

CB 309 MUTAGENESIS AT THE ADENINE PHOSPHORIBOSYLTRANSFERASE LOCUS IN HUMAN LYMPHOBLASTOID CELLS, Kouichi Tatsumi, Akira Fujimori, Akira Tachibana, Izumi Arita and Hiraku Takebe, Department of Molecular Oncology, Faculty of Medicine, Kyoto Univesity, Sakyo, Kyoto 606, Japan

An assay system using microtiter plates has been developed to measure the frequency of mutations at adenine phosphoribosyltransferase (APRT) locus on the human autosomal chromosome #16. An EBV-transformed B-lymphoblastoid cell line, WR10, was chosen for the assay among cell lines derived from heterozygous carriers of hereditary 2,8-dihydroxyadenine urolithiasis (APRT deficiency). The frequency of cells resistant to 100 μM of 2,6-diaminopurine (DAP) was found to be 1.1x 10^{-3} with a mutation rate of 1.65 x 10^{-6}/cell/generation. DAP resistant mutants grew as rapidly as phenotypically wild type WR10 cells, and the reconstruction experiments indicated that the mutant fraction was stable for at least 14 days. APRT activities in 10 DAP-resistant clones were found to vary between 0 and 38% the level observed in aprt^{+} WR10 cells. Exposure of WR10 cells to γ-rays resulted in a dose-dependent increase of DAP^{r} mutant fraction up to 2.5 x 10^{-4} at 2 Gy, whereas induced mutant fraction was only 4.7 x 10^{-5} for 6-thioguanine resistance with the background of 1 x 10^{-5}. We found that WR10 cells were heterozygous also for a SphI restriction fragment length polymorphism at the APRT locus, which allowed the functional and nonfunctional APRT alleles to be differentiated by Southern blot analysis. A majority of spontaneous and γ-rays-induced DAP^{r} mutant clones examined were associated with loss of the entire functional allele.

CB 310 POLYOMAVIRUS-BASED SHUTTLE VECTORS FOR STUDYING MECHANISMS OF MUTAGENESIS IN RODENT CELLS , Maria Zernik-Kobak, Artur S. Levine, Irena Szumiel and Kathleen Dixon, Section on Viruses and Cellular Biology, National Institute of Child Health and Human Development, National Institutes of Health ,Bethesda, MD 20892; Institute of Nuclear Chemistry and Technology, Warsaw, Poland.

Shuttle vectors have been proven to be very useful tools for the analysis of mutagen specificity and mechanisms of mutagenesis in monkey and human cell systems. In order to extend this technology to rodent systems, we have constructed a series of analogous vectors based on polyomavirus which replicates in mouse and hamster cells. In these vectors the polyomavirus genome provides replication functions and the bacterial supF gene serves as mutagenesis target. One of the vectors colled pPySLPT-2 appeared to replicate most efficiently in all cell lines tested.

The pPySLPT-2 vectopr has now been used successfully in mouse lymphoma cells to analyze the difference in mutagenicity between UV repair-deficient (L5178-R) and repair-proficient (L5178-S) lines. When UV-irradiated vector is introduced into these two cell line, replication of the damaged vector is inhibited to a greater extend in the LY-R cells, consistent with the inability of these cells to repair UV photoproducts. Futhermore, the frequency of vector mutants is much higher in the LY-R cells, presumably due to the presence of higher levels of unexcised damage in the DNA template at the time of DNA replication. Interestingly, the spectrum of mutations observed in the repair-deficient mouse cells is very similar to that observed in repair-deficient human cells indicating that mutational mechanisms are very similar in the two species.

CB 400 MECHANISMS OF PROTECTION TO REACTIVE OXYGEN SPECIES BY *BACILLUS SUBTILIS*. David Bol and Ronald Yasbin Department of Biological Sciences, Program in Molecular and Cellular Biology, University of Maryland Baltimore County, Baltimore, Maryland, 21228.

The exposure of organisms to reactive oxygen species occurs by two mechanisms. First, cells are exposed to oxidative stress by normal aerobic metabolism where molecular oxygen is the electron acceptor. Alternatively, hydrogen peroxide and hydroxyl radicals can be generated by exposure of bacteria to ionizing radiation. In response to these toxic agents, aerobically metabolizing organisms activate transcription of genes whose products either protect the organism from the agent or repair the damage incurred by the reactive molecules. One gene that has been identified in the protection of bacteria to the damage resulting from exposure to hydrogen peroxide is the catalase gene. The naturally competent, spore-forming, gram positive bacterium *Bacillus subtilis* has two catalase genes. The most active of these two genes, *kat-1*, is expressed under two conditions. The *kat-1* gene is induced following exposure of cultures to H_2O_2 during mid-exponential growth, and is also expressed at higher levels as the culture approaches stationary phase. Neither mechanism of regulation is dependent upon the regulatory function of the *recE* gene. The induction of *kat-1* has been studied for induction in response to several environmental agents ranging from redox cycling compounds to ionizing radiation. Additionally, these oxidative stress agents have been shown to induce the SOS-like SOB response in *B.subtilis*, which is dependent upon the DNA repair and regulatory functions of *recE*. Thus, *B.subtilis* has two independently regulated defenses against oxidative agents and the agents generating reactive oxygen species.

CB 401 X-RAY-INDUCED PROTEINS AND GENES IN HUMAN CELLS,[1]David A. Boothman, [2]Sam W. Lee,[3]Edward N. Hughes and[4]Arthur B. Pardee. [1]Dept. Radiation Oncology, Univ. Michigan Medical Center, Ann Arbor, Mich. 48109;[4]Div.Cell Growth & Regulation and[2]Cancer Genetics, Dana-Farber Cancer Inst., Boston, MA;[3]Dept. Radiation Oncology, Hosp. Univ. Pennsylvania, Philadelphia, PA. We have identified a pleiotropic protein expression response that is latently activated upon X-irradiation of confluence-arrested human melanoma (U1-Mel) cells. Eight major X-ray-induced polypeptides (XIPs) of 126-275 KDa (i.e., XIP126 thru XIP275) were detected by resolving L-[35S]methionine-labeled whole cell extracts using two-dimensional gel electrophoresis. XIPs were found in unirradiated, log-phase U1-Mel cells, shut off under plateau-phase conditions and resynthesized after X-irradiation. Three classes of proteins were affected by X-irradiation. Class I proteins were induced linearly with increasing X-ray doses. Class II proteins increased linearly with low X-ray doses, but plateaued at 150-250 cGy. Class III proteins decreased with increasing X-ray doses. XIP expression was specific for ionizing radiation, since neither heat shock, hypoxia or alkylating agent treatments induced XIP synthesis in confluence-arrested cells. A strong correlation between the induction of XIP269 and the PLDR capacities of tumor, cancer-prone and normal human cells was noted. Treatment of X-irradiated U1-Mel cells with caffeine partially blocked PLDR and concommitantly inhibited the synthesis of XIP269; a possible role for XIP269 in growth-arrest following X-irradiation will be discussed. We are presently using differential and subtractive hybridization techniques combined with various immunological methods to clone genes which are induced by X-irradiation, including those genes which would correspond to the XIPs recently reported (Cancer Res. 49:2871-2878, 1989).

CB 402 DNA REPAIR, ANTIOXIDANT ENZYME AND MEDIA DEPENDENCE OF THIOL-INDUCED RADIATION PROTECTION IN AEROBIC CELLS. H. Gregg Claycamp, Carmella DeRose, and Kam-Kuen Ho, Radiation Research Laboratory, University of Iowa College of Medicine, Iowa City, IA 52242.
Thiol protection of Escherichia coli against ionizing or far-UV radiation cell killing requires a DNA repair-proficient phenotype. To characterize this phenomenon, we have been investigating overlap of DNA repair with cell mechanisms affording protection against oxidative stress. Methods include treatment of wild-type, DNA repair-deficient or antioxidant enzyme-deficient cells in minimal (MM) or rich (RM) medium containing dithiothreitol (DTT) after which they are washed and held in phosphate buffer prior to irradiation and survival assays. Thiol pretreatment obviates chemical protection during radiation treatment and has been shown to induce DNA repair from DNA damage induced by "autoxidatively-generated" active oxygen species. Results show that thiol-induced ionizing and far-UV radiation protection are DNA-repair-, media- and antioxidant enzyme-dependent. In general, DTT+MM treatments induce protection against either radiation whereas DTT+RM induces little ionizing radiation protection and, paradoxically, sensitizes cells to UV. When chelators are used to slow DTT oxidation in RM, UV sensitization is reversed and ionizing radioprotection resembles the DTT+MM case. Results of studies using DNA repair- and antioxidant enzyme-deficient strains suggest that the target of thiol-induced damage in DTT+MM treatments is DNA, whereas the target in DTT+RM rich medium in the cell membrane.

CB 403 REGULATION OF SUPEROXIDE RADICAL-INDUCIBLE GENES IN E. coli. Spencer B. Farr. Laboratory of Toxicology, Harvard School of Public Health. Boston, MA 02115.
Cells exposed to an increase in the steady state levels of superoxide radicals induce at least 12 proteins and include a novel DNA repair pathway (1,2). Most of these proteins are distinct from those induced by H_2O_2 (5 proteins are similar). In order to study the regulation of $O_2^{\cdot-}$ -inducible genes we have isolated 3 Mu d(bla,lacZ) promoter fusions that respond specifically to $O_2^{\cdot-}$ stress. These fusions have been termed soi::lacZ for superoxide inducible (3). Two of the soi promoters have been characterized. Additionally, a regulatory mutant that affects transcription of several $O_2^{\cdot-}$ -inducible genes has been isolated. Genetic and molecular biological experiments suggest that the soi genes and sodA share a common regulatory pathway. Regulation of $O_2^{\cdot-}$ -inducible genes will be discussed.

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CB 404 DNA-DAMAGE-INDUCIBLE GENES IN MAMMALIAN CELLS, Albert J. Fornace Jr., N.C.I., N.I.H., Bethesda, MD 20892

Based on results in bacteria and yeast, many genes would be expected to be DNA-damage inducible (DDI) in mammalian cells; some may represent specific responses to DNA damage, while others may be general stress responses to cell injury. A variety of mammalian genes, such as metallothionein, collagenase, *c-fos*, ubiquitin, and ß-polymerase[1], have been found to be DDI by our group and/or other investigators. Most of these examples probably represent general stress responses since they were induced by unrelated agents such as heat shock and/or activators of protein kinase C. However, ß-polymerase mRNA was found to be specifically induced only by alkylating agents and similar agents that produce DNA damage repaired by a mechanism involving ß-polymerase[1]. The ß-polymerase gene had several properties in common with bacterial genes that are specifically DDI: low abundance, rapid induction of 2-10 fold, and induction specific for DNA damage. An approach to isolate cDNA clones of other such DDI genes was developed using hybridization subtraction at low ratios of RNA:cDNA[2]. 49 different cDNA clones were isolated that coded for transcripts rapidly induced 2-fold or more by UV radiation in Chinese hamster cells[2]. Many of these transcripts were induced only by DNA-damaging agents; these DDI cDNA clones were divided into 2 classes. In Class I, only UV radiation and other UV-mimetic agents were effective inducing agents, while in Class II other base damaging agents such as alkylating agents were also inducing agents. Characterization of individual DDI cDNA clones will be presented including evidence that a Class I member (DDIA18) encodes a nucleic acid single strand binding protein, that several Class II genes are coordinately regulated indicating that they are members of the same regulon, and that at least one DDI gene is x-ray-inducible.

[1] Fornace A.J. Jr., Zmudka, B.Z., Hollander, M.C., and Wilson, S.H.: Molec. Cell. Biol. *9*: 851-853, 1989.
[2] Fornace, A.J. Jr., Alamo, I. Jr., and Hollander, M.C.: Proc. Natl. Acad. Sci. USA *85*: 8800-8804, 1988.

CB 405 DAMAGE INDUCED SIGNAL TRANSDUCTION IN MAMMALIAN CELLS. Peter Herrlich, Bernd Stein, Hans Jobst Rahmsdorf, Bernd Kaina, Christine Lücke-Huhle and Sabine Mai, Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, P.O.Box 3640, D-7500 Karlsruhe 1, FRG. The conversion of absorbed radiation energy into a genetic response involves a minimum of four but probably many more preexisting cellular components. We will discuss these components using mainly two endpoints: Amplification of SV40 origin-linked DNA and induced gene expression both in response to UV, gamma- and alpha-irradiation. Complex biological reactions such as replication and gene expression are brought about by evolutionarily optimized sets of proteins and, if influenced from outside the cells, they are regulated and triggered by physiological mediators. Adverse non-physiological agents can only feed into a physiological chain of events and the response is likely to be rather uniform no matter which agent elicits the response. The minimum assembly of steps in radiation induced amplification and gene expression consists of i) absorption of radiation energy in DNA or interaction of radicals with the DNA. Radiation can be circumvented by the transfer of irradiated DNA into cells. ii) The structural change of DNA resulting from the absorbed energy or radical interaction is recognized by one or several yet unknown proteins which stimulate a signal transduction pathway. iii) At the other end of the signal transfer, cellular replication and transcription factors are activated. All these steps do not require new macromolecule syntheses. iv) The replication factor acting at the SV40 origin has been identified and shown to bind to a specific sequence. The binding is absolutely necessary as the initial step in amplification. The activated transcription factors select their genes by binding to specific cis-acting promoter sequences. These genes are transcribed and are largely responsible for the phenotype of the genetic response. The induced genes encode themselves transcription and replication proteins, various proteins characteristic of proliferating cells, and both repair and mutator functions. Amplified DNA is itself a source of permanent genetic changes.

CB 406 DNA BINDING PROTEIN INDUCED BY IONIZING RADIATION IN HUMAN CELLS,

Martin. F. Lavin and Surinder P. Singh
Molecular Oncology, Queensland Institute of Medical Research, Herston, Brisbane 4006, Australia.

Exposure of mammalian cells to a number of stress-causing agents leads to activation of existing proteins and preferential transcription of certain genes. A recent report by Glazer *et al* (1989) showed that UV radiation led to induction of several DNA-binding proteins by a mechanism that did not involve *de novo* protein synthesis.

An accompanying abstract (Singh and Lavin 1990) describes the presence of a constitutively active DNA-binding protein in extracts from ataxia-telangiectasia cells. This syndrome is characterized by radiosensitivity and predisposition to cancer. The DNA-binding protein observed in A-T extracts binds to a region of the SV40 enhancer located between the binding site for transactivating factors AP-3 and AP-5. It was only possible to detect the same binding activity in normal cells after exposure to γ-radiation. This binding activity increased with radiation dose over the range 2.5-20 Gy. The protein reached a maximum level 1 hr post irradiation and declined thereafter, being undetectable by 9 hr after irradiation. The radiomimetic agent streptonigrin also activated this protein but neither UV nor heat shock did so. This activity bound to the same nucleotide sequence as the A-T protein and was of the same size, 43kDa. It is not clear at this stage whether this protein is involved in DNA repair or DNA replication.

CB 407 AMPLIFICATION, EXCISION AND REINTEGRATION OF SV40 DNA SEQUENCES IN SURVIVING HAMSTER CELLS AFTER 60-CO-γ-IRRADIATION, Christine Lücke-Huhle, Monika Pech and Peter Herrlich, Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, Postfach 3640, D-7500 Karlsruhe 1, F.R.G. In order to study radiation-induced changes in mammalian cells at the gene level SV40-transformed Chinese hamster embryo cells (Co631) were exposed to 60-Co-γ-radiation and the fate of the integrated SV40 sequences was pursued over a period of 20 days following radiation exposure. Without leading to intact virus γ-irradiation induces SV40 DNA amplification about 25 fold in exponentially growing Co631 cultures and to a lesser extent in density inhibited cells. Because of a retarded amplification kinetics compared to the response following α- and UV-irradiation, colony hybridization could be used to demonstrate SV40 amplification in surviving and nonsurviving cells (= abortive colonies). At 20-30 cell generations after irradiation most of the amplified copies, however, had been lost in surviving cells, but restriction enzyme analysis revealed novel integration sites within the hamster genome: 9 out of 30 clones examined showed distinct changes in their restriction pattern after digestion with Xba I, Eco RI, Kpn I and Hind III. Since all the irradiated cell clones investigated still exbibited the SV40 hybridizing bands of control cell DNA we conclude that none of the original integration sites had been lost after γ-irradiation and the appearance of new SV40-containing fragments in the Southern blot was due to new integration of amplified and excised SV40 DNA.

CB 408 **X-RAY-INDUCED CHANGES IN DNA METHYLATION ARE DETECTABLE USING DNA FINGERPRINTING,** C. Dennis Miller, John J. White, Laboratory of Molecular Genetics, Gerontology Research Center, National Institute on Aging, Baltimore, Maryland 21224

The DNA fingerprinting methodology makes it possible to survey simultaneously genetic alterations in multiple loci scattered throughout the genome. Now used in a wide variety of applications, DNA fingerprinting is a convenient way to document identity of cell lines. We were concerned, however, whether large scale changes in the fingerprint pattern might occur during treatment with X-rays (10-1500R). To study this, HeLa and Rat-1 cells were subcloned following treatment with various doses of X-rays. DNA was isolated from confluent cultures and digested with either *Hinf*1 or *Hae*III, and probed with minisatellite probes 33.15 or 33.6. We found no alterations in DNA fingerprint patterns, presumably due to the low resolving power of the technique. A recent report, however, indicated that epigenetic changes, i.e., hypomethylation of cytosine residues, apparently occur after cells are exposed to gamma radiation. We found that further digestion of *Hinf*1 or *Hae*III cleaved DNA from the irradiated cell lines with *Msp*1 produced altered patterns in some clones when compared to double digests with the methylation insensitive isoschizomer *Hpa*II.

CB 409 PARAQUAT TOXICITY IS MARKEDLY INCREASED IN ESCHERICHIA COLI MUTANTS DEFECTIVE IN THE BIOSYNTHESIS OF POLYAMINES, Kenneth W. Minton[1], Herbert Tabor[2] and Celia White Tabor[2]. [1]Department of Pathology, Uniformed Services University of the Health Sciences and [2]Laboratory of Biochemical Pharmacology, National Institute of Diabetes and Digestive and Kidney Diseases. Paraquat (methylviologen) is used widely as a source of superoxide in a variety of biological systems. We have found that the toxicity of paraquat is markedly increased in Escherichia coli mutants that are defective in the biosynthesis of putrescine or of spermidine when grown in purified media. Strains that cannot make putrescine because of deletions in the genes coding for the biosynthetic enzymes show a 100-fold increase in paraquat toxicity, which is completely corrected by the addition of putrescine and spermidine to the growth medium. A 20-fold increase in toxicity is noted in strains that can synthesize putrescine, but are blocked in the synthesis of spermidine. It is of interest that there is such a marked increase in the toxicity of paraquat in the spermidine-deficient cells, since the spermidine-depleted cells normally only show a 15% decrease in the overall growth rate. The toxicity of paraquat in these cells is bacteriostatic rather than bacteriocidal. However, we do not know the mechanism by which amine deficiency increases the toxicity of paraquat.

CB 410 STIMULATION OF POLY(ADP-RIBOSE) SYNTHESIS BY FREE RADICALS IN C3H10T1/2 CELLS: RELATIONSHIP WITH NAD METABOLISM AND DNA BREAKAGE, Guy Poirier[1], Dominique Lautier[1], Danièle Poirier[1], Annie Boudreau[1], Moulay A. Alaoui Jamali[2], André Castonguay[2] and Guy Poirier[1], [1]Laboratoire du Métabolisme du poly (ADP-ribose), CHUL, Québec G1V 4G2; [2]Laboratoire d'Étiologie et de Chimioprévention du Cancer, Université Laval, Québec, G1K 7P4, Canada.

Poly(ADP-ribose) polymerase activation is coupled with DNA excision repair. We have studied the effect of H_2O_2 and O_2^- produced by xanthine/ xanthine oxidase, on NAD catabolism, poly(ADP-ribose) synthesis and production of DNA single strand breaks in C3H10T1/2 cells. The results have shown a correlation between the induction of DNA single strand breaks, the decrease of NAD pool and the accumulation of polymers. New techniques based on affinity chromatography and reversed-phase HPLC have allowed an accurate determination of polymer contents and showed an active oxygen species-induced stimulation in polymer biosynthesis by 20-fold. However, inhibition experiments performed in the presence of 3-aminobenzamide have shown that the decrease of NAD levels after exposure of cells to active oxygen species was caused by stimulation of poly(ADP-ribosyl)ation and of another cellular process. The involvement of mono(ADP-ribosyl) transferases is under investigation.

CB 411 IDENTIFICATION AND PARTIAL PURIFICATION OF AN UV-INDUCIBLE DAMAGE-SPECIFIC DNA BINDING PROTEIN FROM PRIMATE CELLS, Miroslava Protic, Marija Abramic, Steven Hirschfeld, and Arthur S. Levine, National Institute of Child Health and Human Development, NIH, Bethesda, MD 20892.

Utilizing a DNA band shift assay, primate cell-free extracts, and UV-irradiated oligonucleotide probes, we have identified a DNA binding protein which appears to be specific for (6-4) pyrimidine dimers. In monkey cells pretreated with UV light there is a time and UV-dose dependent increase in the amount of this binding protein. This enhancement is inhibited by actinomycin D, which suggests a requirement for de novo protein synthesis. Cell extracts prepared either from mock-treated or UV irradiated cells were used for protein purification. A damage-specific DNA binding protein(s) was eluated with linear KCl gradient from a phosphocellulose column into 3 peaks of activity. Gel exclusion chromatography assigned the native form of one of the peaks to has a molecular weight of about 200 kDa. Induced levels of this damage-specific DNA binding protein might be responsible for enhanced expression of UV-irradiated DNA we observed in pretreated cells (Protic et al., Somat. Cell. Mol. Genet. 14:351, 1988).

CB 412 CHARACTERIZATION OF AN IONIZING RADIATION-ACTIVATED DNA-BINDING PROTEIN IN ATAXIA-TELANGIECTASIA NUCLEI,

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Ataxia-telangiectasia A-T is a rare human genetic disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency and developmental abnormalities. This disorder is also associated with a predisposition to development of cancer, in particular, leukaemias and lymphomas. Increased sensitivity to ionizing radiation *in vivo* and in cells in culture together with evidence of defective DNA repair in some cases chromosomal instability, specific gene rearrangements and altered DNA topoisomerase II activity all point to the probability of a DNA processing defect in A-T.

We report here the presence of a specific DNA-binding protein capable of binding to the SV40 enhancer, in A-T nuclear extracts. The protected motif was shown by DNase 1 and DMS footprint analysis to be ACAGTCAATCCA, a sequence located in the 5' flanking region of the distal 72bp repeat of the enhancer. South Western analysis demonstrated that the protein was approximately 43,000 daltons in size. It was not possible to detect a similar activity in extracts from unperturbed normal cells. However, exposure of control cells to ionizing radiation revealed the presence of a DNA binding activity which was identical to the A-T protein. Our results demonstrate the presence of a constitutively active protein in A-T extracts which is normally activated by ionizing radiation and presumably plays an important role in response to radiation damage.

CB 413 CHROMATIN INVOLVEMENT IMPLICATED IN ACTIVATION OF HIV-1 GENE EXPRESSION BY DNA DAMAGING AGENTS. Kristoffer Valerie* and Martin Rosenberg, Smith Kline & French Labs, PO Box 1539, King of Prussia, PA 19406-0939, and *Department of Radiation Oncology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0058.

We have recently shown that human immunodeficiency virus 1 (HIV-1) LTR-directed gene expression can be activated by DNA damaging agents, such as ultraviolet light, to levels similar to those obtained by the HIV-1 *tat* gene.

After testing a number of different DNA damaging agents for their potency to activate HIV-1 gene expression we found that only the type which produce the bulky type of DNA lesion would activate, such as UV, mitomycin C, 4NQO, and PUVA. Ionizing radiation, and alkylating agents did not activate gene expression although cell killing was significant also after these treatments. Gene activation by UV was also observed in xeroderma pigmentosum A cells, but required only lower doses, suggesting that the DNA damage itself is the triggering signal and that an intact DNA excision repair system is not required for activation. DNA synthesis does not appear to be involved in the activation process since DNA synthesis inhibitors did not influence HIV gene expression. Furthermore, UV activation is very sensitive to novobiocin, an inhibitor of topoisomerase II, indicating that topological changes in chromatin is required for activation.

Our results suggest that activation of HIV-1 gene expression by UV at early times after radiation (<24h) occurs mainly at a step prior to that normally associated with direct activation by transcriptional factors. This activation is most likely due to a transient decondensation of chromatin which would allow the transcriptional machinery better access to the HIV-1 LTR, thereby increasing gene expression.

Supported in part by NIH grant A124845.

CB 414 PHYSIOLOGICAL STUDIES OF E. coli CELLS WITH ARTIFICIALLY CONTROLLED SOD LEVELS, Linda K.B. Walkup and Tokio Kogoma, Depts. of Cell Biology and Microbiology, University of New Mexico School of Medicine, Albuquerque, NM, 87131. All aerobic organisms have mechanisms to protect themselves from reactive oxygen species which arise even during normal respiration and metabolism. Superoxide dismutases (SODs), which catalyze the conversion of the superoxide radical (O_2^-) to hydrogen peroxide are the first line of defense against O_2^-. E. coli sodA$^-$ sodB$^-$ mutants lack all SOD activity and thus are constitutively stressed when growing aerobically, showing increased rates of mutagenesis and sensitivity to O_2^--generating drugs. A sodA$^-$ sodB$^-$ lac$^-$ E. coli strain was lysogenized with a lambda vector carrying a ptac-controlled sodA coding for a manganese superoxide dismutase. The gene for the lacI^Q repressor carried on a high copy number plasmid was used to repress ptac activity and thus reduce SOD activity to near zero levels. IPTG was added at various concentrations to induce the promoter and thereby vary SOD activity. The effects of abnormal levels of SOD activity on growth, viability, and resistance to superoxide radical-generating drugs were studied. To monitor transcription, a pool of random promoter fusions to lacZ were created in this strain. Beta-galactosidase assays of the pool of lacZ fusions indicated a general increase in transcription as the SOD activity decreased. Chloroquine gel analyses of plasmid DNA indicated that parallel increases in the negative superhelicity of plasmid DNA occur with decreasing SOD activity.

CB 415 REGULATION OF PROTEIN KINASE C BY IONIZING RADIATION, Gayle E. Woloschak, Chin-Mei Chang-Liu, and Pocahantas Shearin-Jones, Biological and Medical Research Division, Argonne National Laboratory, Argonne, IL 60439-4833

Members of the Protein Kinase C (PKC) gene family have been shown to play an important role in tumor promotion and the regulation of cell growth. Experiments were designed to examine the effects of exposure of confluent Syrian Hamster embryo (SHE) cells to different qualities (high and low LET radiations) of ionizing radiation administered at a variety of doses and dose-rates on the expression of PKC-specific mRNA. The results of these experiments have shown that low LET radiations (such as X-rays and gamma-rays) are capable of inducing increased expression of PKC within one hour following radiation exposure. Levels of induction of PKC-mRNA are four- to six-fold increased over unirradiated controls. Dose and dose-rate effects were evident, with higher doses and dose-rates inducing increased accumulation of PKC-mRNA (with doses ranging from 6-200 cGy). Induction of PKC=mRNA occurred at a time when total cellular transcription was reduced following radiation exposure. Similar exposure of the cells to high LET fission-spectrum JANUS neutrons, however, had little effect on PKC-mRNA expression. Modest induction (two-fold compared to untreated cells) occurred when radiations were given at very low dose-rates (0.5 cGy/min). These results suggest that induction of PKC mRNA may play a critical role in the cellular response to ionizing radiation. In addition, they demonstrate that different qualities of radiation may regulate PKC differently. (Work supported in part by the U.S. Department of Energy, Office of Health and Environmental Research, under contract No. w-31-109-ENG-38.)

CB 500 **INDUCTION AND REPAIR OF αDNA BASE AND STRAND DAMAGE IN X-IRRADIATED MONKEY CV-1 CELLS**, Robert Bases and Frances Mendez, Department of Radiology, Albert Einstein College of Medicine, Bronx, NY 10461. Base damage in αDNA from irradiated monkey CV-1 cells was determined by measuring release of 5'-^{32}P-end labelled DNA fragments after digestion with endonuclease III of E. coli. The frequencies (~$4x10^{-7}$/nt/Gy) and base sequence locations of the enzyme sensitive sites were determined. Fragments were released from irradiated DNA at sequence sites of pyrimidines and guanines. The half time of repair of single strand breaks (~$3x10^{-7}$/nt/Gy) was approximately 1.5 h. Repair of base damage was slower, as judged from loss of enzyme sensitive sites in DNA. More than half of the damaged bases were still detectable after 4 h of repair. The patterns of base and strand damage found after 900 Gy to cells were drastically altered by induction doses of only 5 Gy given 4 to 18 h before, consistent with induction of DNA repair processes.

CB 501 ROLE OF DNA POLYMERASE δ IN IONIZING RADIATION CLASTOGENESIS[1], Michael A Bender[2], Ruth C. Moore[3] and Beatrice E. Pyatt[2], Medical Department, Brookhaven National Laboratory, Upton, NY 11973 and [3]The Cancer Institute, Peter MacCallum Hospital,Melbourne 3000, Australia. We had earlier inferred the involvement of DNA polymerase α in repair of X-ray-induced lesions which if unrepaired lead to chromosomal aberrations in G_o, G_1 and G_2 cells from experiments in which the inhibitor aphidicolin (APC) was used as a post-treatment. The enzyme's involvement in the production of chromosomal inter- and intrachanges was inferred from the total absence of exchanges in cells kept in the presence of the inhibitor until they entered mitosis. With the discovery of a second major DNA synthesis polymerase, δ, which is also aphidicolin-sensitive, we have undertaken experiments with the inhibitors N^2-(p-n-butylphenyl)-9-(2-deoxy-β-D-ribofuranosyl) guanine (BuPdG) and 2-(p-n-butylanilino) -6-methyoxy purine (BuAOMe), both specific inhibitors of pol α but not pol δ (kindly provided by Dr. G. E. Wright), in order to determine whether pol α, pol δ or both are responsible for the cytogenetic phenomena. Both BuPdG and BuAOMe were as effective as APC in inhibiting DNA synthesis in human peripheral blood lymphocytes. In G_o experiments (200R + 2 hr 10^{-5}M) we found no effect of BuPdG. In G_2 experiments (100R + 2 hr 10^{-5}M) we found a small but statistically significant increase with BuPdG, but none with BuAOMe. We conclude that pol δ alone is responsible for the cytogenetic phenomena seen with APC.

[1]Research supported by the U.S. Department of Energy under Contract No. DE-AC02-76CH00016 with Associated Universities, Inc.

CB 502 **A PATHWAY REQUIRED FOR THE REPAIR OF GAMMA RAY INDUCED DOUBLE STRAND BREAKS (DSBs) IS ALSO REQUIRED FOR THE EFFICIENT REMOVAL OF DSBs INDUCED BY TOPOISOMERASE II INHIBITORS**, Keith Caldecott, Geoffrey Banks and Penny Jeggo, Department of Genetics, The National Institute for Medical Research, Mill Hill, London, NW7 1AA. The Chinese Hamster Ovary (CHO) cell line *xrs-1* is hypersensitive to gamma radiation. This sensitivity has been attributed to the inability of this cell line to efficiently repair gamma ray induced DSBs. The *xrs* cell lines are cross-sensitive to a variety of DNA damaging agents including several chemicals potentially useful in chemotherapy. Two such drugs, VP-16 and mAMSA, potentiate toxicity through their ability to interfere with topoisomerase II activity. These drugs inhibit the ability of topoisomerase II to reseal the transient DSB it introduces into DNA during normal activity. This results in the accumulation of what are normally transient topoisomerase II-bridged-DSB intermediates (cleavable complexes). *In vivo* and *in vitro* evidence indicate that *xrs* and CHO-KI cells accumulate and remove cleavable complexes from the genome at equal rates. However, our studies suggest that non-enzyme-bridged (frank) DSB arise in cells treated with these drugs and that the *xrs* DSB repair pathway is required for efficient removal of these breaks. Whether cleavable complexes are the source of these breaks is as yet unknown.

CB 503 DNA DOUBLE STRAND BREAKS LEAD TO CELL DEATH, CHROMOSOME ABERRATIONS, AND TRANSFORMATION BUT NOT SISTER CHROMATID EXCHANGES.
Cleaver, J.E.[1], Morgan, W.F.[1], Ong, A.[2], and Borek, C.[2] [1]Laboratory of Radiobiology and Environmental Health, University of California, San Francisco, CA 94143, and [2]Radiation Research Laboratory, Columbia University College of Physicians and Surgeons, 630 West 168th Street, New York, NY.
The damage from exposure of mammalian cells to ionizing radiation involves a complex variety of DNA molecular alterations, including single strand breaks, double strand breaks, and base damage. Unraveling whether specific lesions can cause specific chromosomal and cellular changes is therefore difficult. As an approach to simplifying this problem, we have developed a system by which Chinese hamster ovary and mouse C3H cells can be made permeable by electroporation to restriction enzymes which allows definition of the biological role of DNA double strand breaks alone, separate from single strand breaks and base damage. These enzymes produce DNA double strand breaks during the first few hours after electroporation, and cause chromosome aberrations, cell killing, mutations, and transformation. Restriction enzyme induced transformation by electroporation of PvuII, XbaI, and PstI ($1.9 - 5.66 \times 10^{-4}$ transformants/surviving cell) was about half the level seen from 3 Gray of X rays (8.77×10^{-4}), whereas no transformants were produced by electroporation alone. Sister chromatid exchanges (SCEs) were not, however, induced by restriction enzyme-induced double strand breaks. This is consistent with the observation that other compounds which induce DNA double strand breaks, e.g., ionizing radiations, bleomycin, and neocarzinostatin, do not induce SCEs. Work supported in part by the U.S Department of Energy, contract no. DE-AC03-76-SF01012 (JEC, WFM), and by grant CA-12536 from NCI, and a contract from the National Foundation for Cancer Research (AO, CB).

CB 504 STUDIES OF A RADIATION SENSITIVE V79 CLONE WITH A POSSIBLE DEFECT IN REPAIR SYNTHESIS, Deborah M. Creissen and Colin, K. Hill. Albert Soiland Cancer Research Laboratory, Department of Radiation Oncology, USC School of Medicine, 1414 S.Hope St., Los Angeles, CA 90015.
The V79/UC mutant cell line was isolated following acute exposure of normal V79 cells to 254nm UV light and has been successfully maintained in the laboratory for several years. The V79/UC cells are hypersensitive to the cell killing effects of both far and mid-UV and X-rays, but show normal sensitivity to mitomycin C.
Direct assay of DNA polymerase and the use of specific inhibitors in survival assays indicate a possible alteration in the activity of DNA polymerase alpha in these cells. Studies using alkaline elution to measure the extent of DNA strand breakage following UV-irradiation support the hypothesis of a defect at the repair synthesis stage of DNA repair. Preliminary data from repair synthesis assays indicate significant differences between the two cell lines. The V79/UC cells may represent a unique category of UV-sensitive rodent cell since its properties differ considerably from those descibed in the literature, and thus offers the possibility for cloning a novel human repair gene(s). Attempts to complement the defect in these cells by transfection with human DNA have led to the isolation of primary transformants showing increased resistance to UV light. These transformants are being screened for the presence of human DNA and secondary transfections are underway.
The V79/UC cells also show variable patterns of mutability depending on the agent of mutation and the locus of interest. These changes may be due to alterations in the fidelity of DNA polymerases either during replicative or repair synthesis and experiments to study this possibility are in progress.

CB 505 DNA REPAIR BY E. COLI RR1 WHICH HAS INCREASED RADIATION RESISTANCE DUE TO TRANSFECTION WITH CLONED D. RADIODURANS DNA, G. V. Dalrymple, J. W. Hardin, T. J. Lynch, M. C. Dedrick, Jr., A. J. Moss, Jr., and L. R. Barrows, VA Medical Center, the University of Arkansas for Medical Sciences, and the University of Arkansas at Little Rock, AR 72205, the University of Arkansas at Pine Bluff, AR 71601, the University of Nebraska Medical Center, Omaha, NE 68105, and the Utah Regional Cancer Center, Salt Lake City, UT 84132.
We are able to increase the radiation resistance of E. coli RR1 by transfection with D. radiodurans (DR) DNA cloned into pBR322 (pDR) (Radiat Res, in press). We have extended our initial work by performing studies of DNA Single Strand Break (SSB) repair by means of alkaline sucrose sedimentation analysis. Organisms studied include E. coli RR1 transformed: 1) with pDR, 2) with pBR322 (without DR DNA insert), and 3) without plasmid. Survival curves show the Do value to be about 850 Gy for DR, 450 Gy for E. coli RR1 with pDR, and 350 Gy for E. coli RR1 with and without pBR322. Alkaline sucrose sedimentation showed E. coli RR1 to have complete SSB repair by 2 hr after 1100 Gy. Both E. coli RR1 transfected with pDR and pBR322 showed 285% and 175% rejoining (over-rejoining) at 2 hr after 1100 Gy, respectively. These data suggest a correlation between radiation resistance and enhanced DNA repair by E. coli RR1, as a consequence of the presence of DR DNA.

CB 506 3-AMINOBENZAMIDE (3AB) RESISTANCE RESTORES THE IRS-2 RADIATION PHENOTYPE BACK TO THE PARENTAL V79 PHENOTYPE. L. Dethlefsen[+], J. Marston[+], W. Geary[+], G. Brunet[!], G. Poirier[!], and L. Barley[+]. [+] Sect. of Expt. Oncology, University of Utah, S.L.C., UT 84132, and [!]Lab. of Molecular Endocrinology, Laval University, Quebec, Canada G1V 4G2.
The parental V79 and radiosensitive mutant irs-2 cells were grown for 6+ mo. in increasing concentrations of either 3AB or adriamycin (ADR) and developed significant drug resistance and cross-resistance which is quite stable (>40 doublings in drug-free medium). The radiosensitivity of the irs-2(3AB)^R returned to the V79 response but the V79(3AB)^R was unchanged (i.e., Do = 0.80, 1.52, 1.53, and 1.51 Gy respectively). Analysis of DNA dsb induction and repair by alkaline (pH 7.2) filter elution actually demonstrated less damage in the irs-2 cells than the other 3 lines which were similar (60 Gy: strand scission factors of 0.46 vs$\sim$0.58 resp.) and the residual damage after 2 hr of repair at 37° was$\sim$20% of the induced damage in all lines. The endogenous poly(ADP-ribose) polymerase activity (10 min ^{3}H-NAD incorp.) for irs-2 was equivalent to V79; however, stimulation by x-irradiation was nil in irs-2 but a factor of 2.5 higher at 45Gy in V79. In contrast the endogenous activity of irs-2(3AB)^R was 3.2 x the V79 & after 45Gy was 4.7. The respective values for V79(3AB)^R were 2.5x & 3.5x. The V79(ADR)^R & irs-2(ADR)^R cells showed essentially no change in radiosensitivity or polymerase activity. The multi-drug resistant glycoprotein (P170) expression was elevated 11x in the V79(ADR)^R cells relative to V79 but was unchanged in V79(3AB)^R. In contrast, the irs-2 expression of P170 was 5x the V79 but in irs-2(3AB)^R it was down-regulated back to V79. The expression in irs-2(ADR)^R was equivalent to V79(ADR)^R cells. The polymerase activity appears to correlate with cell survival but not DNA dsb induction or repair. The mechanisms underlying these changed phenomena in irs-2(3AB)^R cells are currently unknown.

CB 507 CORRELATION OF POTENTIATION OF RADIATION-INDUCED DNA SSB'S WITH ENHANCED CELL KILL BY PLATINUM CHEMOTHERAPY, Evan B. Douple, Li-Xi Yang, Julia A. O'Hara and Robin A. Crabtree, Department of Medicine, Dartmouth Medical School, Hanover, NH 03756

V79 cells were treated with cisplatin or paraplatin 1 hour before irradiation under hypoxic conditions, or the chemotherapeutic agent was added immediately after irradiation and present during a 6 hour post-irradiation incubation period. These two protocols produced enhanced cell killing by at least a factor of two greater than that predicted by the additive toxicity of the two agents. Production of DNA SSB's was analyzed for the treatment protocols using alkaline elution and the fluorescent absorption DNA unwinding (FADU) assays. In addition, the production of DNA double strand breaks (DSB's) was assessed using the neutral elution assay. The results of these studies indicate that the production of increased SSB's correlated with the enhanced cell kill when these platinum complexes were combined with irradiation to produce hypoxic cell radiosensitization or post-irradiation potentiated cell kill. On the other hand, there was no evidence for an increase in DSB's when radiosensitization occurred, even at doses of 80 Gy. The increased SSB production occurred using doses of radiation and platinum chemotherapy which are expected to produce platinum-induced adducts and radiation-induced SSB's separated on the average by at least 1.5×10^7 daltons of DNA, or about 25,000 base pairs. (Supported in part by grant CA40500 from the U.S. NIH, NCI and DHHS.)

CB 508 SUBLETHAL DAMAGE IS DISTINGUISHABLE FROM POTENTIALLY LETHAL DAMAGE BY TREATMENTS THAT DIFFERENTIALLY INHIBIT THEIR REPAIR, M.M. Elkind, H. Utsumi, and M.L. Shibuya, Department of Radiology & Radiation Biology, Colorado State Univ., Fort Collins, CO 80523.
Because the delayed plating of plateau-phase cultures of mammalian cells is usually used to demonstrate the repair of potentially lethal damage (PLD), it has been difficult to distinguish unequivocally between the repair of PLD and the repair of sublethal damage (SLD), the latter usually made evident by dose fractionation. However, it had been shown that medium (CM) conditioned by plateau-phase cells [Utsumi & Elkind, *IJRB* **47**, 569-580 (1985)] facilitated the repair of PLD if applied to cells in log-phase growth. By comparing the effects on the 2-dose survival of log-phase V79 cells treated between doses with fresh medium (FM) or CM, we found that the topoisomerase II inhibitor novobiocin, and the poly(ADP-ribose) synthetase inhibitor 3-aminobenzamide, differentially inhibited PLD as opposed to SLD repair. This, and other, evidence support the likelihood that these two forms of damage are distinct. In this context, other inhibitors of topoisomerase II, both DNA intercalative (mAMSA) and nonintercalative (VP-16 & VM-26), acted differently from novobiocin in that they did not differentially affect X-ray survival in FM *vs.* CM. Under the conditions of incubation with novobiocin that were used, ATP levels in CM could not be distinguished from those in FM. (Work supported by the Dept. HHS, US public Health Service, via a grant from the National Cancer Inst. CA 47497.)

CB 509 TRANSFECTION AND EXPRESSION OF THE E.COLI nth GENE IN MAMMALIAN CELLS AND ITS EFFECT ON RADIOSENSITIVITY. L Harrison[1], M Skorvaga[1], A J Watson[1], H Ahern[2], R Cunningham[2], J H Hendry[1], G P Margison[1]. [1]Paterson Institute for Cancer Research, Christie Hospital and Holt Radium Institute, Manchester, M20 9BX, UK. [2]Department of Biological Sciences, University at Albany, New York 12222, USA.

The E.coli nth gene encodes endonuclease III which removes ring saturated, fragmented and ring contracted thymine derivatives from irradiated DNA. nth was ligated into PZIPneoSV(X)I and transfected into Chinese hamster V79 lung fibroblasts. Following selection with G418, Southern and northern analysis showed the presence of the nth gene and message, and western analysis using an anti-endonuclease III rabbit antibody demonstrated the presence of endonuclease III in extracts of 2 clones 1D and 7D. On gamma irradiation 1D and 7D were found to be more radiosensitive than the parental and vector control cell lines, which had equivalent clonal survival.

CB 510 ANALYSIS OF TWO IONIZING RADIATION SENSITIVE HAMSTER CELL MUTANTS, Ian D. Hickson, Stella M. Davies, Sally L. Davies, Adrian L. Harris, Craig N. Robson, Imperial Cancer Research Fund, Molecular Oncology Laboratory, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK.

We have isolated 2 mutants of CHO cells showing enhanced sensitivity to X-rays compared to parental CHO-K1 cells. Mutant BLM-2 is cross-sensitive to bleomycin and other radiomimetic agents, as well as to UV light and some alkylating agents. This mutant is spontaneously hypermutable (~ 4-fold), and has a defect in the repair of both single- and double-strand breaks induced by bleomycin. Enzymes involved in the repair of apurinic sites, or of DNA treated in vitro with bleomycin, are currently being studied in BLM-2 cells. Mutant ADR-3 is defective in the repair of double- but not single-strand breaks induced by X-rays. ADR-3 is cross-sensitive to agents which generate strand breaks via inhibition of the enzyme topoisomerase II, but are resistant to the topoisomerase I inhibitor, camptothecin. The possibility that topoisomerase I activity is abnormal in this mutant is being investigated. Both mutants are recessive in hybrid crosses with CHO-K1 cells with respect to X-ray sensitivity. BLM-2 represents a different genetic complementation group from the X-ray sensitive hamster mutants xrs1-6, irs1-4, EM9 and XR-1. We are attempting to clone the human gene correcting the defect in BLM-2 cells. Transfectants with a corrected phenotype ($X\text{-ray}^r$) have been isolated following transfection with a human cDNA library. Recovery of the transfected DNA is in progress.

CB 511 GAMMA-RAY SURVIVAL OF CLOUDMAN S91 MELANOMA CELLS VARYING IN INTRACELLULAR MELANIN CONTENT, Helene Z. Hill, Section of Cancer Biology, Department of Radiology, NJ Medical School, Newark, NJ 07103

Melanomas often respond poorly to ionizing radiation therapy. The pigment melanin has radical scavenging properties and may be important in the radiation response. Sublines of the Cloudman mouse melanoma which varied in steady-state intracellular melanin were evaluated for survival after varying doses of ionizing radiation. Thirty to 10,000 cells were plated, incubated overnight in RPMI1640 medium plus 5% fetal bovine serum, exposed to gamma rays from a ^{137}Cesium-irradiator, reincubated for 12 days, fixed, stained and evaluated for colonies. The lines studied were S91/amel, S91/M1B and S91/I3 containing 1.2, 2.4 and 3.6 pg/cell of melanin respectively. Although S91/amel gives a positive pigment response, pelleted cells are white. The data from several experiments were averaged for each cell line and the radiobiological parameters α and β were estimated. S91/Amel survival exhibited only an α component (α = 0.726/Gray) and S91/I3 survival exhibited only a β component (β = 0.0646/Gray^2). Survival of S91/M1B, with intermediate melanin, exhibited both α and β components (α = 0.325/Gray, β = 0.0454/Gray^2). These findings suggest that melanin content may influence the survival of melanomas and that radiation therapy may spare more melanotic cells which may also be more proficient at performing DNA repair.

Supported by a grant from the New Jersey Commission on Cancer Research.

CB 512 DNA SYNTHESIS AND CELL REPLICATION IN EPITHELIAL CELLS OF THE RESPIRATORY TRACT FOLLOWING ACUTE RADON PROGENY EXPOSURE. N.F. Johnson, D.G. Thomassen, A.L. Brooks and G.J.Newton, Lovelace Inhalation Toxicology Research Institute, PO Box 5890, Albuquerque NM 87185. In vivo exposures to radon progeny (900 WLM) were used to identify the initial anatomical site of DNA alterations following exposure to radon progeny. DNA synthesis and cell cycle kinetics were measured in nasal, tracheal and lung tissue using immunohistochemical identification of nuclei labeled with BrdUrd and flow cytometry. Responses include cell cycle perturbations, and depression or increase in cell turnover. The changes seen were dependent on tissue location. The colony forming efficiency (CFE) of isolated rat tracheal epithelial (RTE) cells was determined as a function of time after exposure. There was a significant reduction in CFE even at 33 days after exposure. Chromosomal aberrations and transformation frequency were increased in isolated RTE cells obtained immediately after exposure compared to control cells. Comparison of in vivo results with in vitro exposure data show that the dose to the tracheal lining was 0.5-0.7 Gy. These sub-cellular changes were present without identifiable histological lesions. Results show that the biological effects of inhaled radionuclides on epithelial cells can be quantified and compared to similar in vitro endpoints to derive direct calculation of radiation dose to respiratory cells. Research sponsored by U.S. Dept. of Energy O.H.E.R. contract No. DE-AC04-76EV01013.

CB 513 RADIATION-INDUCED DNA STRAND BREAK AND REPAIR IN QUIESCENT AND PROLIFERATING LYMPHOCYTES, Peter C. Keng, and Kim M. Wilson, Cancer Center, Univeristy of Rochester School of Medicine and Dentistry, Rochester, NY 14642

Reported differences in radiation sensitivity seen between resting and stimulated lymphocytes could reflect differences in DNA repair capacity. We have utilized alkaline and neutral filter elution to examine in vitro ionizing radiation-induced DNA single (SSB) and double strand (DSB) break repair in murine splenic lymphocytes. After 3 days of stimulation with phytohemagglutinin (PHA), cultures contained 45% proliferating (P) cells and 55% quiescent (Q) cells as measured by acridine orange staining and flow cytometry. Initial radiation-induced damage (both SSB and DSB) did not differ among any of the populations examined. PHA stimulated cells and freshly isolated resting lymphocytes had similar SSB repair kinetics up to 2 hours after 5 Gy radiation, however at later times, fresh cells had more SSB remaining than did stimulated cells. Fresh, resting cells also repaired DSB less efficiently than did PHA stimulated cells after 50 Gy irradiation. Using centrifugal elutriation, enriched Q and P lymphocyte populations were separated from PHA stimulated cultures for examination of strand break repair. DNA SSB and DSB repair kinetics were the same for P and Q cells up to 2 hours although SSB repaired to a greater extent (15-25% damage remaining) than did DSB (30-40% damage remaining). At later times, Q cells had a higher amount of SSB damage remaining than did P cells. Stimulated lymphocytes had less DNA damage after later repair times which may indicate more efficient DNA repair than resting cells. DNA strand break repair may, in part, reflect some of the unique characteristics of the response of lymphocytes to ionizing radiation.

CB 514 ISOLATION OF A SCHIZOSACCHAROMYCES POMBE RAD9 MUTANT RESISTANT TO GAMMA RAYS AND UV LIGHT AFTER TRANSFECTION WITH GENOMIC DNA, Howard B. Lieberman, Hsiao M. Chu and Maureen Laverty, Center for Radiological Research, Columbia University, College of Physicians and Surgeons, New York, NY 10032

Mutants of S. pombe defective in the rad9 gene are extremely sensitive to ionizing radiation and UV light. In addition, they are less mutable by UV light when compared to the parental Rad^+ strain. The specific role of rad9 in the response of cells to radiation exposure, however, is not well understood.

In order to learn more about the function of rad9 at the molecular level, we initiated a study to clone the wild-type gene. We transfected a S. pombe genomic library, made in the vector pWH5, into a rad9-192 leu1-32 double mutant population and selected cells receiving plasmid (i.e., having a Leu^+ phenotype). We screened 6,000 transformants for the acquisition of resistance to UV light, and we identified one radiation-resistant colony. Subsequently, we found that the cells within this colony were also resistant to gamma rays. Furthermore, plasmid loss correlated perfectly with loss of resistance to both types of radiation. We are now in the process of isolating plasmid DNA from the radiation-resistant transformant for further analyses. (This work was supported in part by NIH Grant CA-12536.)

CB 515 CYTOGENETIC INDICES OF DIRECT VS INDIRECT RADIATION ACTION. L.G. Littlefield,[1] E.L. Frome[2], Oak Ridge Assoc. Univ.[1], Oak Ridge Nat'l. Lab.[2], Oak Ridge, TN 37831
Our laboratory has conducted a series of experiments using dimethyl sulfoxide (DMSO) as a model OH radical scavenger to quantify the fraction of chromosome aberrations induced in lymphocytes by "direct" vs "indirect" modes of radiation action (Littlefield et al., Int. J. Rad. Biol. 53:875, 1988; Meyne et al., Mut. Res. 226:75, 1989). Additional studies employing in vitro irradiations of human, rabbit, or pig lymphocytes provide evidence that:

- For all three species, radioprotection against X-ray-induced chromosome aberrations varies inversely as a function of scavenger concentration in medium during exposure, with maximum protectible fractions of ~60-70% at DMSO concentrations of 0.5-1.0M.
- DMSO provides similar levels of radioprotection for aberration induction in human lymphocytes exposed to X-rays during G_0 vs G_2 of the cell cycle.
- ARA-C inhibits repair of X-ray-induced DNA lesions that lead to chromosome aberrations to an equal extent in non-protected vs maximally-protected lymphocytes suggesting that a similar spectrum of DNA damage is induced by prompt ionization and OH radical attack in cells exposed to low LET radiation.
- From comparisons of dose response parameters in human lymphocytes exposed to various doses of 220 kV X-rays or monoenergetic RARAF neutrons in the presence or absence of DMSO, we estimate that OH radical mediated DNA damage contributes 50% of the dose dependency for intra- and inter-track aberrations for X-rays, but only 40 and 15% respectively of the dose dependency for chromosome aberrations induced by 6.0 and 0.44 MeV neutrons. Sponsored by DOE Contracts DE-AC05-76OR00033 and DE-AC05-84OR21400.

CB 516 DNA REPAIR IN ALKYLATING-AGENT RESISTANT CELL LINES
H. Lohrer, T. Robson, H. Grindley, A. Hall, B. Kaina*.
Cancer Research Unit, Medical School, Framlington Place, Newcastle NE2 4HH, U.K.; *) Institute of Genetics, Kernforschungszentrum, P.O.Box 3640, D-7500 Karlsruhe 1, FRG.///The CHO cell line K1-2 was transfected with plasmid pMTII-BPV, carrying the human metalltothioneinII-A gene (hMTII-A) under the control of its own promoter. The isolated cell line K1-2MT overexpressed the hMTII-A gene already without special treatment, and a further increase in steady state hMTII-A mRNA was found after treatment with cadmium and zinc salts (50 fold) and gamma-ray (20 fold). K1-2MT proved to be as resistant as the parental cell line to ionising radiation, bleomycin, hydrogen peroxide, methyviologen and adriamycin, but was more resistant to the alkylating agents MNNG, MNU, MMC. This resistance was not due to a slowing down in cell cycle of the resistant line, nor to a higher activity of methyltransferase. Also GST activity and levels of GSH did not differ between K1-2 and K1-2MT cells. ^{14}C-MNU was incorporated identically into the nuclear DNA of both cell lines. However the K1-2MT cells exhibited a 5-fold decreased spontaneous mutation frequency of the HPRT gene compared to K1-2, and the mutation frequency was strongly inducible by MNNG in K1-2MT cells than in the parental K1-2 cells. We conclude that MT overexpression does protect K1-2MT cells from cytotoxic effects of some alkylating agents but it does not protect nuclear DNA from damage by alkylating agents.

CB 517 THYMINE GLYCOLS AS A REMNANT LESION RECOGNIZED BY A DNA PROOFREADING MECHANISM IN MAMMALIAN CELLS: TEST OF A HYPOTHESIS, Frank Q.H. Ngo, Jia Xian-Li and Steven A. Leadon*, Laboratory of Radiobiology, Cleveland Clinic Foundation, Cleveland, OH 44195 and *Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720
With partially synchronized Chinese hamster V79 cells, we have demonstrated that DNA strand breaks (SB) are formed when cells progress into S phase following gamma- or neutron-irradiation at G1 phase. Because this SB formation can be selectively inhibited by treatment with aphidicolin or ß-araA, but not by caffeine, novobiocin, nor anisotonic solutions, we postulated that the late-developed SB is a product of DNA proofreading process. However, the origin(s) of the SB in DNA fragment is not known. In this study, we wish to examine whether or not thymine glycols (TG), a known radiation-induced lesion, may be a type of remnant lesions which are actively removed during DNA replication and lead to the formation of SB. V79 cells, partially synchronized in plateau G1 phase, were irradiated with 50 Gy of gamma rays and the cells were stimulated to progress by refeeding with fresh medium (MEM) during postirradiation incubation. The irradiated cultures were divided into four groups and were incubated in MEM, MEM containing aphidicolin, caffeine, or 6-mercaptopurine ribonucleoside, respectively. The amount of TG was determined by the immunoassay of Leadon and Hanawalt (1983) and the removal kinetics of TG for a period of 14 hrs postirradiation for the four groups were compared. We found that there were initially approximately 10.2 TG per 10^6 base pairs and that the amount decreased with incubation time with a kinetics that is essentially identical for all four different groups. By 8 hrs when most of the cells were S phase, the remaining TG were lowered to the background level. Thus, it may be concluded that unrepaired TG may not be the lesion that leads to the late-developed SB. This work is supported by NCI Grant CA33951 (FN), and CA40453 (SL).

CB 518 ON THE CHARACTERISTICS OF IONIZING-RADIATION-SENSITIVE MUTANTS OF MAMMALIAN CELLS, Koki Sato, David J.-C. Chen and Gary F. Strniste, Division of Radiation Hazards, National Institute of Radiological Sciences, Chiba 260, Japan and Genetics Group, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545

We have isolated six ionizing-radiation-sensitive mutants of mouse cells; M10, LX821, LX827, LX830, SX9 and SX10. They were also sensitive to the lethal effect of bleomycin, though heterogeneity in cross-sensitivity was observed among mutants for other chemicals. At least two complementation groups were identified in these mutants for radiation sensitivity. Gamma-ray-induced mutations and chromosome aberrations were higher in M10 cells than in wild-type cells on a per-unit-dose basis but were similar between mutant and wild-type cells on a per-survival basis. DNA synthesis immediately after irradiation was inhibited to similar extents in a dose-dependent manner in both mutant and wild-type cells. This finding forms a striking contrast to that in ataxia telangiectasia cells which show hypersensitivity to ionizing radiation for cell viability but radio-resistant DNA synthesis. On the other hand, the recovery of DNA synthesis was much faster and higher in mutants than in wild-type cells. Mutant cells were proficient in rejoining DNA-single-strand breaks but deficient in repairing DNA-double-strand breaks. Complementation tests between mouse cell mutants and Chinese hamster cell mutants are in progress.

CB 519 **REPAIR OF RADIATION-INDUCED DOUBLE STRAND BREAKS IN THE CIRCULAR DNA OF DOUBLE MINUTE CHROMOSOMES.** M.D. Story and R.E. Meyn, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

We have used the method of pulsed field gel electrophoresis (PFGE), developed for the resolution of very large (>10^6 base pairs (Mbp)) DNA fragments, to analyze the induction and repair of DSBs in human cells. The human colon cancer cell line, DiFi, which has the unique feature of being amplified for the epidermal growth factor receptor (EGFR) gene in the form of double minute (DM) chromosomes, was used in these studies. We have recently shown using the clamped homogeneous electrical field (CHEF) version of PFGE that these DMs will not enter a pulsed-field gel unless fragmented, which suggests a circular structure. It was of interest therefore to evaluate whether the DiFi cells were capable of repairing the DSB's in the DM's. For these experiments, DiFi cells were irradiated with 30 Gy and incubated for various times prior to being prepared for PFGE. Following electrophoresis, the DNA fragments were transferred to nylon membranes and hybridized with ^{32}P-labeled EGFR probe. The autoradiogram for the irradiated but not incubated control lane showed two bands corresponding to 1.6 and 0.80 Mbp. With repair incubation these bands lost intensity as a function of time following the initial dose of 30 Gy. These results indicate that DSB's produced in DM's by radiation can be repaired by DiFi cells and illustrate the usefulness of PFGE for studies of DSB induction and repair in irradiated mammalian cells. (Supported by NIH/NCI CA-26312).

CB 520 REPLICATION OF A DEFINED DNA FRAGMENT CARRYING PYRIMIDINE DIMERS, Kevin S. Sweder, Graciela Spivak, and Philip C. Hanawalt, Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

The mechanism whereby mammalian cells replicate through damaged sequences remains one of the most interesting phenomena yet to be elucidated. This translesion synthesis would be expected to be a major contributor to mutagenesis in mammalian cells exposed to DNA-damaging agents. Cultured rodent cells offer an ideal model system to study lesion bypass because these cells remove pyrimidine dimers selectively from espressed genes, and in particular, only from the transcribed DNA strands (Bohr *et al.*, 1985; Madhani *et al.*, 1987; Mellon *et al.*, 1987). Using techniques to determine the level of pyrimidine dimers within a specific, essential gene, we compared the dimer content of replication products within the *DHFR* locus of repair-proficient (CHO AA8) and repair-deficient (CHO IID3; complementation group 2) cell lines. These studies revealed no differences in the efficiency of dimer removal between the template strand of the replicated DNA and the unreplicated, parental DNA. There were no dimers present in the newly synthesized strand of replicated DNA, based upon susceptibility to T4 endonuclease V. Similar results were obtained for repair-proficient cell lines containing a 50-fold amplified *DHFR* locus (CHO B11). Thus, replication forks appear to move through the damaged DNA in the absence of repair or significant levels of strand exchange.

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Madhani, H.D., Bohr, V.A., and Hanawalt, P.C. (1986) *Cell* **45**, 417-423.
Mellon, I., Spivak, G., and Hanawalt, P.C. (1987) *Cell* **51**, 241-249.

CB 521 VISCOELASTIC ANALYSIS OF X-RAY INDUCED DNA DAMAGE AND REPAIR IN TETRAHYMENA PYRIFORMIS, Elliott Uhlenhopp, Emmanuel Skoufos, Michelle Borg and Rob Van Der Luijt, Department of Chemistry, Grinnell, Iowa 50112.

When cultures of exponentially growing Tetrahymena pyriformis cells were irradiated with a 10 krad split dose of X-rays , DNA double-strand breakage followed by gradual recovery from this damage was observed as indicated by changes in viscoelastic retardation time of cellular lysates. Complex kinetics were observed, indicating the possibility of several waves of repair in which the viscoelastic retardation time, τ, increased over a period of a few hours followed by a subsequent decrease, a pattern which was repeated several times during two days post-irradiation.

To further characterize the molecular repair process, several inhibitors or enhancers were tested. Caffeine had little effect on growth curves or retardation times, but 3-amino benzamide drastically inhibited recovery of DNA molecular weight and conformation. Attempts to stimulate repair by providing additional substrate for the enzyme poly ADP ribose synthetase, either by direct addition of NAD+ or the precursor tryptophan, did not markedly increase the rate or amount of DNA repair. Sodium butyrate gave moderate inhibition of repair, the topoisomerase inhibitor novobiocin decreased repair only modestly, and the protease inhibitor antipaine resulted in somewhat enhanced repair.

DNA and RNA synthesis were somewhat depressed post-radiation, whereas protein synthesis was slightly elevated in the first ten hours after radiation. Although 87.5% of the cells survived the 10 krad split dose of radiation, some of the cells began exhibiting abnormal morphologies between five and fifteen hours post-radiation. Transverse alternating field gel electrophoresis indicated the possible appearance of a discreet size of molecular fragments post-irradiation.

CB 522 PROPERTIES OF AN X-RAY HYPERSENSITIVE MUTANT OF CHINESE HAMSTER OVARY CELL LINE, A.J. Varghese and G.F. Whitmore, Physics Division, Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9

We have isolated a Chinese hamster ovary cell line designated as XRS-T1 which exhibits 3-4 fold hypersensitivity to ionizing radiation. Complementation analysis and studies of the properties of XRS-T1 suggest that it is different from other known radiation sensitive mutants of mammalian cell lines. The new mutant is more sensitive to bleomycin, adriamycin, daunomycin and etoposide than the parent. These drugs are known to produce double-strand breaks in DNA, although by different mechanisms. Detectable differences in sensitivity between the parent and the mutant were not observed with UV light, mitomycin C, chlorambucil, EMS, MMS and melphalan which are either alkylating or cross-linking agents. XRS-T1 cells repaired single-strand breaks almost as efficiently as the parent but were defective in ability to repair double-strand breaks. Treatment with azacytidine did not have an effect on the reversion frequency of XRS-T1. Vector-mediated DNA-double strand break repair analysis in the parental and mutant cell lines revealed a significantly (5 fold) reduced ability to rejoin correctly restriction endonuclease cuts in the radiation-sensitive mutants. The observed cross-sensitivity of XRS-T1 cells to bleomycin, adriamycin and etoposide, and the absence of cross-sensitivity to UV and mitomycin C, indicates a resemblance to ataxia-telangiectasia cells.

CB 523 NEUTRON AND GAMMA RADIATION INDUCE SIMILAR DAMAGE AND REPAIR AS MEASURED USING A NUCLEOID ASSAY. Andrew TM Vaughan, David J Gordon and Stuart Green. Departments of Immunology and Physics, University of Birmingham, B15 2TJ. U.K.

Chinese Hamster Ovary cells AA8 arose from the CHO K1 line as a spontaneous mutant. Irradiation of these cells with a 20 MeV D^+,Be neutron beam gave an RBE of 2.1 at 1% survival. Histone depleted nuclei (nucleoids) were extracted from irradiated cells, stained with the fluorescent dye ethidium bromide and passed through a FACS 440 flow cytometer. Data on the nucleoid forward light scatter was accumulated from 10,000 events per experiment. Both the neutrons and cobalt-60 gamma rays produced a comparable, dose-dependent increase in forward scatter as similar numbers of damaged replicons failed to be rewound by the dye, giving an RBE of 1. With both radiations, the damage was repaired to control levels after 1 hour incubation at 37°C. No differences in repair kinetics were observed during this time. The derived RBE for this assay is comparable to that from DNA double strand break assays and is greater than those measuring single strand breaks. The data highlights the influence of the chosen technique in DNA damage assays and re-emphasises the role of high LET mediated damage in neutron radiobiology.

CB 524 PROLIFERATIVE STATUS AS A DETERMINANT OF DNA REPAIR RATES IN LIVER, Kenneth T. Wheeler and William H. St. Clair, Department of Radiology, Bowman Gray School of Medicine, Winston-Salem, NC 27103. It has been proposed that, 1) the rate of DNA repair is related to cell survival (1), and 2) the rate of DNA repair is determined by a cell's proliferative status (2). To test the latter hypothesis, quiescent liver cells from adult (4-5 month) male Fisher 344 rats were irradiated *in situ* with 18.4 Gy. At various times after irradiation, the rats were killed, the livers dissociated into single cell suspensions and the DNA damage assayed by alkaline elution. The identical procedure was performed 20-24 hr and 30 hr after a partial hepatectomy in which >60% of the liver was removed. Under these conditions, cells labeled with radioactive thymidine begin to appear at ≈18 hr, reach a maximum number at 24-26 hr and exist in all zones from the terminal portal venule to terminal hepatic veins by ≈ 30 hr (3). The percentage of the initial DNA damage remaining 10 min after irradiation was ≈ 30%, ≈ 23% and ≈ 13% for quiescent liver cells and liver cells 20-24 hr and 30 hr after hepatectomy, respectfully; at 45 min after irradiation, the corresponding values were ≈ 16%, ≈ 7% and ≈ 4%. The data strongly suggest that proliferative status is a major determinant of the rate of DNA repair in rat liver. (Supported by NIH grant #CA45156)

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Ionizing Radiation-Induced DNA Lesions; Radiation and Cancer

CB 600 DEVELOPMENT OF THIN GELS CONTAINING VIRAL DNA FOR DETERMINATIONS OF STRAND-BREAK INDUCTION BY ULTRASOFT X-RAYS, Mary Helen Barcellos-Hoff, Gail V. Shirley, Aloke Chatterjee, Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Ultrasoft X-rays are an important tool for understanding the physical and biochemical mechanisms of radiation damage since the production of low-energy electrons significantly contributes to the effect of the primary radiation. However experimental use of soft X-rays is constrained by their excessive rate of absorption. We have developed methodology to produce thin (< 5 μm) agarose gels containing SV40 DNA that will be used to study strand break induction by photons of low energies. To determine the feasibility of this approach, we used ^{60}Co to irradiate supercoiled SV40 DNA (10 μg/ml) in 10 mM tris-buffer prepared as an aqueous solution or in a 0.5% low melting point agarose gel. Strand break induction was evidenced by the production of circular and linear forms of the DNA, which were quantified following neutral gel electrophoresis of the DNA [Roots *et al., Int. J. Radiat. Oncol. Biol. Phys. 11*, 259 (1985)]. The D_{37} for DNA single-strand break induction was 76 Gy in aqueous samples and 78 Gy in gel samples. We also varied the tris concentration and obtained similar results for the aqueous and gel DNA samples. Therefore, we concluded that low concentration agarose gel is a suitable matrix in which to irradiate DNA and is comparable to aqueous solution. A modification of spin-on coating technology that produces 3-μm thick agarose gels containing DNA will be described. We will also report preliminary experiments using these ultrathin DNA samples to evaluate the DNA strand break production by aluminum and carbon monoenergetic X-rays.

CB 601 FORMATION AND STABILITY OF REPAIRABLE PYRIMIDINE PHOTOHYDRATES IN DNA, Robert J. Boorstein, Timothy P. Hilbert, Richard P. Cunningham, and George W. Teebor, New York University School of Medicine, New York, NY 10016.

UV irradiation of poly(dG-dC) and poly(dA-dU) produces pyrimidine hydrates which are repaired by bacterial and mammalian DNA glycosylases (Biochemistry, 1989, *28*, 6164). *E. coli* endonuclease III was used to quantitate the formation and stability of these pyrimidine hydrates in poly(dG-dC). When this copolymer was irradiated with 100 kJ/m^2 of 254nm light at pH 8.0, 2.4% of the cytosine residues were converted to cytosine hydrate (6-hydroxy-5,6-dihydrocytosine) and 0.1% were converted to uracil hydrate (6-hydroxy-5,6-dihydrouracil). To determine the stability of these products, we incubated poly(dG-dC) in solution for between 0 and 24 hours after irradiation. Cytosine hydrate in poly(dG-dC) was stable at 4^{o} and decayed at 25^{o}, 37^{o} and $55^{o}C$ with half lives of 76, 25, and 6 hours. There was no significant accumulation of uracil hydrate. Uracil also did not accumulate in the copolymer as measured with a Uracil-DNA glycoslyase assay. These experiments show that the hydrate of cytosine is much more long-lived in DNA than are the corresponding hydrates of dCMP, dCtd, or Cyt. Like these compounds, it decays mainly by dehydration and not deamination. Our results suggest that cytosine hydrates in DNA are sufficiently stable to promote the development of repair enzymes.

CB 602 AN APPROACH TO ABSOLUTE DETERMINATION OF RADIATION INDUCED DOUBLE-STRAND BREAKS IN DNA AS MEASURED BY NEUTRAL FILTER ELUTION AND PULSE FIELD GEL ELECTROPHORESIS
Björn Cedervall, Klaus Erixon and Rolf Lewensohn, Department of Medical Radiobiology, Karolinska Institutet, Box 60212, S-10401 Stockholm, Sweden.

Pulse field gel electrophoresis (PFGE) allows the determination of the absolute number of radiation-induced double-strand breaks and their distribution. This was possible by running chromosomal size markers in the range 0.1 to 12.6 million base pairs in the gel. Mouse leukemia L1210 cells were x-irradiated with 0 - 50 Gray. Samples were evaluated both with PFGE and neutral filter elution (NFE). Under the assumption that both migration and elution, respectively, will start with the smallest fragments and proceed with increasingly larger fragments, it is possible to match the fraction migrated below a certain size to the fraction eluted. When these fractions are identical, the size of the largest fragments eluted can be read from the gel. We further assume that all columns have eluted up to the same size at a given elution time and that the double-strand breaks are Poisson distributed. From the different fractions eluted, samples can then be assigned aN average number of double-strand breaks.

CB 603 DIRECT AND INDIRECT EFFECTS IN DNA AQUEOUS SOLUTION, Aloke Chatterjee and William R. Holley, Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720. A computer model has been developed to calculate the D_{37} values for DNA strand breaks by ionizing radiation when present in aqueous solution. This model considers track structure, ·OH mediated damage and direct excitation and ionization of sugars and bases. The presence of tris buffer in the solution to scavenge ·OH and moderate its average migration distance to a DNA molecule has been included in the calculation over a wide range of concentrations (10 mM to 1 M). When compared with available experimental data for Co^{60}-γ rays, the following conclusions are reached: (1) in aqueous solution tris radicals do not seem to cause appreciable strand breaks, (2) below $3x10^{-2}$ M tris, only indirect effects are important, and (3) above this concentration, direct effects must also be included in the overall mechanisms.

CB 604 DNA DAMAGE BY HYDROXYL RADICALS AND SINGLET OXYGEN: A COMPARISON USING REPAIR ENDONUCLEASES AS PROBES. Bernd Epe and Dieter Wild, Institute of Toxicology, University of Würzburg, D-87 Würzburg, F.R.G.

We characterize different kinds of DNA damage using repair endonucleases with various substrate specificities. By means of their different recognition potentials characteristic DNA damage profiles are obtained which serve as "fingerprints". Damaging species characterized in cell-free systems may be identified in cells by comparison.

Using this approach we have demonstrated that illumination of Salmonella typhimurium cells with visible light in the presence of methylene blue gives rise to a DNA damage profile which can be attributed to the direct action of singlet oxygen. Exposure of Salmonella typhimurium to hydroperoxides gives rise to another form of damage profile which is also unlike that produced by hydroxyl radicals in a cell-free system. However, the latter dissimilarity does not exclude hydroxyl radicals as ultimately reactive species but has to be attributed to a very rapid repair of the base modifications induced.

Both types of damage are subject to repair by uvrABC-independent pathways. Revertant frequencies observed in parallel in several Salmonella strains indicate a pronounced mutagenicity of the lesions induced by singlet oxygen. Therefore, these might contribute to genotoxic effects observed under conditions of "oxidative stress" even though singlet oxygen is much less reactive with DNA than hydroxyl radicals.

CB 605 STRUCTURE OF A HYDROXYL RADICAL-INDUCED DNA-PROTEIN CROSS-LINK INVOLVING THYMINE AND LYSINE IN NUCLEOHISTONE, Ewa Gajewski and Miral Dizdaroglu, National Institute of Standards and Technology, Gaithersburg, Maryland 20899. Hydroxyl radical-induced formation of a DNA-protein cross-link involving thymine and lysine in calf thymus nucleohistone *in vitro* is reported. Basic amino acids such as lysine constitute a very high proportion of the amino acids of histones, and help histones to bind to DNA in chromatin. For this reason, basic amino acids are likely to participate in DNA-protein cross-linking. For identification of the thymine-lysine cross-link in nucleohistone, hydroxyl radical-induced cross-linking of thymine to lysine was investigated first in an aqueous mixture of thymine and lysine. Hydroxyl radicals were generated by exposure of this mixture to ionizing radiation after N_2O-saturation. The technique of gas chromatography-mass spectrometry (GC-MS) was used to analyze the samples for possible cross-links. One thymine-lysine cross-link was found and its structure was elucidated. Using GC-MS with selected-ion monitoring, this thymine-lysine cross-link was identified in acidic hydrolysates of calf thymus nucleohistone γ-irradiated in N_2O-saturated aqueous solution. The yield of this DNA-protein cross-link was also measured and found to be a linear function of radiation dose between 15 and 200 Gy. This yield amounted to 0.0085 $\mu mol \cdot J^{-1}$. Possible mechanisms for the formation of this DNA-protein cross-link in nucleohistone were proposed.

CB 606 DIRECT MEASUREMENT BY PULSED-FIELD GEL ELECTROPHORESIS OF REPAIR OF DOUBLE-STRAND BREAKS INDUCED BY IONIZING RADIATION, Peter J. Hahn and So Young Anh, Department of Radiology, State University of New York Health Science Center, Syracuse, NY 13210

Double strand breaks induced by ionizing radiation are thought to be the critical lesions responsible for cell death, although most of these breaks are thought to be repaired quickly. However, quantification of double strand breaks and their repair has been limited by technical considerations. We have developed a pulsed-field gel electrophoresis based system that allows direct measurement of DNA size distributions between 10 megabasepairs and .2 megabasepairs and used this to describe double-strand breakage and repair induced by 50 Gy ionizing irradiation. 50 Gy induced 5000 to 10000 breaks per cell which were repaired at a linear rate of 30 breaks repaired per minute per cell for the first 3 hours after irradiation.

CB 607 EFFECTS OF POLYAMINES AND THIOLS ON THE RADIATION SENSITIVITY OF BACTERIAL TRANSFORMING DNA, Kathryn D. Held and Samia Awad, Department of Radiation Medicine, Massachusetts General Hospital, Boston, MA 02114

The bacterial transforming DNA system allows one to irradiate purified DNA under conditions where the radiation chemical reactions occurring in the DNA can be controlled or predicted but the damage to the DNA can be assessed in terms of biological activity. Because polyamines are found in close proximity to DNA in cells, we questioned whether polyamines might modify the effects of ionizing radiation on DNA irradiated in the absence and presence of sulfhydryl-containing compounds. Under both oxygenated and hypoxic conditions the polyamines (spermine, spermidine, putrescine and cadaverine) are radio-protectors of transforming DNA activity, with the degree of protection increasing with polyamine concentration. When N_2-saturated solutions are irradiated, the degree of radioprotection by polyamines correlates with the number of amine groups per polyamine molecule, suggesting that the protection is due to stabilization of the DNA molecule by the polyamines. However, in O_2 the protection seems to be due largely to OH radical scavenging. With the exception of spermine, the polyamines are slightly more protective of oxygenated DNA than of hypoxic DNA. When DNA is irradiated in the presence of both polyamines and thiols, either dithiothreitol or WR1065, the combined protection is greater than that exhibited by either agent alone, but generally less than that predicted on the basis of the two agents acting independently and additively. The results suggest that DNA-bound polyamines reduce the ability of thiols to radioprotect DNA.

CB 608 DAMAGE OF DNA CONSTITUENTS BY HYDROGEN PEROXIDE / IRON COMPARED TO RADIATION,
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Division of Biochemistry and Molecular Biology, University of California, Berkeley, CA 94720.

Thymine- and guanine-containing DNA constituents have been exposed to either Fe2+/H2O2 or Fe3+/NADH/H2O2 Fenton reactions and their products studied by radioactivity, HPLC, TLC, MS, and UV spectra.

The products of dT are a subset of the dT radiation products identified by Teoule and Cadet [(1975) *Bull. Soc. Chim. France* 879-896] and dTMP results in a smaller subset of the Fenton-type dT products. 3'- and 5'-dTMP have the same set of degradation products. The 3' and 5' moiety of the dimer TpT are equally affected. The damages sustained by TMP, TpT, and oligo(dT) are similar. Under anaerobic conditions dTMP and TpT are more reactive, whereas dT is less so. Scavenging of Fenton-type radicals by ethanol inhibits dT degradation completely and dTMP degradation only partially. NADH/Fe^{3+}/H_2O_2 is equally effective as Fe^{2+}/H_2O_2 in damaging TpT, but less so for dTMP and dT.

Half the products observed by exposing dG to Fenton-type reactions were not observed by Berger and Cadet [(1985) *Z. Naturforsch.* 40b, 1519-1531] upon irradiation of dG; conversely most radiation products were not observed. dGMP and dG are equally reactive, but only half the products overlap. Some differences are apparent between the product spectrum of 3'-dGMP and 5'-dGMP. Under anaerobic conditions the reaction is not detectably different for dG, but qualitaive differences exist for dGMP. Scavenging of Fenton-type radicals by ethanol inhibits dG damage substantially and dGMP damage slightly.

Homogeneously distributed, scavengable radicals are apparently responsible for alterations of nucleosides by Fenton-type reactions. However with the nucleotides, the production of the damaging species is perturbed by interaction of iron with phosphate moieties. Hence the products differ significantly from those due to radiation.

Analogous studies of adenine and cytosine are in progress.

CB 609 QUANTITATION OF OXIDATIVE BASE DAMAGE IN DNA INDUCED BY MELANIN IN VITRO, Karen Hubbard-Smith and Helene Z. Hill, Section of Cancer Biology, Department of Radiology, NJ Medical School, Newark, NJ 07103. Melanomas are less sensitive to ionizing radiation than other tumors and it has been speculated that melanin may scavenge free radicals produced by ionizing radiation. In these studies, thymine glycol (TG), a major thymine radiolysis product, was measured in calf thymus DNA using a rabbit polyclonal anti-TG antibody in competitive ELISA assays. Frank strand breaks were measured by conversion of ØX-174 RF DNA from form I to form II. Eumelanin was synthesized by auto-oxidation of DOPA (DOPA-melanin, DM). Melanin at low concentrations reduced the level of thymine glycol in DNA irradiated with 200 Gy of gamma radiation. At high concentrations (> 200 ug/ml), melanin induced thymine glycol in both irradiated and unirradiated DNA. Histidine, a scavenger of hydroxyl radicals and singlet oxygen, reduced the production of thymine glycol by melanin in unirradiated DNA. D_2O, which stabilizes various oxygen species, increased the numbers of antibody-reactive sites induced by melanin. Melanin also produced single strand breaks in ØX-174 RF DNA. These were reduced when either histidine or tryptophan was included in DNA-eumelanin mixtures. These results suggest that melanin may have a significant role in modulating the level of active oxidative radical species in cells.
This work was supported by a grant from the NJ Commission on Cancer to HZH.

CB 610 IMMUNOCHEMICAL MEASUREMENT OF 8-HYDROXYPURINES IN X-IRRADIATED DNA, Hiroshi Ide*, Bi-Xing Chen#, Bernard F. Erlanger#, Grace Sterling* and Susan S. Wallace*, *Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT 05405, #Department of Microbiology, Columbia University College of Physicians and Surgeons, New York, NY 10032. 8-Hydroxyadenine (8HOA) and 8-hydroxyguanine (8HOG) are major purine lesions produced in DNA by ionizing radiation . Hydroxylation of C8 of guanine has also been reported to occur due to aerobic metabolism or oxygen radical-producing agents. As a first step in assessing the biological significance of the 8-hydroxypurines, we have developed a immunochemical assay to specifically and sensitively detect 8HOA and 8HOG in DNA. Antibodies were prepared by immunizing rabbits with corresponding 8-hydroxypurines conjugated to BSA. The specificity of the antibodies to 8HOA and 8HOG was examined by Ouchterlony gel diffusion and hapten inhibition of the antibody reactivity with the 8HOA- or 8HOG-RSA conjugate using ELISA. Antibodies were found to be highly specific for 8HOA and 8HOG. The IC50 of 8HOA and 8HOG was 8 and 0.15 uM, respectively. Preliminary data suggested that 8HOA in X-irradiated poly(dA) can be detected at least as low as 10 Gy. Results with different types of X-irradiated DNA will also be presented and discussed.

This work was supported by National Institutes of Health Grant CA 33657 awarded by the National Cancer Institute, Grant DE-FG02-87ER60510 from the U.S. Department of Energy, and a grant awarded by American Cancer Society, Vermont Division.

CB 611 INDUCTION AND REPAIR OF DNA DOUBLE STRAND BREAKS IN SYNCHRONOUS POPULATIONS OF CHO CELLS AS MEASURED BY PULSE FIELD GEL ELECTROPHORESIS.

George Iliakis, Lorenz Metzger and Orlando Cicilioni, Department of Radiation Oncology and Nuclear Medicine, Thomas Jefferson University, Philadelphia, PA 19107.

The Asymmetric Field Inversion Gel Electrophoresis (AFIGE) method developed by Denko et al (Analytical Biochemistry 178, 172-176, 1989) was used to study induction and repair of DNA dsb in irradiated populations of CHO cells in various phases of the cell cycle. The fraction of activity released from the agarose plug, where the cells were embedded, during the 40h of electrophoresis time was used as a parameter to measure the amount of DNA dsb induced. The method was calibrated by means of ^{125}IdUrd incorporation in phases of the cell cycle similar to those used for radiation experiments. The results obtained indicated considerable variation in the dose response in cells irradiated in various phases throughout the cycle; cells irradiated in G_1 or G_2 showed a larger release of DNA from the plug, per Gy, than cells irradiated in S. This effect may be due to a decrease in the mobility of partly replicated DNA and is similar to that previously reported for synchronous CHO cells using the non-unwinding filter elution technique (Okayasu et al 1988; Int. J. Radiat. Biol. 53, 729-747). Repair of radiation induced DNA dsb was found to be faster in S cells than in cells irradiated in other phases of the cell cycle. These results will be discussed *vis-a vis* results obtained under similar conditions using non-unwinding DNA filter elution.

CB 612 **RADIATION-INDUCES DNA DAMAGE AND REPAIR IN HUMAN LEUKOCYTES**, T.J. Jorgensen, L.R. Rivero, and J.M. Buatti, Department of Radiation Medicine, Georgetown University School of Medicine, Washington DC 20007. Despite observations of differential tumor induction in specific white cell subpopulations, there is little data on the possible differences in patterns of DNA damage or repair among these subpopulations of cells. Several studies have examined the effects of chemical carcinogens on the DNA from peripheral blood cells, but have required 25-50 ml of whole blood to look at a quite heterogeneous white cell population. We are developing a highly sensitive method to measure DNA strand breaks in different subpopulations of human leukocytes. Basically, the assay is the alkali unwinding assay with the modification of using radiolabeled probes to human sequences to measure human DNA in the presence of nonhuman DNA, both in single- and double-stranded DNA fractions following hydroxylapatite chromatography. Nonhuman carrier DNA is used to circumvent variability problems and enhance the sensitivity of the method. Quantitation is achieved by densitometry scanning of slot-blots hybridized with radiolabeled human-sequence-specific probe. Using this approach, radiation dose/response for strand breaks in freshly isolated human leukocytes has been performed, as well as measurements of repair rates following irradiation. Quantitation of strand breaks in the DNA from 300 cells was achieved and correlated with direct scintillation counting of ^{3}H-prelabeled DNA from 50,000 cultured cells treated in the same way. The techniques requires only 12,000 cells for a complete dose/response or repair curve. Concurrent with assay development, we are isolating leukocyte subpopulations by cell sorting (FACS). Sorted cells are mixed with nonhuman cells or carrier DNA for assay. This allows even rare populations to be assayed.

CB 613 PURIFICATION OF DNA FRAGMENTS CONTAINING EXCISION REPAIR PATCHES FROM HUMAN CELLS USING STREPTAVIDIN-BIOTIN, Guylaine E. Larone and Darel J. Hunting, MRC Group in the Radiation Sciences, Faculty of Medicine, The University of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4.

It has previously been shown that permeable human fibroblasts, when supplied with ATP and the four deoxyribonucleoside triphosphates, carry-out all the known steps in the DNA excision repair pathway and furthermore, incorporation of biotinated deoxyuridine monophosphate (biodUMP) in place of TMP has little effect on the repair process. We have used these findings to develop a method for purifying DNA fragments containing excision repair patches. Normal human fibroblasts were damaged with UV radiation, made permeable and allowed to perform repair synthesis in the presence of ATP, dGTP, dATP, 3H-dCTP, and biodUTP. The DNA was purified, sonicated to a number average length of 120 bp, then incubated with streptavidin, a protein which binds biotin very tightly (Kd 10-15) and has a density of 1.35 g/cc in CsTFA compared to 1.6 g/cc for DNA. (The calculated density of streptavidin complexed with a 120 bp DNA fragment is 1.45 g/cc). Isopycnic centrifugation in CsTFA resulted in the separation of the streptavidin-DNA complex from the free DNA with little or no dissociation of the complex. This method can be used with any DNA damaging agent which is repaired by the excision repair pathway(s), including ionizing radiation, and will be used to study the genomic distribution of repair events.

Supported by the MRC of Canada and the FRSQ.

CB 614 REACTION OF SNAKE VENOM PHOSPHODIESTERASE WITH OsO_4-TREATED DNA AND ^{32}P-POSTLABELING DETECTION OF THYMINE GLYCOLS, Michel Liuzzi and Malcolm C. Paterson, Cross Cancer Institute, Edmonton, Alberta T6G 1Z2, Canada.
Covalent formation of either a cyclobutane dimer or a (6-4) photoproduct between adjacent pyrimidines in DNA renders the phosphodiester bond 5′ to the UV-induced cross-link refractory to nucleolytic cleavage by snake venom phosphodiesterase (SVP). Complete resistance to hydrolysis is also observed for the sugar-phosphate bond immediately 5′ to an AP (apurinic/apyrimidinic) site. Hence, exhaustive digestion of depurinated or UV-treated DNA with SVP and calf alkaline phosphatase (CAP) yields lesion-containing oligonucleotides having the general formula d-NpL, where N represents a normal nucleoside and L a lesion. Since these molecules can be phosphorylated at their 5′-end by T4 polynucleotide kinase (PNK) in the presence of [γ-^{32}P]ATP, it is possible to quantitate UV photoproducts or AP sites at the femtomol level in unlabeled DNA. Here we have demonstrated that loss of aromaticity, brought about by saturation of the 5,6 double bond of thymine residues by their reaction with OsO_4, was sufficient to render the 5′-phosphodiester group adjacent to the so-formed thymine glycol resistant to the action of SVP. Similarly, the phosphodiester linkage immediately 3′ to the oxidized thymine residue proved to be refractory to nuclease P1 (NP1)-mediated hydrolysis. Accordingly, thymine glycols were quantitatively recovered in the dinucleoside monophosphates d-TGpN and d-NpTG after treatment with NP1 and SVP, respectively, and subsequent incubation with CAP. Co-digestion with all three enzymes generated free thymidine glycol. It was also observed that d-NpTG was phosphorylated by PNK at the same rate as the parent compound d-NpT, and that d-TGpN reacted at a much slower rate ($\geq$ 20-fold). Thus, digestion of thymine glycol-containing DNA with SVP and CAP, followed by enzymatic phosphorylation of the lesion-containing dinucleotides by PNK, sets the stage for the development of a sensitive ^{32}P-postlabeling assay for measurement of thymine glycols in human tissues at biologically relevant doses of ionizing radiation. It is anticipated that other single base modifications that result in loss of aromaticity, including 5,6-dihydrothymine and ring-fractured thymine residues such as urea, will also be quantitated by this ^{32}P-postlabeling technique.

CB 615 DNA LIGANDS AS RADIOMODIFIERS, Roger F. Martin, Lyn Denison, Glenn D'Cunha, Angela Haigh, Marshall Pardee and David P. Kelly. Molecular Sciences Group, Peter MacCallum Cancer Institute, Melbourne, and Department of Organic Chemistry, University of Melbourne, Australia.
DNA ligands have been synthesised and studied with a view to developing radiomodifiers for cancer radiotherapy. The ligands are all minor groove binding bibenzimidazole analogues, chosen because their sequence selectivity enables evaluation of the effects on DNA strand cleavage, by DNA sequencing gel analysis. One analogue protects V79 cells against ionizing radiation, with a dose modifying factor of 1.7 at a concentration of 20 micromolar. Sequencing gel analysis of experiments with plasmid DNA fragments show both 'global' protection against strand breakage as well as more pronounced protection at the ligand binding sites. There is some evidence suggesting that strand-cleavage that yields 3'-phosphorylglycolate terminii is preferentially protected. Another analogue is an iodine substituted ligand, inspired by the known radiosensitization by halogenation of bases in DNA. This ligand induces DNA strand-breaks upon irradiation of the DNA-ligand complex with UV-A. Sequencing gel analysis indicates that the strand breakage is mediated by formation of a carbon centred free radical on the ligand in the minor groove, and subsequent abstraction of a H-atom from the 5'- deoxyribosyl carbon on DNA. The ligand markedly sensitizes cells to killing by UV-A.

CB 616 PROPERTIES OF DNA-PROTEIN CROSSLINKS (DPC) INDUCED BY IONIZING RADIATION, Nancy L. Oleinick, Song-mao Chiu, Liang-yan Xue, Narayani Ramakrishnan, and Libby R. Friedman, Division of Biochemical Oncology, Department of Radiology, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.
DNA-protein cross-links induced by ionizing radiation have the following properties: (a) the yield is about the same as the yield of DNA double-strand breaks but is enhanced in hypoxic cells; (b) the formation of DPC preferentially involves a sub-set of DNA-binding proteins of the nuclear matrix and their attached DNA sequences; (c) the repair of DPC is slower than the rejoining of DNA strand breaks, occurs at a slower rate in glutathione-depleted cells, and is absent in mitotic cells. To further investigate the factors affecting DPC, we have irradiated V79 cell nuclei, nuclear matrices, and chromatin and measured the yield of DPC. Our results show that greater than 60% of the DNA can be induced to form DPC when isolated relaxed chromatin or when nuclei treated with $CuSO_4$ are irradiated in contrast to only 20% of the DNA when intact cells are irradiated. CsCl sedimentation or urea treatment of DPC reduced while Cu^{++} treatment increased the amount of matrix proteins bound to DNA. Based on these observations we conclude: (1) Chromatin as it exists in the intact cells is a poor substrate for DPC formation; (2) nuclear matrix DNA attachment sites may be centers of $OH^{\cdot}$ production catalyzed by trace metals; and (3) radiation-induced DPC may be a mixture of covalent and non-covalent associations of DNA and nuclear matrix proteins. (Supported by R01CA15378 and P30CA43703 from the NCI, DHHS).

CB 617 HETEROGENEITY IN RADIATION-INDUCED DNA DAMAGE AND REPAIR MEASURED USING THE "COMET ASSAY", Peggy L. Olive, Judit Banath and Ralph E. Durand, British Columbia Cancer Research Center, Vancouver, B.C., Canada V5Z 1L3.

The "comet assay", which measures DNA damage to individual cells, is based on the microelectrophoresis method described by Ostling and Johanson in 1984 and recently adapted for video image analysis in our laboratory. Cells embedded in agarose are lysed in 1 M NaCl,0.03 M NaOH then briefly subjected to an electric field, stained with propidium iodide and viewed using a fluorescence image processing system. Broken DNA migrates further in the electric field and the cell then resembles a "comet" with a brightly fluorescent head and a tail region which increases as damage increases. The ability to measure damage to individual cells allows us to address the question of heterogeneity in damage and repair - do some cells within a population show more damage or slower repair than others? Average damage and heterogeneity in DNA damage were measured in Chinese hamster V79 lung fibroblasts, WiDR human colon carcinoma cells, and in SCCVII mouse tumor cells and macrophages. The "tail moment", a function of the amount of DNA in the tail and the length of the tail, was the most informative feature of the comet image. The average response of the 4 cell types was similar over the range of 0-20 Gy, with sensitivity for detecting damage comparable to the alkali unwinding and DNA precipitation assays used in our laboratory. However, there was considerable heterogeneity in damage detected using this method; approximately 5% of cells exposed to 10 Gy appeared undamaged by our criteria. Similarly, average DNA repair kinetics ($t_{1/2}$ = 5 min at 37°C) were identical for tumor cells and macrophages but some cells within the population appeared to repair damage much more slowly than others. Differences in response of cells through the cell cycle did not appear to contribute directly to the observed heterogeneity in damage.

CB 618 MOLECULAR MECHANISMS OF RADIATION-INDUCED DNA DAMAGE: DIRECT IONIZATION, H-ABSTRACTION AND β-CLEAVAGE.

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Approximately 80% of the primary damage, direct and indirect, induced by ionizing radiation is centered on the bases of DNA. For this primary event to become lethal the radical from the base has to be transferred to the sugar, which eventually can lead to a double strand break.To investigate the transfer of the radical from the base to the sugar new parameters for AMBER were derived from properties of the radicals obtained from quantum mechanical calculations. Molecular dynamics simulations of radical damaged DNA show that introducing a radical on one of the bases of a 12-mer of double stranded DNA d(CGCGATATCGCG) causes a distortion around the point of damage. Such a distortion allows the base with the radical to move close to the deoxyribose and abstract the H_2' hydrogen atom from the sugar to yield a radical centered on deoxyribose. The presence of the radical on the sugar induces a β-cleavage that leads to a strand break. Quantum mechanical simulations of the strand breaking process show that the water molecule near the O_3' of deoxyribose assists in breaking the phosphodiester bond, thus indicating that the mechanism of strand breaking proceeds in a concerted fashion. In this mechanism water molecules that are bound to the DNA assist in the cleavage to yield directly a strand break. This differs from the commonly proposed mechanism of β-cleavage. Other mechanisms of DNA damage can be understood from the combined quantum mechanical and molecular mechanics simulations.

Supported by DOE grant DE-FG02-88ER60675.

CB 619 RADIATION CHEMISTRY OF HALOGENATED PYRIMIDINES: QUANTITATION OF HALOGEN RELEASE, R.O. Rahn, Department of Environmental Health, School of Public Health, University of Alabama at Birmingham, Birmingham, Alabama 35294. Of interest is the use of either specific ion probes or HPLC/electrochemical detection (HPLC/EC) for measuring the radiation-induced release of halogen from DNA substituted with either Iodouracil (IU) or Bromouracil (BU). Model system studies employing either monomers or polymers of IU or BU were undertaken. Specific ion probes allowed measurement down to 10^{-7}M I^- or 10^{-6}M Br^-. The more sensitive (5 to 10-fold) HPLC/EC method utilized a C_{18} column with an ion-pairing agent in the mobile phase and a silver electrode EC detector. Plots of halogen released as a function of concentration of starting material were used to estimate G values. In most cases, such plots did not give a clear indication of a plateau. At an initial concentration of 10^{-3}M, both BrU and IU as well as poly IU gave a G value of 5 for irradiation in air, while poly BrU gave a G value of approximately 3-fold less. The difference in yield between nitrogen and oxygen saturated solutions was 2-fold. Addition of stochiometric amounts of AMP or poly A to either of the homopolymers reduced their G value 2-fold. These results demonstrate the feasibility of using halogen-release measurements to monitor radiation-induced alterations arising from halogenated pyridimines.

CB 620 BASE DAMAGE : SENSITIVITY TO LOCAL ENVIRONMENT, James A. Raleigh, Department of Radiation Oncology, University of North Carolina, Chapel Hill, North Carolina 27599. The free radicals produced in aqueous environments by ionizing radiation are highly reactive and attack nucleic acids at all points. The result is a bewildering collection of molecular lesions at the sugar phosphate and base moieties. The situation is further complicated by the fact that the distribution of products is not fixed but dependent on factors such as target stereochemistry, charge distribution in the target, the presence of redox active agents such as oxygen and the nature of the ionizing radiation itself. While possible, it seems unlikely that any of the lesions are inherently unrepairable and hence uniquely threatening to an irradiated cell. They are, however, a record of free radical damage in cells. As such, selected lesions could be used as probes of cellular repair and of free radical events occurring in irradiated cells. In this context, an examination of the local environmental factors which affect the nature of base lesions will be presented.

CB 621 RADIATION-INDUCED DNA DAMAGE AS A FUNCTION OF THE DEGREE OF HYDRATION, Steven G. Swarts, Michael D. Sevilla and Kenneth T. Wheeler, Bowman Gray School of Medicine, Winston-Salem, NC 27103 and Oakland University, Rochester, MI 48309. It is generally accepted that the types and quantitites of DNA damage produced by direct and indirect action are important for our understanding of the biological effects of ionizing radiation. The types and quantity of radiation-induced DNA lesions can be influenced by the degree of DNA hydration. For example, previous studies have shown that radiation-induced DNA strand breaks and crosslinking depend on both the water content and the presence of oxygen (1). In the study reported here, we have used light scattering, HPLC and GC/MS techniques to examine the DNA lesions produced by ionizing radiation as a function of the water content of salmon sperm DNA (0, 8, 12, 20 and 28 water molecules per nucleotide). In agreement with Lett *et al.*, we found that the molecular weight and formation of DNA gels decreased as the water content and dose increased. Moreover, in our study, the predominant damage involved the release of unaltered DNA bases with little, if any evidence for the induction of base damage. Although it is clear that direct action mechanisms result in strand breaks, our preliminary results suggest that direct action mechanisms may not play a predominant role in the generation of DNA base damage. (Supported by grant #DE-FG05-86ER60404 from the Department of Energy)

1. Lett, J.T., Alexander, P. *Radiat. Res.* 15: 159, 1961.

CB 622 ENHANCEMENT OF TOPOISOMERASE I-MEDIATED UNWINDING OF SUPERCOILED DNA BY RADIOPROTECTANT AGENTS, Charles E. Swenberg, Eric A. Holwitt, L. S. Myers, Jr. and Colleen Loss, Radiation Biochemistry Department, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

The radioprotectant, WR33278, the disulfide of WR1065 (N-(2-mercaptoethyl)-1,3-diaminopropane) is shown to stimulate eukaryotic topoisomerase I unwinding of negative supercoiled DNA using plasmid PIBI30 isolated by alkaline lysis from E. coli. The electrophoretic ladder of isomers produced was similar to that resulting from spermidine in the presence of topoisomerase I. Increase in the concentration of WR33278 increased the degree of stimulation. The effects of the disulfide of WR3689 and the intercalating agent 1-methyl-2-(2-methylthio)-2-piperdinovinyl quinolinium iodide on topoisomerase I interaction with plasmid PIBI30 are discussed and both the interaction time course and drug concentration on DNA unwinding are presented. The potential importance of this process for in vivo radiation protection, enhanced DNA repair, and identification of critical DNA sites damaged by ionizing radiation is discussed briefly.

CB 623 IMMUNOCHEMICAL AND BIOCHEMICAL DETECTION OF SINGLE-STRAND BREAKS AND BASE-DAMAGE IN DNA OF HUMAN WHITE BLOODCELLS IN FULL BLOOD EXPOSED TO IONIZING RADIATION AT BIOLOGICALLY RELEVANT DOSES. Govert P. Van Der Schans, Adriaan A.W.M. Van Loon*, Arie J. Timmerman, Frans J.A. Kouwenberg, Roos H. Groenendijk and Robert A. Baan. TNO Medical Biological Laboratory, Rijswijk and *Laboratory for Radiation Genetics and Chemical Mutagenesis, Sylvius Laboratory, State University of Leiden, Netherlands.
Exposure of cells to ionizing radiation results in damage to the DNA. This damage comprises strand breaks and base modifications. It is important to quantify these various damages to get information about their relative contribution and persistency. In case of (accidentally) irradiated persons analysis of DNA damage of e.g., bloodcells could reveal exposure dose and recovery perspectives. To this purpose we are developing sensitive immunochemical and biochemical methods to quantify single-strand breaks, alkali-labile sites and base damages. The immunochemical method is based on the binding of a monoclonal antibody to single-stranded DNA. The technique is based upon the determination of the percentage single-strandedness resulting from the partial unwinding of cellular DNA under strictly controled alkaline conditions. Strand breaks and alkali-labile sites form initiation points for the unwinding. The extent of unwinding is a measure of the number of such sites. The results are compared with those obtained with "alkaline elution". Base damages can be quantified in a similar way when alkaline unwinding is preceeded by treatment of the DNA with damage-oriented endonucleases (i.c. a *Micrococcus luteus*-extract). With these methods single-strand breaks and base damages can be assayed down to doses as low as 0.5 Gy and 5 Gy, respectively.

CB 624 VARIATIONS IN DNA DOUBLE STRAND BREAK INDUCTION AS A FUNCTION OF CHROMATIN STRUCTURE. Raymond L. Warters and Bradley W. Lyons, Department of Radiology, University of Utah, Salt Lake City, UT 84132.

DNA double strand break (dsb) induction by 250 kV x-rays was measured in hamster cell DNA by pulsed-field-gel electrophoresis and polycarbonate filter elution at pH 7.2. DNA dsb formation was reduced in the presence of DMSO (78% maximum protection at 2 M, and half-maximum protection at 100 mM DMSO). DNA dsb were induced as a linear (i.e., single-hit) function of radiation dose; and were induced 8, and 3, times more efficiently in "naked" DNA, and DNA in fully relaxed chromatin (i.e., nuclei in 10 mM NaCl), than in whole cell DNA. The efficiency of DNA dsb induction in fully condensed chromatin (i.e., nuclei in 150 mM NaCl) was the same as in whole cell DNA. The results indicate that ionizing radiation-induced DNA dsb result from radical (probably hydroxyl radical) attack on the DNA fiber. The association of histone proteins (nucleosomes) with DNA may reduce dsb induction by a factor of as much as 3. Condensation of the chromatin fiber into a higher order (solenoid) structure may protect DNA by an additional factor of 2.5. Thus organization of DNA into chromatin structure at both the nucleosome and higher-order-coiling levels appears to provide substantial protection against radical attack and DNA dsb formation. (This work was supported by NCI grants No. CA 25957 and CA 45154).

CB 625 POSTLABELLING ASSAY FOR DAMAGE TO γ-IRRADIATED DNA, Michael Weinfeld, Krista-June Soderlind and Malcolm C. Paterson, Departments of Radiobiology and Molecular Genetics and Carcinogenesis, Cross Cancer Institute, Edmonton, Alberta, Canada T6G 1Z2
We have developed a sensitive assay for damage to γ-irradiated DNA on the basis that certain lesions, including apurinic sites and saturated pyrimidines and their fragmentation products, render the adjacent 5'-phosphodiester bonds refractory to DNase I and snake venom phosphodiesterase. In the protocol, irradiated DNA is completely digested by these nucleases together with calf alkaline phosphatase. After removal of the protein, lesion-containing "dinucleoside" monophosphates, are end-labelled by incubation with polynucleotide kinase and γ -^{32}P-ATP. (This approach has the advantage that the damage-containing DNA fragments are good substrates for polynucleotide kinase because the nucleoside at the 5'-end of the molecules is unmodified.) The radiolabelled products are then isolated by polyacrylamide gel electrophoresis. Using this technique, we have detected at least five distinct products from DNA irradiated in vitro with 5 Gy of ^{60}Co γ-radiation and these are being further analysed by HPLC. A modified strategy which focuses on apurinic sites has also been developed. It is intended that this latter assay be combined with damage-specific DNA glycosylases to more specifically quantitate particular lesions. Since these assays can detect the lesions at fmol levels, it is anticipated that DNA damage and repair can be quantified in cells treated with biologically relevant radiation doses.

CB 700 GENETIC CHANGES IN MAMMALIAN CELLS TRANSFORMED BY RADIATION, M. Durante and G. Grossi, University of Naples, Italy, and Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland; T.C. Yang and R. Roots, Cell and Molecular Biology Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720. Midterm Syrian hamster embryo (SHE) cell (secondary passage) were employed in our studies of cells transformed by radiation to become neoplastic. We are using three criteria to evaluate possible changes in selected genes: (1) Detection of genetic translocations or other abnormalities by gene sublocalization in metaphase chromosome spreads, (2) Restriction fragment length (RFL) abnormalities, and (3) Alterations in gene expression. Transformants were selected after clonal proliferation in soft agar. Subsequently, neoplasia tests in nude mice were done. Transformants were followed by subculturing for prolonged periods post the initial isolation in soft agar. Specific chromosome abnormalities are often associated with specific types of neoplasia. Often suppressor genes and/or protooncogenes are characteristically involved in these chromosomal changes. To gain knowledge at the molecular level, we are collecting data from a number of independently isolated transformed cells in order to assess if a general (or universal) phenomenon exists for the radiation-induced neoplastic SHE cells. We have detected Pst-1 RFL differences and mRNA differences in the c-myc gene locus only well into the progression process after the initial isolation. However, our initial work is on detection of karyotypic changes using selected genetic probes to identify changes in the banding pattern(s) of the chromosomes. A gene which is identified as altered will then be examined further for changes in DNA sequences and mRNA expression. Our preliminary data show karyotypic changes in the chromosome C series at the onset of neoplasia.

CB 701 ARE RADIATION-INDUCED DNA DOUBLE-STRAND BREAKS RELEVANT FOR CELL TRANSFORMATION? Marlis Frankenberg-Schwager, Dieter Frankenberg and Roswitha Harbich, Gesellschaft für Strahlen-und Umweltforschung mbH, Paul-Ehrlich Str.20, D-6000 Frankfurt 70, FRG.

Densely ionizing radiation has a higher relative biological efficiency (RBE) at inducing both double-strand breaks (dsb) in cellular DNA and cellular transformation. These findings point to a possible involvement of dsb in cell transformation.
Further support for this may be obtained by comparison of the oxygen enhancement ratios (OER) for dsb and cell transformation. An OER-value of about 3 for dsb induction has been found in various eukaryotic cell systems. We have determined the OER for cell transformation. For this purpose, inactivation and transformation of mouse embryo fibroblast C3H 10T1/2 cells irradiated with 60-Co-gamma rays under oxic and anoxic conditions was measured. An OER-value of about 3 was found for both cell inactivation and transformation.

Thus, the DNA dsb is the only molecular lesion known so far whose RBE- and OER-values are in agreement with the corresponding values observed for cell transformation.

CB 702 SISTER CHROMATID EXCHANGE AND IN VITRO DNA REPAIR SYNTHESIS IN DOWN'S SYNDROME, Surabhi Kakati, Robert E. Drury and Mark R. Kielma, Roswell Park Memorial Institute, Buffalo, NY 14263

Certain genetic conditions which predispose individuals to cancer are known to have an increased rate of spontaneous or induced chromosomal damage and/or sister chromatid exchange (SCE). Quite often these are associated with a deficiency in DNA repair synthesis.

In Down's syndrome (DS), a genetic disease, the incidence of acute leukemia is twenty fold higher than in the normal population. We have observed that the rate of SCE in the lymphocytes of DS patients is not different from normal individuals. However, the rate of mitomycin C (MMC) induced SCE is significantly higher than that of normal individuals. The possibility arises that the observed increase in the number of SCE's may be due to cells deficient in DNA repair synthesis (DRS). We have examined DRS using the autoradiographic technique for unscheduled DNA synthesis (UDS). However, no significant difference in the amount of DNA repair synthesis induced by MMC was observed between the DS and normal individuals. This indicates that the high rate of induced SCE in DS is not due to deficiency in DRS.

CB 703 DNA TOPOISOMERASE II, REPLICON INITIATION AND RADIATION SENSITIVITY. William Kaufmann, Jayne Boyer and Laurel Estabrooks, Curricula in Genetics and Toxicology, University of North Carolina at Chapel Hill.
Defective regulation of replicon initiation after radiation exposures characterizes cells from patients with ataxia telangiectasia (AT) and AT cells are hypersensitive to radiation-induced chromosomal aberrations. DNA topoisomerase II (Topo-II) appears to play a role in DNA replication and has been implicated in the pathophysiology of AT. Incubation of human fibroblasts with 10 uM etoposide or 1 uM amsacrine inhibited Topo II activity and produced DNA strand breaks as monitored by alkaline elution chromatography. Removal of the inhibitors led to reversal of the breaks within 30 min. By this time the rate of replicon initiation in treated cells was less than 50% of control. The pathway which down-regulates replicon initiation rate appears, therefore, to be sensitive to the reversible interruption of Topo-II activity. To test whether AT cells fail to down-regulate replicon initiation because of a deficiency in Topo-II activity, we compared Topo-II mediated strand breakage in normal and AT lymphoblastoid cells. Incubation of AT cells with amsacrine and etoposide produced the same levels of DNA breaks as were seen in normal lymphoblastoid cells and diploid fibroblasts. This result implies that the basal levels of Topo-II that can form cleavable complexes with DNA are normal in AT cells. Supported by PHS grants GM07092 and CA42765.

CB 704 PROTO-ONCOGENE EXPRESSION IN PLUTONIUM-INDUCED LUNG CARCINOMAS. G. Kelly, P.R. Kerkof, P.J. Haley, and F.F. Hahn, Lovelace Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque NM 87185. We examined the expression of a number of proto-oncogenes in radiation-induced lung carcinomas to: 1) establish patterns of proto-oncogene expression in radiation-induced lung tumors; 2) correlate these patterns of gene expression with radiation dose delivered to the lung and/or morphological tumor type and 3) characterize those proto-oncogenes demonstrating a tumor associated pattern of expression. Primary pulmonary carcinomas used in these experiments were induced in Beagle dogs following the inhalation of aerosols of $^{239}PuO_2$. The relative levels of 14 proto-oncogenes in 11 radiation-induced and 3 spontaneously-occurring carcinomas of the Beagle dog lung were examined. The pattern of proto-oncogene expression that emerges from this analysis suggests that expression of a member of the ras family of proto-oncogenes, c-myc and one of a number of proto-oncogenes encoding receptor tyrosine kinases is necessary for the histogenesis of these lung carcinomas. An analysis of the c-myc gene and gene transcripts has shown that two of the tumors expressing high levels of c-myc contain transcripts whose size differs from that of the normal canine c-myc transcript, suggesting that a rearrangement mechanism may be responsible for the elevated levels of c-myc RNA. Sponsored by the U.S. Dept. of Energy O.H.E.R. Contract No. DE-AC04-76EV01013.

CB 705 RADIOMIMETIC EFFECTS OF SV40 T ANTIGEN IN HUMAN DIPLOID FIBROBLASTS. F.A. Ray, P.M. Kraemer, D. Peabody, L.S. Cram, C.L. Goolsby, Los Alamos National Laboratory, Los Alamos, NM 87545
To define the role of SV40 large T antigen in the neoplastic transformation of human diploid fibroblasts, we have used a minimal plasmid construct containing the large T sequences. Unlike widely used constructs such as pSV3 neo, our plasmid lacks unique coding sequences of small t antigen as well as any SV40 promoters, SV40 origins of replication or SV40 late genes. The plasmid was co-electroporated into normal human diploid fibroblasts along with a plasmid containing the neomycin resistance gene. T antigen positive clones, characterized immediately after expansion were not immortalized, transformed, or tumorigenic. However, these cells were grossly unstable cytogenetically and suffered enhanced rates of cell death. These lineages gradually acquired neoplastic properties upon prolonged serial passage. We suggest that the primary role of T antigen in neoplastic transformation is to produce ongoing mutagenicity and the continual generation of new genetic variants subject to *in vitro* and *in vivo* selection.

CB 706 RADIO-IMMUNOTHERAPY WITH ALPHA PARTICLE EMITTING IMMUNOCONJUGATES

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Short lived isotopes that emit alpha particles have a number of physical characteristics which make them attractive candidates for radio-immunotherapy. Among these characteristics are high linear energy transfer, high cytotoxicity, short path length, low potential for damage repair and low dependence on dose rate and oxygen enhancement effects. We have constructed immunoconjugates incorporating the alpha particle emitter Bismuth-212 and have assayed the ability of these radio-immunoconjugates to kill antigen-positive cells. Data derived from limiting dilution clonogenic assays and flow cytometric analysis suggest that very few alpha tracks through the cell nucleus are required for clonogenic inactivation. The efficacy of these immunconjugates is dependent on antigen expression, immunoconjugate stability, target cell binding kinetics, and isotope internalization behavior. These immunoconjugates are capable of antigen-specific cure of mice inoculated with malignant ascites. Alpha particle emitting radio-immunoconjuates show great potential for regional and intracavitary targeted radiotherapy.

CB 707 NEUROBLASTOMA: A STUDY OF RADIOSENSITIVE HUMAN TUMOUR CELLS,

T.J.McMillan, J.Eady, A.Holmes, J.Peacock and G.G.Steel. Radiotherapy Research Unit, Institute of Cancer Research, Sutton, Surrey, U.K.

Neuroblastomas are radioresponsive tumours and this is reflected by the high sensitivity to ionizing radiation of cell lines derived from these tumours. Each of the two cell lines examined in this study, HX142 and HX138, demonstrate a sensitivity to ionizing radiation which is comparable to that of transformed Ataxia telangiectasia (A.T.) fibroblasts.
These cells do not exhibit a reduced level of cellular recovery in split-dose or low dose-rate experiments unlike A.T. cells. In addition they show an inhibition of DNA synthesis following irradiation which is comparable to resistant normal cells rather than A.T. fibroblasts. Repair capacity has been assessed using the viral reactivation assay. The level of reactivation for the two neuroblastoma cell lines was found to be in the middle of the range seen for resistant tumour cell lines. This suggests that they do not have a significant repair defect.
Both lines show a higher than average level of DNA double-strand breaks immediately after irradiation, however the extent of this is not sufficient to fully explain the sensitivity of these cells. It therefore seems that the sensitivity may be related to a high level of damage induced in the DNA by the radiation but that some as yet undetermined processes increase the conversion of this damage into lethal events.

CB 708 GAMMA RAY-INDUCED DNA DAMAGE OF HUMAN CELL HYBRIDS ASSOCIATED WITH NEOPLASTIC TRANSFORMATION ASSESSED BY RFLPS OF HUMAN FIBRO-

BLAST CHROMOSOME 11, Marc S. Mendonca[1], Eric J. Stanbridge[2], and J. Leslie Redpath[3], Dept. of Radiological Sciences[1,3], Dept. of Microbiology & Molecular Genetics[2], University of California, Irvine, CA 92717. Restriction fragment length polymorphism (RFLP) analysis and karyotyping of spontaneous tumorigenic segregants of nontumorigenic (HeLa X Human Skin Fibroblast) hybrids have implicated the loss of one copy of human fibroblast chromosome 11 with re-expression of a cell surface protein (p75/150) and tumorigenicity (1,2). Introduction of skin fibroblast chromosome 11 by microcell transfer into HeLa cells or into tumorigenic hybrids results in a suppression of tumorigenicity (3). These observations suggest that it is the loss of a functional tumor suppressor gene(s) located on chromosome 11 which may be responsible for malignancy in this system (1-3). We have been isolating gamma ray-induced p75/150 expressing mutants after a 21 day expression period. Preliminary RFLP analysis of these mutants with a chromosome 11-specific c-H-ras gene probe indicate that loss of chromosome 11 specific markers is also associated with p75/150 re-expression and tumorigenicity. We are proposing that in this system radiation-induced neoplastic transformation is a result of inactivation or loss of a tumor suppressor gene. Through continued analysis of radiation-induced tumorigenic cell lines we hope to be able to better localize the deleted region on chromosome 11 in which this gene(s) lies. This work was supported by NIH Grant CA 39312 (JLR & EJS) and CA 19401(EJS), and the Phi Beta Psi Sorority.

1. Stanbridge et al.,Somatic Cell Genetics 7: 699-712 (1981)
2. Srivatsan et al. Cancer Research 46: 6174-6179 (1986)
3. Saxon et al. EMBO J. 5: 3461-3466 (1986)

CB 709 EVIDENCE FOR INCREASED INITIAL DNA AND CHROMOSOME DAMAGE AND DEFECTIVE G2 REPAIR IN ATAXIA TELANGIECTASIA (AT) CELLS, Tej K. Pandita, Ken Pinkston and Walter N. Hittelman, Dept. of Medical Oncology, Univ. of Texas M.D. Anderson Cancer Center, Houston,Texas 77030, USA. Ataxia Telangiectasia cells exhibit increased sensitivity to ionizing radiation, bleomycin, and VP 16 resulting in increased chromosome aberrations at mitosis. To better understand the basis of this sensitivity, G1 and G2 phase cells from three AT lymphoblastoid cell lines obtained by centrifugal elutriation were examined for the initial amount and rate of repair of DNA double strand breaks (DSB's)(neutral filter elution) and chromosome damage (premature chromosome condensation technique) after X-irradiation. AT cells showed slightly increased levels of initial DNA DSB's compared to normal lymphoblastoid cultures in G1, S, and G2 phase. In contrast, G1 and G2 phase AT cells showed 2-3 fold higher initial chromosome aberration frequencies than normal controls. This would suggest that DSB's are translated into chromosome damage with higher efficiency in AT cells compared to normal cells. All three AT cell lines exhibited decreased rates of DNA repair,responsible in most part by a profoundly decreased slow component of DNA repair in G2 phase. Similarly, G1 AT cells repaired chromosome breaks at a similar rate to normal G1 cells, whereas G2 AT cells had a marked deficiency in chromosome repair. These observations suggest that a higher rate of initial chromosome damage and a decreased rate of DNA and chromosome repair in G2 cells both contribute to the sensitivity of AT cells.

CB 710 ALTERATION OF DNA REPAIR AND CHROMOSOMAL RADIOSENSITIVITY OF LYMPHOCYTES FROM BREAST CANCER PATIENTS TREATED WITH RADIOTHERAPY AND CHEMOTHERAPY. Odile Rigaud, Geneviève Guedeney, Isabelle Duranton*, Marie-Thérèse Doloy, Henri Magdelénat*, Commissariat à l'Energie Atomique, D.P.S., Fontenay aux Roses, *Institut Curie, Paris, France.

The aim of this study was to investigate the genotoxic effects related to medical treatment with DNA damaging agents such as irradiation and cytoxic drugs on peripheral blood lymphocytes(PBL)from breast cancer patients. Fourty-three patients were examined before and after either local radiotherapy or chemotherapy or combined treatments. First, we showed that the local cobaltotherapy induced unstable chromosome aberrations (CA)in PBL whereas no CA were found after chemotherapy. Second, DNA repair and chromosomal radiosensitivity were assessed after a subsequent in vitro irradiation of lymphocytes collected on patients before and after treatment. These two indicators of radiosensitivity were compared to those measured for healthy donors using Student's t-test. Patients tested before treatment had a decreased DNA repair efficiency but their chromosomal radiosensitivity was not altered. When tested after treatment, both DNA repair and in vitro radiation-induced CA were modified according to the kind of treatment. As compared to paired-tested normal controls, DNA repair was altered after radiotherapy but not after chemotherapy. The modifications of in vitro induced dicentrics were not similar to those of acentrics after a given treatment. Interindividual variations were observed for both indicators of radiosensitivity tested by these two assays. These results provide evidence for a genotoxic effect of radiotherapy or chemotherapy on PBL from breast cancer patients. The possibility that these assays should be used for predicting the individual sensitivity to radiation or chemotherapeutic drugs exposure is discussed.

This study was supported by Research Contracts of the Radiation Program of the European Communities (B160065F) and of the Ministère de la Recherche(88C0594).

CB 711 RADIATION-INDUCED ACTIVATION OF THE C-*MYC* ONCOGENE AND A NOVEL TRANSFORMING GENE IN MOUSE C3H10T1/2 CELLS, Mary Jean Sawey and Ann R. Kennedy, Department of Radiation Oncology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

It has recently been shown that both the *myc* and *ras* oncogenes are activated in primary rat skin tumors induced by radiation (Sawey et al., 1987). To further characterize the molecular alterations involved in radiation induced transformation and tumorigenesis a series of *in vitro* radiation transformation experiments were carried out. C3H10T1/2 cells were irradiated with 600 rads X-irradiation and transformed foci resulting from a single irradiated cell were obtained. DNA from the radiation transformed cells were analyzed for the presence of transforming genes (oncogenes) in the NIH3T3 transfection assay. The DNA from Type III foci tested positive in the transfection assay (0.014 foci/ug DNA) whereas DNA from irradiated control, spontaneously transformed (Type II), and Type II radiation transformed 10T1/2 cells, were all negative. Secondary transfectants from a Type III transformant were obtained in Rat 2 cells. The resulting transfectant cells exhibited anchorage independent growth and formed tumors when injected into athymic mice. Southern analysis of transfectant DNA demonstrated that the transforming gene was not from the *ras* gene family and appears to be a novel transforming gene. Southern analysis of rat transfectant DNA with a mouse repetitive probe (ß1) indicated the presence of a mouse specific band (2.5kb) that may represent the putative transforming gene. We are currently carrying out experiments directed at cloning and sequencing this gene. Southern analysis of DNA from the original radiation-induced transformants, using a series of oncogene probes, demonstrated a c-*myc* oncogene rearrangement in the Type III transformants. Molecular analysis is being carried out on cells from various time points during the progression from the normal to the "initiated" to the transformed phenotype, in an attempt to pinpoint at what stage the critical "transforming' lesion is introduced and expressed in the radiation transformed C3H10T1/2 cells.

CB 712 DNA AND CELLULAR REPAIR CAPACITY OF HEMATOPOIETIC PROGENITORS DURING CHRONIC RADIATION-INDUCED LEUKEMOGENESIS, Thomas Seed, Lillian Kaspar, and David Grdina, Biological and Medical Research Division, Argonne National Laboratory, Argonne, IL 60439. Hematopoietic progenitors (GM-CFU) from preleukemic dogs under chronic whole-body gamma irradiation (7.5 cGy/day) exhibit marked clonal and functional changes with both time and preclinical phase of evolving disease. A critical early occurring preclinical event has been identified and involves a coupling by GM-CFU of acquired radioresistance and renewed, but aberrant, proliferative capacity. Currently, the molecular and cellular bases of this critical preclinical event are being evaluated. DNA damage and repair functions are being assessed by using both alkaline elution and unscheduled DNA synthesis assays, while complementary cellular repair functions are being evaluated by split-dose and variable-dose assays. Results indicate that a) the inherent radiosensitivity of the preleukemic progenitor's DNA [in terms of single strand break (SSB) production] remains essentially unchanged during the occurrence of this critical progenitor event; b) the capacity to repair induced SSB's within its DNA (slow repair component) is substantially increased by ~30%; and c) its cellular repair capacity (measured in terms of recoverability following low dose- or split-dose type irradiations), is not only quantitatively enhanced, but modified in terms of its pattern of expression. These results continue to support the concept that these progenitor cell acquisitions are critical in the early stages of chronic radiation leukemogenesis and serve to 'set-the-stage' for subsequent late clonal events. (Work supported by USDOE #W-31-109-ENG-38.)